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THE EFFECTS OF MICRO- AND NANOHYDROXYAPATITE APPLICATION IN METAL CONTAMINATED SOIL ON METAL ACCCUMULATION IN IPOMOEA AQUATICA AND SOIL METAL BIOAVAILABILITY

Azlan Kamari [1,2], Norjan Yusof [3], Che Fauziah Ishak [4], Esther Phillip [5], Galuh Yuliani [6]

[1] *Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjong Malim, Perak, Malaysia*
[2] *Nanotechnology Research Centre, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjong Malim, Perak, Malaysia*
[3] *Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjong Malim, Perak, Malaysia*
[4] *Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*
[5] *Malaysian Nuclear Agency, 43000, Kajang, Selangor, Malaysia*
[6] *Department of Chemistry, Universitas Pendidikan Indonesia, Jl. Dr. Setiabudi 229, Bandung, Indonesia*

Abstract

The potential of microhydroxyapatite (MHA) and nanohydroxyapatite (NHA) to immobilise heavy metals in a 25-year old active firing range soil was investigated. The effects of immobilisation were evaluated in terms of metal accumulation in water spinach (*Ipomoea aquatica*) and soil metal bioavailability. A pot trial was conducted by amending firing range soil with MHA and NHA at application rates of 0%, 1% and 3% (w/w). Both amendments increased biomass yield and reduced plant metal uptake. The bioconcentration factor (BCF) values of the metals were in the order of Zn > Cu > Pb. The bioavailable fraction of Cu, Pb and Zn in firing range soil decreased significantly ($p < 0.05$) following MHA and NHA treatments. No toxicity symptoms were observed in water spinach over the pot trial. Therefore, MHA and NHA are two promising immobilising agents for the remediation of metal contaminated land.

Key words: Contaminated soil, heavy metal, soil stabilisation, water spinach, microhydroxyapatite, nanohydroxyapatite

INTRODUCTION

Soil contamination by heavy metals is a serious environmental problem all over the world [1]. For example, about 3.5 million sites of industrial and mine sites, landfills, energy production plants and agricultural land in Europe were reported to be contaminated by heavy metals [2]. In fact, soil contamination and strategy for soil protection were classified as two important issues for action in the European Community [2]. Meanwhile, 20 million hectares of arable land, accounting 20% of the total agricultural land area in China have been identified for heavy metal contamination [3]. In recent years, the concentration of heavy metals in soil has increased tremendously due to rapid global industrialisation. Waste emissions from industrial production, mining activities, biosolids and manure application, wastewater irrigation, and inadequate management of pesticides and chemicals in agricultural production have significantly contaminated soil and groundwater [4]. The level of heavy metals in soil has also influenced by firing range activities. Firing bullets are mainly composed of Pb-alloy slugs enclosed with Cu-alloy jackets [5,6]. Metal particulates originating from multiple impacts of bullet fragments during range operations can be oxidised and transformed into compounds that can be mobilised in soil environment [7-9]. An analysis on metal concentration in military firing range soil collected from Busan Metropolitan City, Korea by Moon et al. [6] found 11,885 mg/kg Pb. Parra et al. [10] measured 9,600 mg/kg Pb in topsoil samples collected from a firing range in New Mexico, USA. Meanwhile, firing range soil of the Small Arms Training Area, Aiken, USA was reported to contain 3,282 mg/kg Pb and 1,762 mg/kg Cu [11].

Toxic metals are not biodegradable and persist for a long period of time in soils. They are not only harmful to ecosystems and agricultural production, but also a serious threat to human wellbeing. Their presence in soil may pose a great risk to food chain and water supplies. Considerable efforts have been made to remediate metal contaminated soils. There are many techniques available for the remediation of metal contaminated soils, such as mechanical separation, solidification, soil washing, heap leaching, soil flushing and electrokinetic [12,13]. However, many of these techniques are costly and not practical to implement. Soil stabilisation is a cost-effective and promising soil remediation technique, and has been extensively used in immobilisation of heavy metals in contaminated soils [14]. This technique relies on application of the soil amendments to help retain metals in the stable solid phase by sorption, precipitation, complexation, ion exchange or redox process, thereby decreasing mobility and bioavailability of metals [14,15].

Liang et al. [14] studied the effects of biochar and phosphate application on Cd leachibility from a contaminated soil. The Cd concentration in TCLP (toxicity characteristics leaching procedure) extract was reported to reduced by 19.6% and 13.7%, respectively. They also reported that the concentration of Cd in the groundwater was reduced by up to 62.7%. The immobilisation of Pb and Zn in a contaminated soil using water treatment sludge, blast furnace slag and red mud was assessed by Zhou et al. [16]. Amending contaminated soil using the three amendments was reported to reduce $CaCl_2$, CH_3COOH, HCl and EDTA-extractable Pb and Zn significantly. The Pb and Zn uptake by Rhodes grass was found to decrease with amendments application. A significant immobilising effect was reported for 10% (w/w) treatment. Fang et al. [17] has shown that phosphate rock tailing and triple superphosphate fertilizer were able to reduce $CaCl_2$-extractable Pb and Zn by 55.2-73.1% and 14.3-33.6%, respectively.

The overall aim of this work was to evaluate the potential of microhydroxyapatite (MHA) and nanohydroxyapatite (NHA) as immobilising agents for the remediation of metal contaminated soil.

RESEARCH METHOD

In this study, the soil sample was collected from a 25-year old active firing range in Selangor, Malaysia. The soil samples were taken at the surface layer of up to 25 cm depth using a stainless steel trowel. The samples were air-dried for 1 week, thoroughly mixed and passed through a 2 mm mesh sieve. The soil consists of sand (45%), clay (36%) and silt (19%). The soil pH measured in deionised water with a soil:solution ratio of 1:2.5 using a pH meter, was 4.82. The total Cu, Pb and Zn concentrations in the soil determined by aqua regia extraction were 725, 2337 and 364 mg/kg, respectively. In addition to the total fraction, the bioavailable fraction of metals in soil was also determined using ammonium acetate (1.0 mol/L, pH 7) at a soil:extractant ratio of 1:10. The ammonium acetate extractable Cu, Pb and Zn were 318, 1066 and 135 mg/kg, respectively.

MHA and NHA (purity > 97%) were purchased from Sigma-Aldrich. Pots with a diameter of 15.0 cm and a height of 18.0 cm were filled with 400 g of soil. MHA and NHA were added to the soil at 0%, 1% and 3% (w/w), in six replicates. The soils were left to equilibrate for two weeks. As the soil has a poor plant nutrient content, ¼ strength Hoagland's nutrient was added to each pot thrice a week at application rate of 20 mL. The Hoagland's nutrient solution was applied to the soils for two weeks only (equilibration period). The addition of nutrient solution was discontinued when the pot experiment began.

After two weeks, each pot was tipped out and remixed to ensure homogeneity and to prevent the soil samples from becoming anaerobic. A pot experiment was carried out for 8 weeks. Water spinach (*Ipomoea aquatica*) seed was sown two weeks after addition of amendments. The pots were arranged in a randomised block design. The water content of the soils was adjusted to obtain 70% of the water holding capacity by adding deionised water daily, avoiding prolonged water logging. Plants were allowed to grow under natural lighting and temperature. Mean daily temperature and humidity were monitored with a digital thermometer. At the end of the pot experiment, the soil pH and ammonium acetate extractable metal content in the soil were determined, as previously described.

The plants were harvested at 8 weeks of growth. The aerial parts were cut at 1.0 cm above the soil surface to avoid contamination by soil using a pair of scissors, which was wiped after each use. Roots were carefully extracted from the soil and washed thoroughly with deionized water to remove soil particles. Plant tissues were washed thoroughly with deionised water and dried in an oven at 70 ºC for 48 h. After two days, the dry weight of plant tissue was measured. Dried shoots and roots were milled using a grinder. Milled samples were ashed at 450 ºC for 3 h in a furnace and digested in hot concentrated HNO_3. Metal concentrations in the plant digests and soil extracts were measured by flame atomic absorption spectrometry (AAS).

Standard reference plant materials (SRM 1573a Tomato Leaves – National Institute of Standards & Technology, USA, and SRM 1575 Pine Needles – National Bureau of Standards, USA) and certified reference soil material (LGC 6135 Hackney Brick Works Soil – Laboratory of the Government Chemist, UK) were used to verify the accuracy of metal determination. Reference materials were treated and analysed using the same procedures applied for plant tissue and soil samples. The recovery rates were within 90-106% for soil and 86-95% for plant tissue, respectively.

All statistical analyses were performed using Minitab 15 Statistical Software (Minitab Inc., PA, USA). The data were analysed using the general linear model of one-way analysis of variance (ANOVA), followed by Tukey's test at a significance level of $p = 0.05$ to determine least significant difference (LSD) for the comparison of means. Correlation was by Pearson's coefficients at $p < 0.05$.

RESULTS AND DISCUSSION

Plant Growth. The water spinach seeds germinated four days after sowing and no obvious difference in plant growth was observed up to two weeks of the pot experiment. Water spinach grown on compost (uncontaminated soil) were observed to be healthier than plants cultivated on untreated contaminated soil. MHA and NHA treatments resulted in healthy appearance on the plant leaves, whereby the leaves were greener as compared to plants grown on zero treatment (untreated) contaminated soil. Table 1 presents the dry biomass yield of water spinach after 8 weeks of growth. From Table 1, it is clear that the shoot and root yields increased with the rates of amendment application. A pronounced effect was obtained for NHA treatment at 3% (w/w), of which the shoot yield for this treatment was found to be higher than zero treatment by a factor of 3.0. MHA 3% and NHA 1% (w/w) treatments gave almost similar shoot yield, with 178-186% increment in biomass production. The highest percentage of increment in root yield was achieved with NHA 3% (w/w) treatment, followed by application of NHA at 1% (w/w). Although there was an increase in the root yield following application of the MHA, statistical analysis revealed no significant difference was obtained between the MHA 1% and MHA 3% (w/w) treatments.

Table 1. Biomass yield of water spinach.

Treatment	Dry weight (g/pot)	
	Shoot	Root
Compost*	13.26	4.51
Zero	3.38 a	1.49 a
MHA 1%	4.68 b	2.14 b
MHA 3%	6.02 c	2.77 b
NHA 1%	6.27 c	2.95 c
NHA 3%	10.16 d	3.53 d
LSD	1.01	0.59

* Plants grown on compost only (uncontaminated soil). Values represent mean of 6 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

MHA and NHA were beneficial as growing media through improvement of soil fertility and provision of plant nutrient. Hydroxyapatite (HA) is a naturally occuring mineral form of calcium apatite with the formula $Ca_{10}(PO_4)_6(OH)_2$. It is an important material in the manufacture of fertiliser, as a source of phosphorus [18]. Due to its role as a plant nutrient provider, HA has been regarded as one of the key materials in agrochemicals formulations. In general, NHA amendment has resulted in higher biomass yield than MHA. This scenario can be related to the particle size of the HA used. The particles sizes of MHA and NHA are 3 µm and 40 nm, respectively. The smaller size of NHA accelerates the rate of degradation process. And therefore, release the phosphorus to soil-plant environment much faster than MHA. Lower yield of biomass obtained for zero treatment plants can be attributed to metal toxicity. No toxicity symptoms were observed on the plant leaves over the pot experiment. This suggests that water spinach is a robust plant species and has great tolerance to high metal concentrations.

Metal Concentration in Plant Tissue. The concentrations of Cu, Pb and Zn in plant shoots after 8 weeks of growth are given in Table 2. It is apparent that MHA and NHA treatments reduced metal concentrations in the shoot tissue of water spinach. From Table 2, metal concentrations in shoots decreased with the rates of amendments application. Marked reductions in metal concentrations were obtained for NHA treatment at 3% (w/w).

Amending contaminated soil with MHA and NHA increased soil pH from 4.82 to 7.72, therefore reducing metal availability for plant uptake. The pH values of MHA and NHA were determined as 7.53 and 7.24, respectively. The reduction in metal concentrations can also be related to the presence of functional groups (PO_4^{3-} and OH^-) on its surface. These functional groups are able to bind or complex heavy metals [15]. Fourier Transform Infrared (FTIR) analysis has confirmed the presence of functional groups on the surface of amendments, as well as the interaction between functional groups and metals (data not shown). It is clear that the accumulation of heavy metals in plant tissues is greatly affected by several factors such as the nature of the amendment, application rate of amendment, the nature of the metal contaminant, plant species and soil pH.

Table 2. Metal concentration in plant shoots.

Treatment	Concentration (mg/kg)		
	Cu	Pb	Zn
Zero	954 d	125 d	1623 c
MHA 1%	827 c	109 c	1485 c
MHA 3%	575 bc	90 b	1296 bc
NHA 1%	513 b	98 b	1144 b
NHA 3%	418 a	75 a	605 a
LSD	84	15	429

Values represent mean of 6 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

Correlations between metal concentrations in soil and metal concentrations in plant shoot were assessed using two extractants, namely EDTA and ammonium acetate (Table 3). It was found that ammonium acetate gave significant correlation between metal concentrations in soil and metal concentration in plant shoot. In contrast, EDTA exhibited poor correlations. The poor correlation between EDTA extractable metal concentrations and plant tissue metal concentrations may be because EDTA is a good extractant for metal associated with organic matter, which may not be available for uptake by plants [4,6].

Table 3. Correlations between metal concentrations in soil and metal concentrations in plant shoot.

Extractant	Metal	Shoot tissue	
		Correlation coefficient	*p*-value
EDTA	Cu	0.029	NS
	Pb	0.013	NS
	Zn	0.042	NS
Ammonium acetate	Cu	0.633	0.000*
	Pb	0.145	0.001*
	Zn	0.826	0.003*

$n = 65$, NS: Not significance, Pearson's correlation coefficient and significance at $p < 0.05$.

Bioconcentration Factor. The bioconcentration factor (BCF) is defined as the ratio of metal concentration in plant shoots to metal concentration in soil [19]. As discussed by Yoon et al. [20], BCF is a measure of the ability of a plant to accumulate metals from soils. In this study, the influence of MHA and NHA treatments on BCF values of the metals was determined, and the values are given in Table 4. The BCF value of Zn for plants grown on zero treatment soil was calculated as 1.24, suggesting that water spinach has great potential for phytoextraction of Zn from contaminated soil. The BCF values suggest that the ability of water spinach to take up Cu, Pb and Zn from soil decreased significantly with the addition of amendments. This can be attributed to metal binding to functional groups of amendments and reduction in metal availability for plant uptake, as discussed in the preceding section.

Table 4. BCF values for Cu, Pb and Zn.

Treatment	BCF		
	Cu	Pb	Zn
Zero	0.33 d	0.22 d	1.24 c
MHA 1%	0.25 c	0.17 d	0.62 b
MHA 3%	0.24 c	0.14 c	0.53 ab
NHA 1%	0.20 b	0.09 ab	0.42 b
NHA 3%	0.14 a	0.06 a	0.26 a
LSD	0.04	0.03	0.13

Values represent mean of 6 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

Metal concentration in plant shoots and soil greatly affects the BCF values. When comparing to Cu and Pb, more Zn was measured in the plant shoots (Table 2). In addition, the total concentration of Zn in the soil (364 mg/kg) was lower than the 725 mg/kg measured for Cu. Therefore, Zn had a greater BCF value than Cu.

Off-take Values. The effect of MHA and NHA application on metal accumulation in plant tissues was further evaluated in terms of off-take value. The off-take value considers both metal concentration in plant tissues and biomass yield [19,20]. The amount of Cu, Pb and Zn removed by water spinach from soil is given in Table 5. It is also important to estimate the off-take value in kg/ha unit as this will provide an insight into the real effect of soil amendments if applied on a contaminated site [2]. The off-take value (kg/ha) was based on conversion factor of pot area to hectare.

From Table 5, it is observed that the removal of Cu, Pb and Zn by water spinach decreased following MHA and NHA treatments. Overall, Zn was the metal most extracted by plants, whereas Pb was the least. At the end of the pot experiment, it is estimated that 0.39 mg/pot of Cu, 0.07 mg/pot of Pb and 3.78 mg/pot of Zn were removed from the untreated contaminated soil. It is also estimated that the off-take value of Zn could be reduced from 9.45 kg/ha (zero treatment) to 5.30 kg/ha (NHA 1% w/w) and 3.00 kg/ha (NHA 3% w/w).

Table 5. Removal of Cu, Pb and Zn from soil.

Treatment	Off-take (mg/pot)			Off-take (kg/ha)*		
	Cu	Pb	Zn	Cu	Pb	Zn
Zero	0.39	0.07	3.78	0.98	0.18	9.45
MHA 1%	0.28	0.06	3.22	0.70	0.16	8.05
MHA 3%	0.21	0.06	2.49	0.53	0.16	6.23
NHA 1%	0.22	0.05	2.15	0.56	0.13	5.30
NHA 3%	0.17	0.05	1.20	0.43	0.10	3.00

* Estimation was based on conversion factor of pot area to hectare.

Bioavailable Fraction of Metals. The uptake of heavy metals by plants is mainly influenced by the bioavailable fraction of metals, not the total fraction of metals in soil [14,15]. Therefore, the effect of application of MHA and NHA on bioavailable fraction of Cu, Pb and Zn in soil was studied using ammonium acetate extraction. The ammonium acetate extractable metals in soil after 8 weeks of the pot experiment are presented in Table 6.

Table 6. Ammonium acetate extractable metal in soil.

Treatment	Concentration (mg/kg)		
	Cu	Pb	Zn
Zero	280 d	1018 d	118 d
MHA 1%	223 c	902 cd	103 c
MHA 3%	208 bc	745 b	75 b
NHA 1%	166 b	857 c	52 ab
NHA 3%	110 a	633 a	44 a
LSD	53	102	25

Values represent mean of 6 replicates. Letters a, b, c and d show the significant differences between the soil treatments, where letter a represents the lowest mean. Different letters indicate significant statistical differences (Tukey's test at $p < 0.05$).

Amending soil with MHA and NHA decreased the bioavailability of Cu, Pb and Zn significantly, particularly at application rate of 3% (w/w). A lower reducing effect was obtained when amendments were applied at 1% (w/w). For example, the ammonium acetate extractable Pb in soil (1066 mg/kg) decreased to 1018 mg/kg (zero treatment), 857 mg/kg (NHA 1% w/w) and 633 mg/kg (NHA 3% w/w) after 8 weeks of pot experiment. The reduction in the amount of metal extracted after the pot experiment can be related to immobilisation effect of the amendments and uptake by plants.

CONCLUSION AND SUGGESTION

Results from this study highlight the potential of MHA and NHA as immobilising agents for the remediation of metal contaminated land. Due to its smaller size, larger surface area and more active sites, NHA was more effective than MHA in immobilising Cu, Pb and Zn in contaminated soil, and reducing plant metal uptake. Pot experiment however is only one aspect of such utilisation. The effectiveness of both amendments as soil amendments rely on their stability in the soil-water environment. It is necessary to study the biodegradation of MHA and NHA and their effect on metal bioavailability.

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REFERENCES

[1] Qu, J., Luo, C., Cong, Q., Yuan, X. Carbon nanotubes and Cu-Zn nanoparticles synthesis using hyperaccumulator plants. Environmental Chemistry Letters, 2012, 10, 153-158.

[2] Petruzzelli, G. Soil contamination and remediation strategies. Current research and future challenge. EGU General Assembly Conference Abstracts, 2012, 14, 7963-7975.

[3] Xi, J.F., Yu, X.Z., Zhou, L.X., Li, D.C., Zhang, G.L. Comparison of soil heavy metal pollution in suburb fields of different regions. Soils, 2011, 43, 769–775.

[4] Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N.S., Pei, J., Huang, H. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environmental Science and Pollution Research, 2013, 20, 8472-8483.

[5] Dermatas, D., Menouno, N., Dutko, P., Dadachov, M., Arienti, P., Tsaneva, V. Lead and copper contamination in small arms firing ranges. Global NEST Journal, 2004, 6, 141-148.

[6] Moon, D.H., Park, J-W., Chang, Y-Y., Ok, Y.S., Lee, S.S., Ahmad, M., Koutsospyros, A., Park, J-H., Baek, K. Immobilization of lead in contaminated firing range soil using biochar. Environmental Science and Pollution Research, 2013, 20, 8464-8471.

[7] Jorgensen, S.S., Willems, M. The transformation of lead pellets in shooting range soils. AMBIO, 1997, 16, 11-15.

[8] Landsberger, S., Iskander, F., Basunia, S., Barnes, D., Kaminski, M. Lead and copper contamination of soil from industrial activities and firing ranges. Biological Trace Element Research, 1999, 71-72, 387-396.

[9] Chrastný, V., Komárek, M., Hájek, T. Lead contamination of an agricultural soil in the vicinity of a shooting range. Environmental Monitoring and Assessment, 2010, 162, 37-46.

[10] Parra, R., Ulery, A.L., Elless, M.P., Blaylock, M.J. Transient phytoextraction agents: Establishing criteria for the use of chelants in phytoextraction of recalcitrant metals. International Journal of Phytoremediation, 2008, 10, 415-429.

[11] Wilde, E.W., Brigmon, R.L., Dunn, D.L., Heitkamp, M.A., Dagnan, D.C. Phytoextraction of lead from firing range soil by Vetiver grass. Chemosphere, 2005, 61, 1451-1457.

[12] Kim, W-S., Jeon, E-K., Jung, J-M., Jung, H-B., Ko, S-H., Seo, C-I., Baek, K. Field application of electrokinetic remediation for multi-metal contaminated paddy soil using two-dimensional electrode configuration. Environmental Science and Pollution Research, 2014, 21, 4482-4491.

[13] Zhang, W., Tsang, D.C.W., Chen, H., Huang, L. Remediation of an electroplating contaminated soil by EDTA flushing: chromium release and soil dissolution. Environmental Science and Pollution Research, 2013, 13, 354-363.

[14] Liang, Y., Cao, X., Zhao, L., Arellano, E. Biochar- and phosphate-induced immobilization of heavy metals in contaminated soil and water: implication on simultaneous remediation of contaminated soil and groundwater. Environmental Science and Pollution Research, 2014, 21, 4665-4674.

[15] Kumpiene, J., Lagerkvist, A., Maurice, C. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste Management, 2008, 28, 215-225.

[16] Zhou, Y-F., Haynes, R.J., Naidu, R. Use of inorganic and organic wastes for in situ immobilisation of Pb and Zn in a contaminated alkaline soil. Environmental Science and Pollution Research, 2012, 19, 1260-1270.

[17] Fang, Y., Cao, X., Zhao, L. Effects of phosphorus amendments and plant growth on the mobility of Pb, Cu, and Zn in a multi-metal-contaminated soil. Environmental Science and Pollution Research, 2012, 19, 1659-1667.

[18] Kottegoda, N., Munaweera, I., Madusanka, N., Karunaratne, V. A green slow-release fertilizer composition based on urea-modified hydroxyapatite nanoparticles encapsulated wood. Current Science, 2011, 101, 73-78.

[19] Kamari, A., Pulford, I.D., Hargreaves, J.S.J. Metal accumulation in *Lolium perenne* and *Brassica napus* as affected by application of chitosans. International Journal of Phytoremediation, 2012, 14, 894-907.

[20] Yoon, J., Cao, X., Zhou, Q., Ma, L.Q. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of the Total Environment, 2006, 368, 456-464.

C-2

CHROMIUM EXTRACTION FROM SOIL BY USING GREEN MUSTARD (*Brassica juncea)*

Tri Santoso, Baharuddin Hamzah, Irwan Said, *Ririen Hardani

Department of Mathematics and Natural Sciences Education, Faculty of Teacher Training and Education, Tadulako University
** Department of Pharmacy , Faculty of Mathematics and Natural Sciences,Tadulako University*
- Jl.Sukarno-Hatta Km.9 Palu (94118) – Indonesia
Tel. +62.451.481356 Fax. +62.451.429743

ABSTRACT

The extraction of chromium (Cr) from soil by using green mustard (*Brassica juncea)* have been studied. The effect of various experimental parameters have been investigated too. The results showed that the amount of Cr from soil by *Brassica juncea* plants affected by soil pH and plant age, the highest withdrawal occurred at of pH 7.0 - 7.5 at the age of 7 weeks. Cr withdrawal from soil by using green mustard without induction with cadmium showed that the accumulation in roots was 412.287 mg / kg of dry mustard and in the dry weight of leaves was 270.634 mg/kg mustard. The results also showed that there was no influence between the size of the pot with the accumulation of Cr on the plant because of the statistical analysis showed that there was no significant difference between the size of the pot (0.5 kg soil) with the other size larger. While the withdrawal of Cr from soil using cadmium induction showed that the Cr accumulation in roots was 873.30 mg / kg of dry mustard and in the leaves was 545.28 mg / kg dry weight of mustard. Thus, translocation of chromium metal ions from soil into roots and leaves of green mustard with the addition of the cadmium as an inductor may increase significantly which is two times greater than the accumulation of chromium without the addition of inductors.

Key words : extraction, chromium, soil, green mustard, *Brassica juncea.*

1. Introduction

There is a few kind of hyper accumulator plants that can attract Chromium (Cr). So far only *Medicago sativa* and *Dactylis glomerate* that have been identified as hyper accumulator plants of Cr (Shanker, et al, 2005), but both of these plants are very difficult to obtain and has not been developed in Indonesia. Therefore, in order to locate and identify other plants that serve as hyper accumulator of Cr is relatively more easily available and cheap, so this research studied the using of mustard green as hyper accumulator plants of Cr. Long-term goal of this research is to find / produce optimum conditions on the withdrawal of Cr from soil by using green mustard (*Brassica juncea*), with or without cadmium induction, so that this green mustard plants can be used as hyper accumulators of heavy metal chromium.

A hyper accumulator plants are plant species that can move pollutants/metal contaminants into the plant shoots more (with a high concentration) of the translocation in the roots without having symptoms of toxicity and to accumulate more than 10 ppm Hg, 100 ppm Cd, 1000 ppm Co, Cr, Cu and Pb, 10,000 ppm Ni and Zn (Lasat, 2000). Types of hyper accumulator plants that can accumulate Cr are very limited..

Consideration of selection of green mustard plants as an alternative hyper accumulator plant are, it has been shown to be used as hyper accumulator plant of lead (Pb) (Jerald, 1997), green mustard is including in Brassicaceae family, the same family of the most hyper accumulator plants of heavy metals that have been identified and green mustard plants are easily available and cheap so that application in the field are more likely to be done

Chromium, a toxic element, in principle dispersed in nature both in the water, air and soil and environmental industries and metal electroplating industry mainly through atmospheric emissions from industrial activities and residential city. Although Cr is not essential for plant growth, but can be taken by most species of plants. The advantages of this metal is poisoning can induce symptoms such as reduced growth and death of plants (Barcelo and Poschenrieder,1990), the destruction of the membrane (Kennedy and Gonsalves, 1987) and alteration of enzyme activity. Chromium intensively used in electroplating, other anthropogenic activities such as industrial metal, metal plating, mining, production, use and disposal of batteries, metal contaminated waste and sludge disposal, will cause a broad spread of Cr into the environment, including soil (Alloway, 1995).

To make the soil clean from Cr metal, required an effective method. Many conventional methods that can be used such as the methods of flushing, dilution and chemical stabilization. However, these methods are not efficient because it will cause new pollution problems at a time when that will come. For example, the method of rinsing / washing the soil causes the cation / anion leached will only move to a deeper layer of soil to reach groundwater. An alternative method that can be used and has proven effective is to use plants that have very high ability to transport metal pollutants from soil (fitoremediation/fitoextraction).

Phytoremediation approach is divided into five general categories namely phytodegradation, rizofiltration, phytovolatilization, phytostabilization and phytoextraction. Phytoextraction is the use of hyper accumulator plants to remove toxic substances like heavy metals from soil and store it in the bud or leaf (Brooks, et.al., 1979, Jerald, 1997). Intake of metals by the roots is an important stage for the main phytoextraction. The efficiency of this technology depends on many factors, among others, the capacity of root systems which retrieve and transfer the metal into the upper plant parts and the interaction between the soil with its physical-chemical characteristics, microbial and others. These complex interactions are influenced by various factors such as specific sections, climatic conditions, hydrology and geology.

Green mustard plant, *Brassica juncea*, is a heavy metal accumulator plants with high biomass, making it a good candidate for application in phytoplankton remediation strategies (Pilon-switches and Pilon, 2002). Strong -ECS or glutathione synthetase (GS) inγexpression of bacterial. *Brassica juncea* increased synthesis of phytoplankton chelats. This phytochelatin compound that can bind heavy metal ions through brown ties, which in turn can distributed from roots to this part of the other plants.

Research for the use of metal cadmium to induce increased phytochelatin synthetase in leaves of green mustard (*Brassica juncea*) has been done. (Hammer, D. and Keller, C., 2002). The results showed that when the green mustard plants were induced with 25 mM Cd, the accumulation of phytochelatin synthetase increased significantly after more than six days and the concentration of phytochelatin synthetase in leaves of three times higher than in roots.

The use of chelate compounds (EDTA, DTPA) to increase the withdrawl of metals by plants *Salix*, and *Thlaspi Sp* has been carried out (Hammer, D. and Keller, C., 2002). The results showed that the soil is acidic or alkaline, metals Cd decreased by *Salix*, *Sp*.withdrawal, In addition, withdrawal of Cd by *Thlaspi* , *Sp* in the acidic soil significantly increased while the soil is alkaline withdrawal increased very little. So there is no general conclusions can be drawn whether the addition of EDTA can increase Cd withdrawal because it depends on the type of plants used and soil acidity. For that we need also determined the optimum soil pH on the addition of EDTA in the withdrawal of cadmium by mustard green.

1.1. Phytochelatin

Phytochelatin peptides are molecules with the structure (g-Glu-Cys) n-gly, (g-Glu-Cys), nb-ala, (g-Glu-Cys) n-ser, (g-Glu-Cys) n-Glu, (g-Glu-Cys) n-Gln, (g-Glu-Cys) n, where n varies between 2-11 (Grill, et.al., 1985) and was first identified in 1983 in the yeast *Schizosaccharomyces pombe* (Cobbett , 2000)

Phytochelatin synthesized from GSH and catalyzed by the enzyme transpeptidase, called phytochelatin-synthetase, where this enzyme has shown activity only in the presence of heavy metal ions, especially Cd, Ag, Pb, Cu, Hg, Zn, Sn, Au, and As both vivo or in vitro (Cobbett, 2000). Several studies of physiology, biochemistry and genetics have confirmed that the tri-peptide glutathione is a substrate for biosynthesis of phytochelatin.

Although -Glu-Cys)γseveral structural variants of phytochelatin, for example: (γ -Glu-Cys) n-Glu have been identified inγ-Glu-Cys) n-ser and (γ-ala, (βn- several plant species, they are assumed to have an analog functions and synthesized through the same biochemical pathway essential (Rauser, 1999). In tobacco leaves, heavy metal-phytochelatin complexes are reported to accumulate in the vacuole (Vogeli-Lange and Wagner, 1990).

1.2.Metal Ion Mobilization in Plants

Acidification of the rhizosphere and carboxylate exudation are two things that are considered as potential targets for increasing the accumulation of metals (Clemens, et.al., 2002). The degree to which higher plants can attract metal ions in the soil depends on the concentration and bioavailability, modulated/regulated by the presence of organic matter, pH, redox potential, temperature and concentration other elements. Withdrawal of metal ions appear to compete with carriers of the same trans-membrane to nutrients such as K, Ca, Mg, Fe, Mn, Cu, Zn and Ni (Clarkson and Luttge, 1989). Cell membrane plays a role in metal homeostasis, prevent or reduce entry into the cell.

Apoplast of the root epidermis and cortex is permeable to solute. The cell walls of endodermal layer serves as a barrier to diffusion appoplastic into the vascular system. In general, solutes have been drawn into xymplasma roots before it can enter into the xylem (Tastet and Leigh, 2001). Due to the withdrawal of the metal into the xymplasma roots, three processes that determine the movement of metals from the root to the xylem are: metal-metal sequestration in root cells, transport simplastic into the Stele and eventually get into the xylem (Clemens, et.al. , 2002)

On the general environmental conditions, metal ions enter the first time to the roots. Metal ions easily penetrated into the roots through a network of cortical and distributed on the network of the top soil . Immediately after the metal ions into the roots, it can reach the xylem through the lane or path apoplastic and xymplastic (Salt, et al, 1995), complex compound formed by several ligands such as organic acids and / or phytochelatin (Senden, et.al ., 1992). In general, metal ion content in plant parts decreased in the order as follows: roots> stems> leaves> fruits> seeds (Blum, 1997)

1.2. Effect of Soil pH

Solubility of metals depends on a number of characteristics of the soil and is often strongly influenced by soil pH (Harter, 1983). As a consequence of the higher solubility of heavy metals at low pH, making more have been observed in some plant species such as wheat grass (Eriksson, 1989) for metal ions and plant the rice mountain to capture metal ions (eg Cd) of ground rock phosphate (Iretskaya, S.,N., et. al., 1998). However, several studies reported that the withdrawal of heavy metals by plants is influenced not only by soil pH but also by the cation exchange capacity, oxidation reduction potential and chemical composition of soil solution (Bingham, et. al., 1979). On the other hand, soil pH and other factors such as availability of nutrients may also affect root growth characteristics.

Dependence of pH on the release of cadmium, copper and lead from soil has also been observed. The results showed that with decreasing pH (range 2.9 to 7.1) the rate of release of the three metals increased, where the rate of release of cadmium> lead>copper (Sukreeyapongse,O,.et.al.,2002). For phytoextraction strategy develop ment, in addition to parameters such as type and degree of pollution, plant selection associated with the potential withdrawal and time of cleaning is needed, as well as parameters such as other forms of physical-chemical bonding of heavy metals should be considered. The various bond fractions of heavy metals can be determined through a sequential chemical extraction (Ahnstrom and Parker, 1999)

2. Method

2.1.Materials and Instruments

The materials to be used in this research is green mustard (*Brassica juncea*), soil, KH-$_2$PO$_4$ 0,1 M, 0.1 M NaOH, fertilizers TSP, KCl, concentrated HNO_3, H_2O_2 35 %, $Cd(NO_3)_2$. $2H_2O$, Whatman-42 filter paper, universal pH paper, distilled water, buffer solution pH 5, 6, 7.0, 7.5, 8.0.

The instruments will be used in this study is a tool commonly used glass in the laboratory, oven, hotplate, magnetic stirrer, porcelain cup, UV-Vis Spectrophotometer Genesys-10uv, furnace, desiccator, pH meters, stative and clamp, analytical balance , a number of pots of various sizes, syringe, and spoon

2.2.Procedure

2.2.1.Determination of soil volume Effect

To study the effect of soil volume, the optimum pH 7.0 to 7.5 and the optimum age of 7 weeks green mustard are implemented as follows. Plastic pots filled with 500, 1000, and 1500 grams of soil contaminated with Cr 25 ppm. Then the soil pH is set between 7.0 to 7.5 using phosphate buffer and incubated for 6 weeks before the experiment began. In the beginning of the experiment, into the pots experiment added 200, 120, 200 and 100 ppm of N, P, K and Mg as a fertilizer. Green mustard (*Brassica juncea*), which had been grown for 2 weeks (in the same condition) were implanted into the pot experiment and placed in a controlled room (temperature and sunlight intensity only). Every day, the plants were given the same amount of distillated water and crops harvested after 7 weeks.

Once harvested, the plants were washed with double distillated water and dried at 60 ^oC for 48 hours and dry weight recorded. Before analysis, dried samples of crushed and refined a number of samples (0.5 g) was dissolved in a mixture of 5 mL HNO3 (65%) and 4 mL of H_2O_2 (30%). Then the concentration of Cr solution was measured using UV-Vis spectrophotometer. As a control, some mustard plants grown in soil with an appropriate variation of soil pH, but the land is not contaminated with Cr.

2.2.2.Determination of Cd Induction Effect

Basically all the same treatment, only green mustard plants were induced with Cd to increase the production of phytochelatin in the mustard green, so the amount of chromium that can be drawn from the soil will multiply.

Plastic pots filled with a number of land (optimum volume) contaminated with Cr in the form of salt with a certain amount. Then the pH (optimum) of land be set using phosphate buffer and incubated for 4 weeks before the experiment began. At the beginning of the experiment, into the pots experiment added 200, 120, 200 and 100 ppm of N, P, K and Mg as fertilizer.

Green mustard (*Brassica juncea*), which had been grown for 2 weeks (in the same conditions) was induced by Cd 25 mM and planted/transplanted into experimental pots and placed in a controlled room (temperature and sunlight intensity only). Every day, the plants were given water with the same amount and plants harvested after a certain time (optimum age). As a control, we use of green mustard without Cr induction. Once harvested, the plants were washed with water and dried at 60 ^{o}C for 48 hours and dry weight recorded. Then 0.5 gram sample is dissolved in a mixture of 5 mL HNO3 (65%) and 4 mL of H_2O_2 (30%). Then the concentration of Cr was measured using UV-Vis Spectrometer.

Treatment: the influence of pot size and the induction of Cd ions is repetition 6 times, then the data obtained were statistically calculated.

3.Results and Discussion

3.1.Determination of soil volume effect .

Research findings in the first year, which is the optimum pH 7.0 to 7.5 and green mustard optimum age 7 weeks later, in the second year followed up to determine the the influence of soil volume. The results of the research presented in Table 1 and Table 2.

Table.1 The content of Cr (VI) in the leaves in various sizes pot experiment (n= 3)

NO	Heavy of soil (Kg)	The average weight of samples (g)	Absorbance	Average concentration (mg/L)	Average levels For dry weight of samples (mg Cr/Kg)
1	0.5	0.5056	0.983	2.761	273.065
2	1.0	0.5001	0.986	2.769	276.910
3	1.5	0.5008	0.984	2.764	275.972
4	2.0	0.5010	0.983	2.761	275.572

n = the number of repeat analysis

Table.2 The content of Cr (VI) in roots at different size of the pot experiment (n= 3)

NO	Heavy of soil (Kg)	The average weight of samples (g)	Absorbance	Average concentration (mg/L)	Average levels For dry weight of samples (mg Cr/Kg)
1	0.5	0.5050	1.473	4.137	409.181
2	1.0	0.4960	1.456	4.090	412.287
3	1.5	0.5030	1.465	4.115	409.062
4	2.0	0.5040	1.463	4.109	407.693

n = number of repeat analysis

Table 1 and Table 2 above are the result of chromium metal accumulation in green mustard (*Brassica juncea*), which was analyzed by UV-Vis spectrophotometer. Chromium metal accumulation in leaves of green mustard with the highest variation of the pot size is 276.910 ppm in the size of the pot 1.0 kg, while the lowest was 273.065 ppm at 0.5 kg pot size. While the accumulation of chromium in the highest green mustard root is 412.287 ppm in pot size 1.0 kg and the lowest was 407.693 ppm at 2.0 kg pot size. It seems that there is no relationship between the size of the pot with chromium metal accumulation in green mustard because of the statistical analysis showed that there was no significant difference between the size of the pot (0.5 kg soil) with the other size larger. Only real difference is shown between the concentration of chromium greater in roots than in leaves.

3.2. Determination of Cd Induction Effect

At this stage, the conditions of treatment in research adapted to the conditions which have obtained the optimum at pH 7.0 to 7.5 and harvested 40 days of age, while the concentration of cadmium ions are added to the soil is 25 mM. The results in this study can be seen in Tables 3 and Table 4.

Tabel 3. The content of Cr (VI) in leaves of Cd-induced ion

NO	Heavy of soil (Kg)	The average weight of samples(g)	Absorbance	Average concentration (mg/L)
1	0.2577	0.987	2.77	537.93
2	0.2554	0.979	2.75	538.37
3	0.2512	0.984	2.76	550.17
4	0.2514	0.983	2.76	549.17
5	0.2499	0.980	2.75	550.78
Average				585.45

n = number of repeat analysis

Table 4. The content of Cr (VI) in roots of Cd-induced ion

NO	Heavy of soil (Kg)	The average weight of samples(g)	Absorbance	Average concentration (mg/L)
1	0.1568	0.984	2.764	881.39
2	0.1536	0.947	2.660	865.92
3	0.1529	0.942	2.650	865.29
4	0.1535	0.953	2.680	871.98
5	0.1540	0.967	2.720	881.91
Average				873.30

n = number of repeat analysis

The results in the first year of Cr metal accumulation in roots and leaves of green mustard (*Brassica juncea*) is 412.287 mg/ kg and 276.910 mg/kg dry weight. In Table 3 and Table 4 shows that the accumulation of metal ions of Cr in the roots and the leaves on green mustard (*Brassica juncea*), which was induced by cadmium is 873.30 mg/kg and 585.45 mg/kg dry weight. Thus, the inductor of Cd can increase the translocation of metal ions Cr significantly by more than two times than the accumulation of chromium without the addition of inductors. The highest accumulation of cadmium metals contained in the root is an average of 873.30 ppm.

. 4.Conclusions

Based on the results of experiment, observation and discussion that has been done, it can be drawn several conclusions.

1. There is no influence between the size of the pot with cadmium metal accumulation in plant mustard greens because of the statistical analysis showed that there was no significant difference among the size of the pot.
2. Trans location of chromium metal ions from soil into roots and leaves of green mustard (*Brassica juncea*) with the addition of the inductor metal cadmium into the soil to increase significantly which is two times greater

5.References

Ahnstrom Z.S., Parker D.R., 1999, Development and assessment of a sequential extraction procedure for the fractionation of soil cadmium, *Soil Sci.Soc.Am.J.*,63. 1650-1658.

Alloway B.J., 1995, *Heavy metals in soils*, 2nd ed., Blackie Academic and Professional Publishers, London, 368-369.

Barcelo J., Poschenrieder C., 1990, Plant-water relations as affected by heavy metal stress: A review, *J.Plant Nut*., 13, 1-37.

Bingham F.T., Page A.L., Mitchell G.A., Strong J.E., 1979, Effects of limiting an acid soil amended with sewage sludge enriched with Cd, Cu, Ni and Zn on yield and Cd content of wheat grain, *J. Environt. Qual*., 8, 202.

Blum W.H., 1997, Cd uptake by higher plants. In : *Proceeding* of extended abstracts from the fourth International Conference on the Biogeochemistry of Trace Elements, pp.109-110, Berkeley, USA, University of California.

Brooks R.R., Morrison R.S., Reeves R.D., Dudley T.R., Akman Y.,1979, Hyperaccumulation of nickel by *Alyssum linnaeus* (Cruciferae). *Proceedings of the royal society* (London) section B 203, 387-403.

Clarkson D.T., Luttge U., 1989, Mineral nutrition, divalent cathions, transport and compartmentalization, *Prog.Bot*., 51 : 93-112.

Clemens S., Palmgreen M.G., Kramer U., 2002, A long way ahead : understanding and engineering plant metal accumulation, *Trends Plant Sci*., 7 : 309-315.

Cobbett C.S., 2000, Phytochelatins and their roles in heavy metal detoxification , *Plant Physiol*. 123 : 825-832.

Eriksson J.E., 1989, The influence of pH , soil type and time on adsorption and uptake by plants of Cd added to the soil. *Water Air Soil Pollut*., 48, 317-335.

Grill E., Winnacker E.L., Zenk M.H., 1985, Phytochelatins : The principal heavy-metal complexing peptides of higher plants, *Science* 230 : 674-676.

Hammer, D. and Keller , C., 2002, Changes in the rhizosphere of metal-accumulating plants evidenced by chemical extractants, *J. Einviron. Qual.* 31:1561-1569.

Harter R.D., 1983, Effect of soil pH on adsorption of lead, copper, zinc and nickel, *Soil sci. Soc. Am.J*., 47, 47-51.

Iretskaya S.N., Chien S.H., Menon R.G.,1998, Effect of acidulation of high cadmium containing phosphate rocks on cadmium uptake by upland rice, *Plant and Soil* 201, 183-188.

Jerald L.S., 1997, *Phytoremediation*, GWRTAC, 1-8.

Kennedy C.D., Gonsalves F.A.N., 1987, The action of divalent zinc, cadmium, mercury, copper and lead on the trans-root potential and H+ efflux of excised roots, *J. Expt. Bot*. 38, 800-817.

Lasat M.M., 2000, Phytoextraction of metals from contaminated soil : A review of plant/soil/metal interaction and assessment of pertinent agronomic issues, *J. Hazard.Subs.Res*., 2, 5-25.

Pilon-Smits E., Pilon M., 2002, Phytoremediation of metals using transgenic plants, *Crit.Rev.Plant Sci.* 21: 439-456.

Rauser W.E., 1999, Structure and function of metal chelators produced by plants : The case for organic acids, amino acids, phytin and metallothioneins, *Cell Biochem.Biophys*. 31 : 19-48.

Salt D.E., Rauser W.E., 1995, MgATP-dependent transport of phytochelatins across the tonoplast of oat roots, *Plant Physiol*., 107 : 1293-1301.

Senden M.H.M.N., Van Paassen F.J.M., Van der Meer A.J.G.M., Wolterbeek H.T., 1992, Cadmium-Citric acid xylem cell wall interactions in tomato plants, *Plant Cell Environ*.15: 71-79.

Shanker, A.K., Cervantes, C., Tavera, H.L., Avudainayagam, S., 2005, Chromium Toxicity in Plants, *J. Environment International*, 31, 739-753.

Sukreeyapongse O., Holm P.E., Strobel B.W., Panichsakpatana S., Magid J., Hansen H.C.B., 2002, pH dependent release of cadmium, copper and lead from natural and sludge-Amended soils, *J. Environ. Qual*, 31, 1901-1909.

Taster M., Leigh R.A., 2001, Partitioning of nutrient transport process in roots, *J. Exp. Bot*., 52 : 445-457.

Van Assche F., Clijsters H., 1990, Effects of metal on enzyme activity in plants, *Plant Cell Environ*., 13, 195-206.

Vogeli-Lange F., Wagner G., 1990, Subcellular localization of cadmium and cadmium binding peptides in tobacco leaves. Implication of a transport function for cadmium binding peptides, *Plant Physiol*., 92 : 1086-1093.

C-3

BIOSORPTION OF TECHNICAL DIRECT DYES BY ACTIVATED SLUDGE

Dewi Yuanita Lestari and Endang Widjajanti LFX

Chemistry Education Department of UNY

Abstract

This study aimed to determine the effect of adsorbent mass, adsorption time and concentration of dye on adsorption efficiency of activated sludge toward technical direct dyes and to know the effect of the adsorption process of the COD (Chemical Oxygen Demand) value in technical direct dyes solution.

The subject of this study was activated sludge. The object of this study was the activated sludge adsorption efficiency on technical direct dyes. Adsorption process was done by conditioning the variation of the mass of adsorbent, adsorption time and concentration of technical direct dyes. Technical direct dyes solution before and after adsorption were quantitatively analyzed with a COD reactor. Technical direct black dyes solution after adsorption were analyzed quantitatively by UV-Vis spectrophotometer. Adsorption efficiency expressed in terms of concentration of adsorbed dyes divided by the initial concentration of dye solution and multiplied by 100%.

The results showed that: the greater the mass of adsorbent, the higher the adsorption efficiency of activated sludge to technical direct dyes, the longer the time given to the process of adsorption, the higher the adsorption efficiency of activated sludge to technical direct dyes, adsorption efficiency decreases with increasing concentration of technical direct dyes, chemical Oxygen Demand (COD) value technical direct were decrease after adsorption process by activated sludge.

Keywords : activated sludge, technical direct, adsorption

INTRODUCTION

Direct dyes are used on cotton, paper, leather, wool, silk and nylon. Direct dyeing is normally carried out in a neutral or slightly alkaline dye bath at or near boiling point with the addition of either sodium chloride or sodium sulfate [1]

The residual Direct dyes from several industries (e,g. Textille industries, pulp and paper industries, craft industries) are considered a wide variety of pollutants introduced into the natural water resources. The discharge of dye containing effluents into environtment is undesirable because of the toxicity and visibility. Dyes are persistent in nature and strongly absorb sunlight which decrease the intensity of light absorbed by water plants and phytoplankton reducing photoshynthesis and dissolve oxygen of the aquatic ecosystem and result in increase of chemical oxygen demand (COD) [1]

Treatment of the effluent containing residual direct dyes is important for the protection of natural water resources. Biosorption technique is considered to be an effective method for lowering the concentration of direct dyes in waste water. Biosorption involves a combination of active and passive transport mechanisms starting with the diffusion of the adsorbed component to the surface of the microbial cell [2]

A number of materials have been used as bioadsorbent. Activated sludge is one of potential material to treat waste water contaning direct dyes in biosorption technique. Activated sludge is biological flok consists of microorganisms (i.e. bacteria, protozoa). More than 300 species of bacteria were found in activated sludge. The common Genus were: *Zooglea, Pseudomonas,Flavobacterium,Alcaligenes,Bacillus,Achromobacter,Corynbactum,Comomonas, Brevibacterium,*dan*Acinetobacter* [3]. Activated sludge is industrial waste so using activated sludge to reduce dyes concentration in wastewater can support zero-waste principal.

RESEARCH METHOD

Batch experiment were carried out in Beakker glass containing the aqueous technical Direct dyes solution of the desired concentration and the known mount of activated sludge. The mixture was agitated for a minute using manual stirrer and allowed at contact time. The dyes solution was separated from activated sludge by centrifugation. The dyes concentration of supernatant was determinned by using uv-visible spectrophotometer. Batch experiment were performed for technical Direct Black and Blue at different contact time : 1; 1,5; 2; 3; 4; 5; and 24 hours, adsorbent mass : 1; 2; 3; 4; 5 grams, initial concentration: 500; 700, 800; 900 ppm. Chemical Oxygen Demand (COD) of direct dyes solution before and after biosorption were measured using COD reactor.

Adsorption efficiency expressed in terms of concentration of adsorbed dyes divided by the initial concentration of solution and multiplied by 100%.

$$\text{Adsorption efficiency} = \frac{\text{Co} - \text{Ca}}{\text{Co}} \text{ x } 100\%$$

Co = Initial concentration of direct dyes (mg/L)
Ca = concentration of adsorbed dyes (mg/L)

RESULT AND DISCUSSION

1. Biosorption of direct dyes by activated sludge

Biosorption of direct dyes solution by activated sludge consist of two steps : adsorption as non-enzyme system and followed by degradation as enzyme system [4]. In this research, biosorption was done by aerobic system using aerator as oxygen supplier for aerobic bacteria in activated sludge.Oxygen supply in biosorption process increased the lifetime of microorganisms in activated sludge. In general, steps in decolorization by aerobic bacteria were adsorption of matrix (plasma membrane) of aerobic bacteria followed by metabolite production (i.e. enzyme) by aerobic bacteria [5]. Plasma membrane of aerobic bacteria used to flow oxygen, nutrient, and waste in aerobic bacteria cell. The adsorption process could be seen by colour change of bacteria flok in activated sludge from grey to black. Aerobic bacteria used carbon and nitrogen from direct dyes solution as carbon-nitrogen source to support their life so the enzymatic decolorization occurred [6]. The process caused colour intensity and concentration of direct dyes solution decrease.

Some research showed that the better way to decolorize was by combination of aerobic and anaerobic method. In anaerobic process, complex molecule was broken to be simple molecule. The simple molecule can easyly biodegradate to CO_2, H_2O, NH_3 and biomass. Fig. 1 showed the reaction occured in biodegradation. But this research showed that aerobic process was able to reduce concentration of direct dyes solution. The derease of direct dyes concentration in solution could be seen by the decrease of direct dyes solution colour intensity after biosorption using activated sludge. Fig. 2 showed the decrease of colour intensity of direct dyes solution. Fig 2 also showed that without aeration, colour intensity of direct dyes solution was high but under aeration process the colour intensity was lower. It was showed that oxygen supply in aeration process very important to aerobic bacteria in activated sludge. Aerobic bacteria need oxygen to break down organic compound to get energy. The energy was used to grow and multiply cells [7]. Microorganism was able to break azo bonding (-N=N-) in direct dyes so the colour intensity of direct dyes solution was decrease.

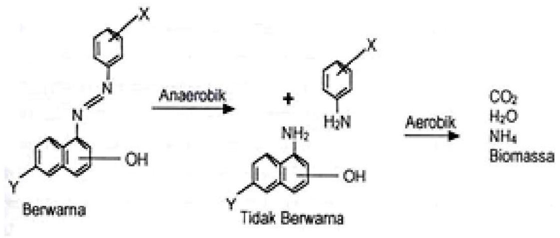


Fig 1. Biodegradation of dye

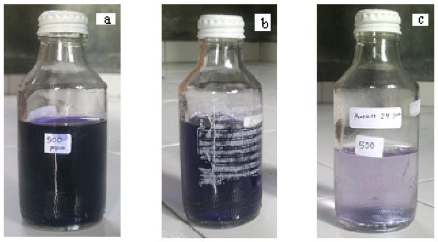


Fig 2. (a) Technical Direct Blue solution before biosorption (b) biosorption without aeration (c) biosorption using aeration

2. Effect of Biosorbent Mass

Fig. 3 showed that adsorption efficiency increase with the increase of biosorbent mass. It occurred both in Direct Black and Direct Blue Dyes.There were correlation between biosorbent mass and number of active sites[8]. The increase in the adsorption efficiency with biosorbent mass is due to greater availability of biosorbent surface area [2] and hence more active sites. The greatest adsorption efficiency values were found in 5 grams biosorbent mass both for Direct Black and Direct Blue dyes. The adsorption efficiency values for Direct Black and Direct Blue dyes recpectively were 40% and 32 %

3. Effect of Contact Time

Contact time was time during adsorption. Fig. 4 showed that increase in contact time both for Direct Black and Direct Blue dyes solution caused the increase in adsorption efficiency. The increase of contact time caused active sites on activated sludge had longer time and also more chance to interact with adsorbate (Direct dye molecules) so the adsorption capability of activated sludge increased[9].

The increase of adsorption efficiency showed that some sites still active adsorbing direct dyes during 24 hours contact time. The greater adsorption efficiency values of Direct Black and Direct Blue solution occurred at 24 hours contact time and the values were respectively 85,51% and 98,73%

It can be observed (fig.4) that the biosorption was rapid within around 5 minutes of initial time, but the adsorption progressed at a lower rate for the remaining time. As at the initial times the bulk solution concentrations were higher, the driving forces were the maximum, leading to maximum rates. But as the biosorption proceeds, the bulk concentration reduced approaching the equilibrium values and the rate decreased [2].

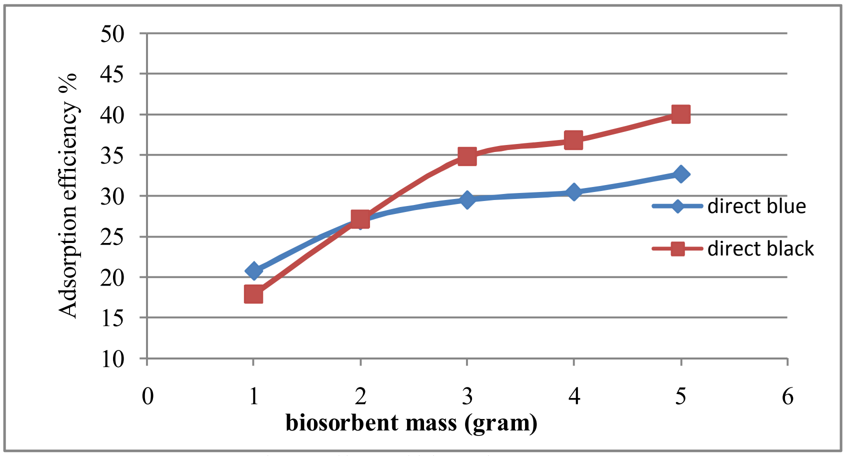


Fig 3 Effect of biosorbent mass

4. Effect of Initial Concentration

Fig 5. Showed that adsorption efficiency decreased with the increased of initial concentration of Direct Dyes solution. The increase of initial concentration cause the actives site were full of Direct dyes molecules. Insufficient number of active sites available for the biosorption of all the dye molecules, lead to higher residual concentration in solution, with increasing initial dye concentrations

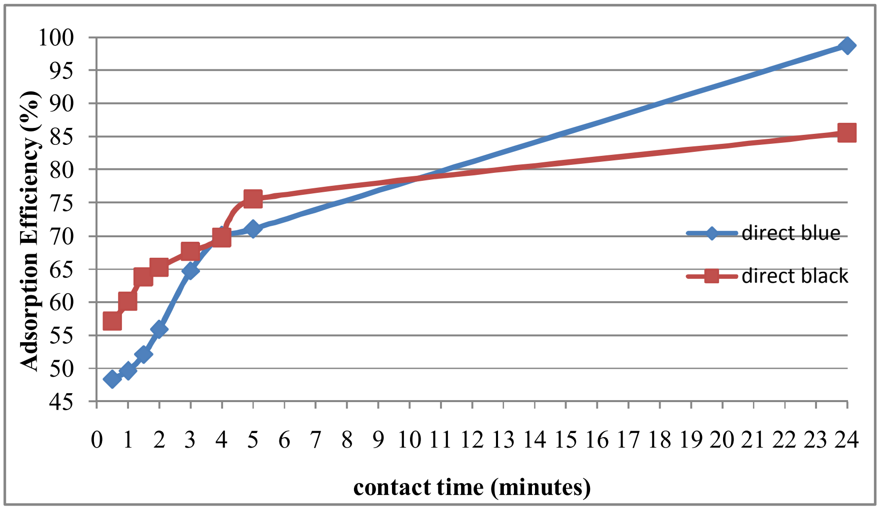


Fig. 4 Effect of contact time

5. COD values

COD value usually use to know whether an environtment was polluted or not. COD value was the amount of oxygen (mg O_2) needed to oxydize organic compounds in one litre aqueous sample. The higher COD value means that the environment was more polluted. Table 1 showed that COD values in 600 ppm Direct Black and Direct Blue solution after 24 hours biosorption decreased. The result showed that aerobic bacteria in activated sludge were able to decrease COD value of Direct Dyes solution. Aerobic bacteria did non-enzyme and enzyme process toward direct dyes so the concentration of dyes decreased. The decrease of direct dyes concentration cause oxygen amount to oxydize Direct dye compounds was lower.

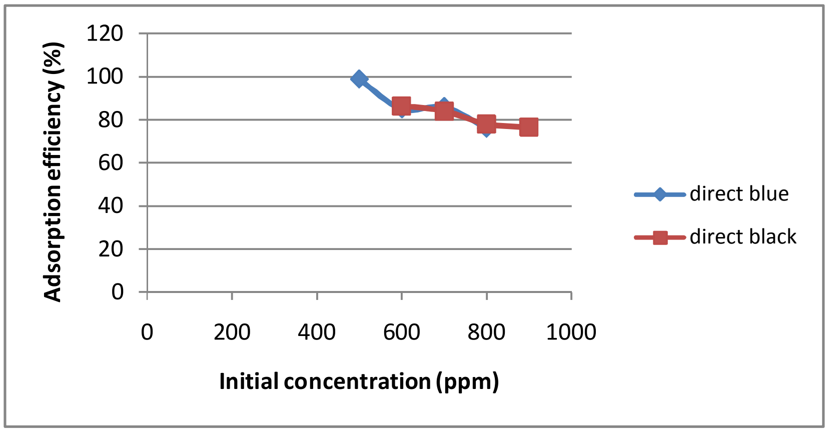


Fig. 5 Effect of Initial Concentration

Table 1. COD values

Dyes	COD value	
	Before Biosorption	After Biosorption
Direct blue	28656,00 mg/L	4776,00 mg/L
Direct black	3184,00 mg/L.	1751,20 mg/L.

CONCLUSION AND SUGGESTION

The results showed that: the greater the mass of biosorbent, the higher the adsorption efficiency of activated sludge to technical direct dyes, the longer the time given to the process of adsorption, the higher the adsorption efficiency of activated sludge to technical direct dyes, adsorption efficiency decreases with increasing concentration of technical direct dyes, chemical Oxygen Demand (COD) value technical direct were decrease after adsorption process by activated sludge.

REFERENCES

[1] Neetu Sharma, D.P. Tiwari, S.K. Singh. (2012). Decolorization of Synthetic Dyes by Agricultural Waste-A review. *IJSER*. Vol 3 Issue 2

[2] Ratnamala and Brajesh. (2013). Biosorption of Remazol Navy Blue Dye from an Aqueous Solution using Pseudomonas putida. *IJSET*. Vol 2. No.1.

[3] (http://www.kelair.bppt.go.id/Sitpa/Artikel/ Tekstil/tekstil.html)

[4] Dewi RS. (2005). Potensi Bakteri Limbah Industri Tekstil Sebagai Agen Pendekolorisasi Pewarna Azo Sumber Pencemaran Warna Perairan. *Prosiding Seminar Nasional dan Kongres Biologi XII*: Yogyakarta, 19-22 April 2005

[5] Awaluddin. (2001). *Biodekolorisasi*. Bogor : Institut Pertanian Bogor

[6] Sharma, P., Singh, L., Dilbaghi, N. 2009. Optimization of Process Variable for Decolorization of Disperse Yellow 211 by Bacillus subtilis using Box-Behnken Design. *Journal of Hazardous Materials*. Vol 164. pp 1024-1029

[7] Hammer, Mark J. (1986). *Water and Wastewater Technology*. 2nd. ed. Canada: John Wiley & Sons, Inc

[8] Puvaneswari, N., Muthukrishnan, J. & Gunasekaran, P. (2006). Toxicity Assessment and Microbial Degradation of Azo Dyes. *Indian Journal of Experimental Biology*. Vol. 44, Hlm.: 618-626

[9] Khopkar, S.M. (1990). *Konsep Dasar Kimia Analitik*. Jakarta: Penerbit Universitas Indonesia

C-4

EFFECT ACTIVATION OF CHEMICAL AND PHYSICAL TO STRUCTURE AND ACTIVATED CARBON QUALITY FROM CHARCOAL OBTAINED BY PYROLYSIS OF COCONUT SHELL

Djefry Tani[1*], Bambang Setiaji[2], Wega Trisunaryanti[2], Akhmad Syoufian[2]

[1] Doctoral Student, Chemical Department, Gadjah Mada University, Yogyakarta
[2] Chemical Department, Faculty of Mathematics and Science Gadjah Mada University
[*]Chemical Department, Manado State University
[*]Phone: +6285228231954, e-mail: djefta@gmail.com

Abtract

Activation of coconut shell charcoal has been done by a combination of chemical and physical activation to determine the effect of chemical activators and activation temperature to changes in the chemical structure and the quality of activated carbon . The study was conducted on charcoal coconut shell of pyrolysis results with variation of concentration and immersion time $ZnCl_2$ as an activator , respectively 0.5 to 2 M for 12 to 24 hours . Then proceed with the activation of physics in reactor the activation of the temperature variation of 500 to 800 ^{o}C with CO_2 gas flow rate 20 mL / min . Evaluation of the chemical structure and the quality of activated carbon performed FTIR , SEM , specific surface area , total pore volume , and average pore with Brunauer - Emmett - Teller (BET) . The results showed the results of the analysis of SPSS through Cofficients test the concentration variables most affect to moisture content , ash content , and iodine absorption from temperature variable and time of activation . The best quality activated carbon produced at a concentration of $ZnCl_2$ of 1.5 M, 24 hours soaking time of 24 hours , temperature 800 ^{o}C and the activation time of 120 minutes . The results as moisture content of 8.47 % , volatile matter content of 18.55 % , ash content 1.44 % , bonded carbon content of 80.01 % and iodine absorption of 769.74 mg / g . The results of FTIR analysis showed the surface of activated carbon containing CO and CH bonds , and SEM analysis showed the number and diameter of pores increased with the higher calcination temperature . Surface area (BET) of 359.286 m^2 / g , pore volume of 1.984 x 10^{-1} cm^3 / g and pore mean of 11.46 Å .

Keywords : coconut shell, activated carbon , ZnCl2 , SPSS , FTIR , SEM , BET

Introduction

Fulfilling active carbon need in the world increase every year. For example in 2007 it reached 300,000 per year. In big countries such as United States, its demand per capita was about 0.4 kg per year and Japan it was about 0.2 kg per year. In Indonesia, active carbon demand was more than 200 ton a month or 2400 ton per year; most of them was still imported for pharmaceutical industry and other industries (Bansal and Goyal, 2005; Fitriani, 2008). The greater demand for domestic active carbon requires searching basic material source as alternative to make active carbon. One of the alternative sources is product of coconut tree in which coconut shell have not been used optimally yet. When the coconut shell is processed further, it will give higher economic value (Mandey, 2007). One of ways to process coconut shell is pyrolisis process. Pyrolisis process will produce coconut shell charcoal, liquid smoke, tar, and uncondensed gases. Charcoal is porous solid material resulted from material containing carbon. Most of its pore is still covered with hydrocarbon, tar and other organic compound. Its component consists of fixed carbon, ash, water, nitrogen and sulphur. Coconut shell charcoal from pyrolisis process is potential to process as active carbon (Scok et al, 2005). Active carbon product from coconut shell charcoal has many micropore, high surface area and high adsorption capacity (Keneeth, 2002; Manocha, 2003).

Process of making active carbon consists of two steps: carbonization process and activation process. Main product resulted from carbonation process is charcoal with side product of tar, liquid smoke and carbon. However, resulted carbon has low surface area and usually less active due to very small adsorption rate. So, it is necessary followed with activation process functioning to develop porosity and adding surface area (Rodenas et al, 2005; Cuhadaroglu and Uygun, 2008).

Activation method commonly used in making active carbon is physical and chemical activation. Physical activation process is charcoal activation to develop pore from active carbon with aid of hot vapor and inert activating gas in high temperature of 800 to 1100^0C (Teng et al, 1998; Hong et al, 2000; Lee and Lee, 2001). Activation temperature is a variable that very influence characteristic of active carbon. In high temperature condition without air will resulted high quality active carbon where volatile components will loss so fixed carbon level is high. Chemical activation is a process of cutting carbon chain from organic compound using chemical material as activator (Derbyshier et al, 1995). Some activators commonly used in making active carbon are KOH, NaOH, $ZnCl_2$, KCl, H_3PO_4 HCl and so on (Sricharoenchaikul et al, 2008; Babel, 2004). However, in other side, making active carbon with only chemical activation has weakness of corrosion process, washing process and cannot regulate resulted active carbon pore (Balci et al 1994; Sricharoenchaikul, 2008; Timur et al, 2006).

This research studied activation process of coconut shell charcoal from pyrolisis process using chemical activation and physical activation using $ZnCl_2$ as activator with CO_2 activating gas. The combination method is expected to make active carbon at lower temperature, easier and at lower cost but with high commercial value. The combined method will result in high quality active carbon with greater surface area and high adsorption capacity. It will contribute to science and technology of making active carbon useful for industry in the future and it can develop optimally distribution of pore dimension according to industry desire.

Experimental

Material

Coconut shell resulted from pyrolisis from PT TNI, $ZnCl_2$, gas CO_2, Kl, $Na_2S_2O_35H_2O$, distilled water, KIO_3, I_2, HCl

Instruments:

A set of pyrolisis tool, a set of activating reactor, 100 mesh and 80 mesh sieve, electronic balance, oven, desiccator, electric furnace, magnet stirring, pH meter, laboratory glass tools.

Procedure

Preparation of coconut shell sample

Coconut shells were cleaned and their dimension were decreased to about 3 x3 cm, and weighted for 1500 gram and used as pyrolisis sample.

Carbon activation step

Coconut shell charcoal of pyrolisis process is grind and pound to make fine powder. Then it was filtered using 100 mesh sieve. Charcoal is activated by being flowed with CO_2. In this step, chemical activation was done by soaking and followed with heating with CO_2 flow.

1. Chemical activation step
   In this step coconut shell charcoal from pyrolysis that has been sieved was soaked in $ZnCl_2$ solution with various concentration and soaking duration (0.5 – 2.0 M and 12 to 24 hours). Charcoal that have been soaked was sieved with vacuum filter and heated in oven at 120^0C for 2 hours to produce dry charcoal then put into desiccator**.**
2. Physical activation step
   Charcoal resulted from chemical step was put into tube furnace activation reactor given with glasswool at lower and upper part, then activated at various temperature (500 to 800^0C) and flowed with CO_2 20 ml/minute for two hours. Then, the charcoal was soaked in 1M HCl solution for 24 hours. Then the charcoal was washed with distilled water to make neutral filtrate (measurement with pH meter). It was sieved and dried in oven at 110^0C for two hours. Active carbon was ready to analyze
3. Active carbon analysis
   Active carbon test including physical and chemical characteristic consist of macro and micro analysis. Macro analysis includes moisture content, volatile content, ash content, fixed carbon content, and adsorption capacity iodine. Result of macro analysis is tested with coefficient test using SPSS. Analysis of micro structure include function group with FTIR, surface structure with SEM, BET surface area, total pore volume and average pore diameter.

Fourier Transform Infra red (FTIR) analysis

Active carbon sample structure of activation result was analyzed using Fourier Transform Infrared (FTIR) Shimadzu model IR-prestige-21 and made pellet KBr. It was used to identify mineral function group.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to analyze micro structure of surface of active carbon before and after activation.

BET surface area analysis

Pore structure analysis and surface area analysis was done using surface area analyzer Nova ver 2.2. Determination of surface area was done with BET method to calculate surface area of active carbon resulted from pyrolisis of coconut shell charcoal. In addition with BET equation, total pore volume and average pore radius may be determined.

RESULT AND DISCUSSION

This research was done to identify effect of chemical treatment using $ZnCl_2$ activator in making active carbon from coconut shell charcoal using chemical and physical activation methods.

Moisture matter of active carbon

Presence of water in carbon related to hydroscopic characteristic of the carbon, in which carbon has greater affinity than water. Data obtained indicated that moisture content of active carbon resulted from this research meet Indonesian Industrial Standard (SII no. 0258-79) maximally of 15%. Its graphical perspective is presented in figure 1.

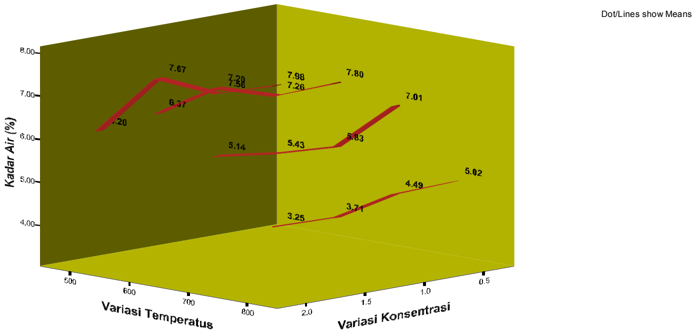


Figure 1. Effect of $ZnCl_2$ concentration and temperature on moisture content of active carbon

Figure 1 reveals that grater concentration of activator solution used in active carbon soaking will result in higher moisture content. Soaking process with activator was done to reduce tar content, so the more concentrated activator the less tar content in carbon. In result, pore in active carbon is increasingly great and much. Greater and more pore lead to increase in its hydroscopic characteristic. It causes adsorption of water from atmosphere by active carbon will increase, which lead to increase in moisture content in the active carbon.

Coefficient test indicates three independent variables on moisture content (%) is temperature (16.159), concentration (12.110) and soaking duration (4.822) (see t count in t column t in coefficient table). Result of multiple regression test using SPSS software indicate that the three variables affect water content.

Ash content

Process of testing ash content will indicate ash of metal oxide in charcoal consisting of mineral that can evaporate in incineration process. Effect of activator concentration on ash content in active carbon is presented in figure 2.

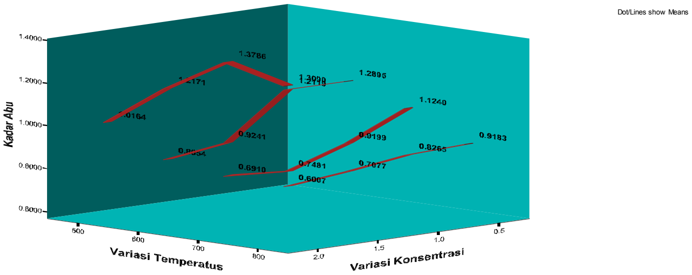


Figure 2. Effect of $ZnCl_2$ concentration and temperature on as content of active carbon

Figure 2 shows that ash content of all active carbon obtained in this research fulfill SII no 0258-79 (maximal 10%) because it was about 0.55-1.44. at activator concentration of 0.5-2.0 M, ash content increased. It relates to forming pores in active carbon. Ash content in active carbon also relates to raw material and its process. High activator concentration will add surface area of active carbon due to great and many pores. Active carbon consists of layers piling to each other making pore. During pore forming, there occur crystal field burning that resulting ash. The more pore made, the more ash produced.

Coefficient test indicated three independent variables affecting ash content are temperature (7.296), concentration (-2.501)and soaking duration (1.096) (see t count in t column t in coefficient table).

Volatile matter content

Volatile substance in active carbon consists of water and tar residue that does not fully disappear in carbonization process. Obtained data indicated that volatile substance content in active carbon obtained in this research still comply with SII no 0259-79 (25%), as indicated in figure 3.

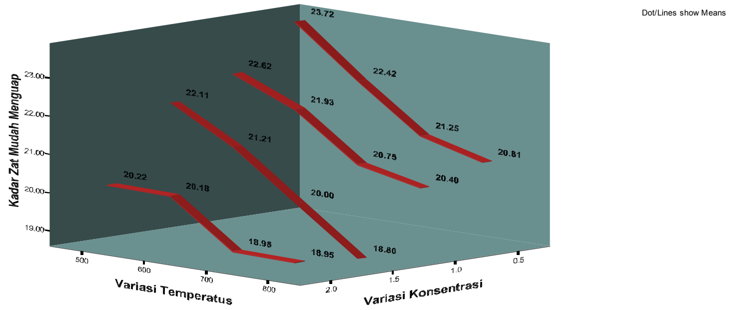


Figure 3. Effect of $ZnCl_2$ concentration and temperature on volatile matter content of active carbon

Figure 3 indicated that higher concentration of activator solution will decrease volatile substance. So, higher ash content indicated lower volatile substance content in active carbon. It is due to absorbed activator will cause erosion in carbon, which reduce efficiency of heating process in activation. In other words, temperature of activation process is low. When activation was done in low temperature, then volatile substance content will increase. Then, decomposition of non carbon compound such as CO, CH_4 and H_2 is expected not perfectly running. The graphic indicated that various concentrations can reduce volatile substance content but their decrease is not great so did no affect the volatile matter.

Based on coefficient test, the three independent variables most influencing volatile matter content are temperature (-4.021), concentration (-3.233) and soaking period (-2.126) (see t count in t column in coefficient table).

Fixed carbon content

Fixed carbon content is carbon obtained based on deduction of all sample weight (100%) on volatile matter and ash content. Fixed carbon content in active carbon was 50%-90%. Therefore, main composer of active carbon is carbon. The data indicated that highest fixed carbon content resulted from carbon activated with 1.5M $ZnCl_2$, with 24 hour soaking period and at 800^0C. Fixed carbon content obtained with various concentration still comply with SII no 0258-79 (at least 65%). The data is presented in figure 4.

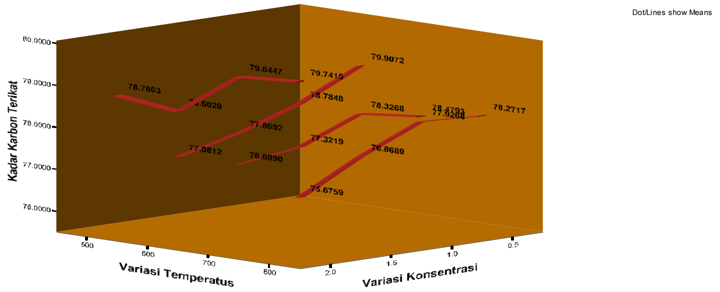


Figure 4. Effect of $ZnCl_2$ concentration and temperature on fixed carbon content of active carbon

Figure 4 indicated that increase in activator solution concentration will increase fixed carbon content. Factors influencing increase in carbon content are ash content and volatile matter content. Lower ash content and volatile matter content in active carbon indicated higher fixed carbon content. Therefore, purity of active carbon will be higher.

Based on coefficient test, the three independent variables most influencing fixed carbon content are temperature (3.418), concentration (3.400) and soaking period (2.023) (see t count in t column in coefficient table).

Iodium number

Adsorption of aqueous iodine is one of main characteristics of active carbon determining quality of active carbon. Test result indicated that active carbon having best adsorption number iodine is that in 1.5 M concentration (769.74 mg/g). The adsorption number on iodine resulted in this research comply with SII no.0258-79 (at least 200 mg/g).

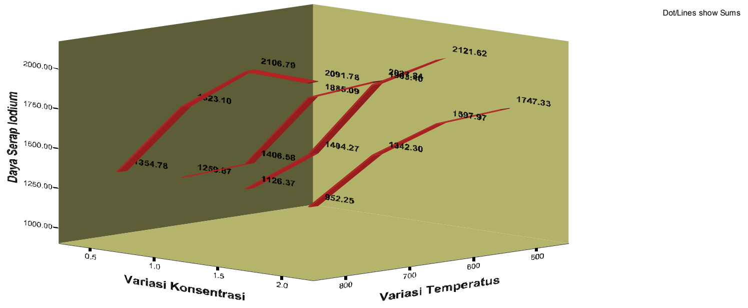


Figure 5. Effect of $ZnCl_2$ concentration and temperature on iodine adsorption number active carbon

Figure 5 reveal high activator solution will increase adsorption number over iodine. It is cause due to tar content in active carbon increasingly decrease with increasing activator concentration at soaking, where soaking process with activator was done to reduce tar content. Therefore, more concentrated activator solution used indicates decrease in tar content in active carbon. It causes greater pores in active carbon or increase in surface area of active carbon. The grater surface area of active carbon, the higher absorption number it has.

Based on coefficient test, the three independent variables most influencing adsorption number iodine are temperature (12.668), concentration (9.417) and soaking period (3.964) (see t count in t column in coefficient table).

Analysis of function group using active carbon FTIR before and after activation with activator Zinc chloride ($ZnCl_2$) at concentration of 1.5 M, 24 hour soaking period and 800^0C

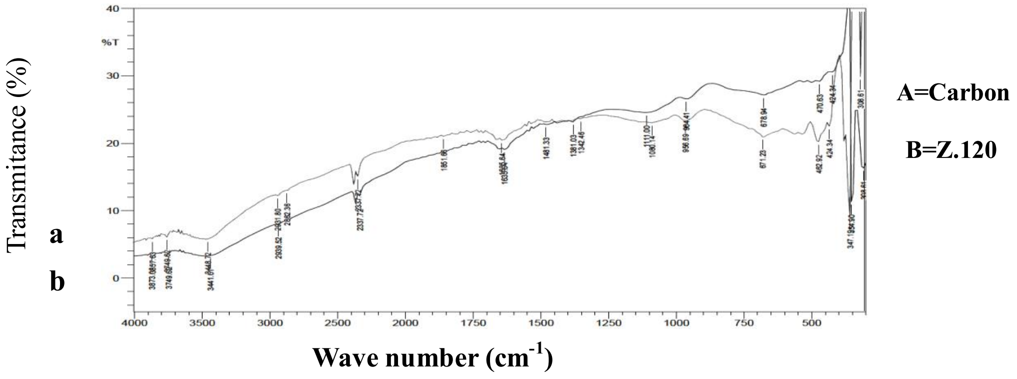


Figure 6. FTIR spectrum of carbon before and after activation with various activation periods

The FTIR spectrum of coconut shell charcoal of pyrolisis without activation informs presence of absorption band in wave number 3487.3-3417.86 cm-1 appearing stretching vibration of O-H group. This vibration is supported with absorption band in wave number 1381.03-1165 that is stretching vibration of C-O and bending vibration of OH from alcohol and carboxylate acid. In addition, in wave number 2337.72 cm^{-1} is stretching vibration of carboxylate acid. Wave number 1581.63-1527.62 cm^{-1} is stretching vibration of C=C in aromatic ring. Absorption band 802.39-747.38cm^{-1} indicated C-H out of plane bending in aromatic ring.

The spectra of the prepared activated carbon resulted from activation with $ZnCl_2$ is relatively similar to shell charcoal without activation. However, its absorption rate decrease or increase according to activation duration so there is shift in wave number. Absorption band in wave number 3448.72 cm^{-1} appear stretching vibration of O-H. The vibration is supported by absorption band in wave number 1342-1080.14 cm^{-1} that is stretching vibration of C-O and bending vibration of OH from alcohol and carboxylate acid. In addition, there is absorption in wave number 2337.72 cm^{-1} that is stretching vibration of carboxylate acid. While in wave number 1635.64 cm^{-1} is stretching vibration of C=C in aromatic ring. Absorption band in wave number 802 cm^{-1} indicate C-H out of plane bending in aromatic ring. Change in function group structure in active carbon may be due to effect of charcoal calcination temperature and duration of activation. Active carbon resulted in activation process has absorption pattern with bonding type –OH, C-H, C –O, and C=C. OH and C=O bonding indicated active carbon tend to be polar so active carbon can be used as adsorbent.

Active carbon analysis with scanning electron Microscope (SEM)

Carbon before and after activation process was analyzed using SEM. Result of imaging using SEM describing carbon surface is presented in figure 7 and 8.

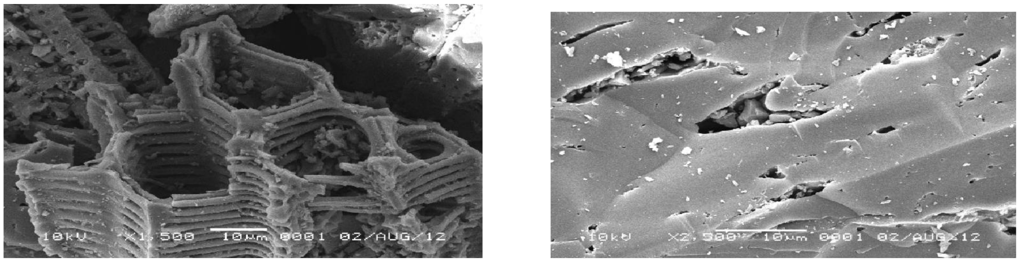


Figure 7. SEM photograph of coconut shell charcoal before activation with 1500 times and 2500 times magnification

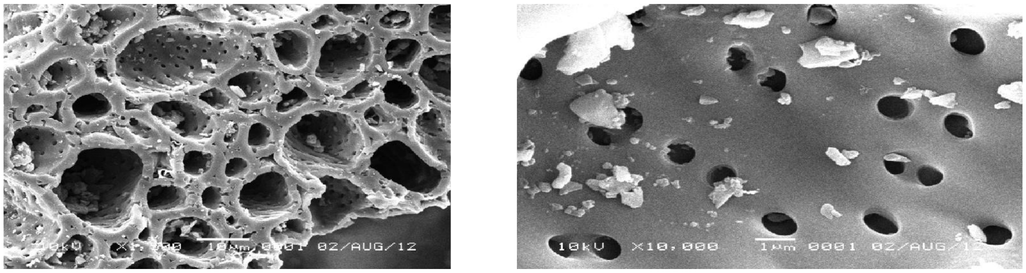


Figure 8. SEM photograph of coconut shell charcoal after activation at 1.5 M $ZnCl_2$ concentration, 24 hours soaking, at 800^0C and 120 minutes activation period with 1500 times and 10.000 times magnification

Figure 7 and 8 indicates shows active carbon before and after activation with activator $ZnCl_2$. Result of SEM before activation indicate much impurity covering pore surface of the carbon (figure 7) compared with after activation (figure 8) in which carbon pore surface more porous. It indicates that activation process can open and shape new pore in carbon. New pore shaping appear in pore diameter distribution

Characterization of active carbon from coconut shell charcoal with nitrogen adsorption-desorption

Adsorption-desorption isotherm for active carbon of coconut shell charcoal before and after activation is presented in figure 9. Based on classification of adsorption type, adsorption isotherm obtained is type IV. Hysteresis indicate mesoporous isotherm. Occurring hysteresis is loop but there is no presented in figure 9 because it occurred in relative small pressure. Coconut shell charcoal resulted from pyrolisis indicated broader hysteresis than active carbon resulted from chemical and physical activation with $ZnCl_2$. However, both samples have hysteresis in low pressure. It provides information about addition of complexity of active carbon pore after activation.

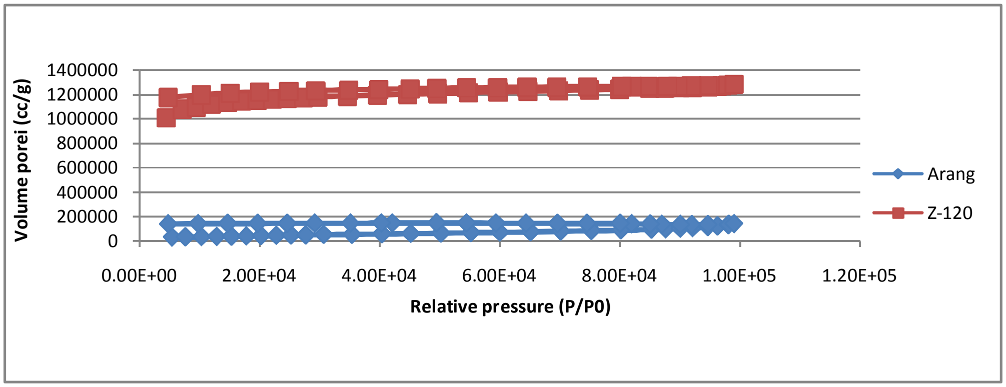


Figure 9. Isotherm of adsorption-desorption of active carbon resulted from activation

Activation of active carbon from coconut shell charcoal have changed its characteristic. It is due to effect of active carbon cavity blocking by cation after activation process. Distributions of active carbon pore dimension before and after activation increase significantly as revealed in figure 10. The figure shows that distribution of active carbon pore dimension after activation with $ZnCl_2$ has peak around pore radius 11.04 Å so dimension of the active carbon pore is mesopore.

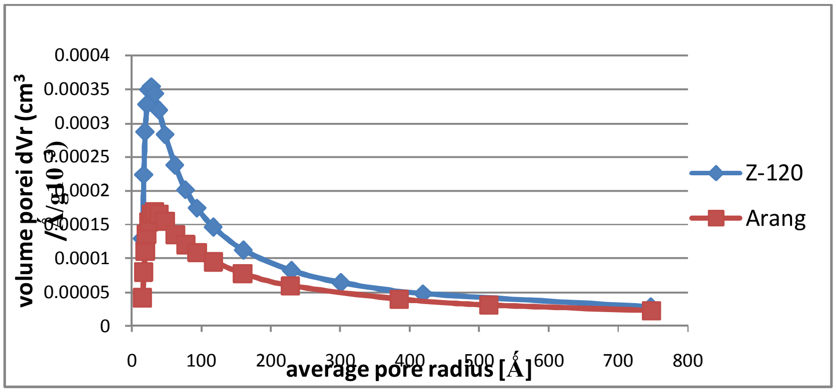


Figure 10. Distribution of active carbon pore dimension before and after activation

Surface area, pore size and pore volume is very important characteristic for material function as adsorbent because it determine measurement of internal surface available for active site. Parameter of active carbon pore structure before and after activation may be seen in table 6. The table indicates that active carbon before and after chemical activation with activator $ZnCl_2$ and physical activation at concentration of 1.5M soaking duration of 24 hour at 800^0C and 120 minutes activation period change pore structures of surface area, pore radius and pore volume.

Table 1. BET surface area, total pore volume, average pore radius of active carbon

Sample	**BET surface area (m^2/g)**	**Totalpore volume (x 10^{-1} cm^3/g)**	**Average pore radius (Å)**
carbon	15,451	0,217	26,478
Z8-1.5-24-120	359,286	1,984	11,045

Table 1 indicates that active carbon surface area before and after activation increased from 15.4561 m^2/gram to 317.1 m^2/gram along with increase in $ZnCl_2$ activator concentration and activation temperature (1.5M and 800^0C). It is due to at 800^0C, volatile substances or non volatile substance will discharge from pore that will form new pore and increase surface area and pore volume and decrease average pore radius.

Conclusion

1. Result of SPSS analysis on coefficient test indicated temperature most influence moisture content, volatile matter, ash content, fixed carbon content, iodine number compared concentration and soaking duration variables.
2. Active carbon resulted from activation process has adsorption pattern with binding types of O-H, C-H, C-O, and C=C. O-H and C=O bonding indicates active carbon tending to be more polar that can be used as adsorbent. Effect of carbonization process and activation can increase aromatic compound as composing hexagonal structure of charcoal and active carbon.
3. Chemical and physical activation of coconut shell charcoal with $ZnCl_2$ activator affect characteristic of active carbon. It may be seen in morphology of surface structure that is more porous and greater surface area.
4. Best active carbon obtained in $ZnCl_2$ concentration of 1.5 M, 24 hour soaking duration, 800^0C and 120 minutes activation duration. The condition resulted in 8.47% moisture content, 18.55% volatile matter, 1.44% ash content, 80.01% fixed carbon and 769.74 mg/g iodine number.
5. Active carbon from activation with various activation has similar pattern, smaller pore diameter and grater pore volume than that without activation. The active carbon has BET surface area of 359.286 m^2/g, pore volume 1.984 x $10^{-1}cm^3/g$ and average pore radius of 11.045 Å.

References

ASTM International., 2006, *Annual Book of ASTM Standards*, Section 5 : Petroleum product, Lubricant, and Fossil Fuels, 05(06) : Gaseous Fuels; Coal and Coke.

Babel, K., Jurewicz, K., 2004, KOH Activated Carbon Fabrics as Supercapacitor Material, *J.Phys. Chem. Sol.*, 65, 275-280

Balcı, S., Doğu, T., And Yücel, H., 1994, Characterization of Activated Carbon Produced from Almond Shell and Hazelnut Shell, *J. Chem. Tech. Biotechnol.,* Vol.60, 419-426

Bansal, R. Ch., and Goyal, M., 2005, *Activated Carbon Adsorption*, Taylor and Francis Group

Cheremisinoff, N.P., 1978, *Carbon Adsorption Handbook*, Ann Arbor Science Publishers Inc, Michigan

Cuhadaroglu, D dan Uygun, O.A., 2008, Production and Characterization of Activated Carbon from a Bituminous Coal by Chemical Activation, *Afr. J. Biotechnol*., Vol. 7(20), 3703-3710

Hong, E.H., Jung, Y.H., and Lee, K. H., 2000, Preparation of Mesoporous Activated Carbon Fibers by Catalytic Gasification*, Korean J. Chem. Eng.*, 17, 237

Jankowska, H., Swiatkowski, A. and Choma, J., 1991, *Active Carbon*, Ellis Horwood, New York

Kenneth, C. L., Nasrin, R. K., Marta, C., Giselle, S, and Thiyagarajan, P., 2002, Microstructural Analysis of Carbons Prepared from Paper Mill Slugle by SANS and BET, *Chem. Mater.*, 14, 327

Lee, S. H., and Lee, C. D., 2001, Influence of Pretreatment and Activation Condition in the Preparation of Activated Carbons from Anthracite, *Korean J. Chem. Eng.,* 18, 26

Mandey, L.L., 2007, *Biokonversi: Teknologi Pemanfaatan Limbah Industri Pertanian*, Sidang Senat Terbuka, Universitas Sam Ratulangi Manado, Manado

Manocha**,** S. M., 2003, *Porous Carbons*, S adhan Vol. 28, Parts 1 dan 2, Feb/Apr, 335-348

Rodenas, L., Castello, L., Amaros, C., Solano, L., 2005., Preparation of activated Carbons from Spanish anthracite Part 1. Activation by KOH, *Carbon,* 39 (5), 741-749

Scok, J.s., Jung, S.C., Ko. Y.C, and D.S. Sun, 2005, *Development of Carbon Dioxide Adsorbents Using Carbon Materials Preparated Coconut Shell*, *Korean J. Chem. Eng 22:291-297*.

Smisek, M. and Cerny, S., 1970, *Active Carbon, Manufacture, Properties and Applications*, Elsevier Publishing Company, New York

Sricharornchaikul, V., Pechyen, C., Aht-ong, D., Atong, D., 2008, Preparation and Characterization of Activated Carbon from the Pyrolysis of Physic Nut (Jatropha curcas L.) Waste, *Energ. Fuels*, 22, 31-37

Teng, H., and Yeh, T. S., 1998, Preparation of Activated Carbon from Bituminous Coals with Zinc Chloride Activation, *Ind. Eng. Chem. Res.,* 37, 58-65

Timur, S., Kantarli, I. C., Ikizoglu, E., Yanik, J., 2006, Preparation of Activated Carbon from Oreganum Stalks by Chemical Activation, *Energ. Fuels,* 20, 2636-2641

C-5

EFFECTS OF CALCINATION TEMPERATURES ON SYNTHESIS OF $LiMn_2O_4$ BY POLYMER MATRIX-BASED ALKALINE DEPOSITION METHOD

Dyah Purwaningsih, Hari Sutrisno, Dewi Yuanita Lestari

Department of Chemistry Education
Yogyakarta State University, Kampus Karangmalang, Yogyakarta, 55281
Email : dyahuny@yahoo.com

Abstract

$LiMn_2O_4$ offers more affordable price than other cathode materials for lithium ion batteries. The other factors are the abundance of the Mn element in nature and its low level of toxicity. Therefore, this study aims to investigate the effects of calcination temperature on the synthesis of $LiMn_2O_4$to obtain better condition for calcination to produce $LiMn_2O_4$. In this study, cubic $LiMn_2O_4$ is synthesized by Polymer Matrix Deposition Method using hydrothermal technique. Precursors used were LiOH, ethylene glycol and $Mn(CH_3COO)_2$. The precursors were synthesized at 150°C for 6 hours and calcined for 4 hours in different temperature variations in order to produce crystals with different sizes and crystalinity. The interval calcination temperature is 500-900°C. The compound was characterized by XRD, TEM and then was analyzed by *U-Fit* program. It is found that at low calcination temperatures, impurities MnO_2 is present but it decreases along with the increase of calcination temperature. The lattice parameters and volume of $LiMn_2O_4$also increase with the increase in heating temperature.The optimum calcination temperature is 700°C.

Keywords: $LiMn_2O_4$, polymer matrix-based alkaline deposition method, calcination temperature

INTRODUCTION

Today battery is almost inseparable from everyday life. Cell phones, digital cameras, laptops, hybrid cars, all require batteries as their energy source. Thus the prospect of battery asthe future energy source becomes strategic and economical. Among the many types of batteries,lithium battery has received great attention in research. In addition to having high power, lithium battery is lightand can be used many times (rechargeable). With the rapid development of technology, lithium battery is supposedly able to produce higher energy become indispensable (Thackeray, 1983).

Materials widely used as lithium battery cathode are $LiNiO_2$ and $LiCoO_2$ layered or the $LiMn_2O_4$ spinel structure (Howard *et al.*, 2003; Sarciaux, 1998). Compared to other lithium compounds such as $LiNiO_2$ and $LiCoO_2$, $LiMn_2O_4$ compound has more affordable price, abundance, and low toxicity level (Ritchie and Howard, 2005). $LiMn_2O_4$ has a spinel structure with the shape of the Li^+ ions fill the spinel tetrahedral cavities so that the flow of electricity or ion exchange electrons received from the anode (reduction reactions) can take place through these cavities (Van der ven *et al*, 2000).

Preparation of spinel $LiMn_2O_4$ is usually performed in a high temperature, between 700-900°C, with the reaction in the form of solid materials including manganese oxide, nitrate or carbonate with lithium hydroxide, nitrate, or carbonate (Zhang, 2011). However, the final product of this reaction often contains too much impurities and the crystalline structure is not well-shaped. To reduce it, various preparation methods such as sol-gel method, spray pyrolisis, hydrothermal (Hwang, 2001; Larcher, 1997; Sarciaux, 1998; Singh, 2010; Wang, 2002; He *at al*, 2003) have been developed.

In this study, we report the combination method using hydrothermal method and polymer matrix deposition techniques to synthesize $LiMn_2O_4$in various calcination temperatures to produce better $LiMn_2O_4$. Hydrothermal method using aqueous solvent takes place at a temperature and autogenous pressure of the bomb hydrothermal. Polymer Matrix Deposition technique uses ethylene glycol as polymer matrix. Precursors used in the synthesis process must be in the liquid phase. The polymers are used so that the precursors can be dispersed evenly in a polymer precursor that can be mixed homogeneously. Thus the reaction is expected to be running perfectly.

RESEARCH METHOD

The procedure was performed by making 50 mL of 0.4 M LiOH solution of LiOH crystals by weighing 0.2444 grams of crystalline LiOH using 50 mL volumetric flask. Then, 50 mL solution of $Mn(CH_3COO)_2$ 0.1 M of crystalline Mn $(CH_3COO)_2.4H_2O$ was prepared by weighing 1.2379 grams of crystalline $Mn(CH_3COO)_2.4H_2O$ using 50 mL volumetric flask. Both solutions were incorporated into a hydrothermal bomb, as much as 10 mL of ethylene glycol was added. The solution was then heated in an oven at 150°C for 6 hours. The resulting solid is then filtered and was put at room temperature for 24 hours. After that, the solid was calcined at 900^{o}C for 6 hours. The solid was then characterized by XRD and TEM. Similar calcination steps were also carried out to the temperature variations (T: 500.600, 700, 800, 900^{o}C).

RESULT AND DISCUSSION

This study was conducted to determine the effect of calcination temperature in the synthesis of $LiMn_2O_4$compounds using alkaline synthesis method based on the characteristics of $LiMn_2O_4$ polymer matrix. The polymer matrix-based alkaline deposition method can be categorized as a liquid phase reaction, which was part of soft chemical (*chimie douce*). This method is a combination of two methods: method of deposition and method of forming the base polymer matrix. The polymer matrix used in this study is ethylene glycol, which serves as a flocculant binder. Thus, after the addition of ethylene glycol, the bonds between the reactant compound will be disconnected and the polymer matrix will envelop the flocculant, so that the flocculants trapped in the matrix become more homogeneous.

The technique used in this study is hydrothermal technique as the formed crystalshave a high degree of purity, controllable stoichiometry, limitable particle size, controllable morphology, uniform and high crystallinity (Thackeray, 1983). Characteristics of $LiMn_2O_4$ compounds include the nature of crystallinity can be seen from the X-ray diffraction pattern.

Conducted at calcination temperature variations of 500, 600, 700, 800 and 900^{o}C,the reaction of $Mn(CH_3COO)_2$ and LiOH then yielded $LiMn_2O_4$. The synthesis performed at a temperature of 150^{o}C for 6 hours synthesis in aqueous solvent with hydrothermal method.

The resulting solidsare reddish black in color. Subsequently, the solids were characterized using X-ray diffraction, whose measurements were performed using an X-ray diffractometer in the region of $10° \leq 2\theta \leq 80°$ with a step size 0.0200 degrees, source of Cu Kα radiation (1.54060 Å), scan speed of 5.00 degrees per-minute, and irradiation performed continuously every 0.24 seconds. Smoothing process was conducted to clarify the X-ray diffraction pattern generated. *U - Fit* program is used to determine the space group and lattice parameters of the compound $LiMn_2O_4$,while TEM is used to determine the particle size of $LiMn_2O_4$.

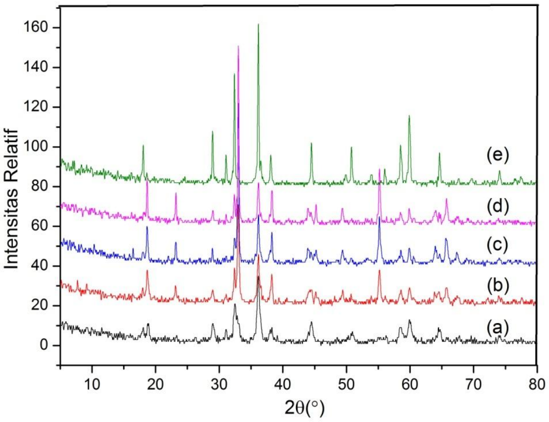


Figure1. X-ray diffraction pattern of solids produced in the calcination temperature variations of 500, 600, 700, 800 and 900°C

Table 1. Crystal plane (*hkl*) of solids generated at each temperature calcination

Solid produced at 500°C		**Solid produced at 600°C**		**Solid produced at 700°C**		**Solid produced at 800°C**		**Solid produced at 900°C**		***(h k l)***
2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	
18,723	21,9	18,699	29,2	18,698	20,3	18,660	22,7			(1 1 1)
								32.4	68.8	(2 2 1)
32,500	56,3					32,399	14,8			(3 0 0)
36,179	100	36,198	47,9	36,179	30,4			36.160	100	(3 1 1)
37,983	15,6									(2 2 2)
44,003	12,5			43,884	7,6	43,903	8			(4 0 0)
		45,198	10,4			45,184	9,1			(4 1 0)
								64.621	20	(4 4 1)
		48,140	6,3							(3 3 1)
		49.419	12,5	49,342	8,9	49,302	9,1			(4 2 0)
50,903	15,6	50,721	8,3	50,760	3,8					(4 2 1)
58,420	18,8									(3 3 3)
				63,943	10,1					(4 4 0)

Solid produced at 500°C		**Solid produced at 600°C**		**Solid produced at 700°C**		**Solid produced at 800°C**		**Solid produced at 900°C**		***(h k l)***
2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	
						65,772	13,6			(5 3 0)
				67,305	6,3					(5 3 1)
69,634	12,5									(6 1 0)
		72,205	6,3							(6 2 0)
74,00	9,4									(6 2 1)
								74.099	8.8	(6 3 0)
		77,620	6,3							(5 4 1)

Table 2. Crystal plane (*hkl*) of $LiMn_2O_4$at each calcinationtemperature

Solid produced at 500°C		**Solid produced at 600°C**		**Solid produced at 700°C**		**Solid produced at 800°C**		**Solid produced at 900°C**		***(h k l)***
2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	
18,723	21,9	18,699	29,2	18,698	20,3	18,660	22,7			(1 1 1)
36,179	100	36,198	47,9	36,179	30,4			36.160	100	(3 1 1)
37,983	15,6									(2 2 2)
44,003	12,5			43,884	7,6	43,903	8			(4 0 0)
		48,140	6,3							(3 3 1)
				63,943	10,1					(4 4 0)
				67,305	6,3					(5 3 1)

Based on the data presented in Fig. 1 and Table 2, there are differentpeaksin each calcination temperature. At 500°C,sharp peaks were formed showing that the calcination temperature forms crystalline solids with high peak intensity. At 600-800°C,the peaks become sharper but their intensity is lower compared to those at 500°C. Meanwhile, at 900°C there is only one peak corresponding to the *hk*l plane $LiMn_2O_4$ but has a high intensity. It can be concluded that the compound calcinated at 500 and 900°C has the best crystallinity compared to other temperatures. Of the five calcination temperatures, one which has the highest number of peaks with *hkl* plane corresponding to the standard $LiMn_2O_4$*hkl*plane is 700°C, i.e. with 5 peaks. Thus, the optimum temperature for the synthesis of $LiMn_2O_4$ by using polymer matrix based alkaline deposition method is 700°C.

In addition to the peaks of $LiMn_2O_4$ compound, the X-ray diffraction patterns also show peaks of impurities, largely in the forms of MnO_2compound. By using the *U-Fit* program and after matched with the standard of MnO_2compound data obtained 2θ and I / Io peaks of X-ray diffraction patterns of MnO_2compound impurity in Table 3.

Table 3. Crystal plane (*hkl*) of MnO_2at each calcinationtemperature

Solid produced at 500 °C		**Solid produced at 600 °C**		**Solid produced at 700 °C**		**Solid produced at 800 °C**		**Solid produced at 900 °C**		***(h k l)***
2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	2θ	I/Io	
29,019	28,1	28,981	12,5	28,923	8,9	28,982	6,8	28.96	33	(3 0 0)
								31.097	16	(0 0 1)
		32,980	100	33,000	100	33,020	100			(1 0 1)
37,983	15,6									(0 1 1)
		40,655	6,3							(0 2 0)
44,500	12,5			43,884	7,6					(4 1 0)
		45,198	10.4	45,183	6,3	45,184	9,1			(2 2 0)
						49,302	9,1			(5 0 0)
		50,721	8,3	50,760	3,8			50.781	23	(4 0 1)
50,903	15,6									(3 2 0)
				53,280	2,5					(5 1 0)
						55,199	30,7			(4 1 1)
								56.003	9	(2 2 1)
		59,882	14,6	59,842	8,9	59,878	8	59.899	40	(6 0 0)
		65,740	14,6	65,640	15,2	65,722	13,6			(0 0 2)
69,634	12,5									(6 0 1)
74,000	9,4									(3 0 2)
77,445	9,4									(3 1 2)

Table 4. Lattice Parameter of $LiMn_2O_4$

Lattice Parameter and Volume of Crystal	**Calcination Temperatures**				
	500°C	**600°C**	**700°C**	**800°C**	**900°C**
a (Ǻ)	8,187912	8,267488	8,230189	8,284771	8,302322
b (Ǻ)	8,187912	8,267488	8,230189	8,284771	8,302322

Lattice Parameter and Volume of Crystal	Calcination Temperatures				
	500°C	600°C	700°C	800°C	900°C
b (Å)	8,187912	8,267488	8,230189	8,284771	8,302322
V (Å^3)	548,9332	565,0940	557,4802	568,6455	572,2670

Based on Table 4, it can be concluded that the lattice parameters of crystalline $LiMn_2O_4$ has a cubic structure *Fd3m*. The lattice parameters for the material prepared at 700°C for $LiMn_2O_4$ was almost identical to the standard value of 8. 245 Å, according to JCPDS card file No. 35-782. Table 4 shows that the lattice parameters of the crystal and the crystal volume have a tendency to rise in line with increasing calcination temperature. This indicates that the higher the calcination temperature,the bigger both the lattice parameters and the volume of the crystal. At low calcination temperatures, the resulting crystals have a relatively lower degree of crystallinity. In the crystal resulting from low temperatures, there are still many crystal defects. The higher the calcination temperature, the less the crystal defects will be and the higher the degree of crystallinity.

The larger the crystal volume and the better the crystallinity, the better the electrical conductivity of the crystal, as when the crystal volume gets bigger, the ions space will also get wider so that the ions will freely move in and out, making the resulting electrical conductivity better. It is therefore expected that the produced $LiMn_2O_4$ will be used as the more durable cathode material of lithium batteries.

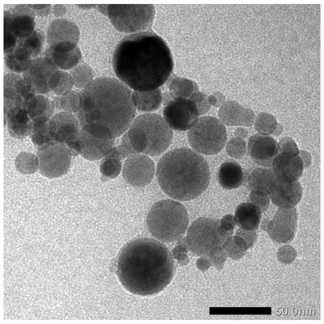


Figure2. TEM of $LiMn_2O_4$

Fig.2 shows that the $LiMn_2O_4$ solids generated from the polymer matrix-based alkaline deposition method with hydrothermal technique have good spherical morphology and uniform particle size between 20-50 nm.

CONCLUSION AND SUGGESTION

Based on the above discussion we can conclude the following:

1. Compound $LiMn_2O_4$ in various calcination temperature, T: 500, 600, 700, 800 and 900 °C can be synthesized with the polymer matrix deposition method using hydrothermal techniques.
2. Compounds synthesized $LiMn_2O_4$ (T: 500, 600, 700, 800 and 900 °C) has a cubic crystal structure with *Fd3m* phase alleged major and MnO_2 phase with minor phases with crystal structures orthorhombic.

A further research on the synthesis of compounds of Li with other methods to obtain the results that have single crystal structure.

REFERENCES

Armstrong, A.R. and Bruce, P.G. (1996) Synthesis of Layered $LiMnO_2$ as an Electrode for Rechargeable Lithium Batteries. *Nature*, 351, 499-500, doi: 10.1038/381499a0

Determination from Powder Diffraction Data. International Union of Crystallography, Oxford Science Publication

He, W.L., Zhang, Y.C., Zhang, X.X., Wang, H., Yan, H. (2003). Low temperature preparation of nanocrystalline Mn_2O_3 via ethanol-reduction of MnO_2, *Journal ofCrystal Growth*., 252, 285-288.

Hwang, B.J., Santhanam, R., Liu, D.G. (2001).Characterization of nanoparticles of $LiMn_2O_4$ syntesized by citric acid sol-gel method. *Journal of Power Source*. 98 : 443-446.

Larcher, D. (1997). Nouvelles voles de synthese et caracterisation de materiaux d'electrodes positives pour accumulateurs au lithium, *These de Doctorat*, L'Universite de Picardie Jules Verne, Perancis

R.C. Ropp. 2003. *Solid State Chemistry*. Netherlands: Elsevier.

Ritchie, A and Howard, W. (2005). Recent Development and Likely Advances in Lithium-ion Batteries. *Journal of Power Sources* 162 (2006) 809–812

Sarciaux. (1998) Les composes MnO2 γet LixMnO2 γ: influence des parameters structuraux et physic-chimiques sur l'insertion electrochimique du lithium. *These de Doctorat*. Universite de Nantes, Perancis

Singh, P., Sil, A., Nath, M., & Ray, S. (2010). Synthesis and Characterization of $Li[Mn_{2-x}Mg_x]O_4$ (x=0.0-0.3) Prepared by Sol-Gel Synthesis. *Original Papers Ceramics-Silikaty*. 54 (1) : 38-46Tarascon, J.M., Coowar F., Amatucci G.G., Shokoohi F., and Guyomard D., (1994). *J. Power Sources*, pp. 1421

Thackeray, M.M., David W.I.F., Bruce P.G., and Goodenough J.B., (1983) *Mat. Res. Bull.*, pp. 461

Van der ven, A., Marianetti, C., Morgan, D., & Ceder, G. (2000). Phase Transformations and Volume Changes in Spinel $Li_xMn_2O_4$. *Solid State Ionics*. 135 : 21-32.

Wang, X., Li, Y. (2002). Selected-Control Hydrothermal Synthesis of α- and β-MnO_2 Single Crystal Nanowires. *J. Am.Chem.Soc*. Vol. 124, No. 12, 2880-2881

Zhang, X., Zheng, H.,Battaglia, V., Axelbaum, R.L. (2011). Electrochemical performance of spinel $LiMn_2O_4$ cathode materials made by flame-assisted spray technology. *Journal of Power Sources*. 196 : 3640–3645.

C-6

SILVER NANOPARTICLE IMPREGNATED ON THE COMPOSITE OF BACTERIAL CELLULOSE-CHITOSAN-GLYCEROL AS ANTIBACTERIAL MATERIAL

Eli Rohaeti[1], Endang Widjajanti LFX[2], and Anna Rakhmawati[3]

[1, 2] *Chemistry Education Department, FMIPA UNY*
[3] *Biology Education Department, FMIPA UNY*

Abstract

The objective of this research were to study the characteristic of silver nanoparticles, the characterictic of silver nanoparticle impregnated on the bacterial cellulose, and the effect of silver nanoparticles toward antibacterial activity of bacterial cellulose and its composites. Bacterial cellulose of rice wastewater is fermented by *Acetobacter xylinum* for 7 days. The water containing bacterial cellulose is removed by heating in the oven. Bacterial cellulose, bacterial cellulose-chitosan composites and bacterial cellulose-chitosan-glycerol composites had been prepared by immersing dry bacterial cellulose in chitosan and glycerol solution. The silver nanoparticles was prepared by chemical reduction with using silver nitrate solution and tri-sodium citric as reducing agent. UV-Vis spectrometry indicated formation of nanoparticles. To achieve antibacterial activity, silver nanoparticles were impregnated into bacterial cellulose-chitosan-glycerol composites by immersing method. Morphology of nanoparticels impregnated in bacterial cellulose-chitosan-glycerol composites was examined by SEM. The antibacterial activity of silver nanoparticles which was impregnated on the bacterial celluloce-chitosan-glycerol was examined by shake flask turbidimetry and clear zone method. The UV-Vis spectroscopy revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption maximum at 419 nm. The SEM image showed that the silver nanoparticles has been impregnated in the surface of bacterial cellulose. The nanoparticles of silver-impregnated bacterial cellulose-chitosan-glycerol composites exhibited antibacterial activity against *S.aureus* and *E.coli*.

Key words: antibacterial activity, bacterial celullose, bacterial cellulose-chitosan, bacterial cellulose-chitosan-glycerol composite, silver nanoparticles.

INTRODUCTION

Nanoparticles are particles that have dimensions less than 100 nanometers. The surface area per unit weight nanoparticles is greater than the width of the particles. This nanoparticle is more reactive than some other molecule. Silver nanoparticles have been shown to have good ability as the anti- microbial against bacteria, viruses and microorganisms eukaryotic structural. Colloidal silver is particularly interesting because it has unique properties and a good conductivity material, chemically stable, can act as a catalyst, and has an antibacterial activity. In low concentrations of silver is not toxic to human cells [1].

Colloidal silver has long been known as an antibacterial. The shape and size of nanoparticles is critical in determining the optical properties, electrical, magnetic, catalytic, and antimicrobial. The smaller size will parikel greater antimicrobial effect . Factors that affect the size of the particles in the synthesis is the temperature, reducing agents, concentration and reaction time. Studies have shown that the particle size, morphology, stability and properties of metal nanoparticles is influenced by the operating conditions of the experiment, the kinetics of the interaction among the metal ions, reductor, and the absorption of stabilizer [2].

Cellulose is the most abundant natural biopolymer on the earth, synthesized by plants, algae, and also some species of bacteria [3]. Bacteria also synthesized cellulose, including the genera Agrobacterium, Rhizobium, Pseudomonas, Sarcina, and Acetobacter [15]. Bacterial cellulose is produced by *Acetobacter xylinum* is markedly different from cellulose obtained from trees and cotton. From the culture medium a pure cellulose network free of lignin and hemicellulose is obtained as a highly hydrated pellicle made up of a random assembly of ribbon shaped fibers less than 100 nm wide. These fibers themselves are composed of a bundle of much finer microfibrils of nanometric size [3, 4]. The unique properties provided by the nanometric structure have lead to a number of commercial products including tires, headphone membranes, special papers, and textiles [4], medical applications including temporary skin substitution [4, 7].

Unlike cellulose from plants, bacterial cellulose (BC) is chemically pure and free of lignin and hemicelluloses. Bacterial cellulose does not require remedial processing to remove unwanted polymers and contaminants, and therefore, retains a greater degree of polymerization [3]. BC has high crystallinity, 2 common crystalline forms of cellulose, designated as I and II. Cellulose I is synthesized by the majority of plants and also by A. xylinum in static culture, parallel ß-1,4-glucan chains are arranged uniaxially. Whereas ß-1,4-glucan chains of cellulose II are arranged in a random manner. They are mostly antiparallel and linked with a larger number of hydrogen bonds that results in higher thermodynamic stability of the cellulose II. [1, 4]Bacterial cellulose is also used as a source of dietary fiber (nata-de-coco) [2], as binding or thickening agents. Interesting composite materials may also be obtained from cellulose. The water uptake depends on the crystalline state of cellulose and the amount of grafted acrylic counterpart [2]. Cellulose phosphate membranes were also studied concerning their biomedical applications. Phosphorylated cellulose seems to be interesting material for bone regeneration and osteointegration.

Bacterial cellulose has unique structural and mechanical properties as compared to higher plant cellulose, BC is expected to become a commodity material in various fields. The BC fibers have a high aspect ratio with a diameter of 20-100 nm. As a result, BC has a very high surface area per unit mass. This property, combined with its highly hydrophilic nature, results in a very high liquid loading capacity. Morever, biocompatibility makes it an attractive candidate for a wide range of applications in different fields, especially those related to biomedical and biotechnology applications [5]. However, in most practical applications, BC may not be of perfect quality and its cost may not be suitable for industrialization either. For economical mass production, it is essential to design a culture aeration and agitation process [10].

Media that can be used as a place to live bacteria is coconut water, wastewater of cassava and rice wastewater and other sources. Rice wastewater is household waste which still contains starch and other compounds content carried by water during the process of washing rice [18], so that the rice wastewater can be used as a medium of live bacteria *acetobacter xylinum*. The use of rice wastewater is an effort to add value to the household waste as materials for natural polymers that can be degraded in the soil so it does not pollute the environment .

Bacterial cellulose had structure linear, highly crystalline, and degree of intermolecular hydrogen bonding is very high, so that many bacterial cellulose had be applied in papermaking, textile, and food industries as cosmetics and biomaterials in the medical field [16]

In the medical field, bacterial cellulose is used as a wound covering material and other medical materials. Antibacterial properties needed to maximize the usefulness of cellulose as a wound covering material because it can reduce bacterial contamination on cellulose and on wounds . The antibacterial properties can be obtained by using a cellulose material deposed silver nanoparticles. Nanoparticles of silver have been known as an antibacterial material because of its ability to make the bacterial cell wall lysis and bacterial death [1]. In addition to the silver nanoparticles can serve as a barrier to the growth of mold and bacteria that often occurs in cellulose . Research on the antibacterial properties of silver nanoparticles and silver nanoprtikel applications in the field of medical and textiles have been carried out [17]. the use of silver nanopertikel have an advantage because they are toxic to microbes but is safe for humans in low concentrations . With the presence of silver nanoparticles that are antibacterial , wound closure will be effective to protect the wound and reduce the occurrence of infections . Preparation of antibacterial cellulose can be combined with several materials to improve the antibacterial properties and mechanical properties of the cellulose . In addition to the deposition of nanoparticles , the antibacterial properties of the cellulose can be obtained with the addition of the chitosan material . Chitosan has long been known as an antibacterial polymer material [9] that are widely applied in the fields of medical and food preservation . The addition of chitosan on cellulose aims to increase the antibacterial properties of the cellulose , cellulose - chitosan composite nanoparticles deposited silver is expected to provide optimal antibacterial properties . There are shortcomings in the manufacture of cellulose - chitosan composite is . The addition of chitosan to cellulose will reduce the mechanical properties of cellulose and antibacterial properties of chitosan itself [10].

Mechanical properties are reduced due to the addition of chitosan on cellulose materials can be overcome with the addition of plasticizers such as glycerol. Glycerol is used as a plasticizer to improve the profile of alginate and chitosan films, preserving the integrity, avoiding holes and cracks [13]. Glycerol was added to a certain concentration to obtain the structure of cellulose are quite elastic so that it can be easily applied as a wound covering material.

In this paper, we focus on preparing bacterial cellulose membranes from rice wastewater with addition chitosan and a plasticizer. Rice wastewater still contains nutrient which needed *Acetobacter xylinum* in synthesizing bacterial cellulose. In this work, new composite membranes were prepared from rice wastewater and chitosan. Their characterization have been done using scanning electron microscopy, X-ray diffraction, tensile tester, FTIR, and antibacterial activity.

RESEARCH METHOD

Preparation of Bacterial Cellulose and Its Composites

20 grams of sucrose, 1 gram of urea, and 1,0 gram glycerol were mixed in 200 mL rice wastewater. Rice wastewater was poured into Erlenmeyer that has been equipped with a magnetic stirrer, then stirred until dissolved. When the pH of the mixture solution was ranged between 5-6, the mixture was acidified by addition of glacial acetic acid to a pH range from 3-4. Subsequently the mixture was cooled briefly and then poured in a warm state into a tray that has been sterilized with alcohol cooled to room temperature is reached. After chilling the mixture was added 40 mL of *Acetobacter xylinum* and trays sealed properly using newspaper and fermented for 7 days at room temperature. After 7 days, the newspaper cover is opened and formed pellicle layer was taken and washed several times with tap water, then with distilled water, then with hot water, then this pellicle layer weighed. Then a solution of 2% chitosan with deacetylation degree of 73.78% is poured onto the pellicle layer and dried in an oven with a temperature between 37- 40 ° C.

Application of Silver Nanoparticle to Bacterial Celluloseand its Composites

100 mL of $AgNO_3$ (aq) $1x10^{-3}$ M is put into the three neck flash and mixed with 0.5 grams of PVA and than refluxed at 100 ^{0}C by stirring. Tri-sodium citric is added until reach pale yellow color. The silver nanoparticle is characterized by using uv-vis spectrophotometer.

Bacterial cellulose and its composite is impregnated with silver nanoparticle over 60 minutes at 145 rpm.

Characterization of Bacterial Cellulose and Its Composites

Analysis of the physical properties of macroscopic and organoleptic. This analysis includes the observation of color, texture, shape and transparency of each sample.

Analysis of functional group using FT-IR instrument. This analysis uses a set of tools FTIR and performed at the Laboratory of Organic Chemistry of the Faculty of Mathematics and Science, UGM. A thin layer is clamped in place and then put the device in the direction of the infrared beam. The result will be recorded onto paper in the form of the intensity of wave numbers.

Analysis of morphological using SEM instrument. SEM image of bacterial cellulose and its composites is measured using SEM instrument. This test is performed in Laboratory at the Center for Borobudur Conservation Agency. Sample is cut in such a way, then the is coated with ion coater apparatus for approximately 5 minutes before vacuum process. Sample is introduced into the electron gun. Then the sample set with microstage to get the right focus. The main key in the ON position and set Accelerate voltage detector sets, 20 kilo volts.

Analysis of mechanical properties. The analysis was performed in Laboratory of Biotechnology, Faculty of Agricultural Technology, UGM. The dried sample material is cut to the size of 11 cm x 2 cm. Results pieces/specimen put in dumbble cutting. Then do the appropriate cutting pattern formed on the sample. Then the sample was measured with a micrometer Mitutoyo thickness and both sides of the cutting result is then attached to a Universal Testing Machine. Power and panel in the ON position. Fill the sample data fit standard size. Specification tool used is taking a knife to the ASTM standard. Then the device is turned on and set to the test speed = 10 mm/min and the specimens were observed to drop out, the test is stopped when the specimen is broken. Data obtained in the form of percent elongation, tensile strength, and F max.

Analysis of thermal properties using Differential Thermal Analysis (DTA). Thermal properties is performed in ATK Yogyakarta. As much as ± 15 mg samples were put into the crucible where the sample that had previously been filled little by standard alumina manufactured by Perkin-Elmer DTA-TGA. Then loaded alumina is also standard that has been put into the crucible and placed on the reference in the DTA-TGA analyzer tool and the weight of the instrument is calibrated using a reference sample in order to obtain the desired weight. Then the condition of temperature measuring devices operated at 30-400^0 C with heating rate 10 ^{0}C per minute and appliance turned on. On the computer, set to a program to read DTA thermogram.

Analysis of crystallinity by X-Ray Diffraction (XRD). Crystallinity was analyzed by using XRD instruments in Department of Geological Engineering, UGM. Samples are cut to size sheet 2x2 cm^2. The sample is then placed in the sample holder and the sample flat on the cultivated sample holder. Further cooling equipment XRD, XRD instrument is turned on and then set conditions for devices with rotary angle $2\theta = 2°$ to $80°$, scan step = 0.04, and a scan speed = 4 °/min.

RESULT AND DISCUSSION

Figure 1 shows that there is a widening of the spectra in the region 3400 cm^{-1} when cellulose was added to the chitosan. The addition of chitosan can improve the reading group at wave number 3400 cm^{-1} due to the – NH_2 in chitosan and – OH in cellulose overlap. All three spectra are still visible in the area around wave number 1570 cm^{-1} indicating the presence of aromatic rings. In the cellulose contained chitosan shows amino group the vibrations at 1558.48 cm^{-1} which are characteristic of chitosan [9]. The characteristic absorption alleged overlap with the aromatic ring absorption at wave number 1570 cm^{-1}, cellulose and chitosan have aromatic rings . In both control no appearance of the spectra in the region 1635 cm^{-1} . These spectra indicate the presence of group C = O. It shows that with the addition of chitosan which has a C = O group in the cyclic as well as the possibility of residual chitin are not going to increase the absorption at 1635 cm^{-1}. Bacterial cellulose has functional group –OH, -CH,

CH_3 bending vibration, and β-1,4-glucan. The cellulose-glycerol-chitosan composites shows –OH, -CH, CH_3 bending vibration, C=O stretching, β-1,4-glucan, -NH stretching (amide I) and –NH bending (amide II). The adding chitosan can cause the presence of C = O stretching (Amide I) and-NH bending (Amide II) that are characteristic of group C = O amide I and amide II NH group contained chitosan. This indicates that chitosan has interacted with bacterial cellulose and glycerol, but amide groups of chitin are not deacetylated perfectly.

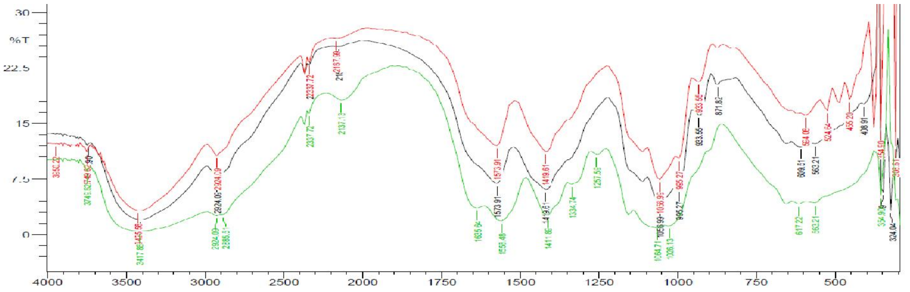


Figure 1. FTIR spectrum of bacterial cellulose-glycerol/BC-G (upper), BC (medium), and bacterial cellulose-glycerol-chitosan/BC-G-Ch (lower)

Table 1 shows absorbance of –OH and –NH in bacterial cellulose and its composites. The adding glycerol and chitosan in preparation bacterial cellulose can cause the decreasing absorbance of the-OH group. This will support the decreasing in percent elongation of bacterial cellulose when added with glycerol and chitosan as well as increasing thermal stability of bacterial cellulose-glycerol-chitosan.

Table 1. The Absorbance of bacterial cellulose from rice wastewater and its composites

Wave number (cm^{-1})	Functional group		Absorbance	
		BC	BC-G	BC-G-Ch
3400	-OH	0.360	0.320	0.329
1635	-NH	-	-	0.116

The addition of chitosan will lead to widening and sharpening of the peak-OH group of cellulose bacteria around the wave number 3400 cm^{-1}. The broadening of this peak indicates a potential for overlap between the-OH group with -NH_2 groups. This is indicates the possibility of overlap between the interaction of hydrogen from the-OH with -NH_2 [9]. Based on Table 1, the visible presence of 1635.64 cm^{-1} and 1566.20 cm^{-1} indicating the presence of C = O stretching (Amide I) and-NH bending (Amide II), shows amide groups of chitin are not deacetylated perfectly [9].

Figure 2 presents a SEM photograph of the surface of bacterial cellulose and bacterial cellulose-glycerol -chitosan. However, the second image is sufficient to prove the existence of differences between chitosan and cellulose. It is proved that chitosan is able to coat the entire surface added from bacterial cellulose.

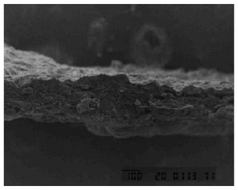


Fig 2.a. SEM Image of Bacterial Cellulose from rice wastewater

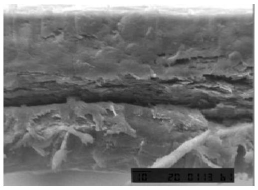


Fig 2.b. SEM Image of Bacterial Cellulose-Glycerol-Chitosan Composite

Table 2. Mechanical Properties of Bacterial Cellulose from Rice Wastewater and Its Composites

Parameter	BC	BC-G	BC-G-Ch
Tensile strength (MPa)	22.48	15.60	17.01
Elongation (%)	22.18	28.12	8.01

Table 2 shows that bacterial cellulose control (BC) has a tensile strength is 22.48 MP , whereas the cellulose-glycerol (BC-G) has a tensile strength that is 15.60 MPa . Decrease in tensile strength is due to the nature of glycerol as a plasticizer that makes rigidity of the material decreases. Result of the decrease in rigidity of this material is lower tensile strength but has the advantage of a higher elongation. Percentage of elongation of BC-G is 28.12 % while in the control cellulose (BC) only 22.18 % . It is proved that the addition of glycerol as a plasticizer cause decreased in tensile strength but increased in elongation [10]. This decrease was due to reduced intermolecular interactions were replaced with polymer bonding interactions with glycerol resulted properties of the polymer becomes more elastic but brittle.

When comparing the cellulose (BC and BC-G) with BC-G-Ch, tensile strength of BC with addition of chitosan will be lower than BC but higher than BC-G. The decreasing of tensile strength of BC-G-Ch is caused chitosan has a high amorphous, whereas the cellulose has high crystallinity. A strong structural material due to its high crystallinity naturally resilient to higher pressure, than the irregular structure of materials and provide lots of space around it. The addition to the amorphous nature of the material which has a high crystallinity will make the strength of the pressure is reduced [14]. The addition of the chitosan, elongation decreased very significantly. Elongation of BC-G's were initially 28.12%, with the addition of chitosan led to a 8.01%. The use of corn starch films happen intermolecular bonds form hydrogen bonds. This bond increases tensile strength but lower elongation [15].

The existence of the rigid structure of the polymer chain will cause the polymer chains become increasingly difficult to move when it is pulled so that elongation will decline [10]. This is reinforced by the results of XRD analysis, a decline in the crystallinity of bacterial cellulose when added to the chitosan. A decrease in the crystallinity showed the addition of amorphous regions in bacterial cellulose. Chitosan is able to reduce the crystallinity of bacteria cellulose due to the presence of amorphous region in chitosan. Bacterial cellulose has a high crystallinity, this is relationship with the high mechanical properties of bacterial cellulose [11]. The addition of chitosan concentration on bacterial cellulose from 12 to 45%, its tensile strength tends to decrease from 130 MPa to 54 MPa while the value of percent elongation dropped from 12% to 6.8% [11].

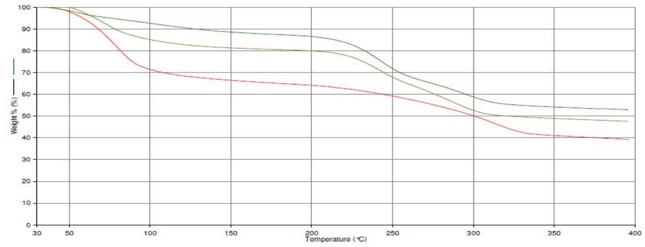


Figure 3. The TGA Thermogram of BC-G (upper), BC (medium), and BC-G-Ch (lower)

Based on Fig. 3, cellulose is added with glycerol and chitosan has a high stability compared with both BC and BC-G. If the data is quantified by taking the final point on the importance of the temperature of 400°C, % mass loss of cellulose (BC), BC-G, and BC-G-Ch respectively 33.54% , 35.56% , and 27.11% . Decrease in thermal stability of BC-G compared to BC might be due to differences in the polymerization of the cellulose. Polymerization on cellulose without glycerol forming linear polymers whereas the cellulose polymer is added to the glycerol producing branched polymers. This is explained by the strong bond that is formed . Bonding in the linear polymer β - 1 ,4 - glycosidic bonds which are covalent, while the branched polymer to form hydrogen bonds. The covalent bond will be much stronger than hydrogen bonds so that the linear polymer is more difficult to decompose than branched polymers [16]. Another possibility, because glycerol is evaporated of polymers. Composite of glycerol-a polymer will evaporated at 200°C temperature [12].

Results from DTA thermogram shows endothermic peak as the release of water molecules. Temperature required for BC-G-Ch release of water molecules is higher compared with both BC and BC-G, because the cellulose pores filled by chitosan, so that water molecules trapped in the cellulose.

BC has degree of crystallinity was 73.65 % , while the cellulose is added glycerol and chitosan having a degree of crystallinity of 50.15 % (Fig. 4). This indicates that chitosan causes a decrease in the degree of crystallinity of cellulose. Chitosan has an amorphous nature, whereas cellulose has a high crystalline properties. The existence of the amorphous nature of the cellulose into the cause deterioration of crystallinity. Decrease the degree of crystallinity affects the mechanical properties of cellulose. Cellulose that has a low degree of crystallinity tend to have a low tensile strength. The addition of chitosan will increase the tensile strength due to the formation of hydrogen bonding between cellulose with chitosan that increases rigidity, but there was also a decrease in the degree of crystallinity of cellulose so that the tensile strength decreases.

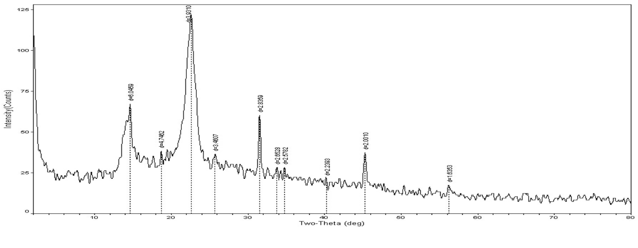


Figure 4. The XRD Diffractogram of BC from rice wastewater-Glycerol-Chitosan Composite

Results of characterization of colloidal silver nanoparticles by using UV - Vis Spectroscopy Shimadzu 1601 is showed by Fig. 5. Figure 5 showed the maximum wavelength of 10^{-3}M $AgNO_3$ solution at 216.20 nm region.

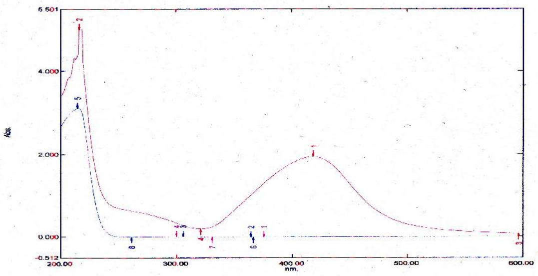


Figure 5. Spectrum of UV - Vis of 10^{-3}M $AgNO_3$ solution and colloidal silver nanoparticles

Colloidal silver nanoparticles showed two largest peaks in the maximum length of 423.40 nm and at 225.80 nm at the maximum length. Based on the studies that have been done by Sileikaite et al (2006) showed that colloidal silver nanoparticles having a maximum wavelength range 350-550 nm with the analysis using visible light spectrophotometer. This research was conducted in the wavelength range of 200-500 nm. Silver nitrate solution has an absorbance spectrum peaks at a wavelength of 215 nm. After the silver nitrate solution is mixed with trisodium citrate, UV-Vis spectra obtained are very much different and the obtained peak absorbance at a wavelength of 350-500 nm. After the reduction process, obtained absorbance of visible light at a wavelength of 419 nm of 0.187. The results of spectrophotometric analysis of the silver nanoparticles at a wavelength of 400-450 nm particles formed is Ag. Based on these data it can be concluded that silver nanoparticles has been obtained by reduction silver nitrate solution.

The addition of silver nanoparticles on cellulose did not change the physical characteristics of cellulose previously. Fig. 6 and Fig. 7 showed SEM image of bacterial cellulose and composite of bacterial cellulose – chitosan impregnated silver nanoparticles.

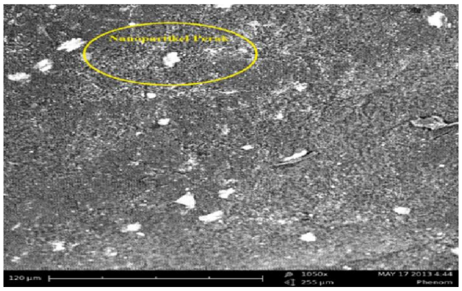


Figure 6 . SEM image of Bacterial Cellulose Composites from rice wastewater impregnated Ag (magnification 1050x)

Based on Fig. 6, we can see the surface morphology of bacterial cellulose impregnated silver nanoparticles with a magnification of 1050x. Fig. 7 showed the top surface morphology of bacterial cellulose - chitosan composite impregnated silver nanoparticles with a magnification of 8700x . Based on SEM image, we can see that silver nanoparticles successfully impregnated on the bacterial cellulose derived from rice waste water. Suspected silver nanoparticles adsorbed on bacterial. Interaction between the bacterial cellulose and silver nanoparticles occurs through chemical bonds to form a covalent bond between the-OH group on the bacterial cellulose with Ag in the silver nanoparticles.

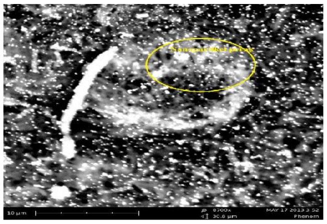


Figure 7. SEM image of Bacterial Cellulose - Chitosan Composites impregnated Ag (magnification 8700x)

Fig. 8 showed antibacterial activity of bacterial cellulose – glycerol – chitosan composite impregnated Ag against *S.aureus* and *E.coli* by using shake flash turbidimetry method. The results of testing the antibacterial activity of all the cellulose samples showed positive results. The clear zone method showed that antibacterial activity of bacterial cellulose – glycerol – chitosan composite impregnated Ag against *S.aureus* lower than antibacterial activity of bacterial cellulose – glycerol – chitosan composite impregnated Ag against *E.coli*. The diameter of clear zone in bacterial cellulose – glycerol – chitosan composite was 0.27 mm against *S.aureus*, and 1.00 mm against *E.coli*.

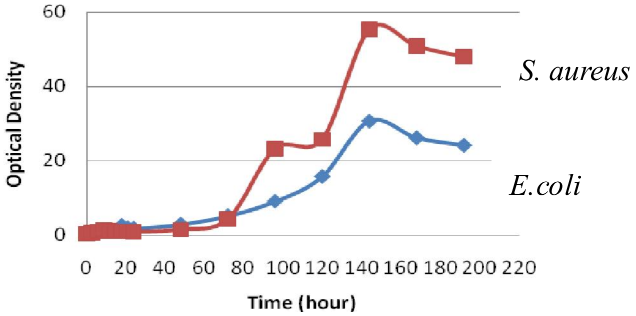


Figure 8. Antibacterial activity of Bacterial Cellulose – Glycerol – Chitosan composite impregnated Ag against *S.aureus* and *E.coli*

Mechanism of the antibacterial properties of silver nanoparticles is the presence of heavy metal ions will react with the thiol group (- SH) on the protein surface . Particles of monovalent silver ions (Ag^+) cation is able to replace the hydrogen (H^+) of the thiol groups of proteins that inactivate sulfhydryl, a decrease in membrane permeability, and ultimately leads to cell death. From these statements interaction between silver nanoparticles with cellulose membrane is important because it affects the release of silver nanoparticles will attack the bacterial wall, so that differences in the effectiveness of the antibacterial activity of each cellulose in testing can be due to differences in the interaction of silver nanoparticles with functional groups on each cellulose.

The interaction that occurs between the silver nanoparticles with functional groups on the cellulose membrane is electrostatic interaction. Electrostatic interaction between functional groups of polymers and nanoparticles depends on the charge of polymer, polymer structure and chemical content in the polymer. Any kind of cellulose samples tested had the charge of electrons (the number of pairs of electrons) and a different structure of cellulose .

CONCLUSION

The UV-Vis spectroscopy revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption maximum at 419 nm. The SEM image showed that the silver nanoparticles has been impregnated in the surface of bacterial cellulose. The nanoparticles of silver-impregnated bacterial cellulose-chitosan-glycerol composites exhibited antibacterial activity against *S.aureus* and *E.coli*.

REFERENCES

[1] Handayana, A., and J. A. Laksmono. (2010). Preparasi Koloid Nanosilver Menggunakan Stabilizer Polivinil Alkohol dan Aplikasinya Sebagai Antibakteri Pada Bakteri *S. aerus* dan E.coli. *Laporan Penelitian.* Fakultas Teknik Universitas Indonesia. Jakarta

[2] Khan, Z. 2011. Silver Nanoparticles: Green Route, Stability and Effect of Additives. *Jurnal of Biomaterial and Nanobiotechnology*. 2(1), pp. 390-399

[3] Zhang, S. and Luo, J. 2011. Preparation and properties of bacterial cellulose/alginate blend bio-fibers, *Journal of Engineered Fibers and Fabrics*, 6(3), pp.69-72

[4] Barud, H. S., Ribeiro, C. A., Crespi, Marisa S, Martines, M. A. U, Dexpert-Ghys, J., Marques, R. F. C, Messaddeq, Y. and. Ribeiro, S. J. L. 2007. Thermal characterization of bacterial cellulose-phosphate composite membranes. *Journal of Thermal Analysis and Calorimetry,* 87(3), pp.815–818

[5] Klemm, D., Schumann, D., Udhardt, U., Marsch, S. 2001. Bacterial synthesized cellulose – artificial blood vessels for microsurgery. *Progress in Polymer Science*, 26(1), pp.1561-1603

[6] Klemm, D., Heublein, B., Fink, H.P., Bohn, A. 2005. Cellulose:Fascinating biopolymer and sustainable raw material. *Angewandte Chemie- International Edition*, 44(1), pp.3358-3393

[7] Czaja, W., Krystynowicz, A., Bielecki, S., Brown, R.M. 2006. Microbial cellulose-The natural power to heal wounds. *Biomaterials*, 27(1), pp.145-151

[8] Yoshinaga, F.; Tonouchi, N. & Watanabe, K. 1997. Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material, *Bioscience, Biotechnology and Biochemistry*, 61(2), pp.219-224

[9] Stefanescua, C., Dalya, W. H. and Negulescu, I. I. 2012. Biocomposite films prepared from ionic liquid solutions of *chitosan* and cellulose. *Carbohydrate Polymers,* 87(1), pp. 435– 443.

[10] Suyatama, N. E., Tighzert, L. and Copinet, A, 2005. Effects of Hydrophilic Plasticizers on Mechanical, Thermal, and Surface Properties of *Chitosan* Films. *J. Agric. Food Chem.*, 53(1), pp. 3950-3957.

[11] Zhong, Q.P, dan Xia, W.S., 2008, Physicochemical Properties of Edible and Preservative Films from Chitosan/Cassava Starch/Gelatin Blend Plasticized with Glycerol, *Biotechnol.* 46 (3), pp. 262–269

[12] Zhijiang, C., Chengwei, H., Guang, Y., 2011, Preparation and Characterization of a Bacterial Cellulose/*Chitosan* Composite for Potential Biomedical Application. *Journal of Polymer Research,* 18(4), pp. 739-744

[13] Yunos and Rahman Yunos, M. Z. B. and Rahman, W. A.W. A. 2011. Effect of Glycerol on Performance Rice Straw/Starch Based Polymer. *Journal of Applied Sciences,* 11(1), pp. 2456-2459

[14] Nasab, M.M. and Yousefi, A.R. 2010. Investigation of physicochemical properties of the bacterial cellulose produced by *Gluconacetobacter xylinus* from date syrup. *World Academy of Science, Engineering and Technology*, 44(1), pp.1258-1263

[15] Rechia, L.M., Morona, J.B.J., Zepon, K.M., Soldi, V., dan Kanis, L.A., 2010, Mechanical properties and total hydroxycinnamic derivative release of starch/glycerol/Melissa officinalis extract films, *Brazilian Journal of Pharmaceutical Sciences,* 46(3)

[16] Steinb□chel. 2005. Biopolymer for Medical and Pharmaceutical Applications volume 1. Wienhelm. Wiley-VCH Verlag GmbH & Co. KGaA
[17] Haryono and Harmami. 2010. Aplikasi Nanopartikel Perak pada Serat Katun sebagai Produk Jadi Tekstil Antimikroba. *Jurnal Kimia Indonesia.* 5(1), pp. 1-6
[18] Harris and Karmas. 1989. *Evaluasi Gizi Pada Pengolahan Bahan Pangan*. Bandung: Penerbit ITB

C-7

DETERMINATION OF GLYCEMIC SCORE OF PROCESSED FOOD FROM WHOLE WHEAT (*Triticum aestivum* L.) FLOUR DEWATA'S VARIETY IN TERMS OF AMYLOSE CONTENT AND STARCH DIGESTIBILITY

Febrine Pentadini*, Silvia Andini, Sri Hartini, Anik Tri Haryani

Chemistry, Science and Mathematics Department, Satya Wacana Christian University
Diponegoro street 52-60 Salatiga, Indonesia
652010002@student.uksw.edu
silvia.andini@staff.uksw.edu

ABSTRACT

Nowadays, whole wheat flour started to be like with Indonesian people. In Indonesia there is Dewata's variety of wheat. Whole wheat flour has average level of glycemic score (55-69). Dewata's whole wheat flour can be processed to be alternative food for diabetic with wet and dry processed. Amylose content and starch digestibility are related to determine glycemic score. Higher amylose content and lower starch digestibility will show low glycemic score. The goal of this research is to determine glycemic score, amylose content, and starch digestibility of processed food from whole wheat flour. The products are noodle and cookies. Amylose content be determined from gelatinization method with iod reagent and measured with spectrophotometer, and starch digestibility use enzymatic method with α-amylase enzyme. Wet and dry processed food are make from substitution of whole wheat flour to wheat flour 0%, 10%, 20%, 30%, 40% dan 50%. Amylose content for noodle is 32.2-38.48% (db) increase with substitution increase. For cookies is more constant 29.4-33.25% (db). Starch digestibility of noodle unchanged with increase of substitution, 10.92-13.15% (db) and cookies 6.67-11.32% (db). Glycemic score of noodle and cookies 20% lower than noodle and cookies without substitution. This score be obtained with incremental area under the blood glucose response curve (IAUC). The conclusion is glycemic score form processed food of whole wheat flour can be alternative food for diabetic.

INTRODUCTION

Now, whole wheat flour more famous and be liked with Indonesian people, because there are more nutrition than wheat flour. Nursantiyah's research (2009) said that wheat flour just made from endosperm component. Whole wheat flour made from bran, germ, and endosperm (Muoma, 2013). In Indonesia there are many variety of wheat that can be cultivate, one of them is Dewata's variety. Wheat Dewata's variety cultivate in Getasan, Semarang. With this cultivation, we need to develop processed food that make from whole wheat flour. Beside that, whole wheat flour has average glycemic score 55-69 (Brand-Miller dan Foster-Powell 1999). Low glycemic score foods are good for diabetic, because that food indirectly convert to blood glucose (Praptini, 2011). Processed food from whole wheat flour can be alternative food for diabetic.

Processed food from Dewata's whole wheat flour that selected are noodle and cookies. Both of them have different process. Noodle is wet processed and cookies is dry processed. Indonesian people like this food as evidence by increasing of consumption by 0,2% every year from 1990 until now (Survei Sosial Ekonomi Pertanian, 2004).

Glycemic score are influenced by amylose content and starch digestibility. Starchy foods are rich in amylose content are associated with lower blood glucose levels and slower emptying of human gastrointestinal tract compared to those with low levels of amylose (Frei *et al*, 2003). Apart of amylose content, other starch properties such as granule size, degree of polymerization, and starch component also influence starch digestibility (Noda *et al*, 2008). Starch digestibility is parameters that show starch's ability to digest in body. Starch digestibility influenced many factor, there are amylose, amylopectin, protein, fat, fiber, and processing. This goal of this research to determine amylose content and starch digestibility from processed food of whole wheat flour and determine the glycemic score.

RESEARCH METHOD

This research is performed in Chemistry's Laboratory, Science and Mathematics Department, Satya Wacana Christian University (SWCU), Salatiga, Indonesia.

Materials and instrument

The main material is whole wheat flour Dewata's variety from Agricultural Department SWCU. Chemical reagent that used is I_2, KI, NaOH, standard glucose, ethanol, standard amylose, DNSA (dinitrosalysilic acid), $NaHPO_4$, Na_2HPO_4, standard maltose (E-Merck grade pro analysis, Germany), α-amylase enzyme (Agricultural Techonology, UGM, Indonesia), and distilled water.

The instrument are water content measurement (OHAUS, USA), incubator (WTB binder, Germany), spectrophotometer (Optizen 2120 UV, Korea), waterbath (Memmert, Germany), digital analytic balance (OHAUS, USA), glucose blood test (Easy Touch GU, Taiwan), dan glassware (pyrex, USA).

Method

Produce whole wheat wet noodle

Producing wet noodle in this research use whole wheat flour that substituted with wheat flour 10 %, 20%, 30%, 40% and 50%. As a control is wet noodle without substitution of whole wheat flour.

Table 1. Formulation of wet noodle

Material	Substitution of whole wheat flour					
	0%	10%	20%	30%	40%	50%
Wheat flour (g)	500	450	400	350	300	250
Whole wheat flour (g)	0	50	100	150	200	250
Egg	1	1	1	1	1	1
Salt (g)	3	3	3	3	3	3
Baking powder (g)	0.5	0.5	0.5	0.5	0.5	0.5

Produce whole wheat cookies

Producing cookies in this research use whole wheat flour that substituted with wheat flour 10 %, 20%, 30%, 40% and 50%. As a control is wet noodle without substitution of whole wheat flour.

Table 2. Formulation of *cookies*

Material	Substitution of whole wheat flour					
	0%	10%	20%	30%	40%	50%
Wheat flour (g)	100	90	80	70	60	50
Whole wheat flour (g)	0	10	20	30	40	50
Sugar (g)	30	30	30	30	30	30
Butter (g)	50	50	50	50	50	50

Amylose Content (Apriyantono *et al.* 1989 dalam Gustiar 2009)

Amylose standard curve

Weighed carefully 40 mg standard amylose, included to capped test tube, add 1 mL ethanol 95%

and 9 mL NaOH 1 M. The tube be heat in waterbath 95^{o}C for 10 minute. Starch gel poured carefully to 100 mL flask and add water. From this solution pipetted 1, 2, 3, 4, dan 5 mL and poured every solution to 100 mL flask. Add 0.2, 0.4, 0.6, 0.8, dan 1.0 mL acetat 1 M and 2 mL iod reagent (0.2 g I_2 and 2 g KI dissolved in 100 mL water) and add water until 100 mL. Wait for 20 minute, and measure the absorbance using spectrophotometer with wavelength 625 nm.

Sample analysis

100 mg sample entered to capped test tube. add 1 mL ethanol 95% and 9 mL NaOH 1 M. The tube be heat in waterbath 95^{o}C for 10 minute. Starch gel poured carefully to 100 mL flask and add distilated water. Pipetted 5 mL starch solution and poured to 100 mL flask. Add 1.0 mL acetat 1 M and 2 mL iod reagent and add water until 100 mL. Wait for 20 minute, and measure the absorbance using spectrophotometer with wavelength 625 nm

Starch digestibility (Muchtadi *et al.* 1992)

1 g sample entered to 250 mL erlenmeyer and add 100 mL water, cover it with aluminum foil and heated in waterbath until the temperature is 90^{o}C while stirring. Cooled the sample and pipetted 2 mL from this solution to capped test tube, add 3 mL water dan 5 mL buffer phosphat 0.1 M pH 7.0. Every sample make twice, the one as blank. Incubate the capped test tube with 37^{o}C for 15 minute. Add incubated solution with 5 mL α-amylase enzyme (1 mg/mL in buffer phosphat pH 7.0) for sample and 5 mL buffer phosphat 0.1 M pH 7.0 for blank. Incubate again for 30 minute. 1 mL result solution from incubating poured to covered test tube that contain 2 mL DNSA. Heat the solution for 12 minute in boiled water and cooled with flow water and 10 mL water. The absorbance can measure in wavelength 520 nm. Standard curve made from DNS's treatment to 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL standard maltose 0.5 mg/mL.

Organoleptic (Idris, 1994)

Organoleptic examination is examination of color, flavor, taste, and texture of food production. The score use line scale. The valuation will do by 30 panelist. The product that wil examination is whole wheat noodle and cookies and wheat noodle and cookies. After that use data analysis with t-Test.

Glycemic score examination (El, 1999 modificate in Gustiar, 2009)

The food that will determine the glycemic score must analyzed as proximate to determine total food that will be consumpt by panelist (contain 50 g carbohydrate). Panelists must do fasting for 10 hours. Panelists that used is 10 human that health with normal BMI (Body Mass Index). 2 hours after consumed the product, the blood will test every 30 minute to measure the glucose blood. For standard, the food product change with standard glucose. Glucose blood (every 30 minute) plotted to 2 X and Y axis. X is time and Y is glucose blood.

Data Analysis

Data will analysis with Randomized Complete Block Design (RCBD) with 6 treatment and 4 repetition. As treatment is concentration of substitution of whole wheat flour. Average of examination analyzed with Tukey HSD 5% (Steel dan Torrie, 1980).

RESULT AND DISCUSSION

Amylose Content

Starch is homopolymer form from glucose with α-glycosidic bond. Starch consist of two different polymers, there are straight compound (amylose) and branched compound (amylopectin) (Muchtadi *et al.* 2006). Amylose is sraight homopolymer α-D-glukosa that connected by α-(1,4) glycosidic bond that soluble in boiled water. Amylose content in starch is divided into 4 level there are lowest amylose content < 10%, low amylose 10-20%, average amylose 20-24%, and high amylose > 25% (Aliawati 2003). Most of scientist claim that amylose is slower to digest (Miller *et al*. 1992; Foster-Powell *et al*. 2002; Behall and Hallfrisch 2002), because amylose is simple polymer with straight chain. This chain make bond of amylose streonger so can't gelatinization easly.

Amylose content of whole wheat noodle is 28-33% and cookies 30-34%. Amylose in cookies is higher than noodle. Although the different is not too high, that is appropriate with Carreira's research (2004) that amylose influence by gelatinization level and processed food that dry processed food has higher amylose that wet processed food. Because wet processed food has fast gelatinization process, so influence total soluable starch. It cause structure of starch gel will weakened by absorbed of water. The weak bond make water easily enter the granule so amylose will soluble in water (Suardi, 2002).

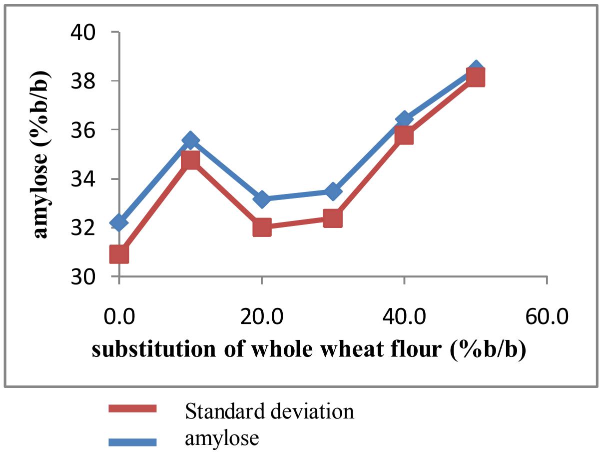


Fig 1. Relation between amylose content and substitution of whole wheat flour in noodle

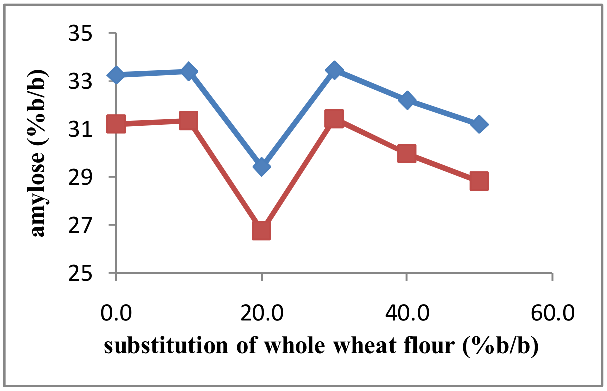


Fig 2. Relation between amylose content and substitution of whole wheat flour in cookies

Starch Digestibility

According to Willet *et al*. (2002), slow absorbed carbohydrate will produce low glucose blood and potential to manage starch digestibility that be affected by amylose composition. This measurement using enzymatic method, the enzyme is α-amylase. Using this enzyme because they can split sample through hydrolysis process to be their smaller unit, such as maltose (Gustiar, 2009). Maltose is sugar that can be absorbed in intestinal. To determine starch digestibility, do the measurement of maltose. Starch digestibility of whole wheat noodle is 10-12% (db) and cookies is 6-11% (db). More adding of whole wheat flour so the starch digestibility is lower. That is appropriate with amylose content in processed food. Amylose content and their composition is have effect to starch digestibility (Indrasari,2008).

Table 3. Comparing starch digestibility in noodle and cookies

Substitution of whole wheat flour (%)	Starch digestibility % (b/b)	
	noodle	Cookies
0	12.37±29.1066 ab	8.59±17.5145 ab
10	10.92±25.6829 a	6.67±13.5918 a
20	11.82±27.8006 a	6,69±13.6377 a
30	12.34±29.0478 ab	8.50±17.3209 ab
40	13.15±30.9360 b	7.50±15.2831 ab
50	12.38±29.1301 ab	11.32±23.0725 b

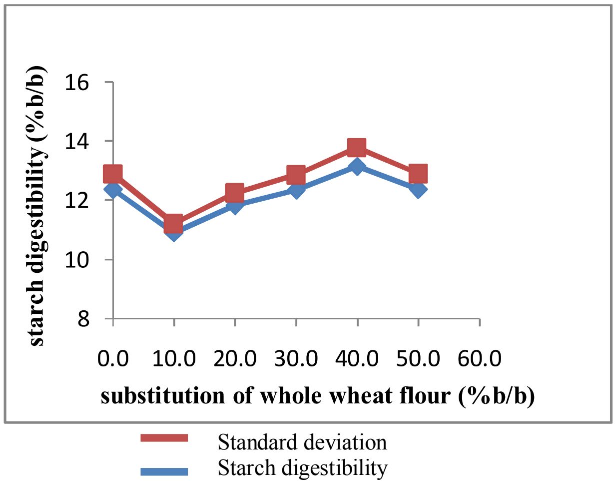


Fig 3.. Relation of between starch digestibility and substitution of whole wehat flour in noodle

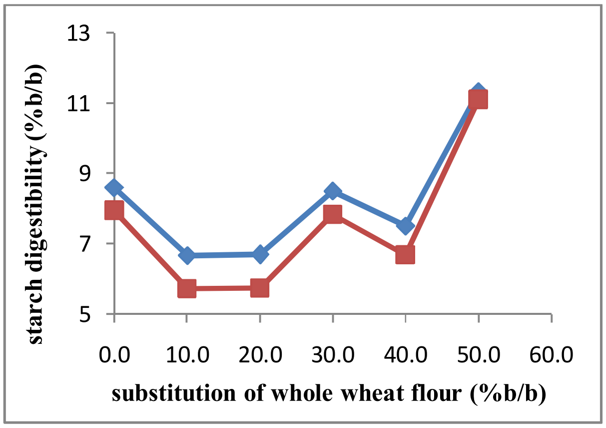


Fig 4. Relation of between starch digestibility and substitution of whole wehat flour in cookies

From this result, starch digestibility for wet processed food (noodle) is bigger than dry processed food (cookies). That is appropriate with amylose content of dry processed food is higher so the starch digestibility of cookies will be lower. Food processing is affect to starch digestibility (Noda *et al*, 2008).

Organoleptic

Table 4. Organoleptic of whole wheat noodle

Parameter	Penambahan tepung gandum utuh (%)					
	0	10	20	30	40	50
Color	4.33 ± 0.2919 c	3.83 ± 0.2869 b	3.67 ± 0.3111 b	3.17 ± 0.2658 ab	2.83 ± 0.3248 a	2.50 ± 0.4139 a
Flavor	3.10 ± 0.3583 a	3.47 ± 0.2898 a	3.63 ± 0.2856 ab	3.50 ± 0.2730 a	3.40 ± 0.2703 a	3.43 ± 0.3757 a
Texture	3.90 ± 0.3154 bc	3.83 ± 0.2955 b	3.90 ± 0.2998 bc	3.27 ± 0.3090 a	2.97 ± 0.3175 a	3.17 ± 0.4053 a
Taste	4.03 ± 0.2296 bc	3.97 ± 0.2296 bc	3,70 ± 0.2432 b	3.50 ± 0.2137 b	3.10 ± 0.3303 a	2.77 ± 0.3205 a

1 : really don't like; 2 : don't like; 3 : neutral; 4 : like; 5 : really like

Table 5. Organoleptic of whole wheat cookies

Parameter	Penambahan tepung gandum utuh (%)					
	0	10	20	30	40	50
Color	4.2000 ± 0.2842 ab	3.5333 ±0.3059 a	4.0333 ± 0.2076 ab	3.4333 ± 0.3114 a	3.0333 ± 0.2497 a	3.0333 ± 0.3600 a
Flavor	3.6333 ±0.3175 a	3.4667 ± 0.3059 a	3.8667 ± 0.3059 b	3.5000 ± 0.2471 a	3.6000 ± 0.2703 a	3.7000 ±0.3556 a
Texture	3.9333 ± 0.3242 bc	3.2667 ± 0.2762 a	4.0667 ± 0.2582 bc	3.7667 ± 0.2889 b	3.3000 ± 0.2622 a	3.7667 ± 0.3351 b
Taste	3.7667 ± 0.3492 a	3.7667 ± 0.2535 a	3.6333 ± 0.2682 a	3.7000 ± 0.3134 a	3.5000 ± 0.2547 a	3.9667 ± 0.3600 bc

1 : really don't like; 2 : don't like; 3 : neutral; 4 : like; 5 : really like

From this result, noodle that can accept with panelists is noodle with 10 and 20% substitution of wheat flour. For cookies is 20% substitution of wheat flour.

Glycemic Score

Glycemic score is leveling of food based on the effect with glucose blood. Food that can increase glucose blood faster has high glycemic score (Miller *et al.* 1992 in Rimbawan dan Siagian 2004). Glycemic score is unique nature, that's influence by their material, processing, and characteristic (composition and biochemistry nature) (Miller, 1992).

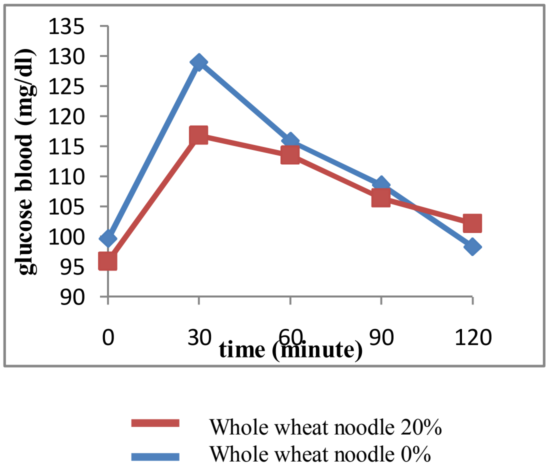


Fig 5. Glucose blood response to noodle

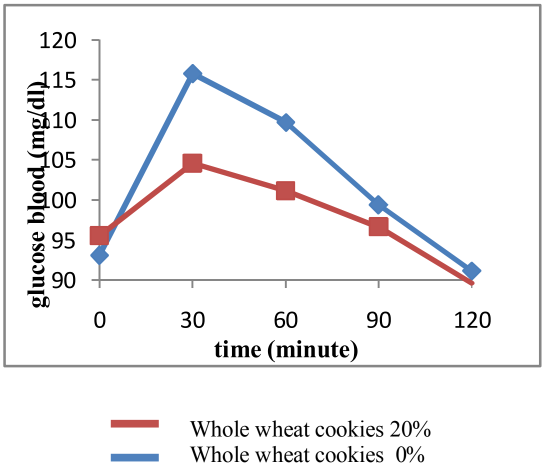


Fig 6. Glucose blood response to cookies

Table 6. Average result of glucose blood response

Sample	Time (minute)					Area under curve	IG
	0	30	60	90	120		
Glucose	102,4	152,1	131,1	108,2	94,9	29403	100
Whole wheat noodle 0%	99,7	129	115,9	108,6	98,3	20397	69,49
Whole wheat noodle 20%	95,9	116,8	113,5	106,4	102,2	19449	66,23
Whole wheat cookies 0%	93,1	115,8	109,7	99,4	91,1	15273	52,11
Whole wheat cookies 0%	95,5	104,6	101,1	96,6	89,6	14622	49,94

Comparing the area under curve from control and acceptance wet and dry processed food with glucose (standard), glycemic score can be obtained.

Glycemic score of whole wheat noodle 0% and 20% are 69.49±1.37 and 66.23±6.14. And glycemic score of whole wheat cookies 0% and 20% are 52.11±2.07 and 49.94±1.90. Glycemic score of whole wheat noodle is in average level (55-69) and cookies in low level (<55).

CONCLUSION AND SUGGESTION

Amylose content of whole wheat noodle is 32.2-28.48% and whole wheat cookie*s* 29.4-33.25%. Starch digestibility of whole wehat noodle is 10.92-13.15% and whole wheat cookies 6.67-11.32%. Through the organoleptic whole wheat noodle and cookies that can accept is with 20% substitution. Glycemic score of whole wheat noodle is 66.23±6.14 is in average level of glycemic score and cookies 49.94±1.90 low glycemic score.

Suggestion for the next research is substitution of whole wheat be expected more than 20% so can get lower glycemis score, but must remains to be accept by people.

BIBLIOGRAPHY

Behall, K.M. and J. Hallfrisch. 2002. Plasma glucose and insulin reduction after consumption of bread varying in amylose content. Eur. J. Clin. Nutr. 56(9):913-920.

Brand-Miller J, Hayne S, Petocz P, dan Colagiuri S. 2003. Low-glycemic index diets in the management of diabetes: A meta-analysis of randomized controlled trials. Diabetes Care, 26, 2261–2267.

Carreira, M.C., F.M. Lajolo, and E.W. de Menezes. 2004. Glycemic index: effect of food storage under low temperature. Brazilian Archives of Biology and Technology 47(4):569.

Foster-Powell K dan Miller B. 1995. International tables of glicemic index. American Journal of Clinical Nutrition. 62 : 871s-893s.

Frei, M., Siddhuraju, P. and Becker, K. 2003. Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. Food Chemistry 83 : 395-402.

Gustiar, Haris. 2009. Sifat Fisiko-Kimia dan Indeks Glikemik Produk *Cookies* Berbahan Baku Pati Garut (Maranta arundinacea L.) Termodifikasi. Bogor : IPB.

Idris, S. 1994. Metode Pengujian Bahan Pangan Sensoris. Fakultas Peternakan. Universitas Brawijaya. Malang.

Ludwig DS. 2000. Dietary glycemic index and obesity. *J Nutr* Supl 130: 280S- 283S

Muchtadi D, Palupi NS, dan Astawan M. 1992. Metode Kimia, Biokimia, dan Biologi dalam Evaluasi Nilai Gizi. Pusat Antar Universitas Pangan dan Gizi.IPB, Bogor.

Muoma, Ike. 2013. Whole Grain Vs Whole Wheat Vs Whole Meal Vs GranaryRefined Bread? Which is best? What to choose?. URL www.iketrainer.co.uk/articles/breads.pdf. Diakses pada 15 September 2013.

Noda, T., Takigawa, S., Matsuura-Endo, C., Suzuki, T., Hashimoto, N., Kottearachchi, N.S., Yamauchi, H. and Zaidul, I.S.M. 2008. Factors affecting the digestibility of raw and gelatinized potato starches. Food Chemistry 110 : 465-470

Nursantiyah. 2009. Gambaran Umum Industri Tepung Terigu di Indonesia dan Ketentuan Pajak Pertambahan Nilai Terkait. Jakarta : UI.

Praptini, P.E. 2011. Menu 30 Hari dan Resep untuk Diabetes. Jakarta : PT. Gramedia Pustaka Umum

Pusat Penelitian dan Pengembangan Sosial Ekonomi Pertanian. 2003. Trend Konsumsi Pangan Produk Gandum di Indonesia. Warta Penelitian dan Pengembangan Pertanian Indonesia , 25, hal. 11-12.

Ratnaningsih. 2010. Pembuatan Tepung Komposit dari Jagung, Ubikayu, Ubijalar dan Terigu (Lokal dan Impor) untuk Produk Mi. Bogor : Balai Besar Penelitian dan Pengembangan Pascapanen Pertanian.

Rimbawan dan A. Siagian. 2004. Indeks glikemik pangan. Penebar Swadaya. Jakarta

Simanjutak, B.H. 2002. Prospek Pengembangan Gandum (*Triticum aestivum L*) di Indonesia. Salatiga : Universitas Kristen Satya Wacana.

Suardi, Suarni, A. Prabowo. 2002. Prosesing Sorgum sebagai Bahan Pangan. Sulawesi Selatan : Seminar Nasional Balai Pengkajian Pertanian

C-8

CHARACTERIZATION OF QUINOLINE AND QUINOLINE CONJUGATED METAL AS THE BASE MATERIAL OFPHOTODETECTOR

I Gusti Made Sanjaya, Dian Novita and Aldo Swaztyznt Saputra

Department of Chemistry, Universitas Negeri Surabaya (the State University of Surabaya)
Email: igmas@fmipa.unesa.ac.id

Abstract

Research has been conducted with the aim to characterize quinoline and quinolineconjugated metal as the base material of photo-detector. The characterization was conducted on the bandgap and infrared, visible, and ultraviolet spectra. This is done through computational chemistry method with NWCHEM 6.3 software. The computation is done by using density functional theory with basis set 6.31G*. The result showed that there was a diminution in the bandgap of quinolone conjugated metal of Mg(E_g ~ 0.16), Ca (E_g ~ 1.25), Cr(E_g ~ 3.51), Fe (E_g ~ 0.37), Ni (E_g ~ 1.18) and Zn (E_g ~ 3.58) than the bandgap of quinoline (E_g ~ 4.58 eV). Photosensitivity of the quinolin conjugated metalseem to shift towards the maximum wavelength in the visible and infrared, ie Mg-quinoline (λ_{max}= 470.57 nm); Ca-quinoline (λ_{max}= 719.98 nm); Cr-quinoline (λ_{max}= 855.76 nm); Fe-quinoline (λ_{max}= 1764.39 nm); Ni-quinoline (λ_{max}= 1325.20 nm); dan Zn-quinoline (λ_{max}= 485.04 nm), compare quinolone photosensitivity that occurs in the ultraviolet region (λ_{max}= 214.39 nm).

Keyword: Quinoline, conjugated metal, photo-detector, characterization.

1. Introduction

Optical sensors is very important in industrial automation systems. Such sensors work based on light sensitivity (Candra, 2006).Optical sensors are generally made by using semiconductor properties of inorganic materials, such as silicon and germanium (Setiawan et al, 2007).The characters of inorganic sensors are rigid, inflexible, limited amount of the deposit, and not easily recycled so it is not environmentally friendly.

Materials of organic compounds have not widely used as a semiconductors which become the raw materials of optical sensors. Though many organic compounds contain group that are active light. Organic semiconductors have such a chromophore groups is very prospective used as raw material of optical sensors.

Many adventages of the use of organic semiconductors as raw material for optical sensors. The structure of organic semiconductor is more flexible because the molecules have Van derWalls bonds. The flexibility of this structure is very important in industrial automation, especially in replacing mechanical works. In addition, the presence of organic material very abundant than inorganic materials which amount are very limited. Organic material is more easily recycled so it does not produce environmental pollution.

Organic materials which are potentially used as organic semiconductors for raw materials of optical sensors, is a material of organic compounds which have conjugated double bonds such as quinoline and others. Conjugated double bonds in organic compounds that allow the absorption of electromagnetic waves to move electrons from ground state to the excited state.

The existence of the chromophore groups and conjugated double bonds lead to organic semiconductors is potentially used as an infrared sensors and a light sensors in the range of UV-

Vis spectra (Pedersen et al, 2004). The sensitivity to light is closely related to the band gap of organic semiconductors that have conjugated double bonds (Brütting, 2005). Organic semiconductors with small band gap have large absorption to the electromagnetic wave.

Organic materials in general have a large band gap. The material is not quite ideal as semiconductors. To improve the properties, organic compounds in the sensor material need to be conjugated with metals. Good use is a metal that has empty d orbitals that electronic transitions can occur more easily (Pamungkas dan Sanjaya, 2013).

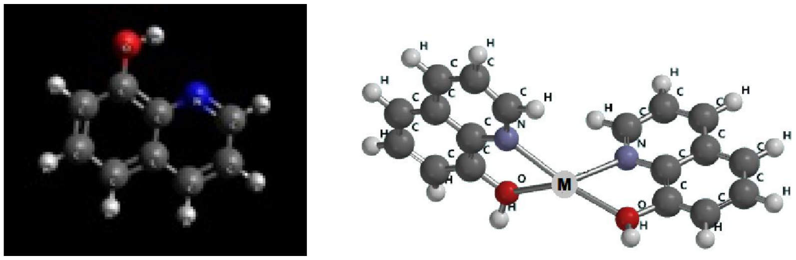
The research is focused on studying the semiconductor character of the derivative compound of quinoline and quinoline conjugated metal as the raw material of the optical sensors. The derivative qompound of quinoline which used as organic semiconductor are 8-hydroxyquinoline and 8-hydroxyquinoline conjugated metal. Under the terms of the ease electrons movement, the metal used in this study are the metals of Group IIA and the metal of periode 3. This selection is useful to determine the contribution of the metals periodically to improve the semiconductor quality of conjugated organic compounds. Studies conducted theoretically through computation chemistry performance.

2. Method

Modeling quinoline and metal quinoline were performed using the Avogadro 1.1.0. The development of the model is done by considering the possibility of coordination covalent bonds involving d orbitals of group IIA metals and metals of period 3. The model is converted into the input file of computation chemistry with program of gabedit 2.4.7. Computing process is then run using NWCHEM 6.3. Computational chemistry is carried out based on density functional theory with the basis set 6-31G*/B3LYP/Restricted Kohn-Sam (RKS). Band gap is determined using the difference between LUMO and HOMO energies. The absorption of light the virtualized with Spartan program.

3. Result and Discussion

Modeling quinolineandmetal-quinolinewhich is done by using Avogadro, shown in Figure 3.1. The notation M in the center is metal marking, which is conjugated to the quinoline structures.



a. Quinoline **b. Metal-Quinoline**

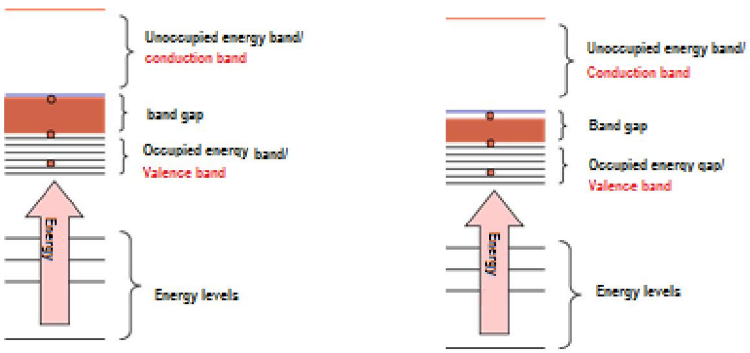
Figure3.1 The basic structure of Quinoline and Metal-Quinoline.

The band gap computation result of quinoline andmetal-quinoline in Figure 3.1showed a decrease in band gap of quinoline into quinoline conjugated metals.

Table 3.1The band gap result of Quinoline andMetal-Quinoline

Compound	HOMO-LUMO Energy (eV)		Band gap (eV)
	HOMO Energy	LUMO Energy	
Quinoline	-1.46458	-6.04240	4.57782
Mg-Quinoline	-2.48919	-2.64952	0.16033
Ca-Quinoline	-12.43888	-13.68823	1.24936
Cr-Quinoline	-12.64305	-16.15032	3.50727
Fe-Quinoline	-13.39013	-13.76018	0.37006
Ni-Quinoline	-14.13851	-15.32044	1.18193
Zn-Quinoline	-11.96226	-15.53794	3.57568

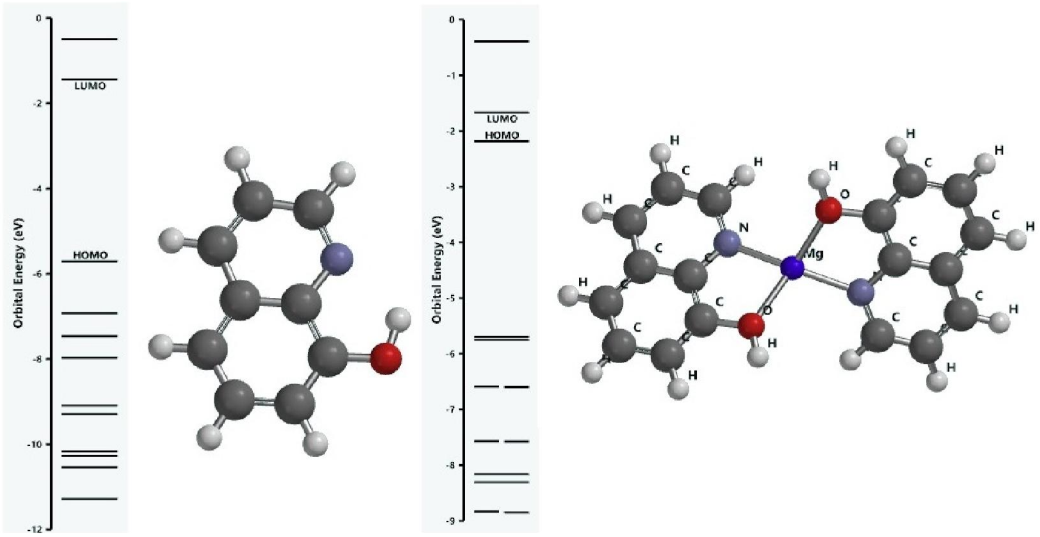
The decrease in the band gap is related to the narrowing gap between the valenceband and the conduction band. This is shown by band gap models in Figure 2. Valence band is indicated by the charging of the pile of orbitals in the HOMO groups and the conduction band is shown with a pile of the orbitals in the LUMO groups (Sanjaya et al, 2013).



(a) (b)

Figure 3.2.Band gap model of(a) quinolineand(b) metal-quinoline

This is made clear by the energy band structure of quinoline computational result and an example of metal-quinoline computational result which is represented by Mg-quinoline as shown in Figure 3.3.



(a) Quinoline (b) Mg-Quinoline

Figure 3.3. Band energy of Quinoline(a) andthe example of Metal-Quinoline (b)

The study could not show a decrease in band gap periodically for quinoline conjugated metals of group IIA and metals of period 3. There are few data on quinoline conjugated metal of group IIA. So it seemed only increase in band gap of Mg-quinoline into Ca-quinoline. While data from computational result of quinoline conjugated metals of period 3 shows the fluctuating band gap width.

Of the seven models of organic semiconductors which is computed, there are two compounds that are close to band gap of the silicon standard semiconductor (Gadisa, 2006),namely Ni-quinoline (E_g ~ 1.18 eV) and Ca-quinoline (E_g ~1.25 eV). There are two compounds that have band gap is smaller than the band gap of silicon, ie Mg-Quinoline (E_g ~0.16 eV)and Fe-Quinoline (E_g ~0.37 eV), so it is expected to work indoor.

Virtualization indicate that the computational results are accurate. These results are very similar to the standard. In Figure 3.4 is shown a comparison between computational result of infrared absorption of quinoline and the experimental result standard of infrared absorption of quinoline.

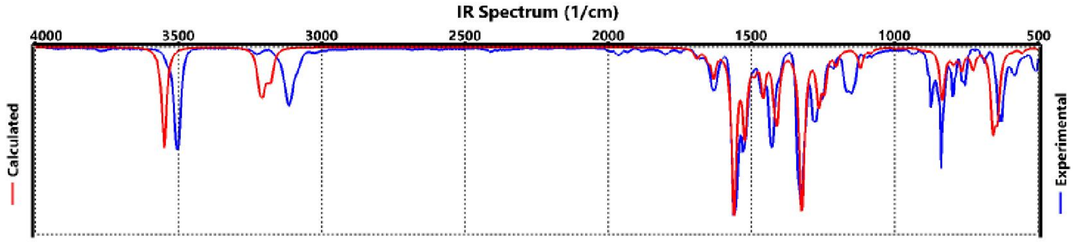
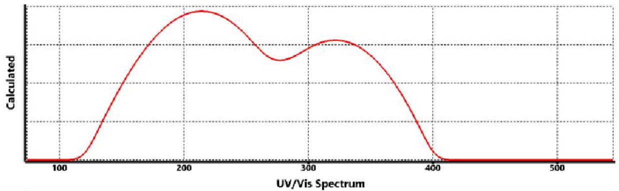
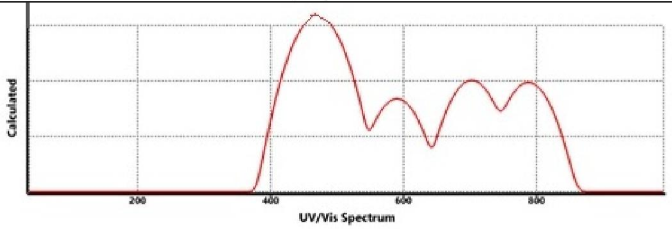
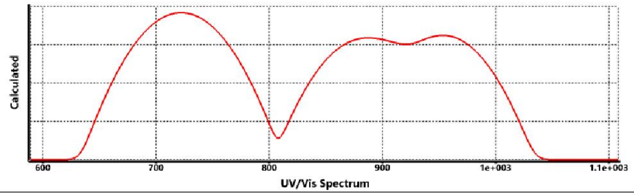
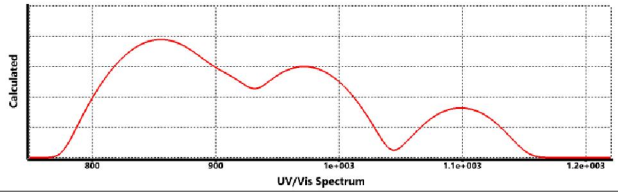
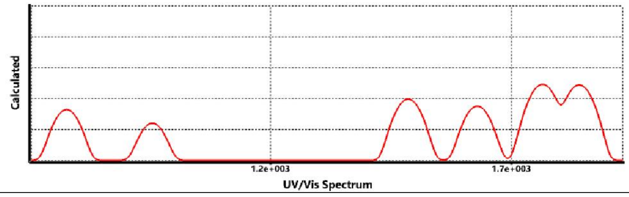
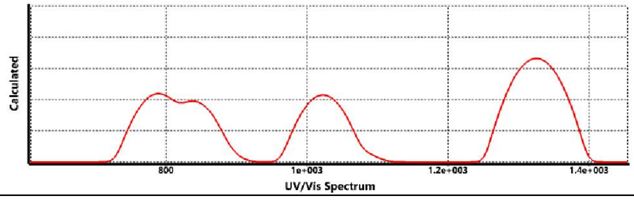
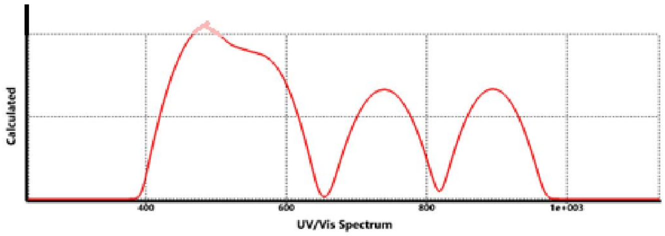


Figure 3.4. Infrared spectrum of quinolinefrom computation and standardof experimental result

The computational results of ultraviolet and visual absorptions of quinoline dan quinoline conjugated metal shown by table 3.2.

Table 3.2.Spectrum Uv-Viz of Quinoline andMetal-Quinoline

Quinoline	:	
Mg-Quinoline	:	
Ca-Quinoline	:	
Cr-Quinoline	:	
Fe-Quinoline	:	
Ni-Quinoline	:	

Zn-Quinoline	:	

Quinoline showed absorption at ultraviolet to violet region with the maximum wavelength in the ultraviolet region,λ_{max}= 214.39 nm. Mg-Quinoline has an absorption in blue to infrared light with a maximum wavelength in blue light area, λ_{max}= 470.57 nm. Ca-Quinoline has an absorption in the red to infrared regions with a maximum wavelength in the red light area, λ_{max}= 719.98 nm. Cr-Quinoline together with Ca-Quinoline has an absorption in the red to infrared region. It has a maximum withlength in infrared area, λ_{max}= 855.76 nm. Fe-Quinoline and Ni-Quinoline have absorption in infrared region with a maximum wavelength of each λ_{max}= 1764.39 nm andλ_{max}= 1325.20 nm. Actually, both of these compounds have also absorption in the red light up to pink light, but its intensity is very small so neglected in this study. The Zn-Quinoline has an absorption in the region of blue light to red light with a maximum wavelength in the blue light region,λ_{max}= 485.04 nm.

Photosensitivity of derivate compounds of quinoline shifted from the ultraviolet light into visible light region and infrared light region because of the conjugated metals. That work well in the visible light region is the conjugated metals Mg, Ca, Cr, and Zn although conjugation metals is partly also working in the infrared regions. While the rest of metal conjugate, is working in the infrared regions.

4. Conclusion

Characterization of the properties of organic semiconductor are made from quinoline and quinoline conjugated metals as the raw material of photo sensor indicate that band gap diminution occurs on the quinoline conjugated metal of Mg (Eg ~ 0.16 eV), Ca (Eg ~ 1.25 eV), Cr (Eg ~ 3.51 eV), Fe (Eg ~ 0.37 eV), Ni (Eg ~ 1.18 eV) dan Zn (Eg ~ 3.58 eV) than the bandgap of quinoline (Eg ~ 4.58 eV).Computational results are quite accurate because the infrared spectra are very consistent with the standard infrared spectra of the experimental result.

Photosensitivity of the quinoline conjugated metal seem to shift towards the maximum wavelength in the visible and infrared, ie Mg-quinoline (λ_{max}= 470.57 nm); Ca-quinoline (λ_{max}= 719.98 nm); Cr-quinoline (λ_{max}= 855.76 nm); Fe-quinoline (λ_{max}= 1764.39 nm); Ni-quinoline (λ_{max}= 1325.20 nm); dan Zn-quinoline (λ_{max}= 485.04 nm), compare quinolone photosensitivity that occurs in the ultraviolet region (λ_{max}= 214.39 nm).

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References

Brütting, W. 2005. Physics of Organic Semiconductors, WILEY-VHC Verlag Gmbh and Co. KGaA, Weinheim.

Candra, R. 2006. *Sensor dan Transduser*. Depok: Universitas Gunadarma.

Gadisa, A. 2006.Studies of Charge Transport and Energy Level in Solar CellsBased on Polymer/Fullerene Bulk Heterojunction,*Dissertasi Doktoral*, Universitas Linköping, Swedia.

Pamungkas, G. dan Sanjaya, IGM. 2013.Kajian Teoritis Untuk Menentukan Celah Energi Porfirin Terkonjugasi Logam Kalsium Menggunakan Teori Fungsional Kerapatan (DFT),*Unesa Journal of Chemistry* Vol. 2, No. 1, hal 54-61.

Sanjaya, IGM., Pamungkas, G., Novita, D., 2013, Karakterisasi Berilium Porfirin Sebagai Bahan Dasar Fotodetektor, , HKI Cabang Sumbar: Seminar Nasional Kimia dan Pendidikan, Padang 7 Desember 2013

Setiawan, Agus, dkk. 2007. *Modul-4 Semikonduktor*. Bandung: Fakultas Pendidikan Matematika dan IPA, Universitas Pendidikan Indonesia.

C-9

STUDY ON POPULATION OF FROM *D. MELANOGASTER* KATUL MEDIA FERMENTED *SACHAROMYCES CEREVISAE* AS SWIFLET WOOF

Ignatius R. S. Santoso

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Manado State University*

ABSTRACT

An identification of *D.melanogaster* population has been developed through artifical medium of fermented whole grain waste product by *Sacharomyces cereviceae*; which it's application and simpel technology is highly economic valuable for swift feed to enhance its quality and quantity. Fermented whole grain waste product has not been used as insect feed. In order to unravel the decreasing of fruits production during dry season which implicated the decreasing of *D.melanogaster* population that use as swift feed . This research aim to identify *D.melanogaster* population from banana, compare to artifical medium. Followed by chemical analysis of protein, carbohydrate, fat, mineral, and calorie value; determine the feed availability with swift nest population as well as determine the nutrition chemical compound in swift nest; mass, thickness, protein, carbohydrate, fat and mineral. The fact that fermented whole grain waste product can growth the *D.melanogaster* which is used for swift feed, swift farmers can develop *D.melanogaster* to increase quality and quantity of swift nest. Based on analysis of variance, natural medium from banana on population of *D.melanogaster* growth. For artificial feed, fermented whole grain waste product, the E treatment of *D.melanogaster* with banana fruit show the best result;

Keywords : *D.melanogaster,* swiftlet nest.

A. INTRODUCTION

The development of agriculture is not free from problems breathing pest and disease control , this damage occurs either on the ground , a warehouse , a lot of fruits and vegetables sold in traditional markets and supermarkets look clean and not deformed , but not the least the buyer fruit complain with poor quality fruit they buy . This fact is very influential in vegetable and fruit traders , because fruits and vegetables tersebuat rapid deterioration and rotting (Novizan , 2002) . One cause damage to fruit crops is the Fruit Fly Drosophila sp which is damaging the flesh of the fruit flies that memyebabkan ripe fruit becomes rotten and falling . Fruit flies can be the same size larger or smaller than house flies , fruit flies is composed of a variety of species , but how to handle it , and produce nearly the same destruction. Efforts to reduce and prevent fruit flies done by keeping the orchard , fruit falling in a vat containing input petisida , buried as deep as 75 cm and the soil surrounding the dismantled in order pupae hidden in the ground out , with oil -containing compound citranela isogenol metly eugenol and amyl alcohol which can bind the male fruit flies . One of the fly Drosophila melanogaster is Drosophilidae familia , these flies are small , at high populations can threaten human health , because of his interest in fruits and vegetables , especially materials that undergo fermentation (Matthews and Matthews , 1978) . Imago of flies , measuring length , 2.5 mm - 4.0 mm (Charles and Noerman , 2005) is usually brownish yellow and brownish black . Melatakan eggs a female can average 50-70 points per day eating an appropriate medium for the larvae is overly ripe fruit and vegetables that have been and will rot or material that is undergoing fermentation . In the dry season production of fruits and vegetables decreases the impact on the population of D. melanogaster fruit flies , fly D. melanogaster is one of the birds' feed (predator) populations of D. melanogaster in order to maintain the waste dry season rice bran (bran) The fermetasi for the development of breeding populations of D. melanogaster . The presence of predators in the world of agriculture is a free-living organism consuming , killing and devouring other organisms directly to the beneficial insects are not enough predators who can be identified from birds to microorganisms that can be separated on the farm . Based on the above meal authors chose to mengiidentifikasi , populations of D. melanogaster and then developed through the artificial medium of rice bran (bran) fermented Sacharomyces cerevoceae , where applications and simple technology for the high economic value of feed bird that can improve the quality as well as the bird's nest swallow . This study aims to determine the populations of D. melanogaster medium banana shoes , which is compared with the artificial media . Followed by chemical analysis of D. melanogaster , protein , carbohydrates , fats , minerals and caloric value and determine the availability of feed to the birds' populations and determine the chemical compounds in the bird's nest covering nutrition , weight , thickness , proteins , carbohydrates , fats , minerals.

B. METHODS

This research was conducted in the District Kamangta Tombulu Minahasa for 12 months . In locations with a height of 550 meters above sea level (asl) with an average temperature range of 26oC-30oC . Materials and Equipment Materials used in this study were rice bran / bran , bananas , shoes , yeast (Sacharomyces cereviceae) , and honey . While the tool used was the fermentor , pH meter , hand reflaktometer , petridish , microscope , auto Clav , mikrokeldhal micro , soklet , bomb calorimeter , AAS , and tools that support the analysis beaker D. melanogaster. The design of experiments The study was designed as a complete randomized trials with repeated three times for media banana shoes , as well as five treatments and three times re -made for the media . The parameters measured by pH , percent sucrose , determining population size medium banana shoes , the media and media metamorphosis in artificial insects are eaten by the percentage of birds' , the chemical analysis of D. melanogaster , (proteins , fats , carbohydrates , minerals , and calorific value) body weight and feed the birds , the thickness and weight of the nest and the nest of proteins , carbohydrates , fats , minerals from bird nest feed treated D. melanogaster. Working procedure

Before developing D. melanogaster , do pembedakan on swiftlet analyze the number of insects in the gizzard . Further develop D. melanogaster in cultured banana shoes , determine the pH and percent sucrose as a medium life cycle of egg , larva , pupa and imago . Moving the D. melanogaster of media banana shoes , artificial media to A , B , C , D , E , after the adult insects analyzed proteins , carbohydrates , fats , minerals , and the caloric value and determine the best medium of artificial media A , B , C , D , E to D. melanogaster insect breeding . Doing hatching birds ' and weighing the birds' weight and feed children who were given the adult insect D. melanogaster from the first day until able to fly for 45 days . Measuring the thickness of the nest , nest weight and analyzes of bird nest (proteins , fats , carbohydrates , minerals) as well as the time required for nest building of birds treated and non- treated .

observation. Made observations on the life cycle of each treatment by calculating the population of D. melanogaster cultured banana shoes , (generation 1 s / d 3rd generation) , long metamorphosis and do the same thing on artificial media . The next rare daily feeding , weighing and amount of feed consumed , accounted for 45 days . Nesting observation for 65 days in the last swallows building harvested weighed , measured the thickness and weight of chemically analyzed nest swiftlet nests treated and non- treated .

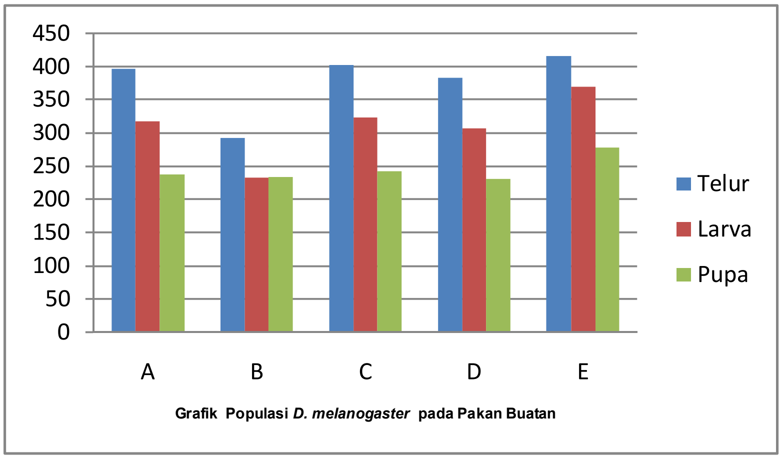
C. RESULT AND DISCUSSION

A. Chemical Analysis Media Alamia Before dimasukakan in fermentator
1). The level of acidity (pH) Natural Foods
Based on the observation level of acidity (pH) of natural food banana shoes , before inclusion in fermentator is 6 . Development of D. melanogaster population started after fertilasi consisting of two periods of the eggs in the embryonic period when fertilasi until such time as the young larvae hatch from eggs more or less within 24 hours , at this time the larvae eat incessantly.
The second period is after the eggs hatch called postembrionik development consisting of stage larva, pupa , and imago .

Table 1 . Development of D. melanogaster populations of banana shoes on artificial food .

D. Population Growth and Long Life of D. melanogaster on Artificial Food .

1) . banana Shoes



Eggs
Based on the analysis of variance , artificial food very significant effect on the E (416 grains) , A (396 points) , C (402 grains) , D (382 grains) , of the (292 grains) , high egg populations . The invention relates to an insect host by substrate color and odor of volatile oil (Chapman , 1971) . The eggs produced by female insects to a couple of times (during the egg stage) have not much effect on the population because the egg is not moving , not eating , and do not proliferate . The eggs of D. melanogaster . Elliptical -shaped objects are small and are usually placed on the surface of the food . Adult females begin to lay eggs on the second day after becoming adult flies and increased in the week to females laying 50-75 eggs per day . Within 10 days maximum of approximately 400-500 pieces of eggs .
eggs ; D. melanogaster vetillin coated by a thin membrane that surrounds the cytoplasm on the outside and thin silvia dianteriornya two tanks . According to (Borror , 1992) , was a very strong thin membrane called the chorion and coated the outside of the egg chorion . flyblow
Based on the analysis of variance , the food was highly significant , medium A (317 head) , B (233.6 tail) , C (322 head) , D (306 animals) , E (369.3 tail) . Types of food , water content ,

and cross-sectional area . Food grain size effect on the development of a type of searangga .
Larvae ; D. melanogaster white , shaped like a segmented worm with a mouth and brownish black , has a pair of spiracles are located on the anterior and posterior ends . D. melanogaster experienced a skin substitute (instars) and lasts for three times where the first instar (I) larvae after hatching , the second instar (II) the appearance of the teeth in the mouth to eat black preparation for the third instar (III) the preparation to form pupae and moving kepinggir - edge fermentator the place dry and then stopped moving Pupa. Based on the analysis of variance , artificial food very significant effect on pupal populations . Media A (237 head) , B (233.6 tail) , C (241.6 tail) , D (229.8 tail) , E (277.3 tail) .
Pupa ; D. melanogaster occurs when larvae instar (III) brown body that contract (shorten) the cuticle be hard and pigmented called fourth instar (IV) is characterized by the Establishment wing head and foot pads . In the pupal stage larvae in a passive state (inactive) . In this time the larvae towards / into adult flies Imago. Based on the analysis of variance , significant artificial food . Reproduction insect nutritional decline due to low population and development is also influenced by the type and amount of food . Media A (166 head) , B (175.33 tail) , C (164.33 tail) , D (166.33 tail) , E (195.33 tail) .
Imago or adult flies aged about 10 days after discharge from the pupa , the wing can not be grown and still a bit pale and after approximately 10 days 9 hours fly females to mate . E. The protein in insects D. melanogaster. In insects as well as other animals one of the major biochemical activity at the cellular level is the synthesis of proteins , where the characteristic features of insects holo metabola is a change in the form of larvae into the imago (adult) at the time of metamorphosis . Fat bodies of insects is a major organ of metabolic processes a wide variety of biochemical substances and their role is crucial , especially in stadia pradewasa . Insects holometabola , during the development of insect larvae fat body responsible for the synthesis of various proteins in insect hemolimfanya synthesized in the fat body of an average protein synthesis at high body fat at the start of the growth of larvae and then decreases . Protein hemolimfa stored as vesibel to transport lipids (fats) , carbohydrates , and hormones . Synthesis and release of specific protein hemolimfa by fat body from larvae that were growing and the disposal and storage of these proteins in the same tissue for further development is controlled by hormones .
For that we should not be surprised when the insect protein in insects D. melanogaster showed high numbers , the protein results in laboratory analysis around 55.19 % . In addition to high-protein insects are relatively low fat content and a clean source of food by eating plants / fruits fresh . Chemical analyst Table D. melanogaster No. Parameter Analysis Method of Analysis Results

1 Protein 55.19 % Makrojeldahl
2 6.75 % Fat Extraction
3 1.80 % Carbohydrates Hydrolysis
4 Sodium (Na) 3.55 ppm AAS
5 Potassium (K) 50.34 ppm AAS
6 Calories 288.71 cal / g calculation
Insect D. melanogaster high protein (55.19 %) as the birds' feed

F. Presentation Given Insects In Bird Treatment and Non- Treatment .

Table . Percentage Insects On The Swallow gizzard Kamangta What Not Provided treatment .

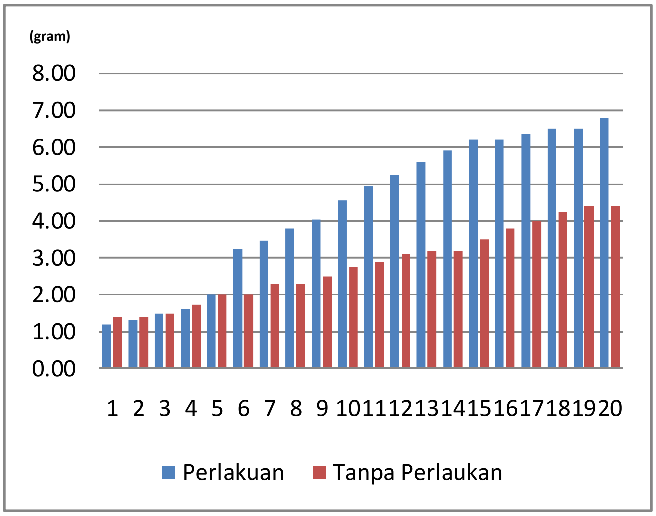
Ordo	**Famili**	**Presentase (%)**
Diptera	Drosophilidae Phoridae	45
Cleoptera	Tenebrionidae	20
Homoptera	Aleyrodidae	10
Isoptera	Kaloptermitidae	10
Lain-lain	-	15*

Table. Percentage Insects On The Swallow gizzard Kamangta treated.

Ordo	**Famili**	**Presentase (%)**
Diptera	Phoridae Psychodidae Drosophilidae	75
Coleoptera	Tenebrionidae Silvamidae	20
Lain-lain	-	5*

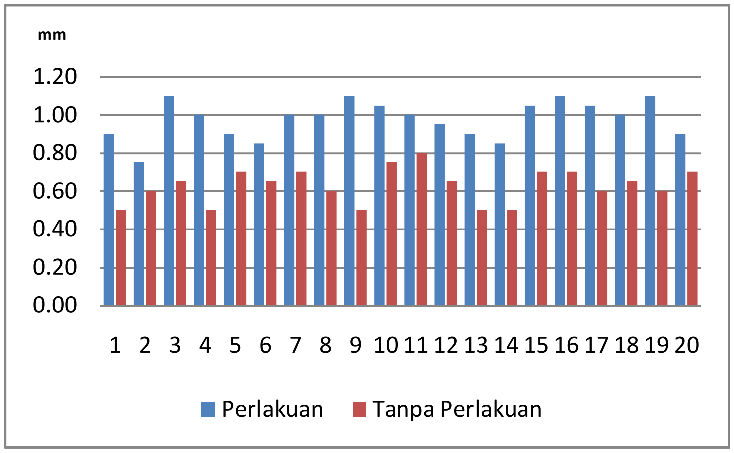
Note *: other; insect was identified as damaged. Swiftlet treated, take the order diptera and coleoptera. Non-free treatment to fly and prey on the order of the Homoptera, Isoptera, diptera, coleoptera and other insects are not detected proving that the location of the bird cage and the ecosystem environment favorable to the development of swiftlet (predators). Observation of the relationship between the number of birds feeding and body weight in birds treated with variable amounts of feed (X) and weight ($\hat{Y}$) r = 0.9 with the regression y = 1.43 x + 1.67 where price x 0.02 , thit = 26, 16> t 0.99 (43) = 1.17 this means that there is a highly significant positive correlation between the amount of feed and weight.

Weight Charts Swallow Treatment and Without Treatment



Based on observations of the thickness of a bird's nest , nest weight with treatment and without treatment with the null hypothesis as follows :

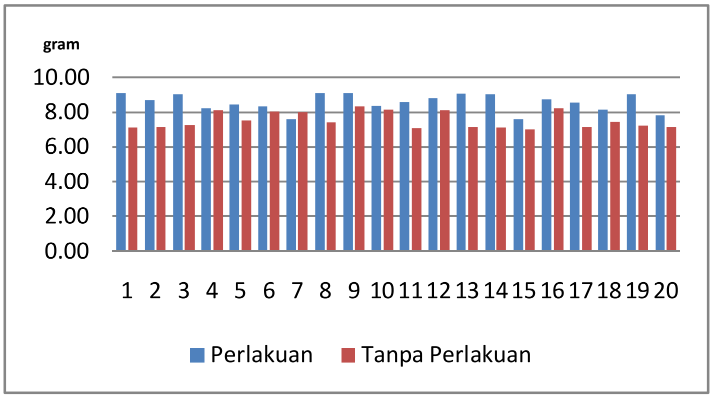
Reject , if thit > ttab
To test this hypothesis , compared to the average thickness of a given bird nest treatment ( ) with a bird's nest without treatment ( ) during the experiment .
= 0.98 with S1 = 0.0968
= 0.62 with S2 = 0.0887
S2 0.00862
thit 12.26
For α = 0:01 , thit = 12:26 > t0 , 99 (38) = 2.42 . This means that the average thickness of the bird's nest in the treatment of very , markedly higher than the thickness of the bird's nest naturally (without treatment) .
The thickness and weight of the bird's nest is very real treatment is higher than the thickness of the non- treatment birds' nests x = 0.01 , thit = 12.26 > 0.99 t (38) = 2.42 . Reject Ho if thit > t0.99 (38) = 2.42, this means that the average weight of edible bird's nest in a very real treatment is higher than the weight of non- treatment birds' nests .
Graph thickness Swallow's Nest Treatment and Without Treatment



Heavy Bird's Nest
Based on observations, it turns heavy bird nest treated higher than the natural bird's nest (without treatment), null hypothesis is formulated as:

Reject, if thit> ttab
To test this hypothesis, compared to the average weight of a given bird's nest treatment () with a natural bird nest / no treatment () during the experiment.
= 8.56 with S1 = 0.4867
= 7.52 with S2 = 0.4588
S2 0.2237
(Vincent, 1991)
thit 6.95 For α = 0:01, thit = 6.95> t0.99 (38) = 2.42. This means that the average weight of birds' nests in the treatment of very, markedly higher than the natural bird's nest weight (without treatment).

Weight Charts Swallow's Nest Treatment and Without Treatment



When food available for the birds in the vicinity of the building energy treated birds to search for food, does not require a long time to find / hunt for food outside the building, especially in the dry season. When hunting birds feed away from buildings, insects gained less than the needs and birds suffering from fatigue, the possibility of getting lost or birds can not return original place of occupancy. Backup saliva prepared for nest building and in particular the protein is reduced impact on the formation of a nest of thin / thick not.

Table. Results of Chemical Analysis of swallow's nests

No	Parameter (%)	Analysis Results		Analysis Method
		Treatment	Without Treatment	
1	Fat	0,30	0,21	Hydrolysis
2	Protein	52,05	52,47	Makrokjeldhal
3	Carbohydrate	16,32	16,33	Hydrolysis
4	Calcium	2,26	0,19	AAS
5	Potassium	2,36	3,18	AAS
6	Magnesium	0,05	0,07	AAS
7	Sodium	0,13	0,18	AAS

CONCLUSION

Insect D. *melanogaster* is an insect living on fruits and media that undergo fermentation , it can be seen clearly when fruits or vegetables and garbage who started / would rot . These insects are interesting to study because living around humans (insects settlement) of the order Diptera Drosophilidae family .

These insects examined in the Village Kamangta with a height of 500-600 m above sea level in fermentator intended bred again swiftlet larvae feed children , imago (adult) for the adult birds' feed .

Some of the scientific information about the contribution of D. *melanogaster* is used to control feed swiftlets (collacalia fuchifaga) of larvae and adults very well with the value of 55.19 % protein , which is bred from natural media (banana shoes) and fermentation of bran / rice bran .

1 . Based on the analysis of variance showed that the natural medium of banana shoes gave a positive response in the growth of insect populations of D. *melanogaster* , for artificial feeding fermented bran treatment E to D. melanogaster best of feed nutrients banana shoe types significantly influenced the development of the fly population D. *melanogaster* fruit and the largest population in the natural medium banana shoes . Besides comparison nisba kelamin1 male and 5 females and D. melanogaster population growth process starts from mating behavior , egg laying , and the process of metamorphosis . 2 . Artificial feed (artifical food) intended to divert the insects D. *melanogaster* from multiplying in the months naturally cultivated , while the bran / rice bran fermented pest activity is expected to result from D. *melanogaster* decreases and reduced and will be a source of feed swiftlets (predators) . Predators can be cultivated in areas mountains at an altitude up to 1000m above sea level between 300m asl apart in coastal areas .

3 . Feed type (insect D. *melanogaster*) as the birds' feed high protein significantly affect the thickness of the bird's nest . For α = 0:01 , thit = 6.95 > t0.99 (38) = 2.42 and weight of the non- treatment nest .

In organoleptic form nests did not differ either in form and color , and the provision of D. *melanogaster* as feed can reduce the duration of post -harvest dry season from 80 days to 65 days .

4 . There is a linear relationship between the amount of feed and weight of children swallow , for α = 26.16 00:01 thit > t0 , 95 (43) = 1.67 means that there is a highly significant positive correlation between the amount of feed (x) and weight (y) the regression Y = 1.43 x + 1.67 . Imago D. melanogaster , have a high protein 55.19 % for α = 4.56 0:01 thit > t0 , 95 (43) = 1.67 weight chicks fed researcher significantly higher than the child's weight bird that fed their parent in other words that the D. *melanogaster* both being fed to birds' . B. Advice This study can be used as a reference for the control of insect pests of fruit fly D. *melanogaster* by exploiting predators (birds') is very beneficial because it targets precisely to the target pest , and does not provide impact resistance on non-target insects than the use of pesticides . Another advantage is the predator birds' nests can produce high economic value of birds and insects D. *melanogaster* easy to breed in a short time (10-13 days) , in addition to the insect pest is below 4 mm, which can lead to pests and diseases in plants and plants can be controlled without the use of pesticides . Voting should be based on the principle of sustainability in order to nest swiftlet population does not experience stress and nest remains sustainable production in post-harvest production , as well as the quality and quantity is maintained nest .

C-10

PREPARATION AND MECHANISTIC STUDY OF ZnO/ZEOLITE AS CATALYST IN 1-PENTANOL DEHYDRATION

Is Fatimah

Chemistry Department, Islamic Univesityof Indonesia
Jl. Kaliurang Km 14, ***Sleman****, Yogyakarta 55581, email: isfatimah@uii.ac.id*

ABSTRACT

Preparation of zinc oxide impregnated zeolite from natural zeolite and its catalytic activity in pentanol dehydration was investigated. The impregnation process was conducted by two main steps; chemical activation followed by wet impregnation using $ZnSO_4.7H_2O$ as precursor and the characterization of prepared material was conducted by X-ray diffraction, scanning electron microscope (SEM-EDX) and surface acidity measured by pyridine adsorption-FTIR spectrophotometric method. As catalytic activity evaluation, pentanol dehydration over prepared material under vapor phase reaction mechanism was conducted by varied temperature of 300,400, 500 and 700^{o}C. For mechanistic study, the product of reactions was analyzed by using gas chromatography-mass spectrometry(GC-MS) . It is found that temperature affect strongly to the reaction mechanism in that at the temperature of above 500^{o}C the etherification was occured. From the comparation with zeolite, it can be noticed that the role of solid acidity also influences the reaction mechanism.

Keywords: Alcohol Dehydration, Natural Zeolite, Impregnatio, Heterogeneous Catalysis.

Introduction

Alcohol dehydration becomes one of important reaction in alternative energy development, for example the conversion of alcohol to ether. For the conversion, several solid acids have been reported to be successful in catalysing the reactions especialy the catalyst with acidity and catalyst thermal stability as the important physical properties for the mechanism[1]. Silica alumina materials such as clay and modified clay as well as zeolite and its modified form have been previously reported. Zeolite with sufficient surface acidity, high specific surface area and higher thermal stability compared to clay is one of the silica alumina mineral for heterogeneous catalysis in alcohol dehydration.The presence of Lewis and Broensted acid sites of zeolite that enhanced by thermal condition is the main reason which theoretically and practically proven. However, in order to improve the activity and selectivity towards a specified product, some modification regarding to the surface functionalization is required. Especially for natural zeolites as raw material in catalyst preparation, major drawback that has been discussed are mainly related with the poor thermal stability character and also their low tolerance to hot liquid water as product of dehydration[2][3]. Based on some researches on the utilization of zinc oxide and its composite forms as highly active in alcohol conversions, this investigation is efforted to improve catalytic properties of zeolite by impregation of ZnO onto zeolite as solid support. Aim of research is to study on the change of physicochemical character in its relation with catalytic activity in 1-pentanol dehydration.Effect of the formation of ZnO In advance effect of temperature to the catalytic mechanism is discussed.

Materials and Method

The zeolite used as raw materialin this research was natural zeolite obtained from Klaten, Jawa Tengah Indonesia.The powder of zeolite was chemically activated by refluxing in hydrochloric acid 0.1M for 6h followed by neutralization before using. Zinc acetate dyhydrate ($ZnO.2H_2O$), ethanol, 1-pentanol were purchasedfrom Merck-Millipore.

ZnO/zeolite catalyst was prepared by impregnation method. Precursor solution prepared from zinc acetate dilluted in ethanol:water solvent was dispersed into zeolite powder slurry followed by stirring for a night. Solvent was then evaporated slowly before drying and calcined at 700^{o}C for 5h. Powder of ZnO/Zeolite was characterized by x-ray diffraction(XRD) analysis collected using Shimadzu X6000 instrument, gas sorption analysis by NOVA1200e and surface acidity determination by pyridine adsorption-FTIR analysis. Sampel in a dessicator was evacuated for 4h before expossed with pyridine vapor for a night and measured by using FTIR spectrophotometer after reevacuation for 1h. An AVATAR FTIR spectrophotometer instrument was utilized for this purpose. Pentanol as feed was vaporized befor was purged into catalyst coloumn at varied temperature. The temperature inside the reactor wascontrolled with a CAL 9500P controller (CAL Controls Ltd.). Product of reaction was condensed and the liquid was then analyzed using a GCMS instrument. Reaction parameters of catalyst activity, total conversion and product selectivity were calculated based on GCMS analysis by using following equation[4]:

$$Total\ Conversion(\%) = \frac{mole\ of\ [1-pentanol]_i - [1-pentanol]_p}{mole\ of\ [pentanol]_i} x100\%$$

$$Catalyst\ Activity(\%) = \frac{mole\ of\ \ 1-pentanol\ reacted\ to\ form\ product}{mole\ of\ \ 1-pentanol\ reacted} x100\%$$

$$Selectivity\ of\ product\ i(\%) = \frac{mole\ of\ [Product-i]}{mole\ of\ 1-pentanol\ reacted} x100\%$$

[1-*pentanol*]$_o$, [1-*pentanol*]$_p$ are peak area of 1-pentanol in feed and product respectively

Result and Discussion

Physicochemical character of catalyst plays role in the catalysis mechanism. As catalyst in alcohol dehydration, the presence of surface acidity and surface active sites will directly drives the interaction amongs reactants. Confirmation on the formation of chrystalline and porous structure from the dispersion of ZnO on zeolite surface was observed by x-ray diffraction and Brunair-Emmet-Teller(BET) isotherm adsorption-desorption profile from gas sorption analysis. XRD pattern of both ZnO/zeolite and activated natural zeolite is depicted in Figure 1.

From the pattern it can be seen that there is no significant change in zeolite structure as shown by the relatively similar intensity of all reflections corresponding to the presence of zeolite structure. Some reflections at 2θ= 9.89; 27.58; and 29.80 are the indications for the mordenite structure and Clinoptilolite structure are confirmed by other reflections at : 22.43^{o}[5]. Those reflections are appeared in ZnO/Zeolite pattern beside of the reflection related to the occurence of ZnO phase at 2θ= (2θ) of 31.3670, 34.0270, 35.8596correspond to the reflection from: 100,002,and 101 crystal planes, respectively(JCPDS card no.0-3-0888)[6], [7].

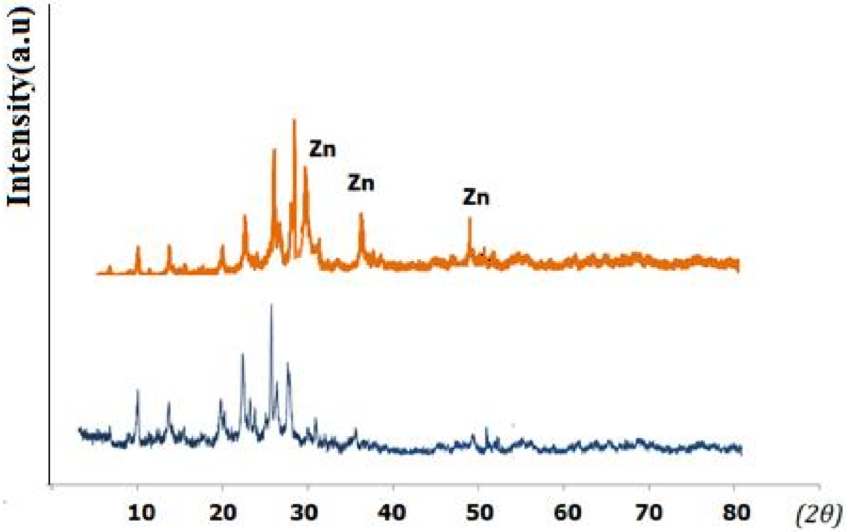


Figure 1. XRD Pattern of ZnO/Zeolite (above) and Zeolite (bellow)

ZnO impregnation also affects to the N_2 adsorption-desorption profile as presented in Figure 2 contribute to the specific surface area, pore volume and pore radius data listed in Table 1. Although was not significantly different, the adsorption capability of ZnO/zeolite is higher compared to zeolite as shown by the higher volume adsorbed at all P/P_o condition. Pore distribution appeared in Figure 2(c) showed that ZnO/Zeolite has higher volume at all pore radius. From the calculation based on the adsorption with BET isotherm data, the higher specific surface area and pore volume were recorded(Table 1) while the pore radius of ZnO/Zeolite is lower than that of Zeolite. This is the indication of the ZnO aggregates formation on surfaces which tends to create aggregate pores.

Table 1. Surface Parameter from BET Surface Area Analysis of ZnO/Zeolite and Zeolite

Catalyst	**Specific Surface area(m^2/g)**	**Pore Radius (Å)**	**Pore volume (cc/g)**
ZnO/Zeolite	80.67	12.21	$38.56\ x10^{-3}$
Zeolite	49.43	14.93	$28.7x10^{-3}$

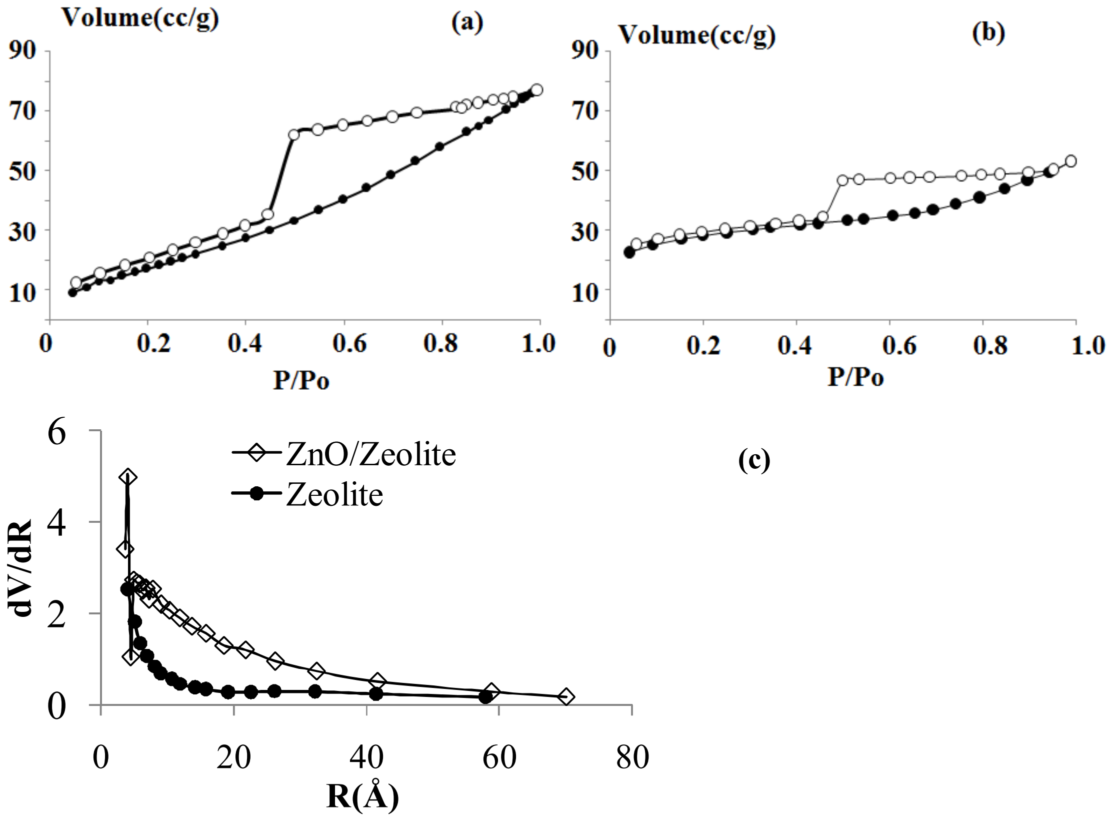


Figure 2. Adsorption-Desorption Profile of ZnO/Zeolite and Zeolite

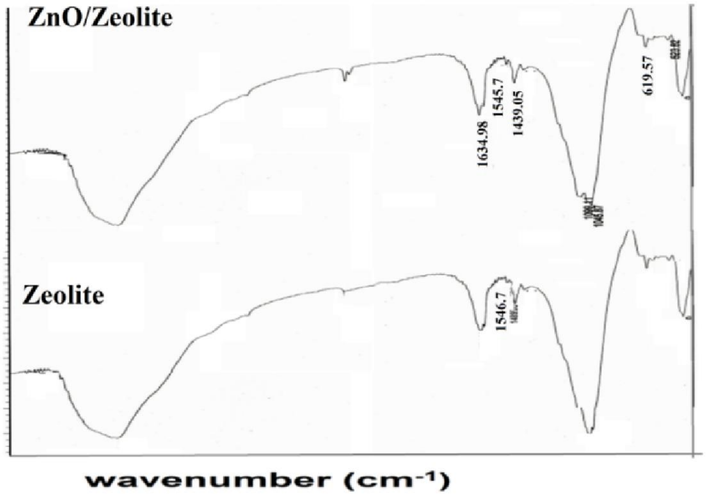


Figure 3. FTIR of ZnO/Zeolite and Zeolite after Pyridine Adsorption

Table 2. Total Acidity and Lewis to Broensted Acidity Ratio of ZnO/Zeolite and Zeolite

Sample	**Acidity (mmol pyridine/g)**	**Total Acidity (mg butylammine/g)**	**L/B ratio**
Activated Zeolite	0.460	0.775	0.99
ZnO/Zeolite	0.760	1.110	1.09

Catalytic activity of ionized zeolite is related to the presence of acid sites from tetrahedral $[AlO_4]^{5-}$ of zeolite frawework in that these sites have character to be both Broensted and Lewis acid. Pyridine adsorption followed by FTIR analysis and also determination of total acidity by using n-butylammine titration were used to observed surface acidity qualitative and quantitatively. Analysis of surface acidity by using pyridine adsorption is based on the interaction between aromatic ring and nitrogen atom of pyridine. Aromatic ring of pyridine can bind to Broensted acid of H^+ and other protons from zeolite structure while nitrogen atom can coordinatively bind to Lewis acid sites i.e from Zn atom supported on surface. Spectra of FTIR is presented in Figure 3 and the data on total acidity and also Lewis to Broensted acidity ratio are compiled in Table 2.

Symmetrical bending of Al-O-Al and Si-O-Si are appeared at 650-720 cm^{-1} while absorbance at the region of 950-1250 cm^{-1} are correspond to asymmetric streching. The interaction between adsorbed pyridine are indicated from some peaks at around 1450.52 $cm^{-1}$and 1546.72 cm^{-1} which correlated to the bonding between N of pyridine and proton (H^+) of zeolite. From the calculation based on FTIR spectra it can be noted that ZnO/Zeolite has higher total acidity and also L/B ratio. The insertion of zinc oxide particles are the main reason for the enhancement of acidity because of the contribution of d-orbitals of dispersed Zn. Lewis acid-base interaction comes from the bonding between electrone pairs of nitrogen to the formation Zn with coordination bonding.

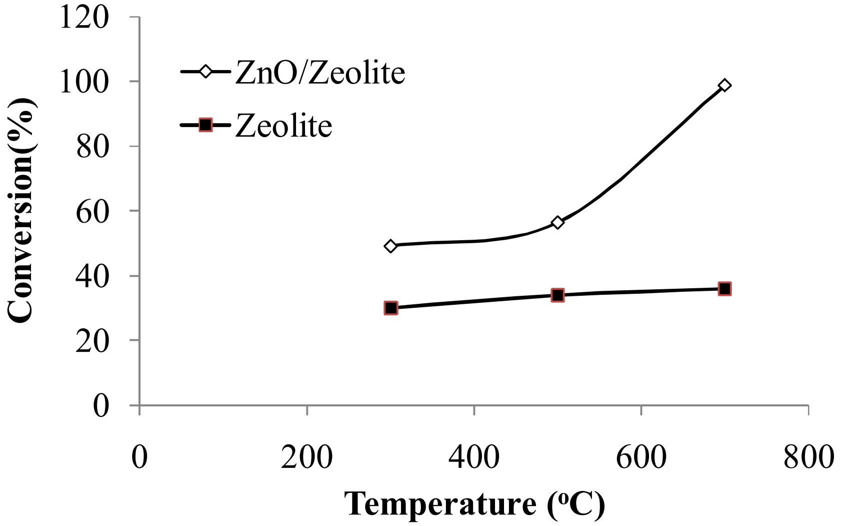
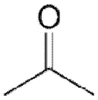
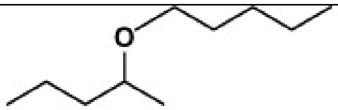


Figure 4. Effect of Temperature on Total Conversion

Catalytic activity test of prepared material showed that the total conversion increased withincreasing reaction temperature(Figure 4). Furthermore, ZnO/Zeolite exhibited a higher conversion at all reaction temperatures, reachingcomplete conversion at 700 °C compared with zeolite. In advance analysis of converted products catalyzed by ZnO/Zeolite were determined by using GCMS analysis as listed in Table 3. It can be seen that at 400°C the product consisted of 2-pentanone which is also produced over activated zeolite at all temperature condition. The yield was gradually changed with increasing temperature whereas at 500°C and 700°C the product of 1-pentyloxy pentene was formed. The product was observed only over ZnO/Zeolite which indicate the role of ZnO as active sites in the catalysis mechanism.

At relative low temperature (400°C) thermal effect seems occured since the product of 2-propanone was found. The route of conversion probably consist of thermal cracking to produce ethylene gas and followed by oxidation to produce 2-propanone. The domination of thermal effect compared to catalytic effect may related to that catalyst was in the inactive condition. As the temperature increased, the product of 1-pentyloxy-2-pentene, penten-1-yl, pent-2-yl ether and di-n-pentyl ether were produced and gradually enhanced by higher temperature. The mechanism of etherification whitin the dehydration was dominantly occured within both varied temperatures. The possible mechanism occurs is presented in schematic mechanism as modified from [8].

Table 3. Product of 1-pentanol dehydration over ZnO/Zeolite at varied temperature

Temperature	**Product of Reaction**	**Structure**	**%**
400°C	2-propanone		48.75
	1-pentanol		50.81
	2-pentanone		0.15
500°C	1-pentanol		43.55
	1-pentyloxy-2 pentene		**11.46**
	Pentanoic acid		0.28
	Heptane		0.58
700°C	1-pentanol		1.24
	Di n-pentil ether		**21.26**
	1-pentyloxy-2 pentene		7.98
	penten-1-yl, pent-2-yl ether		8.10

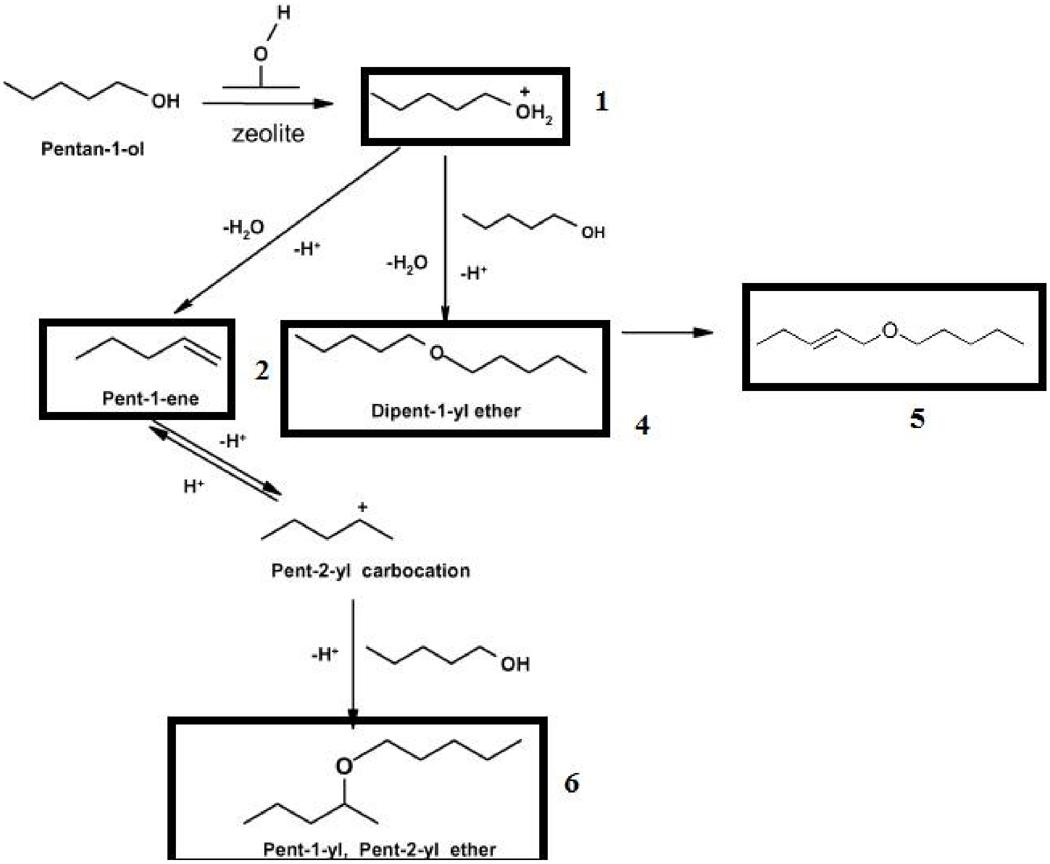


Figure 5. Schematic Possible Mechanism of 1-pentanol Dehydration as modified from Mohammed and El-Esaimi[8]

1-pentyloxy-2-pentene(5) is probably produced from the bimolecular interaction between pent-1-ene(2) and carbocation (1). The ionic interaction has higher possibility and theoretically enhanced since the surface active of Zn on zeolite surface active[4]. The possible route for the formation of this compound attribute to the strong adsorption of intermediate product(1) and (2) by the surface leads to the structural rearangement to form the compound(4). It is also showed by the presence of heptane as a result as well as propanoic acid. At higher reaction temperature of 700°C, it can be seen that penten-1-yl, pent-2-yl ether(6) and di-n-pentyl ether(4) were produced. The product of (4) is the main chemical targetted in this mechanism as reported by several authors [4], [8], [9] while the product (5) was similar compound that was also reported from the 1-pentanol dehydration over zeolite[8]. From elucidating the mechanism it can be concluded that ZnO impregnation onto zeolite surface contributes significantly to the surface activity and that is strongly correlated to the surface acidity improvement.

Conclusion

In this research ZnO/Zeolite has been succesfully prepared with enhanced physicochemical character that support the function of the material as catalyst for 1-pentanol dehydration. An important character of surface acidity presents the active sites in the catalysis mechanism. An important concept presented from the varied temperature is that surface active properties of ZnO/Zeolite affects the mechanism involved via the adsorption of reactant by Zn metal dispersed on solid support. Bimolecular interaction on surface occurs at the temperature 500°C and 700°C while at 400°C thermal effect is relatively dominant.

References

[1] D. E. R. P.A. Zapata, J.Faria, M. P.Ruiz, R.E. Jentoft, "Hydrophobic Zeolites for Biofuel Upgrading Reactions at the Liquid− Liquid Interface in Water/Oil Emulsions," *J Am Chem Soc*, vol. 2, no. x, 2012.

[2] P. P. A.Corma, "Catalytic Activity of Modified Silicates: Dehydration of Ethanol Catalyzed by Acidic Sepiolite," *Clay Miner*, vol. 22, pp. 423–433, 1987.

[3] B. Huang, Q. Liu, J. Caro, and A. Huang, "Iso-butanol dehydration by pervaporation using zeolite LTA membranes prepared on 3-aminopropyltriethoxysilane-modified alumina tubes," *J Memb Sci*, vol. 455, pp. 200–206, Apr. 2014.

[4] J. Tejero, F. Cunill, M. Iborra, J. F. Izquierdo, and C. Fité, "Dehydration of 1-pentanol to di-n-pentyl ether over ion-exchange resin catalysts," *J Mol Catal A Chem*, vol. 182–183, pp. 541–554, May 2002.

[5] L. S. A. Arcoya, A. Gonzales, N. Traviesco, "Physicochemical and Catalytic Properties of a Modified Natural Clinoptilolite," *Clay Miner*, vol. 28, pp. 123–131, 1994.

[6] Y. T. Prabhu, K. V. Rao, V. Sesha, S. Kumar, and B. S. Kumari, "Synthesis of ZnO Nanoparticles by a Novel Surfactant Assisted Amine Combustion Method," vol. 2013, no. February, pp. 45–50, 2013.

[7] N. F. Hamedani and F. Farzaneh, "Synthesis of ZnO Nanocrystals with Hexagonal (Wurtzite) Structure in Water Using Microwave Irradiation," vol. 17, no. 3, pp. 231–234, 2006.

[8] M. M. Mohamed and M. M. Al-Esaimi, "Synergistic catalysis effect in pentanol conversion into di-n-pentyl ether on ZSM-5 supported titania catalysts synthesized by sol–gel," *Mater Chem Phys*, vol. 115, no. 1, pp. 209–216, May 2009.

[9] S. A. H. D.Liu, A.Bhan, M.Tsapatsis, "No TitleCatalytic Behavior of Brønsted Acid Sites in MWW and MFI Zeolites with Dual Meso- and Microporosity," *ACS Catal*, vol. 1, no. 7, pp. 7–17, 2011.

C-11

EFFECT OF PYROLISIS TEMPERATURE AND DISTILLATION ON CHARACTER OF COCONUT SHELL LIQUID SMOKE

Johny Zeth Lombok$^{1)}$, Bambang Setiaji$^{2)}$, Wega Trisunaryanti$^{2)}$, Karna Wijaya$^{2)}$.
$^{1)}$ *Faculty of Mathematics and Science (FMIPA) UNIMA Manado*
$^{2)}$ *Faculty of Mathematics and Science (FMIPA) UGM Yogyakarta*

Abstract

A research on effect of coconut shell pyrolisis temperature and liquid smoke distillation on character of resulted liquid smoke has been done. Objective of the research is to identify effect of coconut shell pyrolisis temperature and liquid smoke distillation on character of coconut shell liquid smoke distillate. Liquid smoke was obtained with pyrolisis using pyrolisis reactor on temperature range of 150 to 450°C. Liquid smoke purification was done by decantation, filtering and distillation at various temperatures: <100 (D-I), 100-120 (D-II), 121-140 (D-III), 141-160 (D-IV), 161-180 (D-V) and 181-200 (D-VI). Liquid smoke characteristic was done by determining physical and chemical characteristic such as density, bias index, pH, acid level and phenol level. The result indicated that temperature of coconut shell pyrolisis and liquid smoke distillation affect concentration and liquid smoke characteristic. Highest liquid smoke concentration was obtained in pyrolisis temperature of 150-200°C (6.07% (v/m)) and the lowest one (0.85% (v/m) at temperature of 351-450^{0}C. highest tar concentration (1.03% (v/m) was obtained at 276-350°C, and the lowest (0.20% (v/m)) at 150-200°C. Charcoal concentration was 28.75% (m/m). Characteristic (pH, acid level and phenol level) of liquid smoke before and after purification process and distillation are various. Highest pH (6.2) was D-I, while the lowest (4.1) at D-VI. The highest acid level (58.40%) was at D-VI, while the lowest (1.86%) was at D-I. at D-I liquid smoke does not contain phenol, while the highest phenol content (3.85%) was in D-VI and the lowest was liquid smoke residue (1.93%)

Keyword: liquid smoke, temperature, coconut shell.

INTRODUCTION

Coconut shell is one of coconut parts and is by product of coconut processing. Coconut farmer in North Sulawesi use it as fuel in making copra and for cooking. Direct burning of coconut shell result in some that is dangerous waste and when it is not managed seriously it can air pollution. Coconut shell can be processed to be product having high economic value. Pyrolisis of coconut shell result in charcoal and smoke that can be condensed to be liquid smoke, tar and uncondensed gasses. Charcoal can be processed further to be briquette or active charcoal while liquid smoke can be used as food preservation agent substituting formalin and as antibacterial agent [12,17].

Wood pyrolisis result in liquid smoke that contains various complex chemical compounds[3]. Coconut shell belongs to hard wood group that containing three main components: cellulose, hemicelluloses, and lignin. Cellulose decomposition by heat results in anhydroglucose, carbonyl compound and furan. Decomposition of hemicellulose is similar to that of cellulose, but resulting in acetate acid and carbon dioxide. Partial pyrolisis of lignin results in various phenolic compounds [20]. Composition of liquid smoke is affected by raw material type, duration of burning and burning temperature. Liquid smoke is used commonly by agent providing aroma, texture and taste of food product, such as mead, fish and cheese [15]. In

Indonesia, liquid smoke is used in smoked milkfish industry in Sidoarjo. Liquid smoke of coconut shell is also used in laboratory scale [7].

In pyrolisis process, to obtain quantity and quality of good liquid smoke require research to look for operational condition to maximize concentration and quality of liquid smoke. Pyrolisis temperature is one of operating parameters to achieve the goal. In addition, to obtain liquid smoke free from tar it is necessary a separation process to obtain good and safe liquid smoke to apply for food product.

RESEARCH METHOD

Materials

Coconut shell from byproduct of copra making, liquid smoke, chemical material with grade pro analyst from Merck: NaOH, $Na_2S_2O_3$, pp indicator, ethanol and reagent Follin-Ciocalteu, zeolite, filter paper Wathman no.42

Coconut shell pyrolisis

Washed coconut shell with its dimension is reduced was put into pyrolisis reactor and closed well. Reactor was heated in high temperature (150-450^{o}C) with heating rate of 30^{o}C per minutes. After reaching desired temperature, it was let 2 hours and smoke flow through evaporation pipe, cooled through cooling media. Then its liquid was discharged through pipe placed in container. After there is no liquid, the heating is stopped and reactor is cooled. Condensate and charcoal was weighted to determine concentration. Condensate concentration is mix of liquid smoke and tar calculated as % volume/weight and charcoal is calculated as % weight/weight

Purification and characterization of liquid smoke

Liquid resulted from pyrolisis is heterogeneous mix of liquid smoke and tar and still contain toxic component. The liquid is stored some days to allow tar and other unsolved compounds to separate or settle, then decanted, filtered with filter paper and zeolite and distilled/re-distillated.

Liquid smoke distillation

Liquid smoke resulted from decantation and filtering (750 ml) was put into 1000 mL round-bottom flake then distillated fractionation using fractionation column of 60 cm equipped with condenser and heated using oil heater at 0-200^{o}C. Distillate was taken in collector in various temperatures: <100 (D-I); 100-120 (D-II); 121-140 (D-III); 141-160 (D-IV); 161-180 (D-V); 180-200 (D-VI). Resulted distillates were characterized by determining physical and chemical characteristic.

Characterization of liquid smoke

Liquid smoke is characterized with standard method according to [10], which includes determination of specific mass, bias index, pH value, total acid analysis and phenol content

a. Determination of specific mass and bias index of liquid smoke

Measurement of specific mass of liquid smoke and tar was done using 10 ml pycnometer, while bias index was determined using refractometer. Procedure of specific mass determination is as follow. Cleaned and dried pycnometer was weighted carefully and its weight was recorded. Distillated water was put into pycnometer until calibration mark and weighted. Its weight was recorded. Distilled water is discharged, and then pycnometer is re dried. Then liquid smoke was put into calibration mark and its weight was recorded. Specific mass of liquid smoke was calculated using following equation:

$$Spesific\ mass = \frac{(sample\ mass\ +\ empty\ pycnometer\ mass) - empty\ pycnometer\ mass\ (gr)}{(water\ mass\ -\ empty\ pycnometer\ mass)\ -\ empty\ pycnometer\ mass\ (gr)}$$

(SNI 06-2388-1998)

b. pH value

pH value of resulted liquid smoke is determined using digital pH meter Metrohm type 692 pH/ion meter by dipping electrode into distilled water and wiped with tissue. Then, electrode is put into liquid smoke sample. pH value appearing in screen is recorded as pH value of liquid smoke.

c. Analysis of total acid[2]

Liquid smoke 5 mL was added with 100 mL distilled water and shake until homogenous. Then 3 drips of pp indicator were added. Then it was titrated with NaOH 0.1 N until light red. Total acid measured is considered as acetate acid. Acetate acid content:

$$Y = \frac{amount\ of\ ml\ titer\ \times\ N\ NaOH\ \times\ 60}{sample\ volume\ (mL\ \times\ 1000)} \times 100$$

d. Phenol content[15}

Sample of 10 mL was placed in reaction tube containing 12 mL ethanol 95% and 5 mL distilled water. Then 0.5 mL reagent Follin-Ciocalteu was added into each tubes. They are let for 5 minutes and added 1 ml $Na_2S_2O_3$ 5% into sample, and shaken in Vortex Shaker, then stored in dark room for 60 minutes. Then, sample was shaken again using Vortex Shaker and its absorbance is measured at 725 nm wave length.

RESULT AND DISCUSSION

Pyrolisis result

Coconut shell from byproduct of copra making has 6.16% water content. Pyrolisis product of 8 kg coconut shell in various temperatures is presented in table 1.

Table 1 Yield recovery of liquid smoke, tar and charcoal

No	Temperature (^oC)	The yield of		
		Liquid smoke (%v/m)	Tar (%v/m)	charcoal (%m/m)
1	150-200	6,07	0,20	28,75
2	201-275	3,21	0,23	
3	276-350	4,58	1,03	
4	350-450	0,85	0,83	

In pyrolisis process, coconut shell is broken and put into pyrolisis reactor connected with smoke link pipe and equipped with tar collector, cooling and liquid smoke collector. Pyrolisis process of coconut shell occurred in pyrolisis reactor after heating in high temperature and took 5 hours. Pyrolisis product of coconut shell consisted of charcoal, tar, liquid smoke distillate and uncondensed gases.

Pyrolisate is created or drip at 150^{o}C and at higher temperature from 200^{o}C tar is formed. End of pyrolisis process is known from no distillate created and from discharged gases or not condensed gas. Charcoal product is carbon composing coconut shell solidified after volatile compound of coconut shell change to be liquid smoke and gas in pyrolisis process. Gas volume was obtained from law of mass conservation assumption, in which mass before reaction is same as mass after reaction [16]. After let sometime the resulted Tar and liquid smoke would be separated by forming two layers due to its phase nature, polarity and different specific gravity; liquid phase was in upper layer and solid phase was in lower layer. Tar is non polar and very concentrated, while liquid smoke is polar because it can mix with water and contain non polar component solved within it. Liquid smoke of pyrolisis process has strong smell and contain smoke particle.

According to [6] and [11] in temperature below 200^{o}C is pyrolisis reaction that disappear water from wood, while above 400^{o}C it is not decomposition of wood component to organic compounds but heating process and charcoal ripening. In addition, the temperature range is maximal burning temperature in liquid smoke making process.

Effect of temperature on liquid smoke and tar concentration

Concentration is one of important parameter to identify result of a process [19]. Figure 1 indicate graphic of association between pyrolisis temperature and % liquid smoke and tar concentration resulted from pyrolisis process of coconut shell. The figure shoes that highest liquid smoke volume is obtained at 150-250°C. In the temperature range, water discharge process occurs in coconut shell.

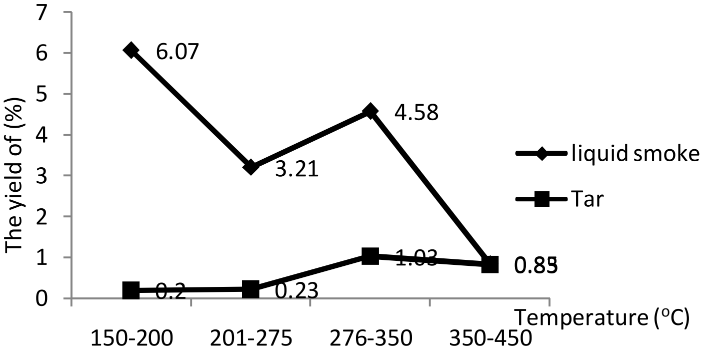


Figure 1 Graph relationships % yield volumes of liquid smoke and tar with pyrolysis temperature

At 201-275°C, it occur decrease in liquid smoke volume due to decrease in water in coconut shell accompanied with discharge of CO and making CO_2, and charcoal residue making. So, result of pyrolisate decrease. At temperature 276-350°C result of pyrolisate rise due to lignocellulose decomposition and lignin disproportion reaction what still result in volatile compound with low molecule weight that is evaporated and condensed to be liquid smoke. Decrease in significant number of pyrolisate is indicated at 350-450°C. It is caused by component formed in pyrolisis process is degraded to result in CO, CO_2, H_2 (low and middle chain hydrocarbon) such as research by [19] stating that higher pyrolisis temperature will decrease amount of organic monomer degradation product. Decrease in degradation product will be accompanied with increase in carbon dioxide and other gas.

Tar volume at 150-275°C is not relatively increase, while at 276-350°C increased and at 351-450°C it decreased again. It may due to addition of vapor product of low volatile compound due to temperature rise, so push of vapor faster to be liquid smoke and result of tar is fewer. Maximal tar volume occur at 276-350°C, because at the temperature range lignocellulose and lignin degradation process occur that resulted in anhydrate and tar. So, maximal tar volume is accompanied with increase in pyrolisate product. At 350-450°C, there occurred decrease in tar volume and also very significant decrease in pyrolisate product due to end of degradation process of coconut shell component.

Total concentration of liquid smoke and tar produced in this research was 14.71% and 2.29%, respectively. Table 1 indicated that solid product of shell charcoal of 28.75 what show in coconut shell there is volatile component of 71.25% that change into pyrolisate, tar and uncondensed gas. Total liquid smoke and tar concentration is 17% so there is 54.25% gas disappear or uncondensed. In this pyrolisis process, percentage of loss gas uncondensed is very high. According to [18], lost chemical component in pyrolisis process is volatile compound and cannot be condensed with water as cooler in form of gas such as CO_2, Co, H_2, CHn and some hydrocarbon.

Compared with some previous research, percentage of liquid smoke concentration in this research was far different. It is due to coconut shell used in this research is coconut shell from copra making remaining that have underwent baking process that has very low water content. In addition, the difference may be due to type of raw material, reactor shape and pyrolisis process.

Concentration percentage also depends on condensation system and instrument used. In this research condensation system sued to form liquid smoke use water flowed continuously as cooler media in order to make heat changing process can occur fast and temperature in cooler system did not increase. Liquid smoke resulted of coconut shell pyrolisis can be obtained maximally when its condensation process run perfectly. Therefore, condenser length used also determine product of liquid smoke. Theoretically, longer condenser may make smoke resulted from imperfect burning in extraction process of smoke distillate will be more optimal [19].

According to [18] liquid smoke concentration resulted from some woods varied from 39.15% to 61.55% with average value of 50.09%. Meanwhile charcoal product varied from 21.5% to 37.62% with average value of 31.54%. According to [14], pyrolisis temperature affect concentration or percentage of pyrolisate resulted. Higher pyrolisis temperature causes higher distillate percentage but with lower charcoal product. However, too high pyrolisis temperature will decrease amount of organic monomer degradation product. Less charcoal product relates to decrease in organic component in coconut shell cell due to higher pyrolisis temperature.

Increase in pyrolisis temperature also increase lost component. It is indicated at pyrolisis execution in which increase in smoke temperature discharged and uncondensed is greater. Based on the result, it may be concluded that liquid smoke concentration resulted in pyrolisis process depend on raw material type, pyrolisis reactor and pyrolisis temperature.

Effect of pyrolisis temperature on liquid smoke and tar specific mass

Specific mass is ratio of mass of a sample and its volume. Specific mass of liquid smoke does not relate directly to liquid smoke quality resulted, but can indicate amount of component within it. Determination of specific mass AC-TK and tar was done using pycnometer. Data of specific mass measurement indicated that change in pyrolisis temperature did not affect specific mass of liquid smoke and tar significantly. In this research, lowest result of analysis of liquid smoke specific mass was 1.022 gr/mL and the highest 1.042 gr/mL. Specific mass of liquid smoke resulted from pyrolisis increase with increase in temperature although its increase very small. Highest specific mass is liquid smoke obtained from temperature 350-450^{o}C, while its lowest in 150-200^{o}C. The lowest specific mass of tar is 1.086 and the highest one is 1.101 gr/mL. Specific mass of tar rise with increase in pyrolisis temperature although its increase is very small as liquid smoke. Specific mass of liquid smoke in each temperature is relatively low than that off specific mass of tar, so increase in pyrolisis temperature did not give significant effect on specific mass of liquid smoke and tar.

Liquid smoke distillation

Liquid smoke distillation was done to remove dangerous compound and not desired such as polyaromatic hydrocarbon (PAH) and tar, with regulation of boil temperature to obtain clean liquid smoke, free of tar and benzopirene. The distillation process used Vigreux column with 60 cm length and 4 cm diameter. Use of Vigreux column is expected to separate component of liquid smoke more effective particularly carcinogenic component so purer liquid smoke may be obtained and its compound is isolated based on its characteristic. Data of volume concentration of distillated result of 750 mL liquid smoke of coconut shell in certain temperature range is presented in graphic of association of % distillate concentration and distillate temperature as presented in figure 2.

Liquid smoke distillation process for 187 minutes resulted in liquid smoke distillate with different time volume and distillate percentage. Data and graphic in figure 2 indicated that D-II has most volume percentage (71.3%) and longest process (125 minutes). While at D-I, the concentration is leas (1.6%) with distillation duration of 15 minutes. D-III to D-V has relative similar concentration (3.7% and 3.6%) with distillation duration of 10, 12, and 15 minutes. D-VI has concentration of 1.7% with 10 minutes distillation period. Remaining liquid smoke residue (un-distilled) was 5.3%. The data indicated that dominant D-II contain water while D-I is composed by volatile compounds having low boil point.

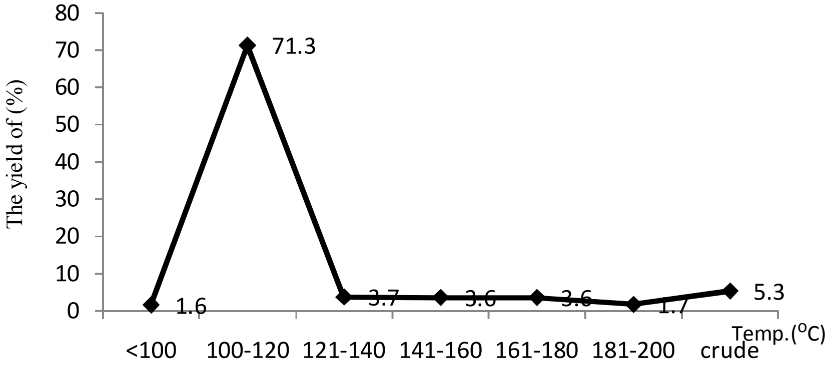


Figure 2 Graph relationships% yield of distillate volume of liquid smoke with distillation temperature

Characteristic of liquid smoke distillate

Liquid smoke quality

Liquid smoke quality in taste and aroma is determined by composition and compound contained within it because the component is made as criteria of liquid smoke quality. According [5] quality of liquid smoke was determined by its chemical compound because the compounds are criteria for taste and aroma as characteristic of some. In this research, test of liquid smoke quality was done with physical and chemical test. Physical characteristic observed include color, aroma, specific mass and bias index, while chemical characteristic include pH, acid content and phenol content.

Specific mass and bias index of liquid smoke distillate

Specific mass of liquid smoke distillate was determined using pycnometer, while bias index was determined using refractometer. Result of specific mass and bias index measurement indicated that distillate collected in various temperatures did not affect specific mass and bias index. Resulted specific mass and bias index of distillate and residue indicated not significantly different value. The lowest specific mass is ACSD (1.01238), while the highest specific mass is liquid smoke residue (1.10369. specific mass of D-I to D-VI are relatively similar (1.07 – 1.09). Bias index of ACSD and distillate indicate not significantly different value (1.344-1.357). The lowest bias index is D-I (1.344), while the highest is in liquid smoke residue (1.673).

pH, acid content and phenol content

pH value is one of parameters of liquid smoke quality and was determined by chemical composition. pH value of liquid smoke related to process level of decomposition of chemical component of coconut shell resulting in organic acid in liquid smoke. When liquid smoke has low pH, quality of liquid smoke resulted is very high because at whole in food preservation process it effect greatly preservation value and storage value of smoked process and its organoleptic characteristic [19].

Result of pH measurement phenol contain and total acid content from liquid smoke of coconut shell pyrolisis before and after purification process and distillation are different. pH of liquid smoke before distillation (LSBD) was 4.82. After distillation at a certain temperature distillate yield with varying pH values. The highest Ph was in D-I (6.20) while D-II to D-VI and residue have no significantly different ph value (4.10 -4.63). First distillate has lowest acidity (highest pH). It is due to distillated compound in the temperature range is a volatile compound having low acidity level. D-I liquid smoke obtained at 100-120°C was predicted having high pH near seven due to the temperature is boil point of water so the distillate has water content, but based on measurement result it has pH 4.63.based on the data, the distillate may contain acid compound particularly acetate acid having boiling point of 118°C. pH value of liquid smoke distillate is increasingly low along with increase in distillation temperature. It may be due to in the temperature water component increasingly less with increase of distilled organic acid. Presence of other compound having high acidity in each distillate is also predicted causing low pH or increase in acidity level of liquid smoke distillate. The lowest pH was D-VI and liquid smoke residue (4.10 and 4.12, respectively). Comparison with pH value of liquid smoke before distillation indicated pH of liquid smoke distillate decreasing, except for D-I having pH 6.2.

Table 2 pH, acid and phenol content of distillate and residual liquid smoke coconut shell

Distillate	pH	Acid Content (%)	Phenol Content (%)
D-I	6,20	1,86	-
D-II	4,63	9,60	2,25
D-III	4,40	29,30	2,42
D-IV	4,36	39,66	2,53
D-V	4,22	56,41	2,82
D-VI	4,10	58,40	3,85
Residue	4,12	48,86	1,93
LSBD	4,82	9,81	2,35

Acid content of LSBD compared with distillate and residue is relatively different. Acid content of D-II liquid smoke is almost same as LSBD. D-I AC-TK has the lowest acid content (1.86%), while acid content of D-III to D-VI increase with increase in distillation temperature. The difference is predicted due to temperature of D-I and DII, boiling point of acid compound has not been reached. At the temperature, distilled component is dominated by volatile compound having low acidity content.

Result of acid content measurement indicated that higher distillation temperature reveal higher acid content. Increase in acid level is expected due to increase on acid component content along with characteristic of acids composing liquid smoke such as acetic acid having boiling point of 118°C, propionate acid (141°C) and butyrate acid (162°C)[4]. Acidity data of liquid smoke distillate suit to pH data in which higher distillation temperature result in distillate with lower pH that means increase in acidity.

According to [19], acid content is one of chemical characteristic determining quality of liquid smoke. Acid compound formed from wood burning process is organic acid compound resulted from pyrolisis process of wood components such as hemicellulose and cellulose in certain temperature. According to [6] some in liquid form also affect total acid of smoke condensate (40%) with 35 acid types. Acidity in some determine natural pH of smoke that have preservation characteristic, particularly in smoked product. Organic acid having important role in liquid smoke is acetic acid. Acetic acid is formed from pyrolisis process of lignin and part of pyrolisis of carbohydrate component, cellulose. Compared with result of research [4] there is not significant different between acid levels from distillate LS-CS purified with re-distillation except in D-I that is collected in temperature below 100°C. Acid content of liquid smoke distillate obtained in this research range from 1.86 to 58.40%, while research [4] indicated range of acid content from 8.68 -55.72%. In this research, first distillate acid content obtained in temperate <100°C (D-I) was 1.86% while research [4] indicated result of 12.34%. the difference may be due to different distillation process condition and temperature of distillate storing range, where in this research D-I is stored in temperature below 100°C so only volatile components have been distilled, while in research [4] D-I is stored up to 100°C so it is possible much water and acid components distillated.

Liquid smoke acidity is also influenced by wood type or raw material used. According to [18] liquid smoke acidity of some woods varied from 4.27 to 11.39% with average value of 6.59%. According to [4] liquid smoke acidity of some woods varied from 4 to 29%. Research

[13] indicated presence of acids till 10 carbon chain but the most is format acid, acetic acid and propionate acid and butyrate acid. Different acid content in liquid smoke may be due to difference on chemical composition particularly cellulose.

Data of phenol content indicates presence of different phenol content between distillate, residue and liquid smoke before distillation. Increase in temperature in distillation process cause increase in phenol content. The research indicates that D-I did not contain phenol. It may due to boiling point of phenol and its derivative is relatively high so it is not distilled below 100°C. Phenol compound begin being detected at distillate collected at 100-120°C although its boiling point is high. It may be due to phenol form azeotrope with other compound such as water and organic acid so it is distillated. The highest phenol content in this research is D-V and D-VI collected in temperature range of 161-200°C (2.82 and 3.85%). It is due to the temperature range is boiling point of phenol compound and its derivative (18°C). Based on result of previous researches, phenol content in this research is not significantly different except phenol content in D-I. [4] reported that liquid smoke re-distillation at 100-200°C resulted in distillate with higher phenol content at 175-200°C (3.1%), while the lowest one is distillate collected below 100°C (1.14%). the result indicted that distillated collected below 100°C did not contain phenol. It is supported with result of identification with GC-MS analysis that did not indicate phenol compound in GC chromatogram or on MS spectrum data [9]. According to [1], phenol containing AC-TK was 2996.48 ppm or about 2.99%, while [21] stated that concentration and composition of phenol in liquid smoke varied between 1.10 to 5.13% depending on type and shape of wood as material of making liquid smoke.

CONCLUSION

1. Concentration of liquid smoke and tar resulted from pyrolisis process of coconut shell is greatly influenced by pyrolisis temperature. Highest liquid smoke concentration is obtained at 150-200°C (6/06%), while the lowest is obtained at 350-450°C (0.85%).
2. Distillation process using fractionation column can separate and purify composing component of liquid smoke of coconut shell. The highest liquid smoke distillate concentration is obtained at temperature range of 100-120°C (71.3%), while the lowest at temperature below 100°C (1.6%)
3. pH value, acid content, and phenol content of liquid smoke before and after purification are various. The highest pH is D-I liquid smoke, while the lowest is in D-VI liquid smoke. The highest acid level is D-VI (58.4%), while the lowest is D-I (1.86%). The highest phenol content is D-VI (3.85%), while the lowest is liquid smoke residue (1.93%). D-I liquid smoke did not contain phenol.

REFERENCES

[1] Arizona, R., E. Suryanto and Y. Erwanto. 2011. The effect of canary shell liquid smoke concentration and storage time on chemical and phisical quality of beef. *Buletin Peternakan.* 35(1):50-56.

[2] Association of Official Analitical Chemist (AOAC). 2005. *Official method of analysis.* 18th edition. Association of official analytical chemist. Inc. Washington DC., USA.

[3] Budianto, S., R. Hasbulla, S. Prabawati, Setyadjit, Sukarno dan I. Zuraida. 2008. Identification and safety test coconut shell liquid smoke for food products. *J. Pascapanen.* 5(1): 32-40.

[4] Darmadji, P. 2006. Antibacterial activity smokes produced from several dry agricultural wastes. *Agris*. FAO of the United Nation.

[5] Gani, A., Z.A. Mas'sud, B.W. Lay, S.H. Sutjahyo dan G. Pari. 2012. Identification of bioactive antifeedant compounds from liquid smoke of urban organic waste pyrolysis product. *J. Tek. Ind. Pert.* (16(3): 111-118.

[6] Girard, J.P. 1992. *Smoking in tecnology of meat and meat products***.** Clermont Ferrand Ellis

Horwood. New York.
[7] Hadiwiyoto, S.P., P. Darmadji dan S.R. Purwasari. 2000. Comparison of hot smoking and use of liquid smoke on fish processing, review the content of benzopiren, phenol, and organoleptic properties of smoke fish. *Agritech*. 20:14-19.
[8] Hamm, R. 1977. *Analisis of smoke and smoked foods*. Pure and Apl. Chem. Pergamon Press. 49: 1655-1666.
[9] Lombok, J.Z., B. Setiaji, W. Sunaryanti dan K. Wijaya. 2012. Characterization and identification of compounds from liquid smoke shell coconut pyrolysis product. *Prociding Seminar ISNPINSA 2.* Semarang. Indonesia.
[10] LTP. 1994. Methods and procedures chemical examination of agricultural products. Dirjen Perikanan, Departemen Pertanian. LTP. Jakarta.
[11] Maga, J.A. 1988. *Smoke in food processing*. CRC Press. Florida.
[12] Milly, P.J., R.T. Toledo and J. Chen. 2008. Evaluation of liquid smoke treated ready-to eat (RTE) meat products for control of listeria innocua. *J. Food Sci.* 73: 179-183.
[13] Porter, R.W., L.J. Bratzler and A.M. Pearson. 1965. Fractionation and study of compounds in wood smoke. *J. Food Sci*. (30):615-619.
[14] Sari, R.N., B.S.B. Utomo dan T.N. Widianto. 2006. Enginering equipment manufacturer liquid smoke for smoke fish production. *J. Pascapanen dan Bioteknologi Kelautan dan Perikanan*. 1 (1):65-73.
[15] Senter, S.D., J.A. Robertson and F.I. Meredith. 1989. Phenolic compounds of the mesocarp of cresthaven peaches during storage and ripening. *J. Food Sci*.1259-1268.
[16] Soldera, S., N. Sebatianuso and R. Bortolomeazzi. 2008. Composition of phenolic compound and antioxidant activity of commercial aqueous smoke flavoring. *J. Agric Food Chem.* 56: 2727-2734.
[17] Toledo, R. T. 2007. *Wood Smoke Components and Functional Properties, International Smoked Seafood Conference Proceedings Alaska Sea Grant College Program.* Anchorage. Alaska USA.
[18] Tranggono, Suhardi, P. Darmadji Sudarmanto dan B. Setiadji. 1997. Identification of liquid smoke from wood various. *J. Ilmu dan Teknologi Pangan*. 1:15-24.
[19] Wijaya, M., E. Noor, T.T. Irawadi dan G. Pari. 2008. Characterization of liquid smoke chemical component and utilization as a biopesticide. *Bionature* 9(1) 34-40.
[20] Wei, Q., X.H. Ma, and J. E. Dong. 2010. Preparation, chemical constituents and antimicrobial activity of pyroligneous acid from walnut tree branches. *J. Anal. Appl*. Pyrol. 87:24-28.
[21] Yu, A. and B. Sun. 2006. Volatile phenolic derivatives and its role in Chinese traditional smoke-cured Meat. *J. Food Tec*. 4:29-31.

C-12

CHARACTERIZATION CHEMICAL COMPOUND BASED PYROLYSIS PROCESS FROM CACAO WASTES

Mohammad Wijaya.M

Department Chemistry Faculty Mathematic and Natural Science Makassar State University
90224
Email : wijasumi @yahoo.co.id

ABSTRACT

The research aim is to produce cacao vinegar through pyrolysis technology of potential chemical compounds from cacao shell wastes. Results of this research are expected to give benefits as follows: Liquid smoke produced from cacao shell wastes through pyrolysis process is able to diversify fertilizer and new products, The results research showedthat thefat contentof fermentedforLuwu district 35.21Results of pyrolysis of cacao waste that gave the highest liquid smoke as much as 40,36% in p.yrolysis temperature of 500^{o}C In terms of the charcoal produced from pyrolysis process containing the highest yield was cacao shell waste Luwu district as 42,08% Identification of GC-MS of cacao shell could provide compounds that mostly derived from acetone 13,01%, acetate acid 73,86%, 2 buthanone 6,60%, methyl ester 2,46%, and propanoic acid 4,07% Usually, Result X-RD analysis for cacao shell char with Luwu District as cristalinity degree 22,83%.contentof Nforcocoa vinegar rindLuwudistrictof175.53ppmand1.39% charcoal, greater thenitrogenelement contentthe betterforplants as fertilizer organic. The technology of integrated cacao vinegar-charcoal production hence deserves its dissemination throughout Indonesian.

Key Words :*Cacao wastes, fermentation, pyrolysis, and Cacao Vinegar*

INTRODUCTION

Cocoa production increased by 325 thousand tonsin 2013, by replanting cocoa, rehabilitation and intensification through Gernas Cocoa, a program of the National Movementof Production and Quality Improvement of Ministry of Agriculture Cocoa is stil considered not optimal, and have not be enable to increase productivity and farmers' welfare people. Low price of cocoa beans, is also a problem for increased production. Results of other studies indicate that cocoar ind fresh sun dried and then milled then be used as animal feed. Cocoa beans are rich in polyphenols. Cocoa and its derivatives (*Cocoa powder, Cocoaliquordanchocolate*) containing varying concentrations polyphenol. The polyphenols in cocoa beans contribute about12-18% of the dry weight of the whole duty (Misnawi. 2012). Potential skin cocoa pods in the degradation of petroleum hydrocarbons contaminated with crude oil, which contained two kg of soil in a plastic bucket 36 contaminated with 250mL of crude oil (Agbor, *etal.*2012).

Pyrolysis is the best method (excelent) to realize a clean an defficient conversion of biomass to the source, where the product gases, liquids and solids have a high value to be used Yemen 2004), in addition to the normal heating method (electrical heating), plasma(Xiu*etal.*2005), heating technology is able to applyfor thepyrolysisof biomass. In general, there arefourstages usedtoconvertplantsinclude bamboo as an other advance of bioethanol pretreatment, hydrolysis enzimatic, ethanol fermentation and purification, to find a way to benefit from natural resource-based biomass for the supply of renewable energy and sustainable development. Application of slow pyrolysis process that can be used to reduce electricity by between 31-33% recovery

The purpose of this study is as follows cocoa bean fermentation process to remove the pulp. .Conduct of liquid smoke from pyrolysis of waste cocoa shell Luwudistrict analysis and testing of chemical compounds contained in the cocoa waste,.

RESEARCH METHOD

Raw Material

Cocoa beans are dried by the drying method used by producers of cocoa. Cocoa beans are sun dried at different times ranging from3-5 days, the drying time is approximately 9 hours per day. Preparation of samples of cocoa beans each treatment, then washed and dried for 3 days, and then analyzedt he fat content.

Methode

Fruit peel waste cocoa and cocoa leaves put into the kiln with the temperature and time setting, capacity (liquid smoke and charcoal). Determine the value of charcoal yield, moisture content, volatile matter, ash content, calorific value and carbon value..

Analysis Date

Cocoa fruit skin samples analyzed Klason lignin content, holoselulosa levels, and Cellulose.,TGA and Analysis charcoal cocoa rind with FTIR, SEM, XRD, andGCMS.

RESULT OF RESEARCH AND DISCUSSION

Based on the research results that have beenfermentedcocoa beans originating from Luwu district has a fat content of 35.21%, with apH of 5.61and FFA of 0.09. Water content in cocoa beanst hat have been fermented by 5.81. In the previous study. Thermal decomposition process has been carried out for the pyrolysis of biomass and its constituent mainly contains cellulose, hemicellulose and lignin are different. The content of hemicellulose, cellulose and lignin on cocoa waste can be seen inTable1.

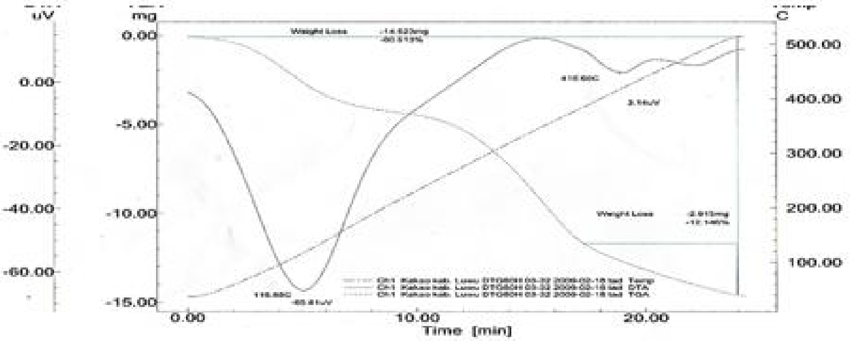


Figure 1.Analysis TGA/DTA for cacao waste

Table1.Content (% b/b) hemicellulose , cellulose and hemicellulose cacao shell

Cacao waste	Content (% b/b)			
	Hemiscelulloce	Celuloce	Lignin	Holoceeluloce
Cacao Shell sawdust	16,81	12,12	64,72	28,93

Based on the decomposition of lignin content in the skin known cacao shell Luwu district 64.72 % , 16.81 % of hemicellulose , cellulose and hemicellulose 12.12 % 27.83 % . (Table 1 and Figure 1) . Lignin is one of the determinants of the components to produce a quality liquid smoke (Nurhayati*et al* . 2005)] . Lignin content depends on the different types of raw materials

. This suggests that the pine wood lignin structures are formed by koniferil alcohol alone , while by teak wood lignin prepared by koniferilsinapil alcohol and alcohol with a certain ratio (Yemen 2004) . Chemical components contained in the liquid smoke is very dependent on the process conditions and raw materials used According Fengel&Wegener . 1995, the chemical composition of teak wood contains 39-57 % cellulose , 7-13 % hemicellulose, and lignin 29-39 % . The chemical composition of corn stalks used containing 42.4 % cellulose , hemicellulose and lignin 29.6 % 21.7 % (Lv. et al . 2010) . The chemical composition of rice straw containing cellulose 34.94 % , 36.06 % hemicellulose and 12.3 % lignin (Tewfik *et al* . , 2011) .

Table 2.Analysis result GC-MS for cacao vinegar shell district Luwu

Cacao Waste	Compound chemical	Prosentase(%)
Cacao Shell Luwu district	Aceton	13,01
	Methyl Ester	2,46
	Asetic Acid	73,86
	2 buthanone	6,60
	Propanoic Acid	4,07

GC - MS analysis was conducted to determine the types of compounds contained in any liquid smoke . Compounds that passed the GC - MS will be separated into its component chemical liquid smoke cocoa rind Luwu district (Table 2) , ie 13.01 % acetone , methyl acetate 2.46 % , 73.86 % acetic acid , 2 butanone 6 , 60 % and 4.07 % propanoic acid . This is due to the presence of liquid smoke kandundan rind of 40.36 % cocoa and cocoa rind charcoal Luwu District of 42.08 % ..and lignin content in cocoa rind Luwu district at 64.72 % , the study was supported by the dominant compound results of the pyrolysis of wood and acacia wood toothpick consisting of acetic acid and vanillin . The chemical composition of the liquid smoke containing acids , especially acetic acid is a derivative of a compound of acetyl groups during pyrolysis (Ratanapisit et al . 2009). The results of XRD analysis for charcoal cocoa rind Luwu district (Figure 2) shows that the degree of kristanilitas of 22.83 % . XRD for natural pits with cellulose ($C_6H_{12}O_6$) , Xylan ($C_{10}H_{12}O_9$, $2H_2O$) or hemicellulose provide crystallographic data with the number of Bragg diffraction of 5.5712 (Zhao *et a*l . 2010] . This is supported by the results of XRD analysis for oil palm waste material for DS do not give a horizontal line , this is due to the amorphous form wherein crystalline forms approaching the bottom line. diffraction angle 38.5353 So to give a ratio of 2.71274 reticular distances N and H celcel .

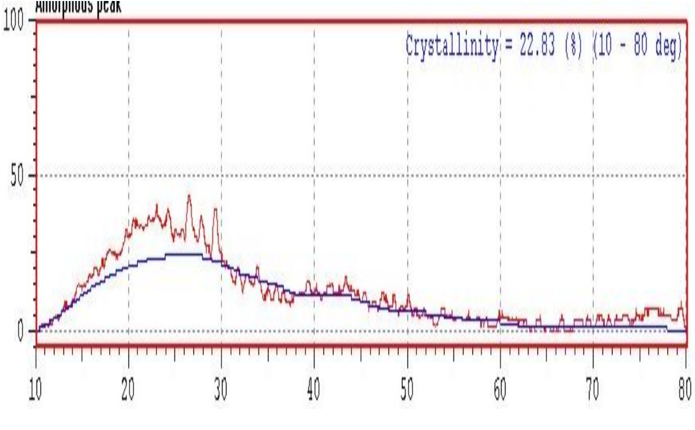


Figure 2. .Analysis result XRD for identification crystalinity degree With charcoal cacao shell sawdust DistrictLuwu

Results of FTIR analysis for charcoal powder cocoa shellLuwu district (Figure3) shows that the wavenumber 1111.000 cm^{-1} indicated dehydration and depolymerization of cellulose and hemicellulose content. Changes in aromatic peak at 1583.49 cm^{-1} indicates the presence of CH , lignin . While the wave number 3473.80 cm - 1 indicates the presence of hydroxyl group absorption from 873.75 to 750.31 cm^{-1} indicate the presence of C = CH (aromatic H) . While the wavenumber 381.21 cm^{-1} indicates the presence of hydroxyl groups (OH) and uptake from 873.75 to 748.38 cm- 1 indicate the presence of C = CH (aromatic H) is supported by the results of this study (Shances*et al* . 2014) . that the FTIR analysis for waste fruits show 3298 , 3275 and 3292 cm^{-1} shows the OH vibrations of alcohol and pectic acid . FTIR analysis is used to change the structure of the waste identification fruits with chemical and biological treatment .. FTIR analysis for sugar cane Strawn suggests that the region 3500-3400 cm^{-1} and the presence of hydroxyl groups hdirogenbond . Between 2900-288 cm^{-1} the presence of aliphatic CH ring . 1430 cm^{-1} shows the deformation of CH2 groups (Melo*et al* . 2013)

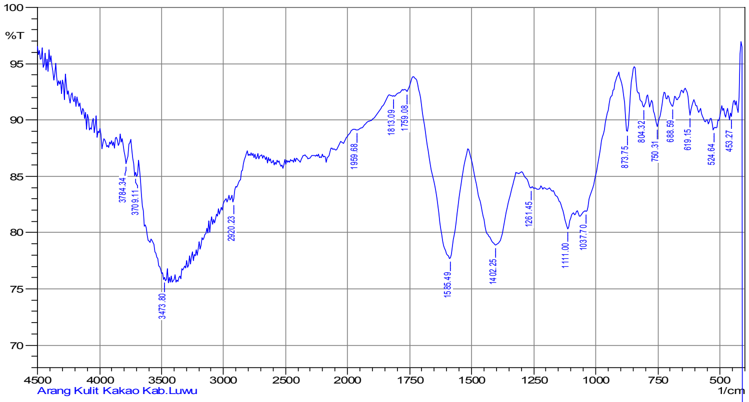


Figure .3Analysis result FT-IR for charcoal cacao shell sawdust .

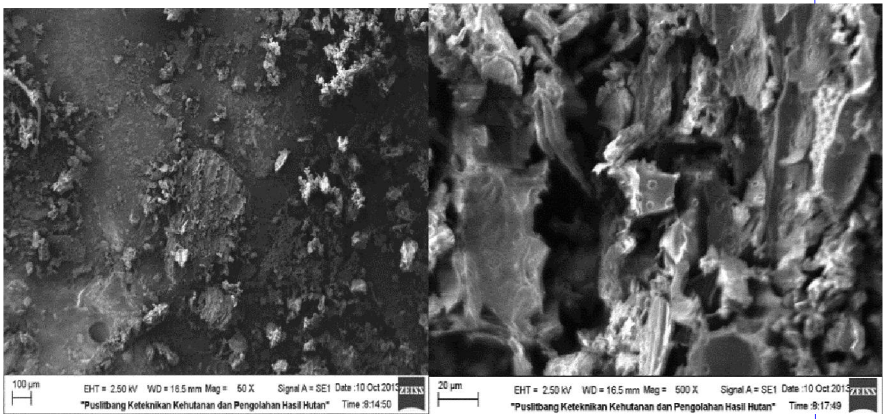


Figure4 .Analysis result SEM micrograph With cacao shell sawdust District Luwu 50x and 500x

Table3 .Content nutrient element into cacao shell waste.

Sampel	pH	C org	N	P	Ca	Mg	K	C/N
Cacao shell Luwu District	6,30	43,92	1,62	0,18	0,51	0,58	1,25	27,11

Information : Analysis C c(%) with Walkley & BlackMethode

N Total (%) with Kjedhal.Methode

pH for saw dust and bark cocoa cocoa shell between6 .30 to 7.80. Element content of organic C b the saw dust largest cocoa District Luwu at 54.88, while the largest N Elements for skin cocoa shell Luwu district of 1.62, 0.18 Elements P, Ca elements and nutrients of 0.51Mg of Element K 0.68and1.25. C / N ratio for sawdust largest cocoa Luwu District 90.63, can be seen inTable3.

CONCLUSION AND SUGGESTION

Based on the objectives and results of the research that has been done a few conclusions can be drawn as follows (1). Lignin contentin cocoa rind Luwu district 64.72%, (2). The results of XRD analysis for charcoal powder cocoa rind Luwu districts hows that 22.83% of kristanilitas degrees. (3). Production of acetic acid obtained from the pyrolysis of waste liquid smoke where cocoa shell Luwu. district to 73.86%.

SUGGESTION.

Further research needs to be done for other raw materials, the temperature is relatively high and then eed fors eparation of compound so ther than the product of acetic acid and other compounds as potential anti-oxidant and anti-bacterial.

REFERENCE

Agbor, R. B, I. A. Ekpo, U. U. Udofia, E.C Okpako1 and E. B. Ekanem. 2012. Potentials of cocoa pod husk and plantain peels in the degradation of total petroleum hydrocarbon content of crude oil polluted soil. Archives of Applied Science Research, 4 (3):1372-1375

Fengel D, Wegener. G. 1995 Wood Chemistry, Ultrastructure, Reaction-reaction. Translate Hardjono Sastrohamidjono, Gadjah Mada University Press fromWood Chemistry.

Lv.G.J, Wu.S.B, and Lou. R. 2010. Characteristic of Corn Stalk Hemicelluloce Pyrolysis in a Tubular Reactor.*J.Biores*. 5(4), 2051-2062.

Melo CA. Coscione AR, Abreu C, Puga A, and Camargo A. 2014. Influence of Pyrolysis Temperature on Cadmiun and Zinc Sorption Capacity of Sugar Cane Straw Derived Biochar. Bioresoureces 8(4), 4992-5004.

Nurhayati T. Desviana, Sofyan K. 2005. TempurungKelapaSawit (TKS) sebagaiBahanBakuAlternatifuntukProduksiArangTerpadudenganPyrolegneousAsap Cair. JurnalIlmu&TeknologiKayuTropis. 3.(.2).

Ratanapisit J, Apiraksakul S, Rerngnarong A, Chungsiriporn J, Bunyakarn C. 2009. Preliminary Evaluation of Production and Characterization of Wood Vinegar from Rubberwood, Songklarakarin. *J Sci Technol*. 31(3) : 343-349.

Shances O, Boldera P, Rou M, Urena P. 2014. Characterization of Lignocelulosic Fruit Waste as Alternative Feedstock for Bioethanol Production.J. Bioresources 9(2) : 1873-1885.

Tewfik SR, Sorour MH, Abulnour AMG, Talaat HA, El Defrawy NM, Farah JY, Abdou IK. 2011. Bio-Oil from Rice Straw by Pyrolysis ; Experimental and Techno-Economic Investigations. *J.AmSci*, 7(2).

Xiu, S, Yi, W. and Li, B. 2005. Flash Pyrolysis of Agricultural Residues using Plasma HetedLaminerEntrated Flow Reactor, Biomass and Bioenergy 29(2),

Yaman S. 2004. Pyrolysis of Biomass to Produce Fuels and Chemical Feedstocks. *J.Energy Conversion and Management*, 45 : 651-671.

Zhao Y, Bie R, Lu J, Xiu T. 2010. Kinetic Study on Pyrolysis on NSSC Black Liquor in a Nitrogen Atmosphere. *J. ChemEngChem*197 : 1033-1047.

C-13

PREPARATION AND CHARACTERIZATION OF POLY(Є-CAPROLACTONE) MICROPARTICLE BLENDS CONTAINING PROPRANOLOL HCl AND CARBAMAZEPINE

Muhaimin[1,*], Burkhard Dickenhorst[2], Roland Bodmeier[2]

[1]Department of Chemistry Education, Faculty of Teacher Training and Education,
University of Jambi, Mendalo Darat, Jambi 36361, Indonesia
[2]College of Pharmacy, Freie Universität Berlin, Kelchstrasse 31, 12169 Berlin, Germany
**Presenting author: muhaimin_73@yahoo.de*

Abstract

The purpose of this study was to investigate physicochemical properties of poly(ϵ-caprolactone) microparticle blends containing drugs with different solubilities (Propranolol HCl [Pro] and carbamazepine [CBZ]). Microparticle blends were formulated with W/O/W emulsion for Pro and O/W emulsion for CBZ. The Pro emulsion (W/O) and CBZ oil phase (O) were dispersed in an external aqueous phase (W), with dispersion time interval (DTI) of 0 and 60 min. Morphology of microparticle blends were characterized by scanning electron microscopy (SEM). Particle size mean of emulsion droplets/hardened microparticles were monitored by focused beam reflectance measurement (FBRM). Encapsulation efficiency (EE) and in vitro drug release in phosphate buffer (pH 7.4) were also investigated. The results showed that the resulting microparticle blends obtained by solvent evaporation method were spherical and having two populations. FBRM data showed that the size of microparticle blends prepared with DTI 60 min and stirring time 4 h was larger than those with DTI 0 min. The encapsulation efficiency were 62.05% to 66.38% for Pro and 70.56% to 73.85% for CBZ in microparticle blends. Drug release in phosphate buffer after 28 days showed that the Pro release (33%) was slower than CBZ release (60%) from microparticle blends with DTI 60 min. This phenomenon was attributed to the interaction of oil phase (CBZ) with hard particles from primary emulsion (Pro), whereby the oil phase had blocked and coated pores on the surface of hard particle from primary emulsion.

Key words: microparticle blends, propranolol HCl, carbamazepine, poly(ϵ-caprolactone), FBRM, solvent evaporation method

INTRODUCTION

Microparticles are widely used in different applications such as the controlled release of drugs, cosmetics and chemical reagents. Several methods are potentially useful for the preparation of microparticles in the field of controlled drug delivery. One of the most common methods for preparing microparticles is the solvent evaporation method (Li et al., 2008). The control of the microparticle preparation processes is essential to produce a desired mean size of the microparticles, size distribution and morphology of the microparticles. The solubility properties of the drugs of the microparticles are important parameters when selecting the emulsion phases for a microparticles preparation process. A low solubility of the drugs in the continuous phase is required for obtaining a high yield. Microparticles can encapsulate many types of drugs including small molecules, proteins, and nucleic acids. Depending on the solubility of the drug, simple or multiple emulsion techniques like oil-in-water (O/W) or water-in-oil-in-water (W/O/W) methods are used (Yang et al., 2001). The microparticle preparation method is a governing factor in the encapsulation and release of drugs. In addition, a complicated array of factors including the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microparticle formulation (e.g., for stabilization of the drugs), porosity, and the microparticle size can have a strong impact on the delivery rates (Yang et al., 2001).

Polymers have been used as a main tool to control the drug release rate from the formulations. Polymers can bind the particles of a solid dosage form. Pharmaceutical polymers are widely used to achieve taste masking; controlled release (e.g., extended, pulsatile, and targeted), enhanced stability, and improved bioavailability. Biodegradable and non biodegradable polymers with good biocompatibility are also used as drug carriers, such as polycaprolactone, PLGA and ethyl cellulose (degradable but non biodegradable).

In most studies reported so far, only one drug was entrapped within controlled release microparticles at a time. Only few attempts have been made on the co-encapsulation of two drugs, especially if the latter exhibits significantly different solubility behavior. Pérez et al. (2003) have successfully incorporated the hydrophilic drug propranolol HCl and/or the lipophilic drug nifedipine separately as well as simultaneously within non-degradable, ammonio methacrylate copolymers(Eudragit RS:RL 4:1 blends) based microparticles. They were prepared with an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. Whereas, Nippe and General (2012) have developed a combination of lipophilic steroidal drugs ethinyl estradiol and drospirenone poly(lactic-co-glycolic acid) (PLGA) microparticles.

Microparticle blends containing two drugs with different solubility have not been reported yet. In the present study, the solvent evaporation method was used to incorporate a lipophilic and a hydrophilic drug within polycaprolactone based microparticle blends. The hydrophilic drug propranolol HCl and the lipophilic drug carbamazepine were used as model drugs. Accurate particle size analysis during solvent evaporation process is a key to study microparticle blends formation from oil-in-water (O/W) and water-in-oil-in-water (W/O/W) methods. For more information about micropaticle blends formation during solvent evaporation process, FBRM can be used to provide in situ/on-line particle characterization in a wide range of applications (Kail et al., 2009; Vay et al., 2012; Wu et al., 2011; Zidan et al., 2010). The great advantage of this technique is that data is acquired on-line and in real time to give particle size data and population trends of particles in suspension, emulsion etc. (Boxall et al., 2010; Wu et al., 2011; Zidan et al., 2010).

The purpose of this study was to investigate effect of dispersion time interval (DTI) and formulation of second primary oil phase on poly(ε-caprolactone) based microparticle blends contained drugs with different solubility (Propranolol HCl and carbamazepine) which prepared by solvent evaporation method.

RESEARCH METHOD

Materials

All materials were of at least reagent grade and used as received: poly (ε-caprolactone) (Mw. 10000) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany); polyvinyl alcohol (PVA, Mowiol® 40–88, Kuraray Europe GmbH, Frankfurt, Germany); propranolol HCl, carbamazepine, sodium chloride, sodium hydroxide, potassium dihydrogen phosphate and dichloromethane (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

Methods

Preparation of Microparticle containing propranolol HCl or carbamazepine

Drug loaded microparticles based on poly (ε-caprolactone) were prepared using an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. The drug loaded systems contained either one drug only (propranolol HCl or carbamazepine). For the

O/W method, 300 mg of poly (ε-caprolactone) were dissolved in 4 ml dichloromethane. 60 mg carbamazepine were dissolved within this organic phase. The organic phase was then emulsified into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) to allow microparticle hardening.

For the W/O/W method, 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication (Sonoplus® HD 250, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of poly (ε-caprolactone). This first emulsion (W/O) was then dispersed into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. A W/O/W emulsion was formed by extensive stirring with a propeller stirrer for 4 h at 500 rpm to allow microparticle hardening. In all cases, after 4 h the microparticles were separated from the external aqueous phase by wet sieving (stainless steel test sieves ISO 3310 - 40, 70, 100 and 160 µm) followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

Preparation of Microparticle blends containing propranolol HCl and carbamazepine

The first primary emulsion containing propranolol HCl (W/O/W) and second primary oil phase containing carbamazepine (O/W). For the W/O/W method, 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of poly (ε-caprolactone). This gave the first primary emulsion containing propranolol HCl. For the O/W method, 300 mg of poly (ε-caprolactone) were dissolved in 4 ml dichloromethane. 60 mg carbamazepine were then dissolved in this organic phase. This process produced the second primary oil phase containing carbamazepine. Following, the first primary emulsion containing propranolol HCl and the second primary oil phase containing carbamazepine were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCl and NaOH at pH 12), with dispersion time intervals (DTI) of 0 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the preparation of microparticle containing single drug process.

Determination of the actual drug loading and encapsulation efficiency

Microparticles (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel GmbH & Co. KG IKA Labortechnik, Staufen, Germany) for 2 h (n = 3). 0.1 ml of methanol extract was diluted in 10 ml of pH 7.4 phosphate buffer. The polymer was separated from aqueous solution by filtration using filter paper (Whatman®, GE Healthcare UK Limited, Buckinghamshire, UK). Propranolol HCl and/or carbamazepine concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 289 nm and 285 nm, respectively (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows:

Actual drug loading (%) = (drug mass in microparticles/mass of microparticles) x 100 %

Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) x 100 %.

For microparticle blends, the amounts of incorporated propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm. The subsequent process steps were similar to the above process.

Particle size analysis

Particle size mean and size distribution of the microparticles were measured by focused beam reflectance measurement. FBRM probe (Lasentec® FBRM D600T, Mettler Toledo AutoChem, Inc., Redmond, WA, USA) was immersed and positioned in the emulsification vessel (WO/W and O/W emulsions mentioned above) to ensure good flow against the probe window and hence allowing a representative sample of the particle system to be measured. The measurement range of the FBRM D600T probe is 0.25 - 4000 µm. In these experiments, FBRM measurements were performed every 10 seconds, during a period of 4 h. All batches were measured in triplicate. The size information was extracted through the iC FBRM® 4.0 software (Mettler Toledo AutoChem, Inc., Redmond, WA, USA).

Microparticle characterization

Optical microscopy

Microparticles were spread on microscope slides and observed with an optical light microscope (Axiotrop 50, Carl Zeiss AG, Jena, Germany) equipped with an image analysis system (INTEQ Informationstechnik GmbH, Berlin, Germany) consisting of a digital camera (type MC1) and the EasyMeasure® software (version 1.4.1).

Scanning electron microscopy

The external and internal morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. To investigate the inner structure, the particles were spread on transparent tape and then cut with a razor blade. All samples were coated under argon atmosphere with gold to a thickness of 8 nm in a high-vacuum (SCD 040, Bal-Tec GmbH, Witten, Germany). Samples were then analysed on the scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

In vitro drug release studies

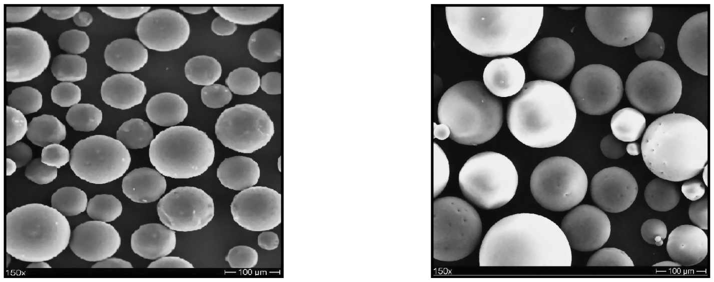
10 mg microparticles/microparticle blends (particle size: < 70 µm) were placed in 10 ml pH 7.4 phosphate buffer (USP XXIV) and shaken at 37 °C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) at 75 rpm. At predetermined time points, 1 ml samples were withdrawn and replaced with 1 ml fresh medium each 7 days, filtered and analyzed. Propranolol HCl and/or carbamazepine concentration was detected UV spectrophotometrically at wavelengths of 289 nm and 285 nm, respectively (n = 3) (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany).

For microparticle blends, the concentration of propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm (n = 3).

RESULT AND DISCUSSION

Morphology and particle size/distribution of microparticle blends

The surface morphology of the microparticles was observed by scanning electron microscopy (SEM). The surface analysis of drug-loaded microparticle blends with different drug solubility prepared by the WO/W (Pro) and O/W (CBZ) reveal that the microparticles were spherical and not aggregated (Fig. 1) with a diameter range of 73 µm to 81 µm. Microparticle blends containing both, propranolol HCl and carbamazepine, prepared by the WO/W (Pro) and O/W (CBZ) methods with DTI 60 min appeared in two population of microparticles, smooth and rough surface (Fig. 1a). While microparticle which prepared with DTI 0 min produced microparticles with pores and smooth surface (Fig. 1b). Micropores were observed on the microparticles surface that it was propranolol HCl loaded poly (ε-caprolactone) microparticles. No pores were observed on the microparticles surface that it was carbamazepine loaded poly (ε-caprolactone) microparticles.



a **b**

Fig. 1. SEM pictures of poly (ε-caprolactone) microparticles blend with dispersion time interval of (a) 60 min and (b) 0 min between Pro (W/O/W) and CBZ (O/W)

The preparation conditions substantially affected the morphology and porosity of the microparticles. In W/O/W method, the microparticles revealed a porous inner structure caused by the inner aqueous phase. The aqueous droplets are precursors of pores and are the result of phase separation occurring in the organic phase during the hardening of the microparticles (Freiberg and Zhu, 2004).

For microparticle blends contained different drugs with different solubility (propranolol HCl and carbamazepine), the size of microparticle blends prepared by W/O/W (propranolol HCl) and O/W (carbamazepine) methods (with DTI 60 min and stirring time 4 h) was larger than the microparticle blends (with DTI 0 min) and microparticles normal (Fig. 2). Based on FBRM data the addition of second primary oil phase contained poly (ε-caprolactone), carbamazepine and dichloromethane (with DTI 60 min) contributed in enhancement of particle size.

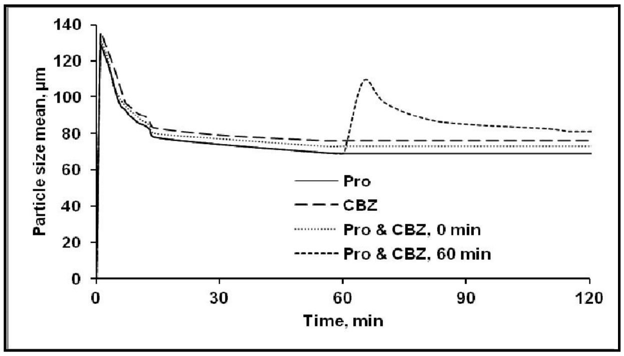


Fig. 2. Particle size mean of poly (ε-caprolactone) based microparticle blends obtained by the FBRM method (before and after primary oil phase addition) during the solvent evaporation process (primary oil phase is added at time = 60 min)

Entrapment efficiency within microparticle blends

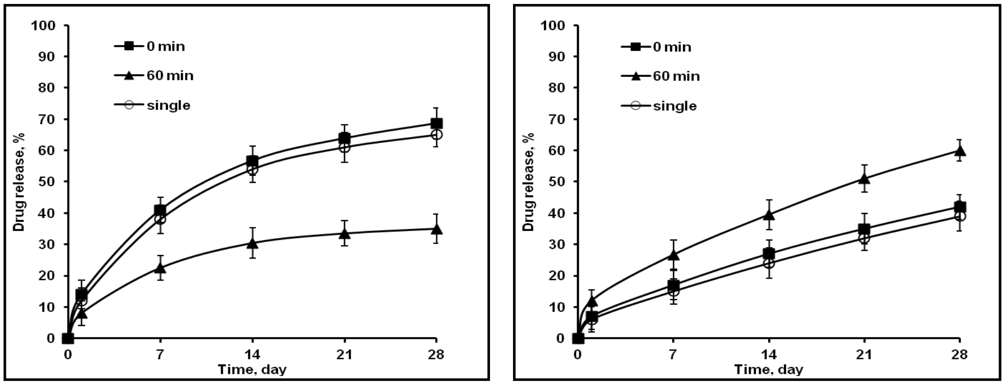
Encapsulation efficiency (EE) was about 62.05% to 66.38% for propranolol HCl and 70.56% to 73.85% for carbamazepine in microparticle blends.

The difference observed in the EE of the two drugs in the microparticle blends can be explained with the different solubilities of the drugs in the aqueous continuous phase used for the two encapsulation techniques. The high solubility of the propranolol HCl in the external aqueous phase and its high volume compared to that of the internal aqueous phase (W/O/W technique) caused the leakage of the drug into the continuous phase. However, after the precipitation of the polymer, the propranolol HCl, due to its hydrophilic nature, still tends to diffuse through the polymeric matrix into the external aqueous phase. Beside that, the degree of ionization of the drug and the pH of the external aqueous phase are critical for the entrapment of ionizable drugs such as propranolol HCl (Pérez et al., 2003). Increasing the pH of the external phase above the pKa of the propranolol HCl results in a decrease of its solubility and, consequently, in an increase of its entrapment in the microparticles.

Release of propranolol HCl and carbamazepine from microparticle blends

Different release rate were observed for propranolol HCl and carbamazepine from poly(ε-caprolactone) microparticle blends in pH 7.4 phosphate buffer (Fig. 3). The propranolol HCl release from microparticle normal and microparticle blends (with DTI 0 min), were faster than carbamazepine release (Fig. 3). Whereas, propranolol HCl release (33%) was slower than carbamazepine release (60%) from poly(ε-caprolactone) microparticle blends (with DTI 60 min) (Fig. 3). Fig. 3 shows that the cumulative percent of propranolol HCl and carbamazepine released from each microparticle blends (the range of ADL Pro ≈ 9.32% to 9.86% and ADL CBZ ≈ 10.69% to 10.97%) at pH 7.4 after 28 days are in the range of 33% to 69% (propranolol HCl) and 41% to 60% (carbamazepine).

In all cases, the resulting release rate(s) of the incorporated drug(s) was/were found to be controlled over periods of at least 28 days. Furthermore, the release of carbamazepine was generally slower than that of propranolol HCl which can most probably be attributed to the much lower solubility of carbamazepine compared to propranolol HCl in the release medium (0.2 mg/ml vs. 250 mg/ml), resulting in lower concentration gradients, the driving forces for diffusion.



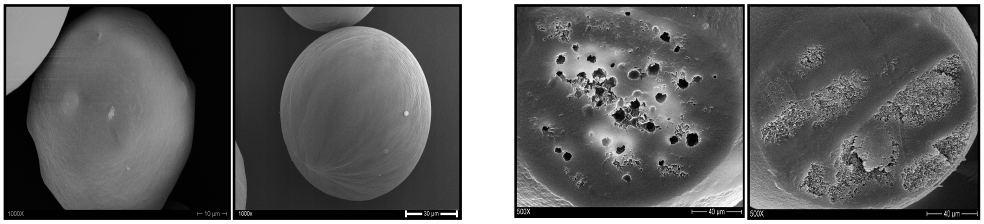
a b

Fig. 3. Effects of dispersion time interval on propranolol HCl and carbamazepine release from polycaprolactone microparticle blends (phosphate buffer, pH 7.4, 37 °C, 75 rpm). [(a) Propranolol HCl and (b) Carbamazepine]

The increase of carbamazepine release from microparticle blend with the W/O/W (Pro) and O/W (CBZ) (DTI 60 min) could be due to the incorporation of carbamazepine on surface of propranolol HCl loaded microparticle. This may have reduced propranolol HCl migration to the surface of the microparticles, and its leakage in the dissolution medium as compared to the microparticles prepared with W/O/W method where the propranolol HCl is either molecularly dispersed or amorphous in the matrix. In addition, the porous membrane observed in the case of the microparticles prepared with W/O/W method favoured a fast release of the hydrophilic propranolol HCl. The incomplete release of carbamazepine from microparticles may be the result of the hydrophobic nature of the drug and its very low water solubility.

On the contrary, the release of propranolol HCl was significantly slowed down in the case of the microparticle blends (DTI 60 min) compared to that of the microparticles normal.

Only 33% of propranolol HCl was released from microparticle blends prepared by W/O/W (Pro) and O/W (CBZ) methods with DTI 60 min. It has to be emphasized that the propranolol HCl was inside of microparticle and carbamazepine was on outer surface of microparticle. Thus only the drug located close to the outer surface could be initially released. The release of surface associated drug creates water-filled channels that allow subsequent diffusion of the drugs located inside the microparticles. A major mechanism for release of propranolol HCl and carbamazepine are diffusion through water-filled pores.



a **b**

Fig. 4. SEM pictures of polycaprolactone microparticle blends with dispersion time interval of 60 min between primary emulsion and primary oil phase (a. higher magnification and b. cross-section).

Based on release data for each microparticle blends, it can be assumed that there is interaction between first primary emulsion (propranolol HCl) and second primary oil phase (carbamazepine) during preparation process of microparticle blends. FBRM data about particle size mean before and after addition of second primary oil phase into single external aqueous phase (Fig. 2) and surface morphology of microparticles blend (Fig. 4a) have indicated it. In addition, cross section of the microparticles revealed a porous inner structure and absence of pores (Fig. 4b). For microparticle blends which were prepared with DTI 60 min the internal structure appeared reducing in the number of pores and size of pores (Fig. 4b). This phenomenon might be attributable to the interaction of second primary oil phase (CBZ) with hard particles from first primary emulsion (Pro), whereby the second primary oil phase (CBZ) had blocked and coated pores on the surface of hard particle from first primary emulsion. This hypothesa supported by optical microscopy pictures. It indicates that the emulsification stage first W/O/W (Pro) and second (CBZ/dye) resulting in two kind of microparticle blends (Fig. 5). This picture showed microparticle with black plaque on the surface and black microparticles.

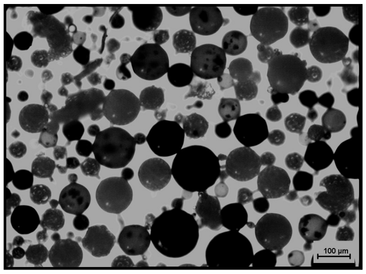


Fig. 5. Optical microscopy pictures of polycaprolactone microparticle blends with dispersion time interval of 60 min. [Microparticles containing dye (black)]

CONCLUSION AND SUGGESTION

The novel microparticle blends containing drugs of different solubility (e.g. propranolol HCl and carbamazepine) offer a high potential for controlled release drug delivery systems. For microparticle blends (with DTI 60 min) containing drugs of different solubility gave propranolol HCl release was slower than carbamazepine release. FBRM studies showed that particle size of microparticle from first primary emulsion (Pro) was smaller than particle size of microparticle after addition second primary oil phase (CBZ) (with DTI 60 min). It was caused second primary oil phase (CBZ) interacted with microparticles from first primary emulsion (Pro). Optical and scanning electron microscopy revealed that microparticle blends (DTI 60 min) were spherical and had two populations. These microparticle blends consisted of microparticles with smooth and rough surface. This phenomenon might be attributable to the interaction of second primary oil phase with hard particles from first primary emulsion, whereby the second primary oil phase had blocked and coated pores on the surface of hard particle from first primary emulsion. Type of dispersion time interval of microparticle blends influenced the physical properties of the microparticle blends.

REFERENCES

Boxall, J.A., Koh, C.A., Sloan, E.D., Sum, A.K., Wu, D.T., 2010. Measurement and calibration of droplet size distributions in water-in-oil emulsions by particle video microscope and a focused beam reflectance method. Ind. Eng. Chem. Res. 49, 1412-1418.

Freiberg, S., Zhu, X.X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1-18.

Kail, N., Marquardt, W., Briesen, H., 2009. Estimation of particle size distributions from focused beam reflectance measurements based on an optical model. Chem. Eng. Sci. 64, 984-1000.

Li, M., Rouaud, O., Poncelet, D., 2008. Microencapsulation by solvent evaporation: State of the art for process engineering approaches. Int. J. Pharm. 363, 26-39.

Nippe, S., General, S., 2012. Combination of injectable ethinyl estradiol and drospirenone drug-delivery systems and characterization of their in vitro release. Eur. J. Pharm. Sci. 47, 790-800.

Pérez, M.H., Siepmann, J., Zinutti, C., Lamprecht, A., Ubrich, N., Hoffman, M., Bodmeier, R., Maincent, P., 2003. Non-degradable microparticles containing a hydrophilic and/or a lipophilic drug: preparation, characterization and drug release modeling. J. Control. Rel. 88, 413-428.

Vay, K., Frieβ, W., Scheler, S., 2012. Understanding reflection behavior as a key for interpreting complex signals in FBRM monitoring of microparticle preparation processes. Int. J. Pharm. 437, 1-10.

Wu, H., White, M., Khan, M.A., 2011. Quality-by-Design (QbD): An integrated process analytical technology (PAT) approach for a dynamic pharmaceutical co-precipitation process characterization and process design space development. Int. J. Pharm. 405, 63-78.

Yang, Y.Y., Chung, T.S., Ng, N.P., 2001. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double emulsion solvent extraction/evaporation method. Biomaterials 22, 231-241.

Zidan, A.S., Rahman, Z., Khan, M.A., 2010. Online monitoring of PLGA microparticles formation using Lasentec focused beam reflectance (FBRM) and particle video microscope (PVM). AAPS J. 12, 254-262.

C-14

PRODUCTION AND CHARACTERIZATION OF ANTI FIM-C *Salmonella typhi* NATIVE PROTEIN ANTIBODY IN DDY MICE

Muktiningsih Nurjayadi[a], Umar Hasan[a], Dea Apriyani[a], Fera Kurnia Dewi[a], Irma Ratna Kartika[a], Fernita Puspasari[b], Dessy Natalia[b]

[a]*Departement of Chemistry, Mathematics and Science Faculty, State University of Jakarta, Indonesia*
[b]*Biochemistry Division, Department of Chemistry, Mathematics and Science Faculty, Bandung Institute of Technology, Indonesia*

Abstract

Typhoid fever is a disease affect many people in developing countries including Indonesia. Prevention can be aided by vaccination to the bacteria; however it still needs further research. This research is aimed to determine the immunogenicity of fim-C native protein *Salmonella typhi in vivo* as a vaccine candidate in ddY mice. Experimental method is used in this research. This research used antigen of pure fim-C native recombinant protein from its previous research. The ddY mice are categorized into five groups, namely experiment group 1 (protein Fim-C+Freund's Complete/Incomplete Adjuvant), experiment group 2 (protein Fim-C *S. typhi*), control group 1 (FCA/FIA adjuvants), control group 2 (PBS1x), and normal group (PBS1x). The results showed that the ddY mice produced antibody response after subcutaneous injection of the Fim-C *S. typhi* native protein +adjuvant or without adjuvant. The antibody responses with Fim-C *S. typhi* native protein as antigen+adjuvant gave higher absorbans than the Fim-C antigen without adjuvant. It is also showed that the antibody titer from the first to fourth injection has gradually increased. These data showed that Fim-C *S. Typhi* native protein had higher immunogenicity. It is concluded that the Fim-C *S. typhi* native protein can be used as a potential recombinant vaccine candidate against typhoid fever.

Key words: *Salmonella typhi*, Typphoi Disease, Fim-C *Salmonella typhi native* Protein Antibody, Recombinant vaccine

INTRODUCTION

Typhoid fever is one of diseases suffered by most people in developing countries, including Indonesia. Typhoid fever is suffered ranging from children to adults. Typhoid fever is easily transmitted to human through food and drink contaminated by *Salmonella typhi* bacteria in the poor standards of environment hygiene. Typhoid fever happens in Indonesia with an average of 900,000 cases per year. The mortality rate is more than 20,000 cases or 91% of all cases ranging in the age of 3-19 years old. The mortality rate increases in every year [Crump and Mintz, 2010; Verry, 2011]. Thus serious action to prevent and overcome typhoid fever in Indonesia is highly needed.

Salmonella typhi bacteria has Fim-C protein, called *fimbriae*. Fim-C protein, a kind of protein on the surface of bacteria cell, acts as important mediators used for interaction or adharence against host cell [Burrows, 2005; Muktiningsih *et al,* 2009]. Proteins on the surface of bacteria cell as Fim-C can be used as an antigen; it also induces immune response well [Verma *et al*., 2009; Toobak *et al.,* 2013; Yang *et al*., 2013; Moreno *et al*., 2013]. It is presumed that Fim-C proteins on *Salmonella typhi* can be potentially used as recombinant vaccine for typhoid fever prevention. Due to its higher level of safety, recombinant vaccine is chosen to be further developed rather than conventional non recombinant vaccine that uses attenuated virus.

In the prior research, researchers team from UNJ has succeeded in Fim-C *Salmonella typhi* gene cloning and sub-cloning on the cloning and expression vectors and its protein purification in both native form and inclusion bodies [Anggraeni R *et al*, 2012; Pratiwi E *et al*. 2013; Muktiningsih *et al*, 2013]. This research is aimed to determine the activity of Fim-C *S. typhi* recombinant protein in its native form as an antigen in mice ddY through immune response analysis. This information is very important in the development of the potential recombinant Fim-C *S. typhi* protein as candidate for recombinant vaccine to prevent typhoid fever in human.

RESEARCH METHOD

A. Production of anti Fim-C *S. typhi* antibodies

Production of anti Fim-C *Salmonella typhi* native antibodies consisted of preparation stage for mice ddY as animal test, preparation stage for Fim-C *S. typhi Native* recombinant protein as antigene, immunization and antibodies production, and mice serum/anti-FimC *S. typhi* antibodies isolation [Harlow and Lane, 1988; Deutsher, 1990; Noer, *et al*., 1992; Jennings, 1995].

A.1. Preparation stage for mice ddY as animal test

Production of anti Fim-C antibodies was conducted in LABTIAP (Agricultural Industry Technology Development and Biomedical Laboratory) BPPT-Serpong. The production of anti-Fim C *S. typhi Native* antibodies performed on 40 male mice ddY strains, age of 5-6 weeks and weigh of 17-24 grams. Mice were obtained from PT Biofarma Bandung. Mice were maintained in cages placed in the treatment room with temperature condition of 20-24^{0}C and 20-70% humidity. Treatment room was made sound-proof and impermeable to keep the air pressure lower than the surrounding so the odor will not come out. Every testing was conducted in different room. 4-8 mice are placed in individual cages of polycarbonate (with *stainless steel cover*) with size of 41,5x27x15 cm^3 each. The individual cages were arranged in 3 level stainless steel racks. During the conditioning process, mice were weighed on day 0, day 3 and day 5. Cage, food, health, and activity checks were also done regularly. The conditioning process is as long as a week.

Mice were grouped into three major groups, namely the experiment group (KP), the sick control group (KS) and the normal control group (KN). Experimental group had two sub groups; group immunized by mixture of Fim-C native protein and Frued complete/Incomplete adjuvant (FCA/FIA) which consists of 8 mice (KP-1) and group immunized by Fim-C native protein which consists of 8 mice (KP2). The sick control had two sub groups; group immunized by FCA/FIA which consists of 8 mice (KS-1) and group immunized by Phosphate Buffer Saline (PBS 1x) which consists of 8 mice (KS-2). The normal control group (KN) immunized by Phosphate Buffer Saline (PBS 1x) consists of 4 mice. In the challenge test, only experiment group (KP) and sick control group (KS) were infected by *S. typhi* bacteria. The normal control group (KN) was not be infected by *S. typhi* bacteria. After the conditioning process, the researcher took blood from mice's eyes as much as 1-2 mL as pre immune serum. A day prior to collect pre-immune serum, mice were conditioned to food-fasting.

A.2. Preparation stage for Fim-C *S. typhi Native* recombinant protein as antigene

As many as 25-100 µg Fim-C *S. typhi native* protein from previous results was disolved witth PBS 1x bufer in *Eppendorf* 1,5 mL tube to have a total volume of 100-250 µL. *Freund's complete adjuvant* (FCA) or *Freund's incomplete adjuvant* (FIA) were added with ratio 1:1. Then performed homogenized mix using vortexs until the mix turned white [Harlow and Lane, 1988; Jenings, 1995].

A.3. Immunization and Antibodies Production

Immunization was carried out on the backs of mice in the front section near head subcutaneously as much as 2-3 points for one-time injection. On the first immunization 20 µg Fim C *S. typhi* native protein that has been prepared as an antigen was mixed with *Freund's complete Adjuvant*. One week after the first injection researchers took blood sample from mice's eyes to prepare the serum. One week after the first injection researchers conducted boosting with 40 g Fim-C mixed with *Freund's incomplete Adjuvant*. The second and third boosting were conducted with the 80 g Fim-C after one week of the second and the third injections to obtain the optimal antibody amount [Harlow and Lane, 1988; Noer, 1992].

A.4. Mice serum/anti-FimC *S. typhi* antibodies isolation

Blood sample from mice's eyes was collected on tubes of centrifuges sterile. Blood sample was incubated at temperature of 37^0C for 30-60 minutes to a visible separation between serum and platelets. Centrifugation was carried out for 10 minutes with the speed of 5000 g at a 4 ^{o}C. Clear liquid/serum was taken out and kept in *Eppendorf*. Then the serum was stored in -20^0C [Harlow and Lane, 1988].

B. Characterization of anti Fim-C *S. typhi Native antibodies by* ELISA

Antibodies used in the formation analysis of anti-FimC *S. typhi* antibodies was mice serum from *Bleed* I-*Bleed* IV. Formation analysis of anti-Fim-C protein *S. typhi* antibodies from day 0 (*pre-immune* serum) until the 6 weeks was obtained by *Enzyme Link Immunosorbant Assay* (ELISA).

Antigen (30–300 ng Fim-C *S. typhi native* protein in50 µL fosfat bufer, 1x PBS) was incubated in *microtiter plate wells* (IWAKI) in room temperature overnight. Every well was washed 3 times each with 1x PBS containing 1 mM $MgCl_2$ and 0,05% (v/v) Tween-20 (wash buffer). After they were washed, researchers added into 150 µL 5% *blotto* (5 g skim milk in 100 mL 1x PBS) in every well. And then the microtiter plate was incubated at 37^0C for 1 hour. After the incubation wells were washed as much as 3 times with wash buffer. As many as 50 µL mice serum (from *bleed* I/*pre-immune* serum) - *bleed* V (fifth weeks), by 100x and 300x dilution were added into each well with prepared ELISA design,and then put them into incubation at 37^0C for one hour. The *microtiter plate* wells were washed with wash buffer 3 times. After washing process, 50 µL secondary antibodies was added into each well (*anti IgG-mice-HRP* dilution 1: 5000 with 1x PBS) [Thermo Scientific Biogen, 2013], and incubated in 37^0C for 1 hour. After the incubation, the *microtiter plate* wells were washed with wash buffer 3 times. As much as100 µL substrate TMB (3,3',5,5'-Tetramethylbenzidine) solution was added into each well and incubated at 37^0C for 30 minutes (produced blue color). After the incubation, the reaction was stopped by adding 50 µL H_2SO_4 2M (produced yellow color). Next, the absorbance reading was conducted using *ELISA-Reader* (*Microplate Reader*) at wavelength 450 nm [Noer, 1992; Deutsher, 1990; Muktiningsih, 2005; Thermo Scientific, Biogen, 2013]. After the formation of antibodies were known to the maximum researchers collected serum in large amount or bleeding terminal as much as 5-10 mL of blood.

RESULT AND DISCUSSION

A.Production of anti-Fim-C *S. typhi* native Antibodies

The results of animal conditioning process and pre-immune serum collection process

Health, weight, diet, and physical condition checks and observation during the conditioning process showed good result. This was demonstrated by the increase in weight on average 3-5 gram/mice. The observed physical condition of feathers and motion also showed patterns of activity that corresponds to standard conditions. The conditioning place and picture of test animals are presented in figure 1.

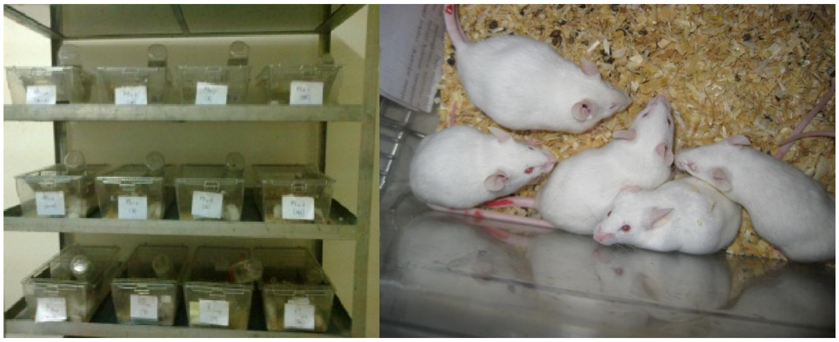


Figure 1. The conditioning place and picture of test animals**.** Mice are grouped into 5 cages. Every cages containing 8 mice for the experimental groups (KP) and the sick control group (KS) and containing 4 mice for normal group (KN).

The retrieval results of pre-immune serum from sinus orbitalis eyes produced 0,5-1 mL blood/mice and it was 0,2-0,5 mL serum of the total blood samples. Serums were stored in frezer with temperture -20^{o}C for further purposes. The pupose of taking *pre-immune* serum is as a negative control to the formation of anti Fim-C antibodies. This is aimed to ensure that there is no interaction occurs between Fim-C protein as antigene with mice antibodies before imunization.

The production results of anti-FimC *S. typhi antibodies*

Production of anti-FimC *S. typhi native* antibodies in experimental and control groups was performed in 6 weeks. The process consisted of the first imunization with 20 µg Fim C *S. typhi native* protein, *boosting*-1 with 40 µg Fim-C *S. typhi* native protein. *Boosting-2* and *boosting*-3 were conducted by using 80 µg Fim-C *S. typhi* native protein. Each step gave a total amount serum 0,2-0,5 mL. After ELISA data was obtained with highest absorbance values, in the sixth week researchers collected blood samples of 4 mice from experimental groups by the maximum as much as 5-10 mL. The blood was prepared to serum containing anti-Fim C *S. typhi* antibodies as many as 2-4 mL/mice.

B. Characterization of anti Fim-C *S. typhi* native antibodies by ELISA

The formation analysis of antibodies used as a primer was taken from mice serum of *Bleed* I-*Bleed* IV. ELISA analysis was performed on 4 mice from each group,that are: experimental group I (immunized by Fim-C *S. typhi native* Protein mixed with Freud complete/incomplete adjuvant-KP-1), experimental group 2 (immunized by Fim-C *S. typhi native* Protein without adjuvant-KP2), control group-1 (immunized by Freud complete/ incomplete adjuvant, KS1), control group-2 (immunized by 1x PBS buffer, KS-2), and normal group (immunized by 1x PBS buffer, KN). The result of development of antibodies formation from 4 mice ddY from each group are presented in table 1. While the analysis results of development formation of anti-Fim C *S. typhi* antibodies is presented in figure 2 and figure 3.

Table 1. The data values of absorbance on development anti Fim-C *S. typhi native* antibodies at experimental group (KP) and the sick control group (KS) at antigene concentration 100 ng and 300 ng

bleed to-	Experimental Group- 1 (KP1)		Experimental Group- 2 (KP2)		The Sick Control Group-1 (KS1)		The Sick Control Group-2 (KS2)	
	100 ng	300 ng	100 ng	300 ng	100 ng	300 ng	100 ng	300 ng
bleed -0	0,0285	0,04	0,01125	0,00975	0,048333	0,009333	0,01	0,01
bleed -1	0,0055	0,01825	0,00275	0,008	0,027333	0,009667	0,0165	0,0165
bleed -2	0,19675	0,23275	0,04125	0,09575	0,029	0,037	0,0365	0,0365
bleed -3	0,2715	0,44175	0,19	0,2225	0,042667	0,037667	0,0335	0,0335
bleed -4	0,27925	0,5175	0,1805	0,20275	0,04	0,043	0,047	0,047

Based on the results of analysis on the development of formation anti-Fim C *S. typhi* antibodies by ELISA shown in Table 1 that Fim-C *S. typhi native* protein with or whithout adjuvants give satisfied immune response. This is shown by an increase absorbance values from *Bleed* 0-*Bleed* 4. The increase of color intensity or absorbance values showed increase in the amount of antibody titer that interacted with Fim-C *S.typhi* antigene. The blue color was from the oxidation TMB substrate (3,3’,5,5’-Tetramethylbenzidine) to 3,3',5,5'-tetramethylbenzidine diimine by *Horse Redish Peroksidase* enzyme which bounded to secondary antibodies. Color formation reaction was stopped by adding 50 µL H_2SO_4 and resulted yellow color and measured at wavelenghts 450 nm [Thermo scientific Biogen, 2013]

Data presented in table 1 or figure 2 and figure 3 give information that induction by protein Fim-C *S. typhi Native* +Adjuvant FCA/FIA as antigene produced higher antibodies than by protein Fim-C *S. typhi Native*+ PBS 1x. This is in accordance with the literature review stated that the Frued complete/incomplete adjuvant (FCA/FIA) can enhance the formation of immune response [Harlow and Lane, 1988; Fiorino *et al*, 2012; Moreno *et al*., 2013]. These results also provide information on specific characters of Fim-C *S. typhi* native protein that if recombinant protein mixed with adjuvants the resulting immune response will be higher than without adjuvants. The information is also essential that Fim-C *S. typhi* native protein without adjuvant can also provide a good immune response.

This information is very important as the scientific foundation to conclude that the withdrawal of recombinant protein molecule of Fim-C *S. typhi native* is candidate vaccine recombinant. The results of literature analysis stated that the recombinant vaccine has many advantages. It (1) has higher safety for patients (2) has higher purity (3) generates more spesific immune response (4) can be produce in large quantities (5) is easy in production and storage, and some of other advantages [Harlow and Lane, 1988; Fiorino *et al*, 2012].

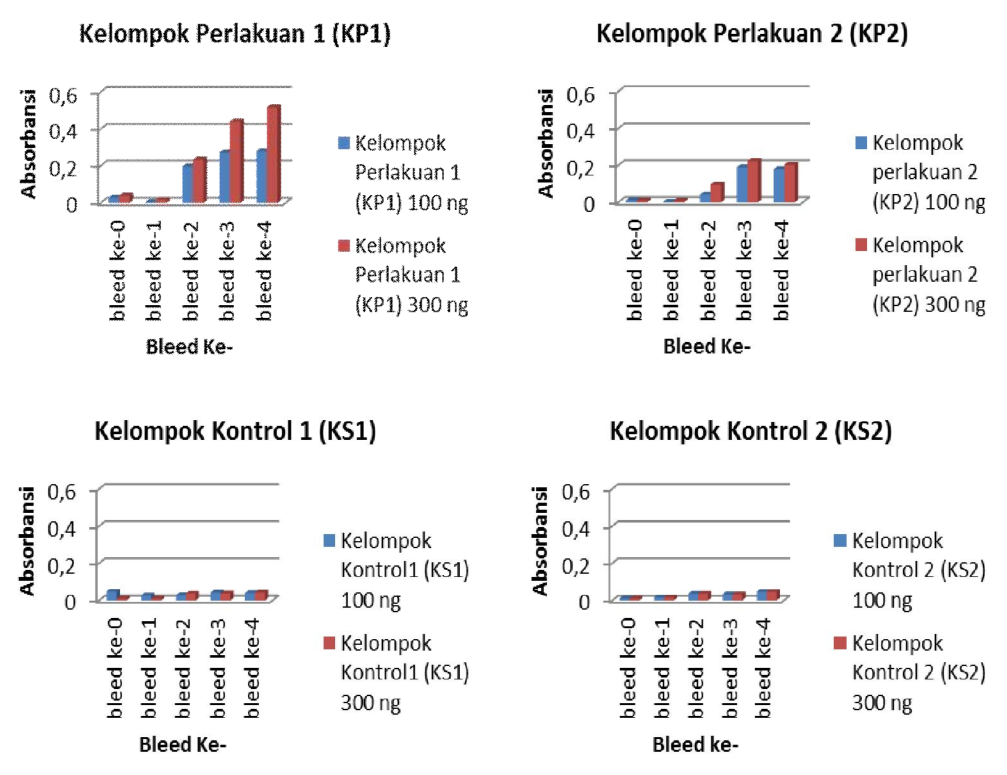


Figure 2. **Graphic analysis of the formation of anti Fim-C *S. typhi native* antibodies in the experimental and the control group.** (A) graphic of formation of antibodies in experimental group-1 (KP-1). (B) graphic of formation of antibodies in experimental group-2 (KP2), (C) graphic of control group-1 (KS1), (D) graphic of control group-2 (KS2). The x-axis showed the development of immunization from each bleed 0-4. The y-axis shows the value of absorbance reading of ELISA reader. Blue and red colored diagram shows the amount of protein FimC *S. typhi* recombinant 100 ng dan 300 ng as antigenes.

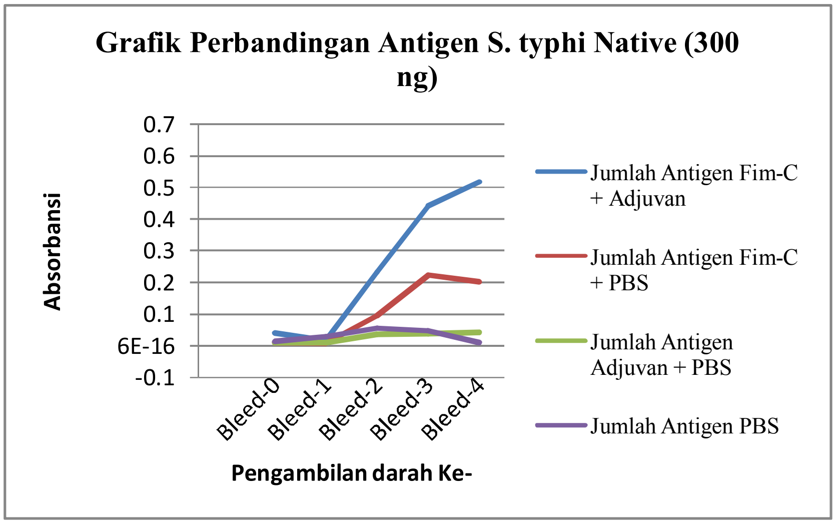


Figure 3. **The graphic result of immunogenicity test on antigens Fim-C *S. typhi Native* protein.** The blue line shows the value of absorbance for a group of mice immunized by Fim-C *S. typhi* native+Adjuvant FCA/FIA as antigens. The red line shows the value of absorbance for a group of mice immunized by Fim-C *S. typhi* native+1x PBS as antigens. The green line shows the value of absorbance for a group of mice immunized by Adjuvant FCA/FIA+1x PBS as antigens. The green line shows the value of absorbance for a group of mice immunized by Adjuvant FCA/FIA+1x PBS as antigens. The violet line shows the value of absorbance for a group of mice immunized by 1x PBS as antigens. Condition of ELISA performed on serum dilutiom 100x and secondary antibodies dilution 5000x.

CONCLUSION AND SUGGESTION

Fim-C *S, typhi* Protein in *native* form has ben succesfully used as an antigen specific in production of anti Fim-C *S. typhi* antibodies *in vivo* in mice ddY. The generated formation of immune response is indicated by the color change of substrate after interaction between Fim-C *S. typhi* antigen with anti Fim-C *S. typhi* antibodies. From Bleed 1-Bleed IV, the establishment of spesific anti Fim-C *S. typhi* antibodies with Fim-C + adjuvant FCA/FIA as antigene gives higher immune response than without adjuvants. The result also provides information that Fim-C *S. typhi* Typhi protein has good nature of imunogenicity because it is able to make higher response immune without the addition of adjuvant and adjuvant FCA/FIA. So that it can be inferred that Fim-C *S. typhi* native Protein can be used as vaccine candidates.

Significant supporting data about vaccine's standardized test and clinical trials for human are still needed to make recombinant protein molecules of Fim-C *S. typhi* native serve as a safe and inexpensive molecular vaccine. The information generated in this study could be made of the scientific basis for the further development of recombinant vaccines for typhoid fever in humans, especially in Indonesia.

REFERENCES

Anggraeni. R., Fera Kurnia Dewi, Irma Ratna Kartika, Fernita Puspasari, Muktiningsih Nurjayadi, Dessy Natalia. 2012. Cloning of Fim-C *Salmonella typhi* gene on pGEM-T *easy* vector to develop recombinant vaccine for typhoid Disease. *Proceeding Gruber Soedigdo Lecture*, 25 September 2012, Bandung, Indonesia.

Burrows, L. L. 2005. Weapons of Mass Refraction. Mol Microbiol; **57**:878-888.

Deutcher, P, M. 1990. *Guide To Protein Purification, Methods In Enzymology*, Vol. 182.

Harlow, E., dan D, Lane. 1998. *Antibodies A Laboratory Manual*. USA : Cold Spring Harbor Laboratory.

Jenings, V., M. 1995. Review of Selected Adjuvants Used In Antibody Production, *ILAR Journal*; 37, 3:119-132.

John A. Crump and Eric D. Mintz, 2010. Global Trends in Typhoid and Paratyphoid Fever. Invited article. *Emerging Infections*. CID 2010:50 (15 january). 241-246

Muktiningsih. 2005. Produk Gen CarA *Salmonella Typhi* Berukuran 42 kDa yang Dideteksi Dengan Antibodi Anti-Protein Fusi. Disertasi Program Pascasarjana, Institut Teknologi Bandung.

Muktiningsih.N., Yoni.F. Syukriani, Irma Ratna Kartika, Murni. S, Catur.D.A. 2009 The Fuction of Salmonella Typhi Reference Spot Bacteria With Molecular Weight 46.7 Kilodalton and Isoelectric Point 6.7. *Proc. The first International Seminar on Science and Technology*, UII-UKM-UMT, 24-25 January. Jogjakarta- Indonesia.

Muktiningsih.N, Endah Dianty Pratiwi, Ulfah Choiriyah, Fera Kurniadewi, Irma Ratna Kartika, Fernita Puspasari,Dessy Natalia. 2013. Purification and Characterization of *Fim*-C *Salmonella typhi* Recombinant Fusion Protein with size 31 kDa As Vaccine Candidate. Proc. The 2nd International Conference of The indonesian Chemical Society (ICICS-2). 22-23 October 2013. Jogjakarta-Indonesia. p.225-235.

Moreno-Eutimio MA, Tenorio-Calvo A, Pastelin-Palacios R, Perez-Shibayama C, Gil-Cruz C, López-Santiago R, Baeza I, Fernández-Mora M, Bonifaz L, Isibasi A,Calva E, López-Macías C., 2013. *Salmonella Typhi* OmpS1 and OmpS2 porins are potent protective immunogens with adjuvant properties. *Immunology.* Aug;139(4):459-71.doi: 10.1111/imm.12093.

Noer, A, S., Marzuki dan Allison, W., S,. 1992. Antipeptide Antibodies To The Carboxy Terminal and the DCCD Binding Region of Human Mithocondrial ATP Synthetase Beta Sub-Unit. *Biochem, Biophys, Acta*. 1099:123-130.

Thermo Scientific**,** Biogen, 2013

Verma, Shailendra Kumar1, Vandana Gautam1, Konduru Balakrishna2, and Subodh Kumar, 2009. Overexpression, Purification, and Immunogenicity of Recombinant Porin Proteins of *Salmonella enterica Serovar Typhi* (*S. Typhi*). *J. Microbiol. Biotechnol.* (2009), 19(9), 1034–1040 doi: 10.4014/jmb.0812.675 First published online 5 June 2009

Verry. 2011. *Waspada Gejala Tifus, Segera Periksa Diri Anda!*. http://www.prodiakalimantan.com/artikel-kesehatan/100-waspadai-gejala-tifus-segera-periksakan-diri-anda.html (Diakses pada tanggal 3 Agustus pukul 21.35 WIB).

Yang Y, Wan C, Xu H, Wei H. 2013. Identification and characterization of OmpL as a potential vaccine candidate for immune-protection against salmonellosis in mice. *Vaccine.* 2013 Jun 19;31(28):2930-6. doi: 10.1016/j.vaccine.2013.04.044. Epub 2013 May

C-15

SYNTHESIS OF STAR POLY(4-VINYLPYRIDINE) ARCHITECTURE BY NITROXIDE MEDIATED POLYMERISATION

Nurulsaidah Abdul Rahim[a*], **Fabrice Audouin**[b], **Johannes G Vos**[b], **Andreas Heise**[b]

[a*]*Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia*
[b]*School of Chemical Sciences, Dublin City University, Dublin 9,Ireland*

Abstract

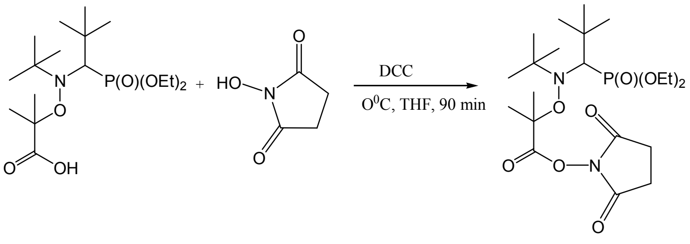
This study proposed multifunctional alkoxyamine 2-({tert-butyl [[1-(diethoxyphosphoryl)-2,2-dimethylpropyl]amino}oxy)-2-methylpropanoic acid (MAMA-SG1) initiators for the 'grafting-from' method to obtain star architecture of poly 4-vinylpyridine (P4VP) from JEFFAMINE®. The structure of macroinitiator was confirmed by amide bond present in NMR and FTIR spectroscopy. Furthermore, the macroinitiator was used to polymerise 4VP. P4VP from JEFFAMINE-SG1 shows a monomodal peak in the SEC chromatogram, indicating more control polymerisation process.

Key words: Nitroxide Mediated Polymerisation, 4-vinylpyridine, star polymer

INTRODUCTION

Nitroxide-mediated polymerisation (NMP) is a powerful tool for the synthesis of macromolecular architectures.[[i],[ii]] NMP is well-documented for the design of new initiators [[iii]], kinetic investigation [[iv],[v]] and preparation of new materials [[vi]]. Block [[vii],[viii]] and graft [[ix]] copolymer as well as star shaped polymers [[x]-[xiii]] prepared from NMP indeed show good structural control producing polymers with narrow molar mass distribution at high conversion. The synthetic approaches to star polymers are classified by two methods which are "arm-first" methods and "core-first" methods. The "arm-first" method involves living macromonomers or macroinitiators which link together in the core with vinylic cross linker such as divinylbenzene (DVB).[[xiv]] On the other hand, the "core-first" methods employs multifunctional initiators that simultaneously initiate the polymerisation of monomers to form the arms of the star polymer.[x]

MAMA-SG1 is an alkoxyamine with high potential nitroxide because it has a high dissociation rate constant and a terminal carboxylic acid group. This functional group allows further modification or transformation processes to open new possibilities for complex molecules that are not accessible with TEMPO nitroxide.[[xv]] In 2008, Vinas *et al.* reported the preparation of MAMA-SG1 bearing a N-succinimidyl ester group **(1)** (Scheme 1).[[xvi]] This alkoxyamine initiator can be attached to OH- and NH_2- functional groups, the later used as macroinitiator for block copolymer preparation.[xvi,[xvii]] Silica nanoparticles were also grafted with activated MAMA-SG1 **(1)** to polymerise styrene from the surface.[[xviii]]



(1)

Scheme 1: Synthetic route to activated MAMA-SG1 with NHS.[xvi]

Star polymers with MAMA-SG1 was explored by Dufils and his co-workers by intermolecular radical addition of alkoxyamine onto olefins.[xix] They synthesised 3- and 4-arms initiators and star polystyrene from this reaction was controllable with dispersity not exceeding 2. Meanwhile, Robin *et al*. synthesised trifunctional alkoxyamine SG1 with 1,3.5-tris(2-hydroxyethyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-tri-one.[xx] Well-defined star polymers of *n*-butyl acrylate (*n*BuA), styrene (S) and block copolymers poly(BuA-*b*-PS) were reported as products from this trialkoxyamine initiator.

In this study, we introduce star macroinitiator with SG1 end functionality using polyetheramine JEFFAMINE® T-403 grafted with NHS activated MAMA-SG1. Then, P4VP was prepared by core first method. The chemical structure were characterised with FTIR and ^{1}H NMR.

RESEARCH METHOD

Materials

All solvents employed were HPLC grade or better and used directly as received unless otherwise stated. Methyl methacrylate, MMA (Aldrich, 99%) and 4-vinylpyridine, 4VP (Aldrich, 95%) were distilled from calcium hydrate before used. 2-({tert-butyl [[1-(diethoxyphosphoryl)-2,2-dimetylpropyl]amino}oxy)-2-methylpropanoic acid (MAMA-SG1) and JEFFAMINE® T-403 were kindly gifted of TU/e, Eindhoven and HUNSTMAN, UK respectively. PPI (polypropylene imine) dendrimers, generations 2, were purchased from SyMO-Chem BV (The Netherlands). 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N*,*N*-Diisopropylethylamine (DIPEA) were purchased from Sigma-Aldrich.

Synthesis of activated alkoxyamine, 2-methyl-2-[N-tert-butyl-N-(1-diethoxyphosphoryl-2,2-dimethylpropyl) aminoxy]-N-propionyloxysuccinimide (MAMA-NHS (1))

This compound was synthesised following a literature procedure.[xvi] MAMA-SG1 (0.5 g, 1.31 mmol) and N-hydroxysuccinimide (NHS) (0.18 g, 1.57 mmol) were dissolved in 2 mL THF and deoxygenated by nitrogen bubbling for 15 minutes. Then, a degassed solution of 0.3 g N,N'-dicyclohexylcarbodiimide (DCC) in 0.5 mL THF was added. After stirring at 0 °C for 1.5 hours, the precipitated N,N'-dicyclohexylurea (DCU) was removed by filtration and washed with a minimum of cool THF. The filtrate volume was reduced under vacuum to one third and kept at -20 °C for 2 hours in order to precipitate the residual DCU. After filtration, the precipitation was performed in pentane. The obtained solid was further washed with water to remove NHS and dried under vacuum. The alkoxyamine was obtained as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 1.17–1.33 (m, 24H), 1.82 (s, 3H), 1.88 (s, 3H), 2.82 (s, 4H), 3.31 (d, 1H), 3.95–4.35 (m, 4H). Mixture with DCU 2.00-1.15 (m, 8H). ^{31}P NMR (400 MHz, $CDCl_3$) (ppm): 24.89. Yield: 200mg (0.40 mmol).

Synthesis of P4VP from JEFFAMINE functionalise MAMA-SG1

Activated alkoxyamine **(1)** (24 mg, 0.05 mmol) and JEFFAMINE® T-403 (20 mg, 0.045 mmol) were added to a Schenk flask and degassed for 15 minutes by nitrogen bubbling. Then, degassed 0.05 mmol DCC in 2 mL DMF was added and stirred at room temperature for 3 days. The precipitated DCU was removed by filtration. Then, the filtrate solution was precipitated in pentane and dried in vacuum oven overnight. The end product was obtained as a yellow sticky material, grafted JEFFAMINE **[2]**. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 0.85 (s, $\mathbf{CH_3}$-CH_2- from JEFFAMINE), 1.01-1.36 (m, -$\mathbf{CH_3}$ from MAMA-SG1), 1.57-1.86 (m, $\mathbf{CH_3}$-C-C(O)), 2.61 (m, -N-**CH**-P from MAMA-SG1), 3.3-4.12 (m, -$\mathbf{CH_2}$- from JEFFAMINE and MAMA-SG1), 8.14 (s, **NH**(CO)) .

Grafted JEFFAMINE **(2)** (30 mg; 0.02 mmol) and 4VP (1.21 g, 11.5 mmol) was added to a Schlenk flask and immersed in a pre-heated oil bath at 110°C for 24 hours. The reaction was quenched by cooling the mixture in an ice-water bath and the polymer was precipitated in diethyl ether, followed to dry in a vacuum oven overnight. Solid white polymer was obtained. Yield: 500 mg; M_n: 119000 g/mol; PDI: 1.4

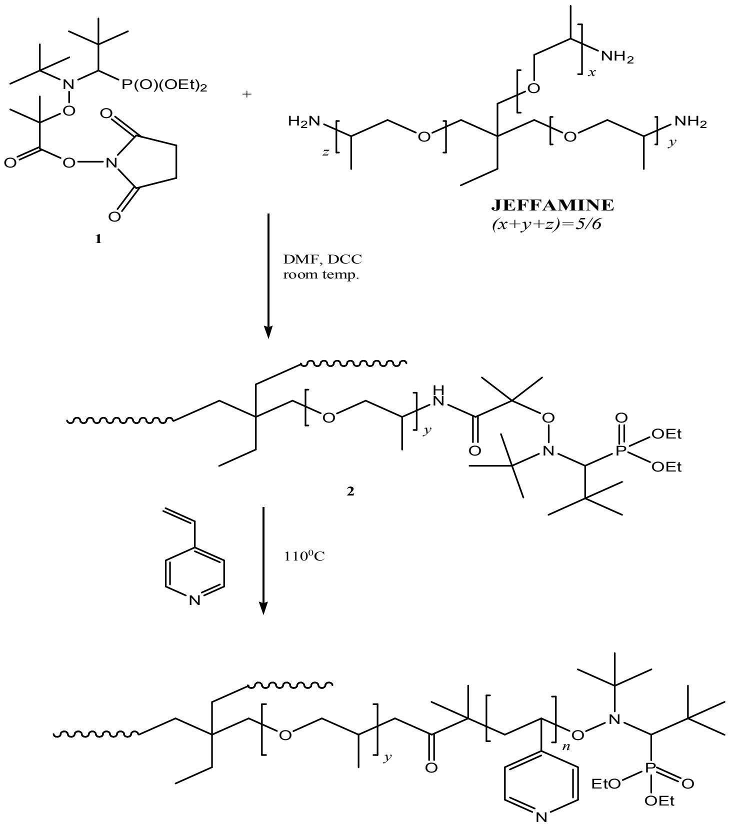
Methods

^{1}H NMR analyses were performed in $CDCl_3$ solution, at 25°C using a Bruker Avance 400 (400 MHz) spectrometer. The chemical shift was calibrated using the solvent peak ($\delta = 7.26$ ppm). The Fourier Transform InfraRed (FTIR) spectroscopy was carried out in the solid state on a Perkin Elmer Spectrum 100. GPC analysis using DMF (0.1 M LiBr) as eluent (elution rate: 1 min/mL) was performed using two PSS GRAM analytical (300 and 100 Å, 10 l) columns on an Agilent 1200 series equipped with a Wyatt Optilab rEX refractive index detector thermostat at 40 °C and a Wyatt DAWN HELEOS-II multi angle light scattering (MALS) detector. Molecular weights and PDI were calculated from the MALS signal using the ASTRA software (Wyatt) and a dn/dc value of 0.225 mL/g (P4VP) [xxi] in DMF. Before analysis, samples were filtered through a 0.45 µm PTFE filter (13 mm, PP housing, Whatman).

RESULT AND DISCUSSION

The synthesis of $sP(4VP)_3$ was carried out according to Scheme 2. It involves (1) functionalisation of MAMA-SG1 with NHS, (2) Grafting the activated alkoxyamine (MAMA-NHS) to JEFFAMINE® and (3) polymerisation.

For MAMA-NHS **(1)**, the reaction of MAMA-SG1 with NHS in the present of N,N’-dicyclohexylcarbodiimide (DCC) was carried out at 0 °C in THF for 1.5 hours. Then, the product was precipitated in pentane as a white powder. The carbonyl band in FTIR spectra shifted from the free acid MAMA-SG1 at 1718 cm^{-1} to the NHS ester 1741 cm^{-1}. The succinimidyl C=O group appears at 1816 and 1781 cm^{-1} (Figure 1a). The FTIR spectrum in Figure 1b shows the new band at 1625 and 1572 cm^{-1} assigned to the amide bond (-CONH-) *i.e.* the C=O stretching and N-H bending vibration band.



Scheme 2: Schematic route of star poly 4-vinylpyridine with JEFFAMINE®

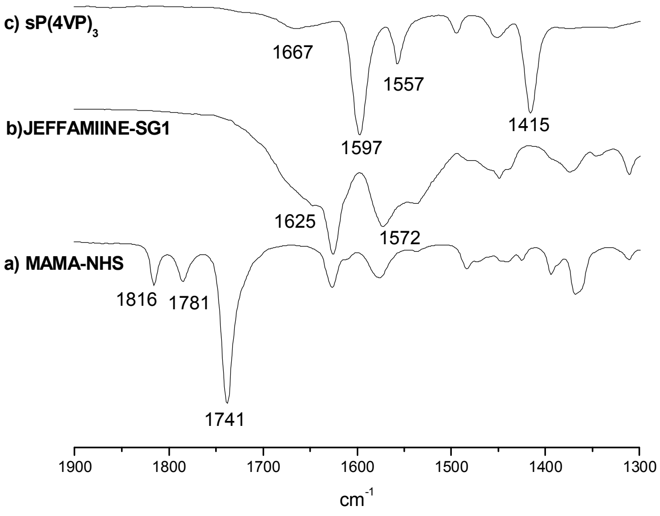


Figure 1: FTIR spectrum of (a) activated alkoxyamine, MAMA-NHS); (b) JEFFAMINE® functionalise SG-1; (c) star polymer 4-vinylpyridine, sP(4VP)$_3$

In addition, ^{1}H NMR confirmed the structure of MAMA-NHS with the peaks of the methyl (**b**, **c**, **e**, **g**), methylene (3.95-4.35 ppm) and –CH (3.31 ppm) from MAMA-SG1 as well as the methylene of the succinimidyl ester appearing at 2.82 ppm (Figure 2a). Then, the activated alkoxyamine was grafted to JEFFAMINE® at room temperature in DMF to form star initiator **(2)** as a yellow sticky material. The ^{1}H NMR spectrum shows characteristic peaks of both the

JEFFAMINE® bands (0.85 ppm for free $–CH_3$, 3.20 – 4.20 ppm for CH and CH_2) and the SG-1 (**b**, **i**, **k**, **m** for $-CH_3$ groups). Moreover, the amide linker can be identified in the spectrum at 8.14 ppm (Figure 2b).

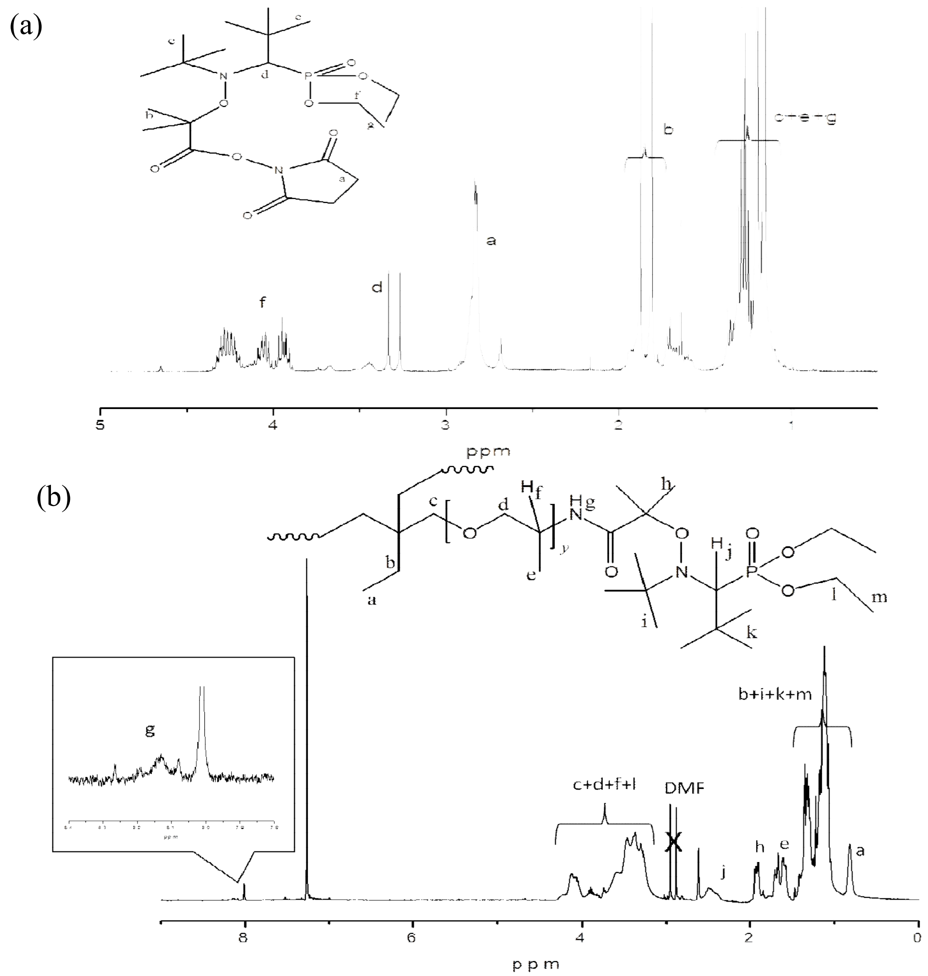


Figure 2: ^1H NMR of activated alkoxyamine MAMA-NHS (a) and JEFFAMINE® functionalised with NHS-alkoxyamine (b).

P4VP with the functionalised JEFFAMINE®-SG1 macroinitiator (**2**) was synthesised at 110 °C yielding white polymer product at 98% conversion. Figure 1c shows the position of the amide bond in $sP(4VP)_3$ shifted from 1625 to 1666 cm^{-1} with the presence of P4VP bands at 1597, 1557 and 1451 cm^{-1}. A molecular weight of 170000 g/mol, M_w, measured by SEC equipped with a MALS detector was obtained compare to the M_n theoretical of 106000 g/mol (Figure 3). The SEC chromatogram shows a monomodal peak with a dispersity of 1.4. Termination reaction are never inhibit during NMP leading to disproportionation and combination reactions between radicals. According to Vinas *et al.*, the dissociation rate constant (k_{d1}) of C-ON bond homolysis in (**1**) is 5 s^{-1} (E_a = 103 kJ mol^{-1}) which is 15 times higher than MAMA-SG1 (k_{d1} = 0.32s^{-1}. E_a = 112 kJ mol^{-1}).[xvi,[xxii]] The higher k_{d1} value can be ascribe to a long-range polar effect of NHS moiety in the activated MAMA-NHS. Besides that, we also observed a broad tailing at the low molar mass side of the chromatogram probably due to autopolymerisation of 4VP at high temperature. Baumann and Schmidt observed that the rate of autopolymerisation of 4VP is in order 11%/h at 125 °C.[xxiii] A summary of the experiment is provided in **Error! Not a valid bookmark self-reference.**.

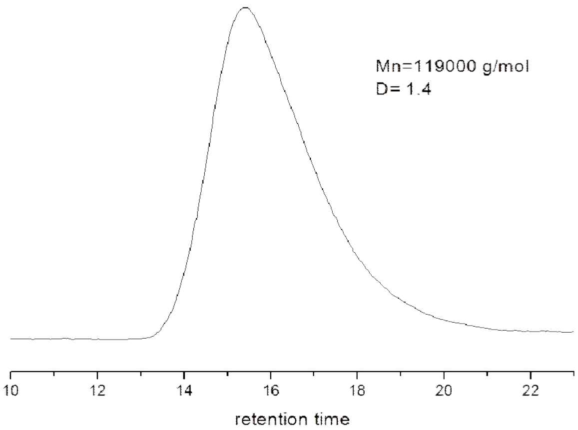


Figure 3: SEC traces (RI signals) of $P(4VP)_3$ with JEFFAMINE® star initiator at a ratio $[M]_0/[I]_0=370$

Table **Error! No text of specified style in document.**.1: Experimental results of star polymerisation of 4-vinylpyridine

Entry	**time, min**	**Conversion [a] (%)**	**$M_{n\ theo}$ [b] g/mol**	**$M_{n\ GPC}$ [c] g/mol**	**$M_{w\ MALLS}$ [c] g/mol**	**D [c]**
$sP(4VP)_3$ [d]	300	98	106000	119000	170000	1.4

[a] Conversion calculated by ^1H NMR through integration of CH_2 peaks of 4VP (5.94 and 5.46 ppm) and P4VP (2.10-1.41 ppm). [b] $M_{n\ theo}$ calculated by ^1H NMR, M_n= (molar fraction x $[M]_0/[I]_0$ x M_w monomer) + M_w macroinitiator. [c] Measured by DMF GPC with multi angle light scattering detector (MALLS). [d] Experimental condition: $[I]_0/[4VP]_0$= 1/370 per arms.

CONCLUSION AND SUGGESTION

Multifunctional initiators as the core molecules was synthesised from JEFFAMINE® to obtain 3- arms for the polymerisation of 4VP. This structure of the alkoxyamines was confirmed spectroscopically. $s(P4VP)_3$ was synthesised from the macroinitiator with high molecular weight (~10^5 g/mol). However, low molecular weight products were obtained in the polymerisation either due to autopolymerisation or termination reactions at the early stage of the polymerisation. Further investigation to control the rate of polymerisation and to synthesise well-defined polymer will be necessary to optimise this procedure.

REFERENCES

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[i]. Hadjichristidis N, Iatrou H, Pitsikalis M, Mays J, Prog Polym Sci 2006;31:1068.

[ii]. Nicolas J, Guillaneuf Y, Lefay C, Bertin D, Gigmes D, Charleux B, Prog Polym Sci 2012;38:63 .

[iii]. Benoit D, Chaplinski V, Braslau R, Hawker CJ, J Am Chem Soc 1999;121:3904.

[iv]. Gigmes D, Bertin D, Lefay C, Guillaneuf Y, Macromol Theory Simul 2009;18:402.

[v]. Bertin D, Gigmes D, Marque SRA, Tordo, Chem Soc Rev 2011;40:2189.

[vi]. Hawker CJ, Bosman AW, Harth E, Chem Rev 2001;101:3661.

[vii]. Rahim NA, Audouin F, Twaley B, Vos JG, Heise A, Eur Polym J 2012;48:990.

[viii]. Benoit D, Harth E, Fox P, Waymouth RM, Hawker CJ, Macromolecules 2000;33:363.

[ix]. Flakus S and Schmidt-Naake, G. Macromol Symp 2009;43:275.

[x]. Miura Y and Yoshida Y, Polymer 2002;34:748.

[xi]. Abraham S, Choi JH, Ha CS, Kim I, J Polym Sci Part A: Polym Chem 2007;45:5559.

[xii]. Lu CH, Wang JH, Chang FC, Kuo SW, Macromol Chem Phys 2010;211:1339.

[xiii]. Li J, Zhang Z, Zhu X, Zhu J, Cheng Z, e-Polymers 2010;145:1.

[xiv]. Tsoukatos T, Pispas S, and Hadjichristidis N, J Polym Sci Part A: Polym. Chem 2001;39:320.

[xv]. Diaz T, Fischer A, Jonquières A, Brembilla A, Lonchon P, Macromolecules 2003;36:2235.

[xvi]. Vinas J, Chagneu N, Gigmes D, Trimaille T, Favier A, Bertin D, Polymer 2008;49:3639.

[xvii]. Habraken GJM, Peeters M, Thornton PD, Koning CE, Heise A, Biomacromolecules 2011;12:3761.

[xviii]. Chevigny C, Gigmes D, Bertin D, Jestin J, Boué F, Soft Matter 2009;5:3741.

[xix]. Dufils PE, Chagneux N, Gigmes D, Trimaille T, Marque SRA, Bertin D, Tordo P, Polymer 2007; 48:5219.

[xx]. Robin S, Guerret O, Couturier JL, Gnanou Y, Macromolecules 2002; 35:2481

[xxi]. Convertine AJ, Sumerlin BS, Thomas DB, Lowe AB, McCormick CL, Macromolecules 2003; 36:4679.

[xxii]. Ananchenko G, Beaudoin E, Bertin D, Gigmes D, Lagarde P, Marque SRA, Revalor E, Tordo P, J Phys Org Chem 2006;19:269.

[xxiii]. Baumann M, Schmidt-Naake G, Macromol Chem Phys 2000;201:2751

C-16

ANTIFUNGAL POTENTIAL TEST OF GLYCOSIDE COMPOUND FROM ROOT WOOF OF *Pterospermum subpeltatum* C. B. ROB

Pince Salempa[a], Alfian Noor[b], Nunuk Hariani[b], Sudding[a], Muharram[a]

[a] *Chemistry Department, Mathematic and Natural Science Faculty, Makassar State University, Makassar, Indonesia*
[b] *Chemistry Department, Mathematic and Natural Science Faculty, Hasanuddin University, Makassar, Indonesia*

Abstract

A steroidal compound, 3-O-β-glucopyranosyl-β-cytosterol is one of steroidal from chloroform fraction of root wood of *Pterospermum subpeltatum* C. B. Rob (Sterculiaceae). The structure of compound was determined based on IR, ^{1}H, dan ^{13}C-NMR spectra. This compound active against *Artemia salina* with the toxicity of LC_{50} 160,93µg/mL and it also active as antifungal against *Candida albicans* with inhibition diameter 11,0 mm

Keywords: 3-O-βglukopiranosil-β-sitosterol, steroid, *Pterospermum subpeltatum*, antifungal

1. Introduction

Poor sanitary conditions and weather are often characterized by very high temperatures and humidity can cause infections in wounds. It generally occurs in tropical developing countries. synthetic antibiotic therapy is not always possible due to high cost. To solves this problem people use plants that grow in their area as a traditional medicine, even without scientific support.

Indonesian traditional medicines from plants have been widely used as a form of treatment. Through intensive research has been known the bioactive compounds, which are secondary metabolites compound in these plants. For example, derivatives compounds of cassane furano diterpen and norcassane from the seeds of Bagore (*Caesalpina crista*, Linn) as an antimalaria. These bioactive compound can be further developed through the modification of the molecular groups or bonds contained in the compound become more active or more secure and can be used as precursor in guiding us to find new drugs (Attamimi, 2004).

Chemical investigation of the cinchona tree was first reported by French researchers Caventou and Pelletier (Cragg, in Ahmad, 2007) and further investigations have been found more than 30 classes of chemical compounds different alkaloids including quinine and quinidine, each of which has been used for the treatment of malaria disease and cardiac disorders (Ahmad, 2007). The results of the investigation consistent with the use of cinchona bark *Cinchona sp*. by people in the Amazon, South America for the treatment of malaria.

In Indonesia, the development of drugs from natural ingredients have great potential because of the tropical climate. Tropical plants are believed have the ability to manipulate diverse chemical compounds which have various interesting bioactivity. The ability of one caused by a self-defense mechanism against the environment. In general, these plants live under the harsh environmental conditions both climatic factors and disorders of herbivore, insects, and diseases. Tropical plants can produce natural chemical compounds that potentially as an insecticide and anti-fungi. As an example*, Sterculia africana* containing of antifungal compounds (Hamza et al., 2005), *Azardirachta indica* as insecticides (Ahmad, 2007).

One of Indonesian tropical plant is Sterculiaceae, which is one of a quite large family, consisting of 70 genus and about 1500 species (Gressier et al., 2008). The genus *Pterospermum* also included in the family Sterculiaceae are efficacious as medicinal plants such as the bark of *P. javanicum* to treat dysentery, toothache, boils, and sprains.

Leaves of *P. diversifolium* used to reduce itchy and skin medicine. The roots of this plant are used as fish poison (Ogata et al., 1995). According to Heyne (1987), leaves of *P. acerifolium* also used as an itchy medicine in Central Sulawesi.

Camporese (2003) reported on the antibacterial activity of hexane extract and methanol extract of stem bark of *Guazuma ulmifolia* (Sterculiaceae). n-hexane extract can inhibit the growth of *E. coli*, while the methanol extract inhibited the growth of *Pseudomanas aeruginosa*. Reid et al. (2005) studied the antibacterial activity of various extracts of *Cola greenway* and reported that the ethyl acetate fraction actively inhibit the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus.*

In this study reported the bioactivity, isolation, and structural determination of 3-O-β-glucopyranosyl-β-cytosterol from chloroform fraction of *Pterospermum subpeltatum* root wood. The molecular structure of the compound was determined based on the interpretation of spectroscopic data which include IR, ^{1}H, dan ^{13}C-NMR spectra.

2. Research Method

2.1. General

The melting point was determined with Fisher John melting point apparatus. The infrared spectra obtained with Perkin Elmer FTIR spectrophotometer, while ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR obtained with Jeol spectrophotometer.

2.2. Materials

The material used is the root wood of *Pterospermum subpeltatum* C. B. Rob, obtained from Mamuju, West Sulawesi and had been determined in Herbarium Bogoriene, Biological Research and Development Center, LIPI Bogor.

2.3. Isolation and Purification

2.3.1. Extraction and Isolation

10 kg of dry weight root wood *Pterospermum subpeltatum* C. B. Rob mashed then maceration with methanol for 1 x 24 hours for several times. Maserat obtained was evaporated until 3 L of thick maserat obtained with dry weight of 1,047 kg. The maserat further partitioned with solvents. Chloroform fraction (35.7 kg) was fractionated by vacuum column chromatography (VCC) with eluent n-hexane, ethyl acetate: n-hexane, ethyl acetate, acetone: ethyl acetate, acetone, and methanol, with increasing its polarity.

Merger fractions obtained were monitored by TLC yielded eight major fractions. Fraction B7 generating 25 g cream-colored floured compound, the test results showed a steroid group with melting point 284-285°C

2.3.2. Biological Test

Toxicity tests performed using baby shrimp *Artemia salina* corresponding with the method of Meyer et al, and anti-microbial testing done by using Nutrient Agar method.

3. Result and Discussion

Compound 1 obtained as cream-colored powder, with melting point 284 - 285°C. IR (KBr) showed an absorption band in 3377 cm^{-1} wavelength indicated the existence of free OH group supported by the peak at 1070 cm^{-1} for the strecthing of C – O. Other absorption at 2954 and 2931 cm^{-1} for aliphatic C- H that supported by peaks at 1463 cm $^{-1}$ (CH_2) and 1367 cm^{-1} (CH_3).

Analysis of ^{13}C-NMR spectroscopy data, showed 35 signals with the degree of protonation determined through DEPT-135 experiments. Twenty nine carbon signals that consist of 6 methyl at δ 11,7 (C-18), 18,9 (C-19), 18,6 (C-21), 19,8 (C-26), 19,1 (C-27), and 11,8 (C-29) ppm; 11 methylen at δ 36,8 (C-1), 29,3 (C-2), 28,6 (C-4), 31,4 (C-7), 20,6 (C-11), 39,2 (C-12), 23,9 (C-15), 27,8 (C-16), 33,3 (C-22), 25,4(C-23), and 22,6 (C-28) ppm; 9 methyne at δ 76,9 (C-3), 121,3 (C-6), 31,4 (C-8), 49,6 (C-9), 56,2 (C-14), 55,4 (C-17), 35,5 (C-20), 45,1 (C-24), and 28,7 (C-25) ppm, and 3 quaternary carbon at δ 140,4 (C-5) 36,2 (C-10), and 41,9 (C-13) ppm, form a steroid skeleton steroid types cytosterol. While 6 other carbon signals consisting of 5 methyne at δ 100,8 (C-1), 73,5 (C-2), 76,8 (C-3), 70,1 (C-4), and 76,8 (C-5) ppm; and 1 methylen at 61,1 ppm (C-6), form a monosaccharide skeleton types glucopyranoside.

Analysis of ^{1}H-NMR spectroscopy data, compound 1 showed some peculiar signals, those are at δ 5,32 ppm (H-6, 1H, brd, J=5,0 Hz) that showed an alkene proton are influenced by two vicinal protons with each position at σ 1,93 ppm (H-7_{α}, 1H, m) and δ 1,52 ppm (H-7_{β}, 1H, m) at δ 3,46 pp (H-3, 1H, tt), J=6,7 and 11,6 Hz) that showed the methyne proton binding the oxy group, and at the δ 0,65 ppm (H-18, 3H, s) and δ 0,92 ppm (H-19, 3H, s) each showed methyl proton that bound to a quaternary carbon. The proton signals indicate that steroid skeleton substituted by two methyl groups and one oxy group. At the aliphatic region there are also two groups of signals, which are the group which indicated an alkane unit, at δ 1,29 ppm (H-20, 1H, m); δ 0,89 ppm (H-21, 3H, d, J,=6,8 Hz); δ 1,32 pm (H-22_{α}, 1H, m), and δ 0,97 (H-22_{β}, 1H, m); δ 1,13 ppm (H-23, 2H, m); δ 0,93 ppm (H-24, 1H, m); δ 1,62 ppm (H-25, 1H, okt, J=6,8 Hz); δ ,81 ppm (H-26, 3H, d, J=6,8 Hz); δ 0,79 ppm (H-27, 3H, d, J=6,8 Hz); δ 1,23 ppm (H-28, 2H, m); and δ 0,82 pp (H-29, 3H, t, j=6,7 Hz), and signals that indicate a unit of glucopyranosyl, include at δ 4,21 ppp (H-1, 1H, d, J=8,0 Hz); δ 2,88 ppm (H-2, 1H, td, J=8,0 and 5,0 Hz), δ 3,11 ppm (H-3, 1H, td, J=8,5 and 3,7 Hz), δ 3,0ppm (H-4, 1H, td, J=8,5 and 5,0 Hz), δ 3,06 ppm (H-5, 1H, ddd, J=9,5; 6,0 and 1,8 Hz), δ 3,64 ppm (H-6_a, 1H, dd, J10,5 and 6,0 Hz) and 3,39 ppm (H-6_b, 1H, m). The relation of bonding in the structure was evidenced by long distance correlation of ^{1}H-^{13}C from HMBC spectrum. HMBC spectrum showed a long distance correlation between the signal of proton and the perculiar carbon, that is at δ 4,21 ppm (H-1') with carbon at δ 76,7 ppm (C-3); δ 0,65 ppm Me-18) with δ 36,2 ppm (C-10); δ 0,92 ppm (Me-19) with δ 41,9 ppm (C-13); δ 0,89 ppm (Me-21) with δ 55,4 ppm (C-17); and δ 0,81 ppm (Me-26) and δ 0,27 ppm (Me-27) with δ 28,7 ppm (C-25), this indicates that the position of O-piranosil group at C-3 and each successive methyl carbon at position C-18, C-19, C-21, C-26 and C-27. HMBC correlations of compound 1 is shown in Figure 1. Data of 1H and 13C NMR spectroscopy (1D and 2D) of compound 1 is shown in Table 1. Based on analysis data above, compound 1 can be summarized as 3-O-β-glucopyranosyl-β-cytosterol. NMR data of compound 1 has similarities with derivative stigmasterol compounds previously reported (Alam M. S., 1995).

Toxicity test against baby shrimp *A. salina* show the value of LC_{50} = 16,93 μg/mL and active against fungi C. albicans with inhibition diameter 11 mm

Table 1. ^{1}H, ^{13}C, and 2D NMR Spectrum of Compound 1

No.	**δ_H (multi, J in Hz)**	**δ_C**	**δ_C (lit)**	**COSY H ↔ H**	**HMBC C ↔ H**
1	1,78 (1H, *m*)	36,8	37,1	2	18
	0,99 (1H, *m*)				
2	1,80 (1H, *m*)	29,3	28,2	1,3	-
	1,51 (1H, *m*)				
3	3,46 (1H, *tt*, *J* = 6,75 & 11,65 Hz)	76,7	79,8	2,4	1'
4	3,46 (1H, *br d*, *J* = 3,05 & 10,55 Hz)	38,3	39,7	3	-
	3,46 (1H, *br t*, *J* = 11,60 Hz)				
5	-	140,3	140,4	-	18
6	5,32 (1H, *br d*, 5,0 Hz)	121,1	121,8	7	-
7	1,93 (1H, *m*)	31,4	31,8	6, 8	-
	1,52 (1H, *m*)				
8	1,41 (1H, *m*)	31,4	31,8	7, 9,	-
9	0,86 (1H, *m*)	49,6	50,1	14	18
10	-	36,2	36,6	8, 11	18
11	1,38 (1H, *m*)	20,6	21,0	-	-
12	1,97 (1H, *m*)	39,2	39,7	9, 12	19
	1,16 (1H, *m*)			11	
13	-	41,9	42,2		19
14	0,98 (1H, *m*)	56,2	56,7	-	19
15	1,50 (1H, *m*)	23,9	24,1	8, 15	-
	1,02 (1H, *m*)			14,16	
16	1,79 (1H, *m*)	27,8	28,2		-
	1,29 (1H, *m*)			15, 17	
17	1,05 (1H, *m*)	55,4	56,0		19,21
18	0,65 (3H, *s*)	11,7	11,7	16, 20	1, 5, 9, 10
18	0,92 (3H, *s*)	18,9	19,2	-	12, 13, 14, 17,
20	1,29 (1H, *m*)	35,5	36,0	-	20
21	0,89 (3H, *d*, *J* = 6,75 Hz)	18,6	18,7	17, 21,	19, 21
22	1,32 (1H, *m*)	33,3	33,8	22	17, 20, 22
	0,97 (1H, *m*)			20	21
23	1,13 (2H, *m*)	25,4	26,0	20, 23	
24	0,93 (1H, *m*)	45,1	45,7		-
25	1,62 (1H, *okt.*, 6,75 Hz)	28,7	29,1	22, 24	29
26	0,81 (3H, *d*, *J* = 6,75 Hz)	19,8	19,7	23, 25,	26, 27
27	0,79 (3H, *d*, *J* = 6,75 Hz)	19,1	18,9	28	25, 27
28	1,23 (2H, *m*)	22,6	23,0	24, 8,	25, 26
29	0,82 (3H, *t*, *J* = 6,75 Hz)	11,8	11,9	27	29
1'	4,21 (1H, *d*, 8,0 Hz)	100,6		25	24, 28
2'	2,88 (1H, *td*, 8,0 & 5,0 Hz)	73,3		25	3
3'	3,11 (1H, *td*, 8,5 & 3,7 Hz)	76,6		24, 29	-
4'	3,00 (1H, *td*, 8,5 & 5,0 Hz)	69,9		28	-
5'	3,06 (1H, *ddd*, 9,5; 6,0 & 1,8 Hz)	76,6		2'	-
6'	3,64 (1H, *br dd*, 10,5 & 6,0 Hz)	60,9		1', 3'	-
	3,39 (1H,*)			2', 4'	-
				3', 5'	
				4', 6'	
				5'	

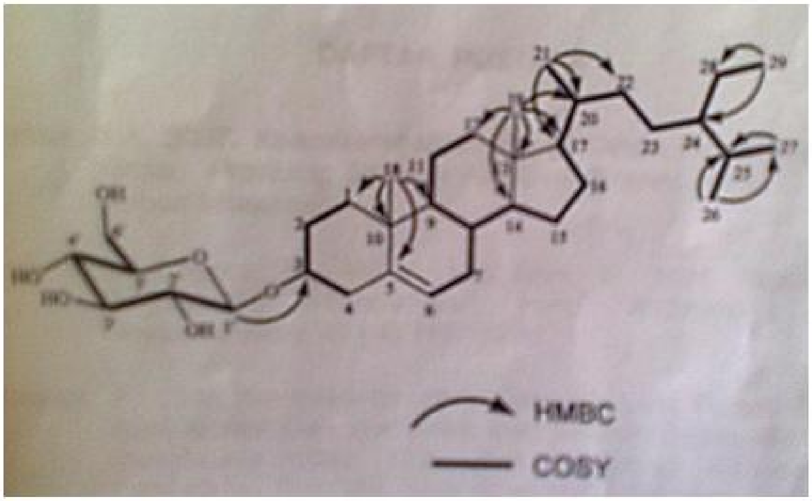


Figure 1. 3-O-β-glucopyranosyl-β-cytosterol

4. Conclusion and Suggestion

The compound of 3-O-β-glucopyranosyl-β-cytosterol have been found for the first time from *P. subpeltatum* and potential as an antifungal with the inhibition diameter 11 mm against *Candida albicans* and also active against Arternia salian LC_{50}: 160,93 µg/mL. Clinical trials of compounds found in plants such *P. subpeltatum* based activities, to be developed and can be biosynthesized.

Bibliography

Achmad, S. A. 2007. Keanekaragaman Hayai Dalam Pembelajaran Ilmu Kimia. *Prosding Seminar Nasional Jurusasn Kimia Universitas Negeri Makassar*.

Alam, M. S., Chopra, N., Ali, M., and Nıwa, M. 1995. Oleanen and Stigmasterol Derivates from *Ambroma augusta*. *Phytochemistry*, **41 (4)** 1197-1200.

Attamimi, F. 2004. Senyawa-Senyawa Baru Cassane Furano Diterpen Hasil Isolasi Dan Identfikasi Dari Ekstrak Daging Biji Bagore (*Caesalpina crista*, Lınn) dan Potensinya Sebagai Obat Antimalaria. Unpublish Dissertation, Chemistry Department PPS Unhas, Makassar.

Anderson, J. E., Goetz, C. M., and Mc. Laughlin, J. L. 1990. A Blind Comparison of Simple Bench-top Bioassays and Human Tumor Cell Cytotoxicities as Antitumor Prescreen. *Phytochemical analysis*. **6**, 107-111

Anjaneyulu, A. S. R., Raju. 1987. Cyclotreterpenes from The Heartwood of *Pterospermum heyneanum*. *Phytochemistry* **26 (10)**, 2805-2860.

Boer, E., Lemmens, R. H. M. J. 1998. Plant Resources f South-East Asia: Timber tress: Lesser-known timber No. 5 (3). Bogor Indonesia.

Camporese, A., Balick, M. J. F., Arvigo, R., Esposito, R. G., Marsellino, N., De Simone, F., Tubaro, A., 2003. Screening of anti-bacterial medicinal plants from Belize (Central America). *Journal of Ethnopharmacology*, **87,** 103-107.

Gressler, V., Caroline, Z., Stoker, C. Z., Dias, G. O. C., Dalcol, I. I., Burrow, R. A., Schmidt, J., Wesshohann, L., And Morel, A. F. 2008. Quinolone Alkaloids from *Waltheria dourandinha. Phytochemistry* **69 (4)** 994-999.

Hamza, O. J. M., van den Bout-van den Beukel, C. J. P., Mateem M. I. N., Moshi, M. J., Mikx, F. H. M., Selemani, H. O., Mbwambo, Z. H., Van der Ven, A. J. A. M, and Verweij, P. E. 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of funganl infection. *Journal of Ethnopharmacology.* **108 (1)** 124-132.

Harbone, J. B. 1984. Metode Fitokimia dan Penuntun Cara Modern Menganalisis Tumbuhan. ITB Bandung.

Heyne, K. 1987. Tumbuhan Berguna Indonesia III. Badan Litbang Kehutanan, Jakarta.

Jawetz, E, Melnick and Adelberg. 2001. *Microiologi Kedokteran*, Edisi 20. Buku Kedokteran. ECG. Jakarta 159-160.

Lyu *et al.* 2005; in Berenguer, B., Trabadela, C., Sanchez-Fidalgo, S., Qullez, A., Mino, P., De la Puerta, R., and Martin-Calero, M. J. 2007. The Aerial Parts of *Guazuma ulmifolia* Lam. Protect Against NSAID-Induced Gastric Lesions. *Journal of Ethnopharmacology*. **114 (2)** 153-160.

Meyer, B. N., Ferrigny, N. R., Putnam, J. E. Jacobsen, L. B., Nicols, D. E., Mc Laughlin, J. L. 1982. Brine Shrimp, A Covenient General Bioassay for Active Plant Contituent. *Journal of Medical Plant Research.* **45.** 31-34.

Ogata, Y. (Committe Members). 1995. *Indeks Tumbuh-Tumbuhan Obat di Indonesia*. PT. Esai Indonesia. Edisi II.

Reid, K. A. Jager, A. K., Light, M. E., Mulholland, D. A., Van Standen, J. 2005. Phytochemical and pharmalogical screening of sterculiaceace spesies and isolation and antibacterial compounds. *Journal of Ethnophamacology*, **97**, 285-291.

Salempa, P., Noor, A., Soekamto, N. H. Harlim, T. 2009. Bioaktivitas fraksi n-heksan dan senywa β-sitosterol dari kayu akar *Pterospermum subpeltatum* C. B. Rob. *Farmakologi* **4 (2)**, 45-60.

C-17

TEST METHOD VERIFICATION OF Fe AND SiO_2 IN INDUSTRIAL WATER BY UV-VIS SPECTROPHOTOMETRY AT PT KRAKATAU STEEL

Reni Banowati Istiningrum[a], Intan Permatasari[a], Idrus Bambang Iryanto[b]

[a]*Professional Program of Chemical Analyst , Islamic University of Indonesia*
[b]*PT Krakatau Steel Cilegon*

Abstract

It has been conducted the test methods verification of Fe and SiO_2 in industrial water using UV-Vis spectrophotometer at PT Krakatau Steel. Verification parameters tested were linearity, precision, accuracy, and detection limit. The results show the value of linearity, precision, accuracy and detection limits for testing Fe are respectively 0.996; 3.803%; 87.94% and 0.048 mg/L while for SiO_2 consecutive tests was 0.998; 2.014%; 100.755% and 0.010 mg/L. The results of test methods verification of Fe and SiO_2 meet the requirements and can be accepted as a method for routine testing in the chemical laboratory of PT Krakatau Steel.

Key words: verification, industrial water, Fe, SiO_2, UV-Vis spectrophotometer

INTRODUCTION

Water is one of the utilities that support industrial process. In the industry, especially the chemical industry, water has several functions: water as the industrial household, process water, cleansing water and water steam generator (boiler feed water) (Widyatmiko, 2005). According to Mussey (1961), process water is water that comes into contact with an end product or with materials incorporated into an end product; cooling water is water used exclusively for cooling; boiler-feed water is water introduced into boilers for conversion to steam; and sanitary and service water is water used for drinking, showers, general cleaning, and flushing wastes.

PT Krakatau Steel is the first and largest integrated steel industry in Indonesia, located in Cilegon, Banten. The products of PT Krakatau steel are hot rolled coils, cold rolled coils an wire rod steel wich are the raw material for the next industries. Large quantities of water are required to produce steel, typically 180-200 m^3 of water per ton of steel produced.

Water is used in the steel industry for direct contact cooling and cleaning of the steel in process, for cooling the process off gases, for product rinsing, and for process solution makeup; however, the vast majority of the water used in steel making is for non-contact cooling of associated processing equipment. Water is also used for steam and power generation, potable uses, and dust and moisture control (Lehrman et.al, 1999).

Because water has an important role in the steel industry, it will need special requirements such silica and Fe content. High concentration of silica in boiler feed water is harmful as silica tends to deposited as glassy and hard scale in boiler and cooling water systems. While high concentrations of Fe can causes the boiler and water line deposits as iron oxide (Fe_2O_3 or Fe_3O_4). Iron and steel industries in the United States use a water supply with a maximum SiO_2 and Fe content for cooling and processing water in steel plants are 30 and 6.3 ppm; for cooling and processing in hot-rolling mills are 21 and 0.4 ppm, and for boiler feed in steel plants are 30 and 6.3 ppm (Walling and Ottis, 1967). Meanwhile, according to the American Society of

Mechanical Engineers (ASME) boiler feed water quality requirements are a maximum of 0.1 ppm for Fe, while the requirements for boiler water is a maximum of 150 ppm for silica.

Therefore, it is necessary to determine the water used to supply in an effort to control the quality of water that can be prevented greater losses. To analyze the content of SiO_2 and Fe can be done by using UV-Vis Spectrophotometry method. The standard method used in chemical laboratory of PT Krakatau Steel for determination of iron content is in accordance with ASTM D.1068.84 while for SiO_2 content is in accordance with SOP.3133 QP02 019.

Because these methods are routinely used, it should be conducted the methods verification to verify the performance of both standard methods. In addition, methods verification are needed to prove that the laboratory is able to demonstrate the analysis methods to produce valid data. This paper aims to verify the test method of Fe and SiO_2 in the industrial water of PT Krakatau Steel with the parameters linearity, precision, accuracy and detection limit.

RESEARCH METHOD

Materials

All chemicals are analytical grade and used without further purification. Hydrochloric acid (HCl), hydroxylamine hydrochloride ($NH_2OH.HCl$), ammonium acetate (CH_3COONH_4), 1,10-phenanthroline ($C_{12}H_8N_2$), ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$), oxalic acid ($H_2C_2O_4$), and ascorbic acid ($C_6H_8O_6$).

Test Method Verifikasi of Fe

Linierity test is done by determining the correlation and determination coefficient for calibration curve of Fe standard solution. Series of standard solutions prepared from stock solutions of iron 10 mg/L by adding 0; 1; 2; 3; and 4 mL in a 100 mL volumetric flask. Then added with 2 mL HCl : water (1:1 v / v), 1 mL hydroxylamine hydrochloride 10%, 10 mL ammonium acetate 10%, 10 mL fenantrolin 0.1% and filled with distilled water to the mark. The solution was allowed to stand 10 minutes and the absorbance was measured by UV-Vis spectrophotometry at 510 nm.

Precision test was done by measuring the iron content in the sample seven times. A total of 50 mL of industrial water sample put into a 100 mL volumetric flask and then added 2 mL HCl: water (1:1 v/v), 1 mL hydroxylamine hydrochloride 10%, then heated to boiling. The solution was cooled and added 10 mL ammonium acetate 10%, 10 mL fenantrolin 0.1% then filled with water to the mark. The solution was homogenized and the absorbance was measured at 510 nm. % RSD values calculated to determine the value of precision.

Accuracy test was done by determining the value of %recovery. Procedure used was the same with precision test procedures but 10 mL of stock solution of iron 10 mg/L was added as a spike. Detection limit was done by measuring the absorbance of the reagent blank seven times. The procedure was performed the same as the test procedure of precision but was not use industrial water samples.

Test Method Verifikasi SiO_2

Linierity test was done by determining the correlation and determination coefficient for calibration curve of SiO_2 standard solution. Series of standard solutions prepared from the silicate stock solution 10 mg/L by adding 0; 5; 10; 20; 30 and 40 mL in 250 mL volumetric flask. Then added with 5 mL ammonium molybdate 5% and aged 4 minutes. Then added 5 mL oxalic acid 5% and allowed to stand 2 minutes. Furthermore, added with 5 mL ascorbic acid 4% and distilled water to the mark. Solution was homogenized and the absorbance was measured by UV-Vis spectrophotometry at 815 nm.

Precision test was carried out by the same procedure to the linearity test by replacing the standard solution to 10 mL industrial water sample. Absorbance measurements performed seven times. Accuracy test procedure used was the same as precision test procedures but 10 mL of the standard solution silicate 10 mg/L added as a spike. Absorbance measurements were also carried out seven times. The absorbance of the reagent blank were measured seven times by the same procedure with precision test but without samples water for determining the detection limit.

RESULT AND DISCUSSION

The principle of the determination of Fe with ASTM D.1068.84 method is the reduction of Fe^{3+} in the sample to Fe^{2+} by hydroxylamine hydrochloride in acidic conditions. Fe^{2+} ions then reacted with 1,10-fenantrolin form reddish orange complex compounds and absorbance was measured at 510 nm. This complex compound is very stable at pH 4-6 which is maintained by the addition of ammonium acetate. The reaction is as follows:

$$4Fe^{3+} + 2NH_2OH \longrightarrow 4Fe^{2+} + N_2O + H_2O + 4H^+$$
$$Fe^{2+} + 3C_{18}H_8N_2 \longrightarrow [Fe(C_{18}H_8N_2)_3]^{2+}$$

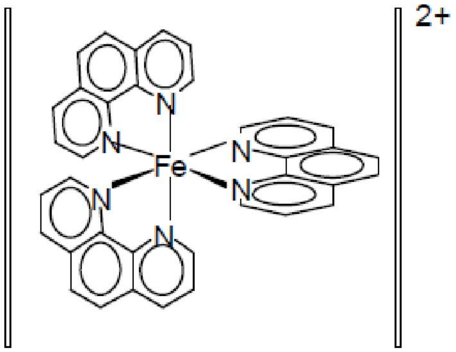


Figure 1. The structure of the iron-1,10-phenanthroline compound

The common aqueous forms of silica are H_4SiO_4 and $H_3SiO_4^-$ (APHA, 1997). In the presence of magnesium, it can form scale deposits in boilers and in steam turbines. Determination of silicate in water based on the reaction between silicate and ammonium molybdate in acid media to form yellow form ß-molybdosilicic acid (silicomlybdate). This complex is reduced by ascorbic acid to form heteropoly molybdous-blue complex and the absorbance was measured at 815 nm. Interference from phosphate, which forms a phosphomolybdate complex was eliminated by the oxalic acid introduced to the sample before the addition of the ascorbic acid reagent.

Linearity test

Linearity test for analysis method conducted to demonstrate the capability of the method to produce a linear relationship between the concentration of the instrument response from a series of standard solutions. Linearity of an analysis procedure can also be defined as the ability (within a given range) to generate the data analysis that is proportional to the concentration of analyte in the sample. Linearity should be determined by using a minimum of five standards whose concentration span 80 –120% of the expected concentration range.

The parameters used to indicate that relationship is linear correlation coefficient. The results may not show a significant deviation from linearity, which means the value of the correlation coefficient R> 0.99 in the working range (80-120%). In addition, the acceptance criteria of linearity was also determined from intercept where the value of the intercept does not differ much from zero, or less than 2% (McPolin, 2009).

Linearity test in Figure 2 shows the value of the coefficient of determination R^2 0.996 for Fe and 0,998 for SiO_2. It means that the correlation coefficient were 0.998 for Fe and 0,999 for SiO_2. Figure 2 also shows that the intercept were less than 2%. These data indicate that the method of determination of Fe and SiO_2 by UV-Vis Spectrophotometry meet the criteria to guarantee the quality of the analysis data.

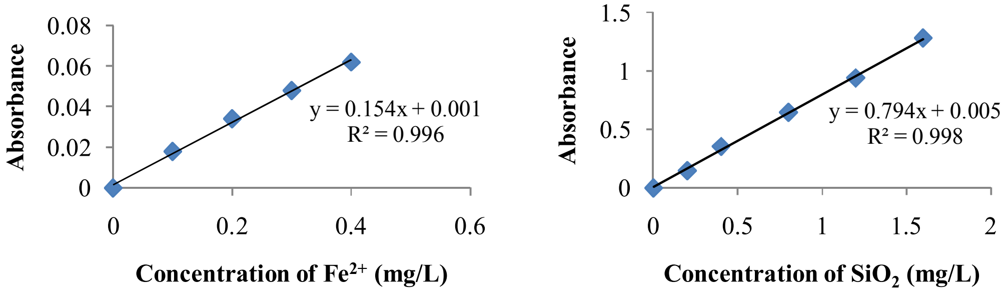


Figure 2. Calibration curve of Fe and SiO_2 standard solution

Precision test

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (APVMA, 2004). Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Parameter used to determined precision value in this paper was repeatability. Repeatability expresses the precision under the same operating conditions (samples, analyst, instrument, laboratory) over a short interval of time. Repeatability is also termed intra-assay precision (Huber, 2010). Repeatability value can be used for evaluating consistency of analyte and the difficulty of the methods. % RSD was used to determine the precision parameters criteria which criteria applied in PT Krakatau Steel was the % RSD <2% (high precision), 2-5% (moderate precision), and> 5% (low precision).

Table1. Repeatability data for test methods of Fe and SiO_2 content in water samples

	Absorbance		Concentration (mg/L)	
No	Fe	SiO_2	Fe	SiO_2
1	0.017	0.533	0.220	16.782
2	0.018	0.531	0.233	16.719
3	0.019	0.512	0.246	16.120
4	0.018	0.513	0.233	16.152
5	0.019	0.512	0.246	16.120
6	0.018	0.531	0.233	16.719
7	0.018	0.533	0.233	16.782
Mean			0.235	16.485
SD			0.00897	0.33248
%RSD			3.819	2.017

The data in Table 1 show that the % RSD value for seven repetitions in testing Fe and SiO_2 in the range 2-5% This means that both methods have moderate precision. Then, repeatability limit (r) can be calculated from %RSD using the equation (Haider, 2006):

$$r = 2.83 \text{ x } \%RSD$$

Thus, r value for testing Fe was 10.81% while for testing SiO_2 was 5.71%. r value were used to judged the equivalency of two test result within one laboratory where if they differ by more than the r value they shall be judged not equivalent. r value is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

Accuracy test

Accuracy (bias) is a measure of the difference between the expectation of the test result and the accepted reference value (true value) due to systematic method and laboratory error. The accuracy of an analytical method may be determined by any of the following ways:

1. Analyzing a sample of known concentration (for example, a control sample or certified reference material) and comparing the measured value to the true value.
2. Compare the results of the method with results from an established reference method (the uncertainty of the reference method is known).
3. If certified reference materials or control samples are not available, a blank sample matrix of interest can be spiked with a known concentration by weight or volume. The resulting mixture is assayed, and the results obtained are compared with the expected result.
4. If a blank sample matrix are not available, a standard addition method can be used. Standard addition method (spiked sample). Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample.

Table 2. Accurcy data for test methods of Fe and SiO_2 content in water samples

No	Concentration of sample (mg/L)		Concentration of spike (mg/L)	Concentration of spiked sample (mg/L)		recovery (%)	
	Fe	SiO_2		Fe	SiO_2	Fe	SiO_2
1	0.220	16.782	1	1.090	17.821	87.00	103.90
2	0.233	16.719	1	1.116	17.600	88.30	88.10
3	0.246	16.120	1	1.103	17.254	85.70	113.40
4	0.233	16.152	1	1.155	17.222	92.20	107.00
5	0.246	16.120	1	1.142	17.128	89.60	100.80
6	0.233	16.719	1	1.090	17.695	85.70	97.60
7	0.233	16.782	1	1.103	17.726	87.00	94.40
Mean						87.93	100.74

This paper used the last method to assess accuracy by calculate the percentage recovery of spiked samples. Table 2. shows the data of accuracy assessment of testing Fe and SiO_2 in industrial water. The acceptance criteria for accuracy was determined from %recovery value, which must be in the range of 85-115%. From Table 2. it can be concluded that both method have meet the criteria with the %recovery values for Fe and SiO_2 test method are 87.93 and 100.74%, respectively.

Detection Limit

The detection limit (DL) is the smallest number analyte in a sample that can be detected which still gives significant response compared with a blank (Harmita, 2004). Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental as follow:

1. Based on visual evaluation: the detection limit is determined by the analysis f samples with known concentrations of analye and by establishing the minimum level at which the analyte can be reliably detected.
2. Based on signal-to-noise. Determination of signal-to-noise rati is performed by comparing measures signals from samples with known low concentration of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected.

3. Based on standard deviation of the response and the slope. The detection limit may be expressed as:

$$DL = 3.3\,\sigma/S$$

where σ is standard deviation of the response, S is the slope of calibration curve
4. Based on the standard deviation of the blank. Measured of magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these response.
5. Based on the calibration curve. A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of regression line or the standard deviation of y-intercept of regression lines may be used as the standard deviation (Kalra, 2011).

Table 3. Detection limit data for test methods of Fe and SiO_2 content in water samples

No	Absorbance of blank		Concentration of blank (mg/L)	
	Fe	SiO_2	Fe	SiO_2
1	0.006	0.006	0.039	0.008
2	0.005	0.004	0.032	0.005
3	0.005	0.005	0.032	0.006
4	0.004	0.004	0.026	0.005
5	0.005	0.003	0.032	0.004
6	0.003	0.005	0.019	0.006
7	0.004	0.006	0.026	0.008
Mean			0.029	0.006
SD			0.00637	0.00153
DL			0.049	0.011

This paper used method which based on standard deviation of blank to measured DL which can be calculated as:

$$DL = \bar{x} + 3\sigma$$

where $\bar{x}$ is mean of blank concentration and σ is standard deviation of the blank. The data of DL assessment is shown in Table 3. The detection limits of UV-Vis Spectrophotometer instrument used in this research were 0.049 mg/L for Fe and 0.011 mg/L for SiO_2. It means that analyte whose concentration less than DL may not be detected.

Verification parameters assessment included linearity, precision, accuracy and detection limit indicate that the method of determining the content of Fe and SiO_2 using UV-Vis Spectrophotometer according to ASTM 019 D.1068.84 and SOP.3133 QP02 have met the criteria. Therefore both methods can be used as a method for routine procedures and produce valid data in order to control the quality of the water industry in PT Krakatau Steel.

CONCLUSION AND SUGGESTION

1. The value of correlation coefficient for linearity test, %RSD for precision test, % recovery for accuracy test and limits of detection for the determination of Fe with ASTM D.1068.84 method are respectively 0.996; 3.803%; 87.94%; and 0.048 mg/L.
2. The value of correlation coefficient for linearity test, %RSD for precision test, % recovery for accuracy test and limits of detection for the determination of SiO_2 with SOP.3133 QP02 019 method are respectively 0.998; 2.014%; 100.76%; and 0,010 mg / L.
3. Parameter value of method verification indicates that the test method of Fe and SiO_2 by UV-Vis spectrophotometry meet the requirements and can be used as a routine method for testing water samples in the laboratory chemical industry PT Krakatau Steel Cilegon

REFERENCES

APHA, 1997, *Standard Methods*, 22nd ed., Method 4500-SiO_2, American Public Heatlh Association

APVMA, 2004, *Guidelines For The Validation Of Analytical Methods For Active Constituent, Agricultural And Veterinary Chemical Products*, Australian Pesticide and Veterinary Medicines Authority, Australia

Haider, S.I., 2006, *Validation Standard Operating Procedures: A Step by Step Guide for Achieving Compliance in Pharmaceutical, Medical Device and Biotech Industries*, CRC Press, Taylor & Francis Group, Florida

Harmita, 2004, Petunjuk Pelaksanaan Validasi Metode dan Cara Perhitungannya, *Majalah Ilmu Kefarmasian*, Vol.1 No.3

Huber, L., 2010, *Validation of Analytical Methods*, Agilent Technologies, Germany

Kalra, K., 2011, *Method Development and Validation of Analytical Procedure*, InTech, Rijeka, Croatia

Lehrman, A., Blumenschein, C.D., Doran, D.J., and Stewart, S.E., 1999, *Steel Plant Fuels and Water Requirements*, AISE Steel Fondatioon, Pittsburgh

McPolin, O., 2009, *Validation of Analytical Methds for Pharmaceutical Analysis*, Mourne Training Services, Northern Ireland

Mussey, O. D., 1961, *Water requirements of the copper industry*: U.S. Geol. Survey Water-Supply Paper 1330-E, p. 181-219

Walling, F.B and Ottis, L.E., 1967, *Water Requirement of the Iron and Steel Industry*, US Government Printing Office

Widyatmiko, E.D.S., 2005, *Prarancangan Suatu Industri Kimia*, Seminar Nasional MIPA, UNY 8 Februari 2005

C-18

CHALCONES: THE PROMISING COMPOUNDS TO PROVIDE NEW WAYS FOR CANCER TREATMENT

Retno Arianingrum

Department of Chemistry Education, Faculty of Mathematics and Natural Science,
Yogyakarta State University

Abstract

Chalcones (1,3-diphenylpropen-1-ones), a biosynthetic product of the shikimate pathway, belonging to flavanoid family are precursors of open chain flavonoids and isoflavonoids, which are abundant in edible plants. They have a wide variety of cytoprotective and modulatory functions, which may have therapeutic potential for multiple diseases, especially as antitumor drugs. Several natural and synthetic chalcones and their derivatives appear as promising anticancer activities. Their chemical structure evaluation will be critical to assess their therapeutic utility. Those for which the mechanism of action is well defined can serve as lead compounds for the design of new, more promising molecules. The present review highlights the recently natural synthesized chalcones and their derivatives possessing important pharmacological activities as anticancer and how the mechanism pathways of these compounds in inhibiting cancer cells.

Key words: chalcones; cancer treatment.

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by both external factors (tobacco, infectious organisms, chemicals, and radiation) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote the development of cancer. Cancer is treated with surgery, radiation, chemotherapy, hormone therapy, biological therapy, and targeted therapy (American Cancer Society, 2012).

In the United States and many other parts of the world, cancer is a serious public health issue. Currently, 1 in 4 deaths in the United States is due to cancer. It is estimated that 1,529,560 new cancer cases and 569,490 deaths from cancer are projected to occur in the United States in 2010 (Jemal et al., 2010). According to the data base of health research (Riskesdes, Health Department of Republic of Indonesia), the prevalence of tumors in Indonesia was 4.3 per 1000 population (Depkes RI, 2007). Besides this, it is also predicted that cancer will overtake heart disease as the world's top killer by 2010 and this trend would make more than a double the global cancer cases and deaths by 2030 . So it is the time to better understand the prognosis, diagnosis and treatment of cancer .

Chalcones (α,β-unsaturated ketones) are promising candidates in the new era of medicines on account of their wide spectrum of antitumor, antibacterial and anti-inflammatory activities (Sasayama et al., 2007; Ye et al. 2004; Ye et al., 2005; Lee et al., 2006; Haraguchi et al., 1998; Hsieh et al., 1998; Flechtner et al., 1979; Chang et al., 2007; Rauf et al., 2005; and Pandey et al., 2007). However, the mechanisms of actions of this class of compounds are not yet fully understood, especially in anticancer. The purpose of this review is to provide an overview of the anticancer activity of naturally occurring and synthetic chalcones. This review highlights more recent pharmacological screening of these compounds, their mechanisms of action and relevant structure-activity relationships.

DISCUSSION

1. Structure and Synthesis of Chalcones

Chalcones, one of the major classes of natural products with widespread distribution in spices, tea, beer, fruits and vegetables, have been recently subject of great interest for their pharmacological activities (Dicarlo et al., 1999). Chalcones are precursor compounds for flavonoid synthesis in plants. Naturally occurring chalcones are found mostly in their hydroxylated forms, and have been reported to possess antiinflammatory, antimicrobial, antioxidant and anticancer properties (Echeverria et al., 2009; Nowakowska et al., 2007; Miranda et al., 1999; Shah et al.,2008; Boumendjel et al., 2009; Katsori et al., 2009; Dimmock et al, 1999 and Go et al., 2005).

Chalcones have crystal structure. They are α,β-unsaturated ketones consisting of two aromatic rings (ring A and B) having diverse array of substituents (figure 1). Rings are interconnected by a highly electrophilic three carbon α,β-unsaturated carbonyl system that assumes linear or nearly planar structure. They contain the ketoethylenic group (–CO–CH=CH). Chalcones possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings (Rahman, 2011).

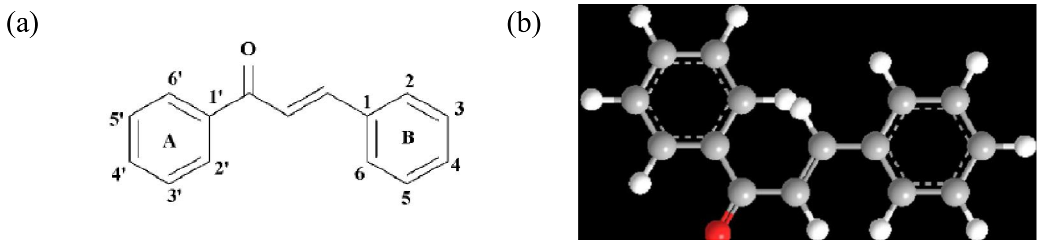


Figure 1. (a) Structure of chalcone, (b) The energy minimazed 3D structure of chalcone

Chalcones can be readily synthesized in laboratory by the Claisen-Schmidt reaction which is very easy and simple to conduct as well as inexpensive. The simplest chalcone can be prepared by an aldol condensation between a benzaldehyde and an acetophenone in the presence of sodium hydroxide as a catalyst (Arty, 2010).

2. The Potential of Chalcones in Cancer Treatment

a. Inhibition of activation NF-□B by chalcone

Cancer is regulated by a number of genes, which are in turn regulated by transcription factors. Among these transcription factors, NF-κB plays a major role in development and progression of cancer because it regulates more than 400 genes involved in inflammation, cell survival, cell proliferation, invasion, angiogenesis, apoptosis, cell cycle and metastasis. Incorrect regulation of NF-□B may cause inflammatory and autoimmune diseases, viral infection and cancer (Yadav et al., 2011and Pahl, 1999).

In tumor cells, NF-κB is active either due to mutations in genes encoding the NF-κB transcription factors themselves or in genes that control NF-κB activity (such as IκB genes); in addition, some tumor cells secrete factors that cause NF-κB to become active. Blocking NF-κB can cause tumor cells to stop proliferating, to die, or to become more sensitive to the action of anti-tumor agents. Thus, NF-κB is the subject of much active research among pharmaceutical companies as a target for anti-cancer therapy (Escárcega et al., 2007).

NF-κB consists of homo- or heterodimers of the Rel family proteins, p50/NFκB1, p52/ NFκB2, p65/RelA, and c-Rel. In most cell types studied to date, in resting stage NF-κB dimers are retained in the cytoplasm through a physical association with inhibitor proteins, termed IκBα (Baeuerle et al., 1988). The classic form of NF-□B is a heterodimer p50 and p65 subunits. Following cell activation, IκBα becomes hyperphosphorylated on distinct serine residues, and a mounting body of evidence indicates that this hyperphosphorylation targets the inhibitor for proteolytic degradation (Finco et al., 1995). The degradation of IκB eventually leads to its dissociation from NF-κB dimers, thereby allowing the movement of the latter towards the nucleus, where they may bind with high specificity to enhancer sequences in the 5′ regulatory region of target genes (figure 2).

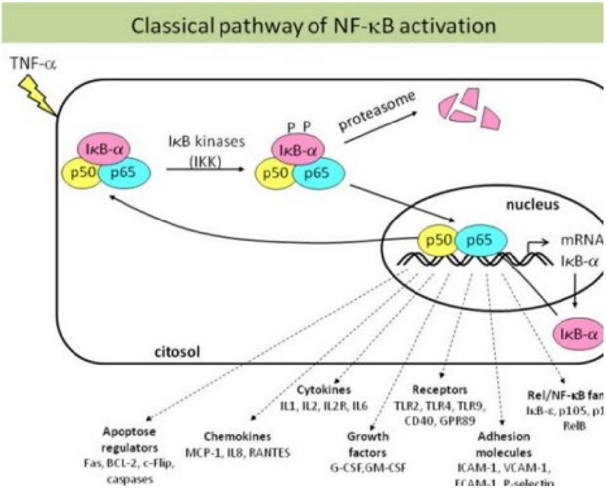


Figure 2. The classical pathway of NF-kB activation (http://redox.fc.ul.pt/research.html.)

NF-kB is often excessively activated in human solid tumor dan leukumias. Activation NF-kB in cancer cell may enhance cancer progression through the activation of cancer cell growth, apoptotic resistance, and increased metastatic activity. To treat or prevent diseases such as cancer that have an etiology based in inflammation, the agents that inhibit the inflammation and have no side effects be required. Among the candidates is chalcone, which is known as a potent anti-inflammatory agent.

Chalcone and some derivate chalcones have been known to inhibit the activation of NF-□B such as 2′,5′-dihydroxy-4-chloro-dihydrochalcone; Broussochalcone A; 3,4,5-trimethoxy-4′-fluorochalcone; 3′,4′,5′,3,4,5-hexamethoxy-chalcone; Xanthoangelol D; 4-Hydroxyloncho-carpin; Flavokawain A, B; 2′-hydroxychalcone; Cardamomin; Isoliquiritigenin;1,3-diphenyl-2-propenone (chalcone); 2′-hydroxy-3-bromo-6′-methoxychalcone; 2′-methoxy-3,4-dichloro-chalcone; Butein; Cardamonin; Hydroxysafflor yellow A; Licochalcone A; 2′,4′,6′-tris(methoxymethoxy) chalcone; 3-hydroxy-4,3′,4′,5′-tetramethoxychalcone; Xanthohumol; and Isoliquiritigenin 2′-methyl ether (Yadav et al., 2011) through several mechanisms. Flavokavains A and B obtained from Piper methysticum cause inhibition of both IκB degradation and subsequent translocation of p50 and p65 NF-κB subunits from the cytoplasm to the nucleus (Folmer et al., 2006). Butein (3,4,2′,4′-tetrahydroxychalcone), natural compound obtained from stem-bark of cashews (Semecarpus anacardium), blocked the phosphorylation and degradation of IκBα by inhibiting IKK activation which is direct and involved cysteine residue 179. This correlated with the suppression of phosphorylation and the nuclear translocation of p65 (Pandey et al., 2007).

b. Chalcones arrests cell cycle progression and induces apoptosis

Both normal cells and cancer cells have growth through a cell cycle. Regulation of cell cycle determines process of cell growth. In cancer cells occurs abnormal regulation of the cell cycle. Cell Cycle consists of proliferative phase, in the resting state (no cells divides, Go), and not permanently divide. Cells that are dividing divided into 4 (four) major phases: the gap phase 1 (G1), synthesis phase (S), gap phase 2 (G2), and mitosis phase (M) (Foster et al., 2001 and Vermeulen et al., 2003). In normal cell, the cell cycle dependent on growth signals from the environment. If the signal is not sufficient growth, the cells are in the G1 phase of the cell cycle exit can enter the Go phase (Van den Heuvel, 2005).

G1 phase is the phase when the cells prepare for DNA replication that will occur in the S phase, when DNA synthesis is complete the cell then enters the G2 phase, in this phase the cells prepare for cell division, when the cell is ready to enter the mitotic phase (M). Cells that are in the G1 phase may decide to enter into S phase or enter the Go phase. Go phase is the phase for on-proliferating cells (Lodish et al., 2000 and Vermeulen et al., 2003). The move from one phase to the next phase of the cell cycle is regulated by three main groups, namely cyclin proteins (cyclin D, cyclin E, cyclin A, and cyclin B), cyclin dependent kinases (CDKs, especially CDK4, CDK6, and CDK2), and cyclin dependent kinase inhibitors (CKIs) (King, 2000).

Disturbance in the cell cycle regulator will cause disruption of the program of cell cycle. In cancer cells, the cell cycle cannot be regulated so that cells become divided continually. Therefore, the development of research on cancer, cell cycle regulators is the potential for targeted anticancer drugs.

Cancer cells are also able to avoid apoptosis mechanism. Apoptosis is cell death program that occurs as a result of the induction of the cell itself. Apoptosis can occur due to intrinsic factors when cells undergo irreversible damage DNA. Triggers apoptosis caused by extrinsic factors involving the role of tumor necrosis factor receptor, called the death receptors, viz. TNF - 2 receptor CD95 (Fas/APO-1), and TRAIL receptor (Lodish et al ., 2000). Proteins that play a role in the regulation of apoptosis are p53, Bcl-2 family proteins, Apaf, Caspase inhibitors pro apoptosis protein (as well as receptors that respond to death signals). Cells undergoing apoptosis has several characteristics, among others, increased expression pro apoptosis proteins (Bax , Bid and Bak) and suppression antiapoptosis protein expression (Bcl - 2 and Bcl - xL), increased levels of cytosolic cytochrome C, caspase activation, PARP1 activation, DNA fragmentation, and cell membrane damage. Accumulation of these characteristics led to the emergence of a variety of apoptotic bodies that result from cell fragmentation (Gerl and Vaux, 2005) .

Chalcone have been found to act through the intrinsic as well as extrinsic apoptosis pathway to prevent tumor progression. Basic structure of chalcone (1,3-diphenyl1-2-propenone) has proven to have a chemopreventive effect in human breast cancer cell lines: MCF-7 and MDA-MB-231 (Hsu et al. 2006) and human bladder cancer cell lines: T24 and HT-1376 (Shen et al. 2007). The research showed that chalcone inhibits the proliferation of T24 and HT-1376 cells by inducing apoptosis and blocking cell cycle progression in the G2/M phase. Chalcone significantly increases the expression of p21 and p27 proteins, and decreases the levels of cyclin B1, cyclin A and Cdc2, thereby contributing to cell cycle arrest. In addition, chalcone increased the expression of Bax and Bak, but decreased the levels of Bcl-2 and Bcl-XL and subsequently triggered mitochondrial apoptotic pathway (release of cytochrome c and activation of caspase-9 and caspase-3). The induction of mitochondrial pathway and inhibition of the nuclear factor kappa B survival system may play important roles in the antiproliferative activity of chalcone in T24 and HT-1376 cells.

Many natural chalcones have been shown to induce apoptosis in different types of cancer cells through a wide variety of mechanisms. Among the most important of these are xanthoangelol, flavokawain B, xanthohumol, isoliquiritigenin, flavokawin A, isobavachalcone, cardamonin, licochalcone A, and butin (Table 1). These triterpenoids have a common target, Bcl-2 protein, which can induce apoptosis in cancer cells.

Table 1. Molecular targets of known synthetic and natural chalcones for anticancer and anti-inflammatory activities chalcones

Chalcone	Targets
2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone	NF-κB, KDR tyrosine kinase, Bim, Bcl-2, caspase-3, erbB-2 receptor, PARP, TNF-α, IL-6, IL-1β, iNOS
4-Hydroxylonchocarpin	NF-κB, MMP-2, iNOS
Broussochalcone A	NF-κB, iNOS, PKC, NADPH oxidase
Butein	NF-κB IAP2, Bcl-2, Bcl-xL, cyclin D1, c-Myc, COX-2 MMP-9, DR5, STAT3, ICAM-1, Bax, caspase-3, EGFR TIMP-1, E- selectin, iNOS, JNK, IL-8, MMP-7, Mcl-1, hTERT, ATM, Chk1/2, cdc25C, Cdc2, Sp-1, VEGF, CXCR4
Cardamomin	NF-κB, TNF-α, COX-2, Akt, DR4/5, Bcl-xL, CHOP
Cardamonin	NF-κB, COX-1/2, TNF-α, iNOS, mTOR, P70S6K, 4E-BP1
Flavokawain A	NF-κB, Bax, Bcl-xL, XIAP, survivin, p27, p27, CDK1/2, Myt1, Wee1, cyclin B1, cdc25C
Flavokawain B	NF-κB, DR5, Bim, Puma, survivin, GADD153, PARP, Bid, caspase-8, Bak, cytochrome C, Bcl-2, iNOS, COX-2, TNF-α, caspase-3/9, Bax, XIAP
Hydroxysafflor yellow A	NF-κB, TNF-α, ICAM-1, IL-1β, IL-6, IL-10, VEGF, p53, Bcl-2/ Bax ratio, HIF-1α, VHL, ET-1, iNOS
Isobavachalcone	caspases -3/9, Bax, A
Isoliquiritigenin	LOX-5/12, caspase-3/8/9, p53, p21, Fas/APO-1 receptor, FasL, Bax, NOXA, NF-κB, Bcl-xL, cIAP-1/2, COX-2, iNOS, cytochrome C, PARP, quinone reductasse, GADD153 ICAM-1, VCAM-1, Bcl-2, MMP-2, ATM, Chk2, topoisomerase II, HO-1, IL-1β, TNF-α, cyclin B1/D1/E, CDK4, p27, cdc25c, IRF3, IP-10, RANTES, Nrf2, mTOR, VEGF, TLR4, uPA, MMP-9, TIMP-1
Kanzonol C	MMP-2
Licochalcone A	NF-κB, COX-1/2, Bax, Bcl-2, STAT3, CD31, Ki-67, VEGFR2, iNOS, CCL2/MCP-1, CXCL1/KC, mTOR, TNFα, topoisomerase-1, cyclin B1/D1/E, Rb, cdc2, CDK4/6
Naringenin chalcone	IL-2, IL-4, IL-5, IL-13, INF-γ, TNF-α, MCP-1, p38 MAPK
Xanthoangelol	NF-κB, caspases -3/9, VEGF, thromboxane B2, ET-1
Xanthohumol	NF-κB, Bax, p53, Akt, survivin, Bcl-xL, XIAP, cIAP1/2 cyclin D1, c-myc, VEGF, PARP, caspase-3/7/8/9, Bcl-2 Keap1, E- cadherin, TLR4, MD2, STAT1α, IRF-1, IL-1β p21, p53, Bcr- Abl, IL-2, IFN-γ, MCP-1, GRP78, Hsp70, PERK, ATF6, CHOP, Mcl-1, XBP-1, IL-8, IL-12

Chalcones influence invansion, metastasis and angiogenesis

Tumor progression is a complex and multifaceted process that involves initiation, growth, invasion, and metastasis. Tumors would not grow beyond the limits of diffusion without stimulating a vascular system for the delivery of nutrients. Key stimulators and inhibitors of angiogenesis along with the tumor microenvironment regulate tumor growth. The relative concentrations of these stimulators and inhibitors determine endothelial cell phenotype, with the change from a quiescent to angiogenic phenotype referred to as the "angiogenic switch." The tumor vasculature also provides tumor cells with a pathway through which to enter the circulation and metastasize.

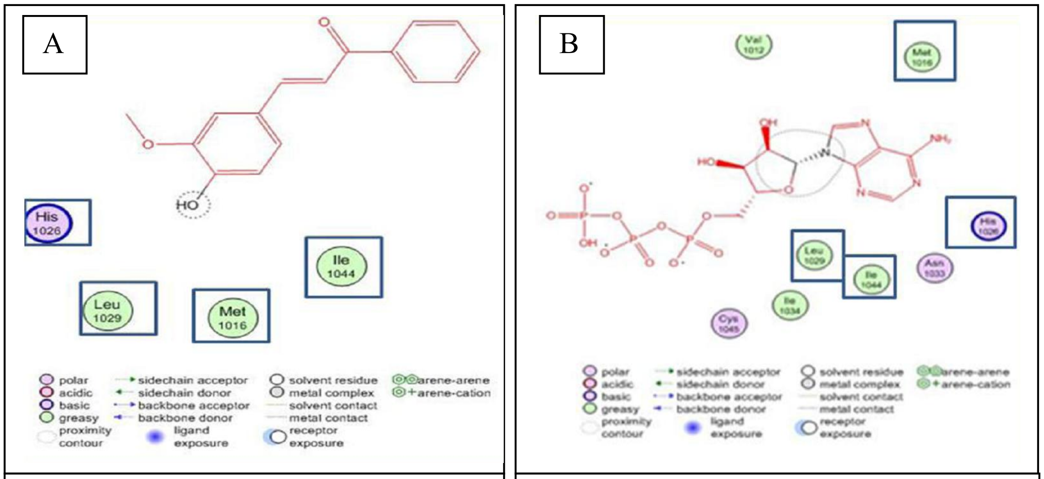
In metastasis the cancer cells migrate from their origin to other parts of the body, via either the bloodstream or lymphatic system. Migration and invasion of tumor cells are promoted by the loss of interaction of adherens junctions with the cytoskeleton, subsequent changes in the activities of Rho family small GTPases (most prominently Rac1, Cdc42, and RhoA), and the concomitant reorganization of the actin cytoskeleton (Noren et al., 2000 and Sahai et al., 2002). Among the factors influencing invasion, which affects whether or not a tumor will metastasize, are matrix metalloproteinase (MMPs) and ICAM-1. MMPs (specifically MMP2 and MMP9) are endopeptidases that degrade the basement membrane components separating the cells from their surrounding tissue and enabling them to move freely and spread to other tissues (Noujaim et al., 2002).

Vascular endothelial growth factor (VEGF) plays a unique role in physiological and pathological angiogenesis. VEGF promotes endothelial cell proliferation and migration, increases vascular permeability and inhibits apoptosis of endothelial cells lining newly formed vessels. There are numerous splice isoforms of VEGF that bind with varying degrees of affinity to VEGF receptors (VEGFR) on the surface of endothelial cells. Most of the angiogenic effects attributed to VEGF are a result of activation of VEGFR-2, which signals through the phosphatidyl-inositol 3 kinase (PI3K)/Akt pathway (Zachary, 2003).

A few chalcones derived from natural sources have been shown to inhibit tumor cell invasion and metastasis by targeting one or more molecules (Table 1). Butein induces down-regulation of MMP-9 gene in human leukemia cells in vitro (Pandey et al., 2007). The different compounds, kanzonol C, 4-hydroxylonchocarpin, paratocarpin, stipulin and dorsamanin A, are potential, naturally-occurring antitumor drugs that inhibit MMP-2 secretion from brain tumor-derived glioblastoma cells (Ngameni et al., 2006).

Chalcones also have potential to inhibit tumor angiogeneis, an important consideration because the growth of human tumors and development of metastases depends on the de novo formation of blood vessels (McMahon, 2000). These vessels enhance tumor growth by providing oxygen and nutrition. They also help tumor cells to migrate, invade, and metastasisze. Inhibition of the VEGF tyrosine kinase signaling pathway blocks new blood vessel formation in growing tumors, leading to stasis or regression of tumor growth. Xanthoangelo inhibits tumor-induced neovascularization, inhibiting the formation of capillary-like tubes by vascular endothelial cells and inhibiting the binding of VEGF to vascular endothelial cells.

Recently, the molecular docking study of chalcone derivate i.e. 3 - (4'-hydroxy-3'-methoxyphenyl)-1-phenyl-2-propene-1-on showed that the binding energy of this compound with VEFGR (2P2I) almost had the same energy binding compared with ATP binding on VEFGR. Docking results between these compounds with the target protein VEGFR tyrosine kinase receptor showed the similarity of amino acids involved in their interaction (figure 3). It is expected this compound has potential as a cancer chemopreventive agent especially as anti angiogenesis (Arianingrum et al., 2013).



Docking score energy: -86.9007

Docking score energy: -86.9007

Figure 4 . Interaction of 3 - (4'-hydroxy-3'-methoxyphenyl)-1-phenyl-2-propene-1-on (A) and ATP (B) with VEGFR (2P2I), showed the similarity of amino acid involved in their interaction (marked with boxes).

Structure Activity Relationship of Chalcones

Chalcone compounds have ortho- (i.e. 2′, 3′- and 3′,4′-) and para- (i.e. 2,5′-) substitutions. Chalcones possess conjugated double bonds and a completely delocalized Π-electron system on both benzene rings. Molecules possessing such a system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions. The basic structure of chalcone (1,3-diphenyl-2-propenone) has NF- κB inhibitory activity at the concentration of 50 μM (Shen et al., 2007).

Chalcones with substituents that increase the electronic density of the B-ring, such as methoxy, butoxy or dimethylamine groups, did not show significant activity in the inhibition of the nitrite production. The B- ring has a flexible ring structure and can easily convert cis-chalcone to trans-chalcone or vice versa. Because of this reason, some of the chalcones that have a single substitution at the B-ring, like xanthohumol, isoliquiritigenin, butein, cardamonin, 2′,5′-dihydroxy-4- chloro-dihydrochalcone, work best at higher concentrations (Israf et al., 2007); Pandey et al., 2007; Harikumar et al., 2009; Huang et al., 2001; Kumar et al., 2007). At the same time some natural and synthetic chalcones with trimethoxy in the B-Ring act by inhibiting nitrite production. If there is trimethoxy chalcone at the A-ring with fluoro, chloro, bromo substitution in on the B-Ring, like 2′-hydroxy-3-bromo-6′-methoxychalcone, 2′-methoxy-3,4-dichlorochalcone, Flavokawain A, or Flavokawain B, then they are better inhibitors of NF-κB (Folmer et al., 2006 and Kim et al., 2007).

Srinivasan et al (2009) synthesized chalcones with trimethoxy substitutions in the A-ring and hydroxyl substitutions in the B-ring. They found that the synthetic compound 1, 2, 3, and 4 inhibited NF-κB even at lower concentrations between 1-8 μM. All the chalcones showed NF-κB inhibition containeda highly electrophilic α,β-unsaturated carbonyl moiety. This α,β-unsaturated carbonyl moiety can act as an electrophile and react with free sulfhydryl groups of thioredoxin and cysteine residues in proteins . Foresti et al (2005) and Srinvisan et al (2009) indicated that electrophilic phytochemicals could give rise to thiyl radicals leading to alkene reduction through a covalent Michaelis addition of nucleophiles, such as SH from cystin from DNA, which binds to NF-κB.

CONCLUSION AND SUGGESTION

From the above review, it can be said that chalcones and their derivatives display a wide range of anticancer activities in particular to their role in suppression of NF-κB-mediated inflammation and cancer. Chalcones are easy to synthesize, further enrich the structural diversity of the template through the introduction of features normally associated with ligand-receptor interaction, namely hydrophobic groups, hydrogen bond donor and acceptor features. Chalcones are highly multifunctional and thus are promising as agents in the treatment of cancer because of their ability to block the NF-κB activation, induce apoptosis, and to inhibit proliferation, invasion, metastasis and angiogenesis. So these natural and synthesic chalcones may serve as lead compound for cancer drug development.

REFERENCES

American Cancer Society. 2012. *Cancer Facts & Figures 2012*. Atlanta: American Cancer Society: 1-6.

Arianingrum, R., Hermawan, A., Meiyanto, E., and Purnomo, H., 2013, Molecular Docking Studies and Anticancer Activity of Chalcone Derivate Compound MPHC on MCF-& Doxorubicin Resistent Cell. Proceeding in 44th World Chemistry Conggress 11-16 August, 2013, Istanbul Turkey, 427.

Arty, I.S., 2010, Synthesize and Citotoxicity Test of Several Compounds of Mono Para Hidroxy Chalcone, *Indo. J. Chem*., 10 (1), 110-115

Baeuerle PA, Baltimore D. 1988. Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-kappa B transcription factor. Cell.: 53:211–7.

Boumendjel, A.; Ronot, X.; Boutonnat, J. 2009. Chalcones derivatives acting cell cycle blockers: Potential anticancer drugs? *Curr. Drug Targets*, *10*, 363–371.

Chang, H.J.; Yoon, G.; Park, J.S.; Kim, M.H.; Baek, M.K.; Kim, N.H.; Shin, B.A.; Ahn, B.W.; Cheon, S.H.; Jung, Y.D. 2007. Induction of apoptosis by the licochalcone E in endothelial cells via modulation of NF-B and Bcl-2 family. Biological and Pharmaceutical Bulletin, 30, 2290- 2293

Departemen Kesehatan Republik Indonesia. 2007. Riset Kesehatan Dasar, Badan Penelitian dan Pengembangan (Balitbang), Jakarta.

Dicarlo, G.; Mascolo, N.; Izzo, A.A.; Capasso, F. 1999. Flavonoids: Old and new aspects of class of natural therapeutic drugs. *Life Sci.*, *65*, 337–353.

Dimmock JR, Elias DW, Beazely MA, Kandepu NM 1999. Bio- activities of chalcones. Curr Med Chem;6:1125–49.

Echeverria, C.; Santibanez, J.S.; Donoso-Tauda, O.; Escobar, C.A.; Ramirez-Tagle, R. 2009. Structural antitumoral activity relationships of synthetic chalcones. *Int. J. Mol. Sci.*, *10*, 221–231.

Escárcega RO, Fuentes-Alexandro S, García-Carrasco M, Gatica A, Zamora A .2007. The transcription factor nuclear factor-κB and cancer. *Clinical Oncology (Royal College of Radiologists (Great Britain))* 19 (2): 154–61Flechtner, T.W. Carbohydrate triflates: reactions with bases. Carbohydr. Res. 1979, 77, 262-266. 10. Chang, H.J.; Yoon, G.; Park, J.S.; Kim, M.H.; Baek, M.K.; Kim, N.H.; Shin, B.A.; Ahn, B.W.; Cheon, S.H.; Jung, Y.D. Induction of apoptosis by the licochalcone E in endothelial cells via modulation of NF-B and Bcl-2 family. Biological and Pharmaceutical Bulletin 2007, 30, 2290- 2293.

Finco TS, Baldwin AS.1995. Mechanistic aspects of NF-kappa B regulation: the emerging role of phosphorylation and proteolysis. Immunity : 3:263–72.

Folmer F, Blasius R, Morceau F, Tabudravu J, Dicato M, Jaspars M, et al. 2006. Inhibition of TNFalphainduced activation of nuclear factor kappaB by kava (Piper methysticum) derivatives. Biochem Pharmacol. : 71:1206–18.

Foresti R, Hoque M, Monti D, Green CJ, Motterlini R. 2005. Differential activation of heme oxygenase-1 by chalcones and rosolic acid in endothelial cells. J Pharmacol Exp Ther. :

312:686–93.

Foster, J.S., Henley, D.C., Ahamed, S., and Wimalasena, J., 2001, Estrogen and Cell Cycle Regulation in Breast Cancer, *Trend Endocr. Metab*. 12(7):320-327

Gerl, R., and Vaux, D.L., 2005, Apoptosis in The Development and Treatment of Cancer, *Carcin*., 26 (2), 263-270.

Go ML, Wu X, Liu XL. 2005.Chalcones: an update on cytotoxic and chemoprotective properties. Curr Med Chem :12:481–99.

Haraguchi, H.; Ishikawa, H.; Mizutani, K.; Tamura, Y.; Kinoshita, T. 1998. Antioxidative and superoxide scavenging activities of retrochalcones in Glycyrrhiza inflata. Bioorg. Med. Chem. : 6, 339-347.

Harikumar KB, Kunnumakkara AB, Ahn KS, Anand P, Krishnan S, Guha S, et al. 2009. Modification of the cysteine residues in IkappaBalpha kinase and NF-kappaB (p65) by xanthohumol leads to suppression of NF-kappaB-regulated gene products and potentiation of apoptosis in leukemia cells. Blood. : 113:2003–13.

Hsieh, H.K.; Lee, T.H.; Wang, J.P.; Wang, J.J.; Lin, C.N. 1998. Synthesis and anti-inflammatory effect of chalcones and related compounds. Pharm. Res. : 15, 39-46.

Hsu, Y L, P L Kuo, W S Tzeng, and C C Lin. 2006. Chalcone Inhibits the Proliferation of Human Breast Cancer Cell by Blocking Cell Cycle Progression and Inducing Apoptosis. *Food and chemical toxicology□: an international journal published for the British Industrial Biological Research Association* 44(5): 704–13.

Huang YC, Guh JH, Cheng ZJ, Chang YL, Hwang TL, Lin CN, et al. 2001. Inhibitory effect of DCDC on lipopolysaccharide-induced nitric oxide synthesis in RAW 264.7 cells. Life Sci.: 68:2435–47.

Israf DA, Khaizurin TA, Syahida A, Lajis NH, Khozirah S. 2007. Cardamonin inhibits COX and iNOS expression via inhibition of p65NF-kappaB nuclear translocation and Ikappa-B phosphorylation in RAW 264.7 macrophage cells. Mol Immunol.; 44:673–9.

Jemal A, Siegel R, Xu J, Ward E. 2010. Cancer statistics. CA Cancer J Clin. ; 60:277–300.

Katsori, A.M.; Hadjipavlou-Latina, D. 2009.Chalcones in cancer: Understanding their role in terms of QSAR. *Curr. Med. Chem*., *16*, 1062–1081.

Kim YH, Kim J, Park H, Kim HP. 2007. Anti-inflammatory activity of the synthetic chalcone derivatives: inhibition of inducible nitric oxide synthase-catalyzed nitric oxide production from lipopolysaccharide-treated RAW 264.7 cells. Biol Pharm Bull. ; 30:1450–5.

King, R. J. B., 2000, *Cancer Biology*, Pearson Education, Second Edition, England. p.1-7,228-231, 263-264

Kumar S, Sharma A, Madan B, Singhal V, Ghosh B. 2007. Isoliquiritigenin inhibits IkappaB kinase activity and ROS generation to block TNF-alpha induced expression of cell adhesion molecules on human endothelial cells. Biochem Pharmacol. ; 73:1602–12.

Lee, Y.S.; Lim, S.S.; Shin, K.H.; Kim, Y.S.; Ohuchi, K.; Jung, S.H. Anti-angiogenic and anti-tumor activities of 2-hydroxy-4- methoxychalcone. Biol. Pharm. Bull. 2006, 29, 1028-1031.

Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P, Baltimore, D., and Darnell, J., 2000, Molecular Cell Biology, 4th edition, New York: W.H. Freeman

McMahon G. VEGF receptor signaling in tumor angiogenesis. Oncologist. 2000; 5 1:3–10.

Miranda, C.L.; Stevens, J.F.; Helmrich, A.; Henderson, M.C.; Rodriguez, R.J.; Yang, Y.H.;Deinzer, M.L.; Barnes, D.W.; Buhler, D.R. 1999. Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupus*) in human cancer cell lines. *Food Chem. Toxicol*., *37*,271–285.

Ngameni B, Touaibia M, Patnam R, Belkaid A, Sonna P, Ngadjui BT, et al. 2006. Inhibition of MMP-2 secretion from brain tumor cells suggests chemopreventive properties of a furanocoumarin glycoside and of chalcones isolated from the twigs of Dorstenia turbinata. Phytochemistry. ; 67:2573–9.

Noren NK, Liu BP, Burridge K, Kreft B. 2000. p120 catenin regulates the actin cytoskeleton via Rho family GTPases. J Cell Biol. ; 150:567–80.

Noujaim D, van Golen CM, van Golen KL, Grauman A, Feldman EL. 2002. N-Myc and Bcl-2 coexpression induces MMP-2 secretion and activation in human neuroblastoma cells. Oncogene. ; 21:4549–57.

Nowakowska, Z. 2007. A review of anti-infective and anti-inflammatory chalcones. *Eur. J. Med. Chem. 42*, 125–137.

Pahl, H L. 1999. Activators and Target Genes of Rel/NF-kappaB Transcription Factors. *Oncogene* 18(49): 6853–66. http://www.ncbi.nlm.nih.gov/pubmed/10602461.

Pandey MK, Sandur SK, Sung B, Sethi G, Kunnumakkara AB, Aggarwal BB. 2007. Butein, a tetrahydroxychalcone, inhibits nuclear factor (NF)-kappaB and NF-kappaB-regulated gene expression through direct inhibition of IkappaBalpha kinase beta on cysteine 179 residue. J Biol Chem. ; 282:17340–50.

Rahman, M A. 2011. Chalcone□: A Valuable Insight into the Recent Advances and Potential. 2011(3): 1–16.

Rauf, S.; Gooding, J.J.; Akhtar, K.; Ghauri, M.A.; Rahman, M.; Anwar, M.A.; Khalid, A.M. Electrochemical approach of anticancer drugs-DNA interaction. J. Pharm. Biomed. Anal. 2005, 37, 205-217.

Sahai E, Marshall CJ. 2002. RHO-GTPases and cancer. Nat Rev Cancer. ; 2:133–42.

Sasayama T, Tanaka K, Mizukawa K, Kawamura A, Kondoh T, Hosoda K, Kohmura E. 2007. Trans-4-lodo,4-boranyl-chalcone induces antitumor activity against malignant glioma cell lines in vitro and in vivo. J. Neu-Onc. 85, 123-132.

Shah, A.; Khan, A.M.; Qureshi, R.; Ansari, F.L.; Nazar, M.F.; Shah, S.S. 2008. Redox behavior of anticancer chalcone on a glassy carbon electrode and evaluation of its interaction parameters with DNA. *Int. J. Mol. Sci. , 9*, 1424–1434.

Shen, Kun-hung, Jiunn-kae Chang, Ya-ling Hsu, and Po-lin Kuo. 2007. Chalcone Arrests Cell Cycle Progression and Induces Apoptosis through Induction of Mitochondrial Pathway and Inhibition of Nuclear Factor Kappa B Signalling in Human Bladder Cancer Cells. 24: 254–61.

Srinivasan B, Johnson TE, Lad R, Xing C. 2009. Structure-activity relationship studies of chalcone leading to 3-hydroxy-4,3′,4′,5′-tetramethoxychalcone and its analogues as potent nuclear factor kappaB inhibitors and their anticancer activities. J Med Chem. ; 52:7228–35.

Van den Heuvel, S., 2005, *Cell-cycle Regulation*, WormBook, oi/10.1895/wormbook.1.28.1, Http://www.wormbook.org

Vermeulen, Katrien ., Bockstaele, D.R., and Berneman, Z.N., 2003. The Cell Cycle: a Review of Regulation, Deregulation and Therapeutic Targets in Cancer", *Cell. Proliff.*, 36: 131-149.

Vivek R. Yadav, Sahdeo Prasad, Bokyung Sung, and Bharat B. Aggarwal. 2011. The Role of Chalcones in Suppression of NF-κB-Mediated Inflammation and Cancer. *Int Immunopharmacol* 11(3): 295–309.

Ye, C.L.; Liu, J.W.; Wei, D.Z.; Lu, Y.H.; Qian, F. 2004. In vitro anti-tumor activity of 2, 4-dihydroxy- 6-methoxy-3, 5-dimethylchalcone against six established human cancer cell lines. Pharmacol. Res. , 50, 505-510.

Ye, C.L.; Liu, J.W.; Wei, D.Z.; Lu, Y.H.; Qian, 2005. F. In vivo antitumor activity by 2, 4-dihydroxy-6- methoxy-3, 5-dimethylchalcone in a solid human carcinoma xenograft model. Canc. Chemo. Pharm., 55, 447-452.

Zachary I. 2003. Vascular endothelial growth factor and antiangiogenic peptides as therapeutic and investigational molecules. IDrugs 6:224–231)

C-19

ELECTROCOAGULATION OF DETERGENT *WASTEWATER USING ALUMINIUM WIRE NETTING ELECTRODE (AWNE)*

Riyanto[1*] and Afifah Hidayatillah[1]**

[1]*Department of Chemistry, Islamic University of Indonesia,*
Jl. Kaliurang KM 14,5 Sleman Yogyakarta Indonesia 55584
**Corresponding author, tel: (0274) 896439 ext 3012; fax: (0274) 896439,*
Email addresses: riyanto@uii.ac.id
***Co-author, email: blue.archer16@yahoo.com*

Abstract

Electrocoagulation of detergent wastewater using aluminium wire netting electrode has been carried out. The electrocoagulation method was performed in a two electrodes system using aluminium wire netting as an anode and cathode electrode. Detergent wastewater is characterized by chemical oxygen demand (COD) concentrations and absorption spectra using spectrophotorometer UV-Visible. Electrocoagulation is carried out in electrochemical cell containing 100 mL detergent wastewater, without supporting electrolyte. In this study electrocoagulation of detergent using applied voltage 5, 10, 15 and 20 Volt with various electrolysis time. The result, of the study showed aluminium wire netting electrode has higher degradation of detergent wastewater.

Keywords: electrocoagulation, detergent, waste water, aluminium wire netting

INTRODUCTION

Water pollution with detergents, is of great importance to satisfy the increasing demands for water for various uses. These detergents compounds do not decompose or degrade in aquatic systems. These detergents are very harmfully and toxic. The accumulation of some detergents in waste water represents a serious environmental problem. The removal of detergents from aqueous solutions is very important from the environmental point of view (El-Said 2004).

Detergents are substances or preparations containing soaps or other surfactants intended for water based laundry or dishwashing processes. Detergents may be used in any form (liquid, powder, paste, bar, cake, molded piece, shape, etc.), widely for household laundry products, domestic and industrial cleaners, cosmetic products, and industrial purposes. Surfactants are organic substances, used in detergents, intentionally added to achieve cleaning, rinsing and/or fabric softening due to its surface-active properties (Bruns and Jelen 2009). They consist of one or more hydrophilic and hydrophobic groups of such nature and size that they are capable of forming micelles Surfactants belong to a group of chemicals of high environmental relevance due to their large production volumes. They are mainly discharged into the environment by the wastewater pathway, either after treatment in a wastewater treatment plant or directly where no treatment system is available. Environmental compartments which may be influenced by surfactants are the freshwater environment (water body and sediment), the soil if surfactant-loaded sewage sludge is added, and the marine environment (Bruns and Jelen 2009). Surfactants are widely used for domestic and industrial purposes, primarily as detergents in cleaning applications.

Surfactants removal operations involve processes such as chemical and electrochemical oxidation (Lissens, *et al.*, 2003; Mozia, *et al.,* 2005), membrane technology (Sirieix-Plénet, *et al.,* 2003; Kowalska, *et al.,* 2004; Fernández , *et al.,* 2005), chemical precipitation (Talens-Alesson, *et al.,* 2002), photocatalytic degradation (Ohtaki, *et al.,* 2000; Zhang, *et al.,* 2003), adsorption (Ogita, *et al.,* 2000; Lin, *et al.,* 2002; Adak, *et al.,* 2005) and various biological methods (Dhouib, *et al.,* 2003; Chen, *et al.,*2005). Among the currently employed chemical unit processes in wastewater treatment, coagulation-flocculation has received considerable attention for yielding high pollutant removal efficiency. This process can be directly applied to wastewaters without being affected by the toxicity in the wastewater and can constitute a simple, selective and economically acceptable alternative.

Electrochemical technologies such as electrolysis have been successfully employed for the treatment of many wastewaters on an industrial scale, for example, oil and grease (O&G) containing wastewaters. The electrochemical technologies have reached such a state that they are not only comparable with other technologies in terms of cost but also are more efficient and more compact (Dae et al. 2013). The electrochemical oxidation of detergent to CO_2 occurs without chemical agent and with a significant rate the potential region of oxygen evolution. It is commonly assumed that electrogenerated hydroxyl radicals are very active in the degradation of organic molecules. This species is the most powerful oxidant in water.

This paper reports a study of the electrocoagulation of detergent wastewater. Electrochemical degradation of organic pollutants, presence in the wastewater needs specific electrodes (Aboulhasan et al. 2006). Electro coagulation experiments on detergent were carried out with aluminium wire netting electrode. Electrocoagulation involves the in situ generation of coagulants by dissolving electrically either aluminum or iron ions from aluminum or iron electrodes, respectively. The metal ions generation takes place at the anode, hydrogen gas is released from the cathode. The hydrogen gas would also help to float the flocculated particles out of the water. This process sometimes is called electroflocculation (Songsak, 2006).

EXPERIMENTAL

Materials

All solutions were prepared by dissolving their analytical grade reagent (Merck) in deionised distilled water. AWN electrodes (Aldrich Chemical Company) and H_2SO_4 from Merck was used for preparation of an AWN electrode. All solutions for COD analysis were prepared from Merck using deionised distilled water.

Sampling procedures

Samples of effluent are collected from domestic laundry wastewater in Sleman, Yogyakarta, Indonesia. The generated effluent is discharged into the sea without any treatment. Sampling of the detergent wastewater is carried out according to standard methods for the examination of wastewater.

Electrode preparation

A metal electrode made of aluminium was used, and the length of each electrode with a width of 10mm was 10mm. Electrodes are made as tube (Figure 1B for anode and 1C for cathode), so as to have more surface area.

Electrochemical measurements

Electrochemical measurements were carried out in a two electrode using DC Power Supply. Aluminium wire netting electrodes were used as anode and cathode electrodes with difference size electrode (Figure 1B and 1C). The electrochemical process of detergent wastewater was performed at room temperature (without electrolyte). The electrochemical coagulation studies by potential constant were performed in 100 mL capacity glass electrochemical cell. The experiments were performed in a two electrodes system using *AWNE* as a anode and cathode electrode.

Analytical procedures
The detergent wastewater degradation results were analyzed using Spectrophotometer UV-Visible Hitachi U-2010 at wavelength 200-400 nm. The chemical oxygen demand (COD) was determined by common photometric tests using Spectrophotometer UV-Visible Hitachi U-2010 according to Standard Methods (SNI 6989.2-2009).

RESULT AND DISCUSSION

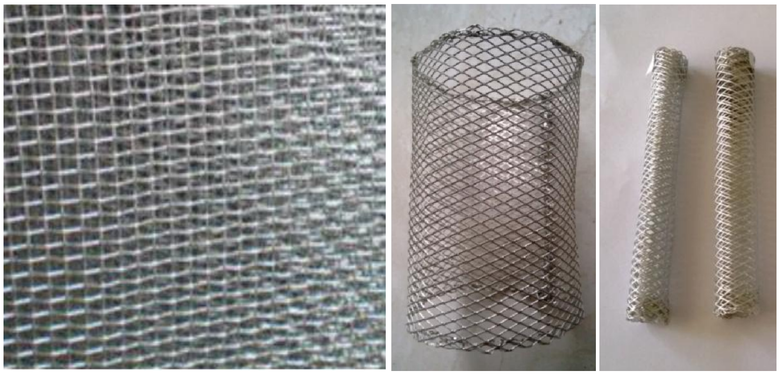


Figure 1. Physical structure of Aluminium Wire Netting Electrode (AWNE) of electrode surface (A); anode (B) and cathode (C)

Figure 1 showed type of aluminium used in studies is woven mesh or aluminium wire netting (AWN). Woven mesh was more effective in increasing current than expanded mesh and solid electrode (Yimin et al. 2010). The larger current per applied voltage produced by AWNE result from larger active surface areas than those calculated or different effects of structures on electro coagulation of detergent. Based on superior performance of the aluminium woven mesh, solid electrode was not examined in further studies. According to (Yimin et al. 2010), the impact of mesh configuration on current was further examined through correlations between mesh number, wire diameter, pore size and surface area and current densities. In this analysis, larger correlation coefficients indicate the factor to be more relevant to variations in current generation. Electrodes are made as tube (Figure 1B and 1C), so as to have more surface area.

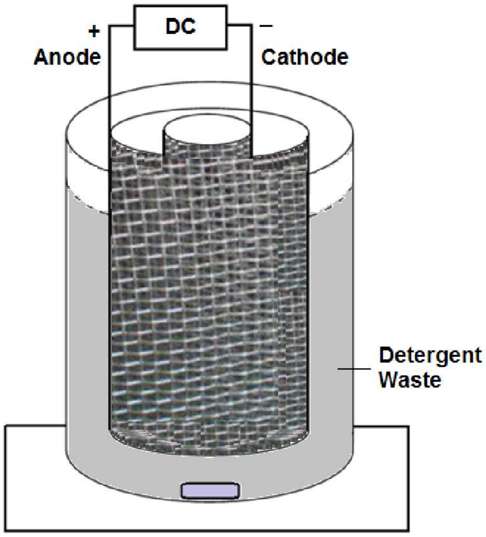


Figure 2. Schematic diagram of electrocoagulation system

The electrocoagulation system in this study is comprised of three parts: reactor for electrolysis, power supply, and AWN electrode in Figure 2. The electrolysis reactor consists of a total liquid volume of 200 mL. Reactor for electrolysis was composed of a cathode and anode. The electrode gap between the cathode and anode was 1.0 cm. A metal electrode made of aluminium was used, and the length of each electrode with a width of 10 mm was 10 mm. The continuous electrolysis system was designed to adjust the potential constant. The electrolysis experiment was performed under constant voltage of 5-20V. DC power supply was used in the system.

Figure 3. showed of scheme of electrocoagulation organic compounds using Al anode. The Al^{3+} or Fe^{2+} ions are very efficient coagulants for particulates flocculating. The hydrolyzed aluminum ions can form large networks of Al–O–Al–OH that can chemically adsorb pollutants. Aluminum is usually used for water treatment and iron for wastewater treatment (Comninellis 1994). The advantages of electrocoagulation include high particulate removal efficiency, compact treatment facility, relatively low cost and possibility of complete automation. The chemical reactions taking place at the anode are given as follows (Songsak, 2006).

For aluminum anode:

$$Al \rightarrow Al^{3+} + 3e^- \quad (1)$$

At alkaline conditions

$$Al^{3+} + 3OH^- \rightarrow Al(OH)_3 \quad (2)$$

At acidic conditions

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+ \quad (3)$$

In addition, there is oxygen evolution reaction

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (4)$$

The reaction at the cathode is

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (5)$$

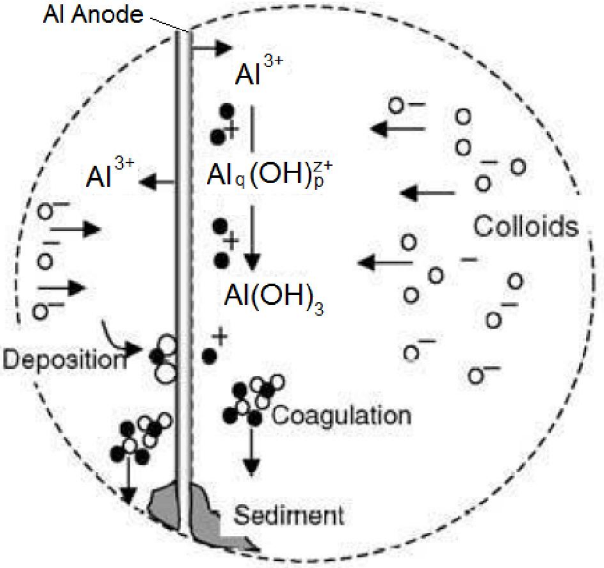


Figure 3. Scheme of electrocoagulation, modified from Den and Huang (2005)

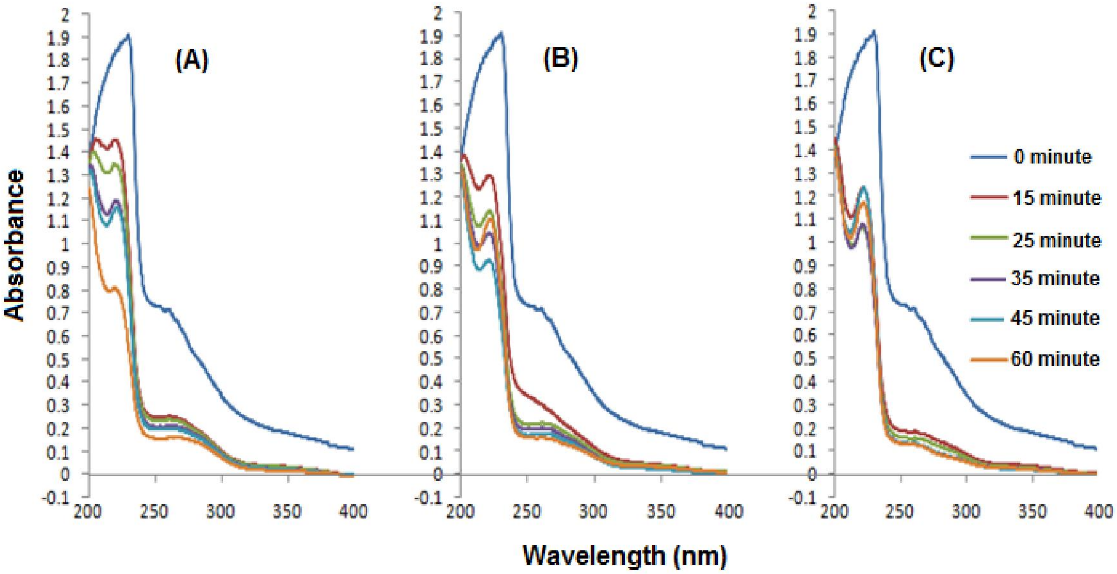


Figure 4. UV scan results at 200–400nm of the electro coagulation at different electrolysis time, with aluminium wire netting electrode of: (A) 10 Volt (B) 15 volt and (C) 20 volt.

Figure 4. showed UV scan results of selected electrolysis runs at a potential of 10, 15 and 20 Volt. It can be concluded that electrocoagulation of the detergent took place from the disappeared peak of 240 nm in the electrolysis process. Electrolysis then stopped at different stages with various carboxylic acids. These results showed that high efficiency both for phenol oxidation to benzoquinone, and benzoquinone oxidation, which is related to the aromatic rings opening can be obtained on anodes (Dae et al. 2013).

Figure 4A, showed a maximum decrease of absorbance with electrolysis time 60 minutes. While, Figure 4B, showed a significant decrease at electrolysis time 15 minutes. The decline occurred only up to 45 minutes. Based on Figure 4, the optimum conditions of wastewater electrolysis at potential and electrolysis time are 15 Volt and 45 minutes, respectively. The longer of the contact time of the wastewater with electrodes will be the efficiency removal. The ability of the aluminium electrode in detergent wastewater is limited so despite prolonged contact time. The effect of the formation of $Al(OH)_3$ at the anode surface will be cause covered electrode surface. In addition, the reaction at the anode is also inhibited so that the oxygen binding of surfactant alkyl benzene is also reduced. Decreased surfactant as an organic compound can be determined by analysis of wastewater COD. Although not very accurate to say so, but examination of COD had to know include organic ingredients. The chemical oxygen demand (COD) was determined by Spectrophotometer UV-Visible Hitachi U-2010 according to SNI 6989.2-2009.

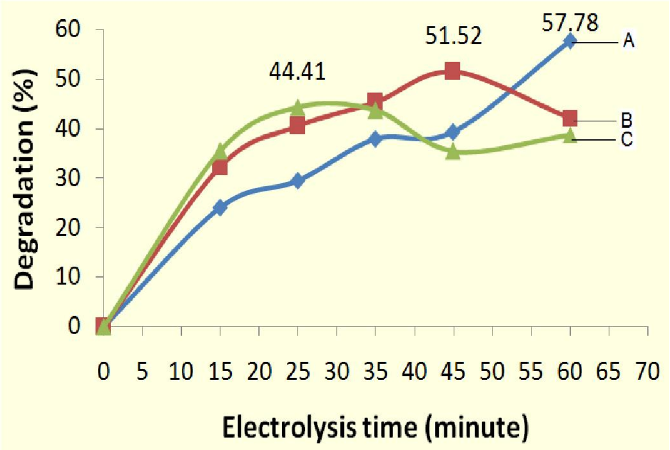


Figure 5. Percent degradation after electrocoagulation of detergent wastewater with various electrolysis time and potential at 10 Volt (A), 15 Volt (B) and 20 Volt (C)

Table 1. Results of the COD determination experiments performed before and after electrocoagulasi with electrolysis time 60 minutes

Detergent Wastewater	Potential (Volt)	COD (mg/L)*
Initial Detergent Wastewater	0	2270
After electrolysis	10	407
After electrolysis	15	523
After electrolysis	20	587

*Average of three determinations

The chemical oxygen demand (COD) is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. For samples from a specific source, COD can be related empirically to BOD, organic carbon, or organic matter (Songsak, 2006). Table 1 showed the electrocoagulation studies performed to good chemical oxygen demand (COD) removals. The specific removals in COD are very dependent on the time necessary to perform the electrocoagulation and, in general, the electrocoagulation times increased with stirring. For the applied current densities, and since the initial COD contents of the samples were high, the degradation process, apart from the final stage of the assays with UP samples, must be controlled by current. The values of COD showing that, in fact, they performed anodic oxidations must have been controlled during most of the assay by current. The electrocoagulation of laundry wastewater using aluminum electrodes, maximum percent degradation and COD are 57.78% and 82.07%, respectively (Figure 5).

CONCLUSIONS

According to the obtained results, the application of combined electrochemical techniques, namely electrocoagulation is very good for degradation of laundry wastewater. The combined treatment, COD removals for laundry wastewater samples were always higher than 82%. In general, the use of stirring increases the time needed to start, with a visible rate, the precipitation of the flocs formed in the electrocoagulation. On the other hand, the electrocoagulation time is reduced by an increase in the applied potential, due to a higher rate of aluminium oxidation. The result of the study showed aluminium wire netting electrode has higher degradation of detergent wastewater. The electrocoagulation of laundry wastewater using aluminum electrodes, maximum percent degradation and COD are 57.78% and 82.07%, respectively.

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REFERENCES

Aboulhassan, M. A. Souabi, Yaacoubi and Baudu, 2006, Removal of Surfactant from Industrial Wastewaters by Coagulation Flocculation Process, *Int. J. Environ. Sci. Tech.,* 3 (4): 327-332, 2006.

Adak, A.,Bandyopadhyay, M. and Pal, A., 2005. Removal of anionic surfactant from wastewater by alumina: a case study. *Colloid. Surface*., 254, 165-171.

Bruns, U.M. and Jelen, E. 2009, Anaerobic Biodegradation of Detergent Surfactants, *Materials*, *2*, 181-206.

Chen, H. J., Tseng, D. H. and Huang, S. L., 2005. Biodegradation of octylphenol polyethoxylate surfactant Triton X-100 by selected microorganisms. *Biores. Technol*., 96, 1483-1491.

Comninellis, C. 1994, Electrocatalysis in the electrochemical conversion/combustion of organic pollutants for waste water treatment. *Electrochimica Acta,* Vol. 39: 1857-862.

Den, W. and Huang, C. 2005, Electrocoagulation for removal of silica nano-particles from chemical–mechanical-planarization wastewater. *Colloids and Surfaces A: Physicochemical Engineering Aspects* Vol. 254: 81–89.

Dae G. K., Woo Yeol Kim, Chan Young Yun, Dong Jin Son, Duk Chang, Hyung Suk Bae, Yong Hyun Lee, Young Sunwoo, Ki Ho Hong, 2013, Agro-industrial Wastewater Treatment by Electrolysis Technology, *Int. J. Electrochem. Sci.,* 8, 9835 – 9850.

Dhouib, A., Hamad, N., Hassaïri, I. and Sayadi, S., 2003. Degradation of anionic surfactants by *Citrobacter braakii. Proc. Biochem*., 38, 1245-1250.

Fernández, E., Benito, J. M., Pazos, C. and Coca, J., 2005. Ceramic membrane ultrafiltration of anionic and nonionic surfactant solutions. *J. Mem. Sci.,* 246, 1-6.

Kowalska, I., Kabsch-Korbutowicz, M., Majewska-Nowak, K., Winnicki, T., 2004. Separation of anionic surfactants on ultrafiltration membranes. *Desalination*, 162, 33-40.

Lissens, G., Pieters, J., Verhaege, M., Pinoy, L. and Verstraete, W., 2003. Electrochemical degradation of surfactants by intermediates of water discharge at carbon-based electrodes. *Electrochemi. Acta*, 48, 1655-1663.

Lin, Y., Smith, T. W. and Alexandridis, P., 2002. Adsorption of a polymeric siloxane surfactant on carbon black particles dispersed in mixtures of water with polar organic solvents. *J. Coll. Interf. Sci.,* 255, 1-9.

Mozia, S., Tomaszewska, M. and Morawski, A.W., 2005. Decomposition of nonionic surfactant in a labyrinth flow photoreactor with immobilized TiO2 bed. *Appl. Catal. Environ*., 59, 155-160.

Ohtaki, M., Sato, H., Fujii, H. and Eguchi, K., 2000. Intramolecularly selective decomposition of surfactant molecules on photocatalytic oxidative degradation over TiO2 photocatalyst. *J. Molecul. Catalysis.*, 155, 121-129.

Ogita, M., Nagai, Y., Mehta, M. A., and Fujinami, T., 2000. Application of the adsorption effect of optical fibres for the determination of critical micelle concentration, *Sensor. Actuator*., 64, 147-151.

Saad M. El-Said, 2004, Adsorptive Removal Of Some Detergents From Waste Water Using Friendly Environmental Materials, *Ass. Univ. Bull. Environ. Res*. Vol. 7 No. 2, 137-147.

Sirieix-Plénet, J., Turmine, M. and Letellier, P., 2003. Membrane electrodes sensitive to doubly charged surfactants. Application to a cationic gemini surfactant. *Talanta*, 60, 1071-1078.

Songsak K. 2006, *Restaurant wastewater treatment by electrochemical oxidation in continuous process*, Dissertation, Department of Chemical Technology, Faculty of Science, Chulalongkorn University, Thailand.

Talens-Alesson, F. I., 2001. Binding of pesticide 2,4-D to SDS and AOS micellar flocculates. *Colloid. Surfac*., 180, 199-203.

Yimin Z. Matthew D. Merrill, Bruce E. Logan, 2010, The use and optimization of stainless steel mesh cathodes in microbial electrolysis cells, *International Journal of Hydrogen Energy*, 35, 12020-12028.

Zhang, T., Oyama, T., Horikoshi, S., Zhao, J., Serpone, N. and Hidaka, H., 2003. Photocatalytic decomposition of the sodium dodecylbenzene sulfonate surfactant in aqueous titania suspensions exposed to highly concentrated solar radiation and effects of additives. *Appl. Environ.*, 42, 13-24.

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CHARACTERIZATION K_3PO_4/NaZSM-5 USING XRD AND FTIR AS A CATALYST TO PRODUCE BIODIESEL

Samik[1], Ratna Ediati[2], and Didik Prasetyoko[3]

[1]*Chemistry Department, Mathematical and Natural Sciences Faculty,*
Surabaya State University (samikunesa@gmail.com, Tel.: +6285731160005), Indonesia
[2,3]*Chemistry Department, Mathematical and Natural Sciences Faculty,*
Institute of Technology Sepuluh Nopember, Indonesia

Abstract

Synthesis of NaZSM-5 and impregnation of K_3PO_4 catalyst with concentration 5, 10, and 15 wt.% on the resulting NaZSM-5 as a catalyst to produce biodiesel have been done in this study. This study is an experimental study, the research stages include the synthesis and characterization NaZSM-5 and K_3PO_4/NaZSM-5. Characterization by X-Ray Diffraction (XRD) was conducted to determine the X-ray diffraction patterns and the percentage crystallinity of the samples. X-ray diffraction pattern of the resulting sample showed the emergence of diffraction peaks with high intensity at $2\theta = 7.8$; 8.8; 23.0; 23.2, and 24.3° are specifically indexed to the structure of the MFI topology. The percentage crystallinity of the sample becomes smaller with increasing number of K_3PO_4 impregnated on NaZSM-5 synthesis. Characterization of the samples by Fourier Transform Infra Red (FTIR) aims to determine the type of functional groups present in a compound. FTIR spectra of samples NZ, NZK5, NZK10 and NZK15 have absorption bands in the region 1220 and 547 cm^{-1} which shows the absorption characteristics of vibration T - O - T on NaZSM-5 type zeolite which distinguish the other and sensitive to changes in structure. The results of characterization using XRD and FTIR showed that the structure of NaZSM-5 remained unchange after impregnated with K_3PO_4.

Keyword: K_3PO_4/NaZSM-5, the diffraction pattern, the percentage crystallinity, absorption characteristics

INTRODUCTION

Biodiesel, a mixture of fatty acid alkyl esters, has been developed as one of the most promising alternative fuel for fossil fuels regarding the limited resources of fossil fuels and the environmental concerns [1]. Almost all commercial biodiesel is currently produced by using homogeneous catalyst (i.e., KOH, NaOH and H_2SO_4). Even though homogeneous catalyzed biodiesel production processes are relatively fast and achieve high conversions, the removal of the homogeneous catalyst after reaction may be a significant problem. This is a concern, since aqueous quenching causes the formation of stable emulsions and saponification, making separation of alkyl-esters difficult, resulting in the generation of large amounts of waste water, and need a high cost in operation especially for waste water treatment [1-3]. For this reason, heterogeneous catalyst is likely to be used replacing homogeneous catalyst in the near future.

Heterogeneous base catalysts are more effective than acid catalysts and enzymes. From the economic standpoint, it would be ideal if solid base catalysts could work efficiently at temperatures below 150^oC and low pressure. On the other hand, solid acid catalysts, enzymes and non-catalytic supercritical transesterification have been largely ignored in biodiesel research due to pessimistic expectations in terms of reaction rates, undesirable side reactions and high costs [4].

Several heterogeneous base catalysts that used for biodiesel production are ZnO-Al_2O_3/ZSM-5 yield 99,00% [2], K_3PO_4 yield 97,30% [5], KF/Ca-Al hidrotalcite yield 97,14% [6], Mg/MCM-41 yield 85,00% [7], KI/silika mesopori yield 90,09% [3]. In this experiment, K_3PO_4 is chosen as catalysts because yield of biodiesel 97,30%, relatively insoluble in methanol and oil [5]. NaZSM-5 supported K_3PO_4 were prepared by impregnation. Thus the aim of this work is to evaluate character of NaZSM-5 and K_3PO_4/NaZSM-5 by XRD and FTIR.

RESEARCH METHOD

Synthesis

The NaZSM-5 crystals were prepared by procedure similar to that described by Khalifah [8]. The seeding gels were prepared by dissolving 1.28 gram sodium aluminate in a solution (12.6 mL TPAOH and 62.5 mL water). After its complete dissolution, 28.2 ml TEOS was added in a solution. This solution was stirred for 15 min and heated at 60 ^oC for 6 h. After gel formed, was added 11.952 gram CTABr little by little and was stirred until its complete dissolution. Subsequently the crystallisation of the seeding gels was carried out using autoclave for 24 h at 150 ^oC. Finally the solid was separated by filtration, washed with distilled water until pH neutral and dried, firstly for 24 h at 60 ^0C and subsequently for 24 h at 110 ^0C. The occluded surfactant (CTA^+) and the organic template (TPA^+) were removed by calcination in air at 550 ^oC for 10 h.

The K_3PO_4 solution was impregnated on NaZSM-5 by impregnation with concentration of K_3PO_4 at 5, 10, and 15 wt.%. The catalyst was dried at 110 °C for 24 h followed by calcining at 550 °C for 10 h so that produce K_3PO_4/NaZSM-5 catalyst. The resulting NaZSM-5 and K_3PO_4/NaZSM-5 catalyst with concentration of K_3PO_4 at 5, 10, and 15 wt.% are labeled NZ, NZK5, NZK10 and NZK15.

Characterisation

The X-ray diffraction patterns and the percentage of crystallinity of NZ, NZK5, NZK10 and NZK15 were characterized by Phillips Expert X-Ray Diffraction (XRD) with Cu K_α radiation (40 kV, 30 mA) at scale $2\theta = 5\text{-}40^o$ and rate of scan 0,04 ^{o}/second. The infrared spectra were recorded using the KBr pellets and Shimadzu Instrument Spectrum One 8400S Fourier Transform Infrared (FTIR) Spectrometer in the range between 4000 and 400 cm^{-1}.

RESULT AND DISCUSSION

X-ray Diffraction

The X-ray diffraction pattern of K_3PO_4, the resulting samples (NZ, NZK5, NZK10 and NZK15) and ZSM-5 was producted by Zhu et al. [9] are shown in Fig. 1. The XRD pattern of the resulting sample showed the emergence of diffraction peaks that similar with high intensity at 2θ = 7.8; 8.8; 23.0; 23.2, and 24.3°.

This XRD resulting is similar as the XRD patterns of ZSM-5 was reported by Zhu et al. [9] with high intensity at 2θ = 7.8; 8.8; 23.1; 23.4, and 24.4° are specifically indexed to the structure of the MFI topology. On the basis of this characterization, NZ, NZK5, NZK10 and NZK15 included the structure of the MFI topology and not be found the another ZSM-5 type.

Fig. 1 also show diffraction pattern of the K_3PO_4 with high intensity at $2\theta = 31.8^0$. The presence of K_3PO_4 impregnated on NaZSM-5 does not show new peak of K_3PO_4 at NZ, NZK5, NZK10 and NZK15, suggesting that the structure of NaZSM-5 is not destroyed during the process of catalyst preparation and K_3PO_4 has well dispersed on NaZSM-5. Zhang et al. [10] observe nothing diffraction peak of PtSnNa catalyst after impregnated on ZSM-5, suggesting that the structure of ZSM-5 is not destroyed during the process of catalyst preparation. Some parts of Na^+ and Sn^{4+} could enter the zeolite main channels, while Pt particles were located mainly on the external surface of the zeolite.

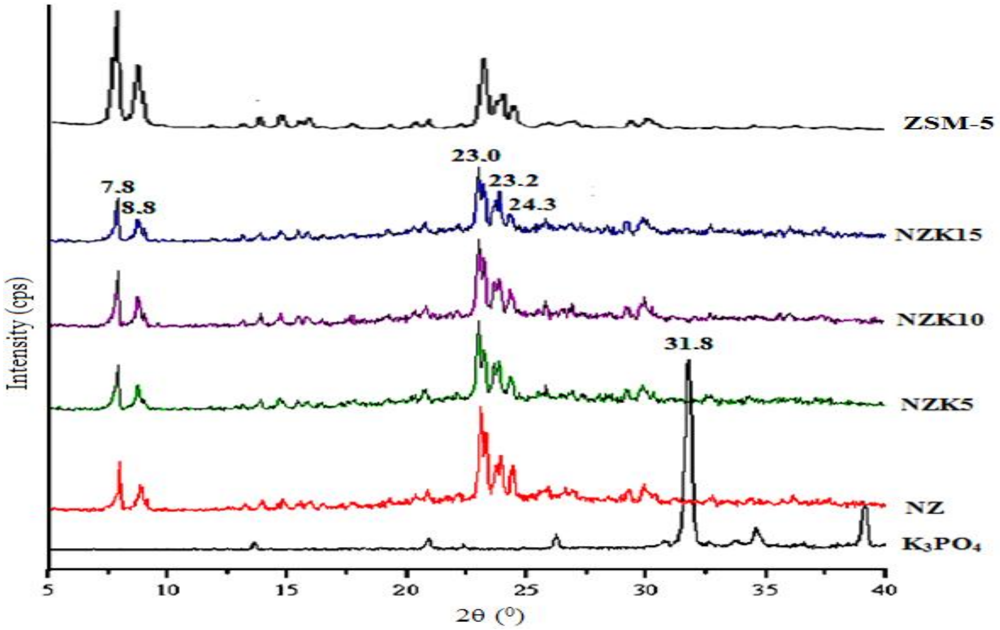

Fig. 1. The X-ray diffraction pattern of K_3PO_4, the resulting samples (NZ, NZK5, NZK10 and NZK15) and ZSM-5 was producted by Zhu et al. [9]

The intensities of NaZSM-5 characteristic peaks were decreasing caused by increasing of K_3PO_4 concentration. The phenomenon decreasing of the intensities of ZSM-5 characteristic peaks also reported by Kim et al. [2] after ZnO-Al_2O_3 and SnO-Al_2O_3 was impregnated on ZSM-5.

Table 1 show relationship percentage crystallinity with percentage K_3PO_4 impregnated on NaZSM-5, that calculated based on intensity main peak at $2\theta = 23°$ using highest intensity sample (NaZSM-5) as comparator standard that assumed has percentage crystallinity = 100. The percentage crystallinity of the samples becomes smaller with increasing number of K_3PO_4 impregnated on NaZSM-5 synthesis.

Table 1. Relationship percentage crystallinity with percentage K_3PO_4 impregnated on NaZSM-5

Sample	2θ	Intensity, I	I/Io	% Crystallinity
NZ	23.10	376.70*	1.00	100
NZK5	23.00	334.18	0.89	89
NZK10	23.01	314.18	0.83	83
NZK15	23.00	271.50	0.72	72

* comparator standard (Io)

FTIR Spectra

FTIR spectra between 400–1300 cm^{-1} of K_3PO_4 and the resulting samples (NZ, NZK5, NZK10 and NZK15) are shown in Fig. 2. In this range all resulting samples possess one broad band at 1000–1200 cm^{-1}, and three bands around 795, 547, and 455 cm^{-1}. FTIR spectra of samples NZ, NZK5, NZK10 and NZK15 have absorption bands in the region 1220 and 547 cm^{-1} which shows the absorption characteristics of vibration T-O-T on NaZSM-5 type zeolite which distinguish the other and sensitive to changes in structure.

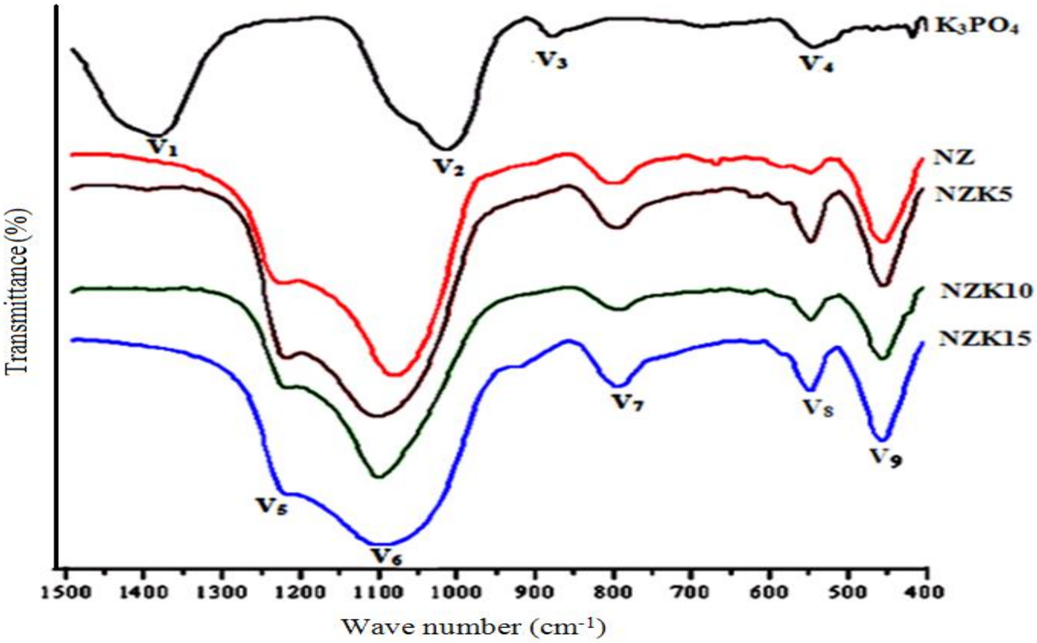

Fig. 2. FTIR spectra of K_3PO_4 and the resulting samples (NZ, NZK5, NZK10 and NZK15)

Traditionally [8,11], the bands in the range at around 1000–1200 and 455 cm^{-1} are attributed to the structure insensitive internal tetrahedron asymmetric stretching vibrations T-O-T and bending vibrations Si-O-Si, respectively. The band at around 795 cm^{-1} can be attributed to both structure insensitive internal tetrahedron and structure sensitive external tetrahedron symmetric stretching vibrations Si-O-Si. The structure sensitive band, appearing in the presented spectra around 547 cm^{-1}, is attributed to five-ring units in the structures of pentasil zeolites like ZSM-5 (Table 2).

FTIR spectra of K_3PO_4 have absorption bands in the region 1396, 1000 and 551 cm^{-1}. The bands at 1396 and 879 cm^{-1} is attributed to vibrations of carbonate ion from CO_2 gas adsorbed by K_3PO_4 [12-14]. The bands at 1000 and 551 cm^{-1} is attributed to stretching vibrations and bending vibrations of phosphate ion on K_3PO_4 [12].

Building on FTIR spectra, the presence of K_3PO_4 impregnated on NaZSM-5 does not show new bands of K_3PO_4 at NZ, NZK5, NZK10 and NZK15. So, the analysis resulting of characterization using XRD and FTIR show that the structure of NaZSM-5 remained unchange after impregnated with K_3PO_4.

Table 2. Wave number of of K_3PO_4, ZSM-5 and the resulting samples (NZ, NZK5, NZK10 and NZK15)

Bands	Wave number (cm^{-1})						Absorption characteristics
	K_3PO_4	ZSM-5 Gonçalves, et al [11]	NZ	NZK 5	NZK 10	NZK 15	
V_1	1396	-	-	-	-	-	Vibrations of carbonate ion
V_2	1014	-	-	-	-	-	Stretching vibrations of phosphate ion
V_3	879	-	-	-	-	-	Vibrations of carbonate ion
V_4	551	-	-	-	-	-	Bending vibrations of phosphate ion
V_5	-	1220	1222	1219	1215	1215	Structure insensitive internal tetrahedron asymmetric stretching vibrations T-O-T
V_6	-	1100	1103	1103	1099	1099	Structure insensitive internal tetrahedron asymmetric stretching vibrations Si-O-T
V_7	-	795	802	795	795	795	Structure insensitive internal tetrahedron and structure sensitive external tetrahedron symmetric stretching vibrations Si-O-Si
V_8	-	546	547	547	548	548	Five-ring units in the structures of pentasil
V_9	-	450	455	455	459	459	Bending vibrations Si-O-Si

CONCLUSION AND SUGGESTION

Characterization by XRD and FTIR of the NaZSM-5 and K_3PO_4/NaZSM-5 catalyst with concentration of K_3PO_4 at 5, 10, and 15 wt.% was investigated. The XRD pattern of the resulting sample (NZ, NZK5, NZK10 and NZK15) showed the emergence of diffraction peaks that similar with high intensity at 2θ = 7.8; 8.8; 23.0; 23.2, and 24.3° that are specifically indexed to the structure of the MFI topology and not be found the another ZSM-5 type. The percentage crystallinity of the sample becomes smaller with increasing number of K_3PO_4

impregnated on NaZSM-5 synthesis. FTIR spectra of resulting samples have absorption bands in the region 1220 and 547 cm^{-1} which shows the absorption characteristics of vibration T-O-T on NaZSM-5 type zeolite which distinguish the other and sensitive to changes in structure. The analysis resulting of characterization using XRD and FTIR show that the structure of NaZSM-5 remained unchange after impregnated with K_3PO_4.

REFERENCES

[1] Zhang, L.,Sun, S., Xin, Z., Sheng, B. and Liu, Q., (2010), "Synthesis and Component Confirmation of Biodiesel from Palm Oil and Dimethyl Carbonate Catalyzed by Immobilized-Lipase in Solvent-Free System", *Fuel*, Vol. 89, 3960–3965.

[2] Kim, M., Yan, S., Salley, S.O. and Ng, K.Y.S., (2009), "The Effect of Sodium on Catalytic activity of ZnO-Al_2O_3/ZSM-5 and SnO-Al_2O_3/ZSM-5 for tranesterification of vegetable oil with methanol", Catalysis Communication, Vol. 10, 1913-1919.

[3] Samart, C., Sreetongkittikul, P., and Sookman, C., (2009), Heterogeneous Catalysis of Transesterification of Soybean Oil Using KI/Mesoporous Silica, *Fuel Processing Technology*, Vol. 90, 922–925.

[4] Helwani, Z., Othman, M.R., Aziz, N., Kim, J. and Fernando, W.J.N., (2009), "Solid heterogeneous catalysts for transesterification of triglycerides with methanol: A review", *Applied Catalysis A: General*, Vol. 363, 1–10.

[5] Guan, Guoqing, Kusakabe, Katsuki, and Yamasaki, Satoko, (2009), Tri-Potassium Phosphate as A Solid Catalyst for Biodiesel Production from Waste Cooking Oil, *Fuel Processing Technology*, Vol. 90, 520–524.

[6] Gao, Lijing, Teng, Guangyuan, and Wei, Ruiping, (2010), Biodiesel from Palm Oil Via Loading KF/Ca – Al Hydrotalcite Catalyst. *Biomass and Bioenergy*, Vol. 34, 1283 – 1288.

[7] Georgogianni, K.G., Katsoulidis, A.P., Pomonis, P.J., and Kontominas, M.G., (2009), Transesterification of Soybean Frying Oil to Biodiesel Using Heterogeneous Catalysts, *Fuel Processing Technology*, Vol. 90, 671–676.

[8] Khalifah, S.N., (2010), *Sintesis Katalis ZSM-5 Mesopori dan Aktivitasnya pada Esterifikasi Minyak Jelantah untuk Produksi Biodiesel*, Tesis, Institut Teknologi Sepuluh November, Surabaya.

[9] Zhu, H., Liu, Z., Kong, D., Wang, Y., Yuan, X. and Xie, Z., (2009), "Synthesis of ZSM-5 with Intracrystal or Intercrystal Mesopores by Polyvinylbutyral Templating Method", *Journal of Colloid and Interface Science*, Vol. 331, 432–438.

[10] Zhang, Y., Zhou, Y., Li, Y., Wang, Y., Xu, Y., and Wu, P., (2007), "Effect of Calcination Temperature on Catalytic Properties of PtSnNa/ZSM-5 Catalyst for Propane Dehydrogenation", *Catalysis Communications*, Vol. 8, 1009–1016.

[11] Gonçalves, M.L., Dimitrov, L.D., Jordao, M.H, Wallau, M and Urquieta-Gonzalez, E.A., (2008), "Synthesis of Mesoporous ZSM-5 by Crystallisation of Aged Gels in The Presence of Cetyltrimethylammonium Cations", *Catalysis Today*, Vol. 133–135, 69–79.

[12] Pye, C.C. and Rudolph, W.W., (2003), "An Ab Initio, Infrared, and Raman Investigation of Phosphate Ion Hydration", *J. Phys. Chem. A*, Vol. 107, 8746-8755

[13] Nakamoto, K., (1978), *Infrared and Raman Spectra of Inorganic and Coordination Compounds,* Third Edition, John Wiley & Sons, Inc., New York.

[14] Silverstein, Bassler, Morril, (1984), Penyidikan Spektrometrik Senyawa Organik, Erlangga, Jakarta

C-21

ADSORPTION RATE CONSTANT AND CAPACITIES Of LEAD(II) REMOVAL FROM SYNTHETIC WASTEWATER USING CHITOSAN SILICA

Sari Edi Cahyaningrum and Dina kartika

Chemistry Department, Faculty of Mathematics and Natural Sciences Surabaya State University, Surabaya
email: muhacahya@ yahoo.co.id

Abstract

The adsorption rate constant and capacities for the removal of Pb(II) from synthetic wastewater using chitosan silica were investigated. The adsorption kinetics model is used to determine the rate of adsorption is the order of the one nearing equilibrium and pseudo second-order. The results showed that the metal ion adsorption of Pb(II) reaches equilibrium at the 60 minute interaction by chitosan-silica bead. Adsorption kinetics model that is suitable for both the pseudo second-order rate constants. The adsorption followed the Langmuir and Freundlich isotherms but could best be approximated with the Langmuir model.

Key words: adsorption, Pb(II), chitosan silica

INTRODUCTION

Water is an essential matter to human and other living organisms. Water is polluted in many ways like effluents from leather and chemical industries, electroplating industries and dye industries. Effluents from textile, leather, tannery, electroplating, galvanizing, pigment and dyes, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sector contains considerable amount of toxic metal ions (Bradbury, M. H., & Baeyens, B.,2002). These toxic metals ions are not only potential human health hazards but also to other life forms .Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, nickel, copper and aluminium poisoning: gastrointestinal (GI) disorders, diarrhoea, stomatitis, tremor, heamoglobinuria causing a rust–red colour to stool, ataxia, paralysis, vomiting and convulsion,depression, and pneumonia when volatile vapours and fumes are inhaled . Several methods have been used to purify the water like sedimentation, filtration, ultra filtration, precipitation, ion exchange, electro coagulation, electro dialysis, and reverse osmosis in cleaning wastewater .Hence, the disadvantages associated with traditional methods of heavy metal removal like incomplete metal removal, high energy requirement, generation of toxic sludge and other waste products have made it important to look of other cost-effective treatment methods(Chiou & Li, 2004) .Moreover, traditional methods of heavy metal removal are not so efficient when metals are present in concentrations less than 100mg/L. Hence, attention is being focused to the development of alternative methods of heavy metal removal like bioremediation. One of such processes is adsorption (Kalyani, S., et.al., 2009).Chitosan derivatives have been extensively investigated as adsorbents (Amit & Mika, 2009). Among them are chitosan derivatives containing nitrogen, phosphorus and sulfur as heteroatoms, and other derivatives such as chitosan crown ethers and chitosan ethylenediaminetetraacetic acid

(EDTA)/diethylenetriaminepentaacetic acid (DTPA) complexes (Varma, Deshpande, & Kennedy, 2004). Recently, chitosan composites have been developed to adsorb heavy metals and dyes from wastewater. Different kinds of substances have been used to form composite with chitosan such as montmorillonite (Wang &Wang, 2007), polyurethane (Won, Lee, Jeong, Min, & Lee, 2009), activated clay (Chang & Juang, 2004), bentonite (Wan Ngah, Ariff, & Hanafiah, 2010), poly vinyl alcohol, poly vinyl chloride, kaolinite (Zhu, Jiang,& Xiao, 2010), oil palm ash (Hameed, Hasan, & Ahmad, 2008) and perlite (Kalyani, Ajitha, Srinivasa, & Krishnaiah, 2005). In this research chitosan modified with silica, this composites have been proven to have better adsorption capacity and resistance to acidic environment.

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RESEARCH METHOD

Materials

Silica gel (100–200 mesh size); chitosan was supplied by IndoChem.,Indonesia. Glutaraldehyde was obtained from Shanghai Shengong Bioengineering Corp. All other chemicals used in the experiment were in analytical grade.

Preparation of chitosan - silica gel beads blend

Chitosan and glutaraldehide mixed solutions were prepared by dissolving 500 mg chitosan and glutaraldehid 5% in acetic acids solution. Ten grams silica gel (75–100 _m) was added into the solution and immersed overnight before drying in vacuum condition. The chitosancoated gel was suspended in dimethylsulphoxide (DMSO) and stirred vigorously in a flask. After dissipation, NaOH was added until pH reached above 10.0. The macrospores on the bead surface, filtered the solution. After thoroughly washed with pure water, the cross-linked matrix with some active aldehide groups was shaken in a 0.85M ammonia solution at 60 ◦C for 4 h. Then, the matrix was washed with purewater to remove the residual ammonia.

Adsorption Pb(II) in variation contact time

The kinetic experiments were carried out in a batch-type for using a 50-mL Erlenmeyer in shaker at room temperature. The volume of the solution of the reacting suspension was 10 mL, the initial Pb(II) ion concentration was 100 mg/L, the pH 5.0, and to each solution 10 mg chitosan-silica as added and then stirred continuously. At a selected time period, the sample was immediately filtered membrane filter and the concentration of Pb(II) remaining in the supernatant was analyzed using AAS. Sample and blank solutions were analyzed under the same condition. The amount Pb(II) sorbed was considered to the difference between the initial and the remaining amount of Pb(II) in the reacting solution. From the data obtained, the value of sorption and desorption rate constants were then calculated based on the assumption that the sorption obeyed. The data obtained was then analyzed using two different adsorption kinetics models, i.e pseudo-second order Ho and first order reaching equilibrium proposed here.

Adsorption capacity and energy

As much as 10 mg of chitosan-silica was interacted with and stirred in 10 mL of solutions containing the various concentrations of 50, 100, 150, 200, 250, 300, 500, and 1000 mg/L Pb(II) for as long as 1 h. After separating the supernatants, the concentrations of Pb(II) in the supernatant were determined using AAS. Under the same condition with the sample solution, the blank solution was also analyzed. Based on the data obtained, the capacity (b) and equilibrium constant (K) of sorption was calculated based on the Langmuir isotherm sorption model, and the energy (E) of soprtion was then calculated from the equation of $E = RT\ln K$

RESULT AND DISCUSSION

Preparation of the metal chelating support was performed in three steps consisting of chitosan coating, crosslinking, and metal chelating. The interaction mechanism of silica with chitosan has been described (Dewi, Rifa). The cross-linking by the glutaraldehide molecules could increase the stability of the coated chitosan layer. Some loss of the primary amino groups in cross-linking step could be recovered by amination of the residue aldehyde groups. BET analysis showed that specific surface area was 26.89m2/g and pore volume was 0.2063mLg−1 with macroporous structure (pore diameter ≥50 nm, 67.8%). The low pore volume might be ascribed to the special interconnected porous structure formed by the chitosan layer on the surface. Such porous surface might favor the accessibility for the target macromolecular binding. The SEM analysis showed that morphology chitosan-silica more porous so that this adsorbent has adsorption capacity more biger than chitosan flake

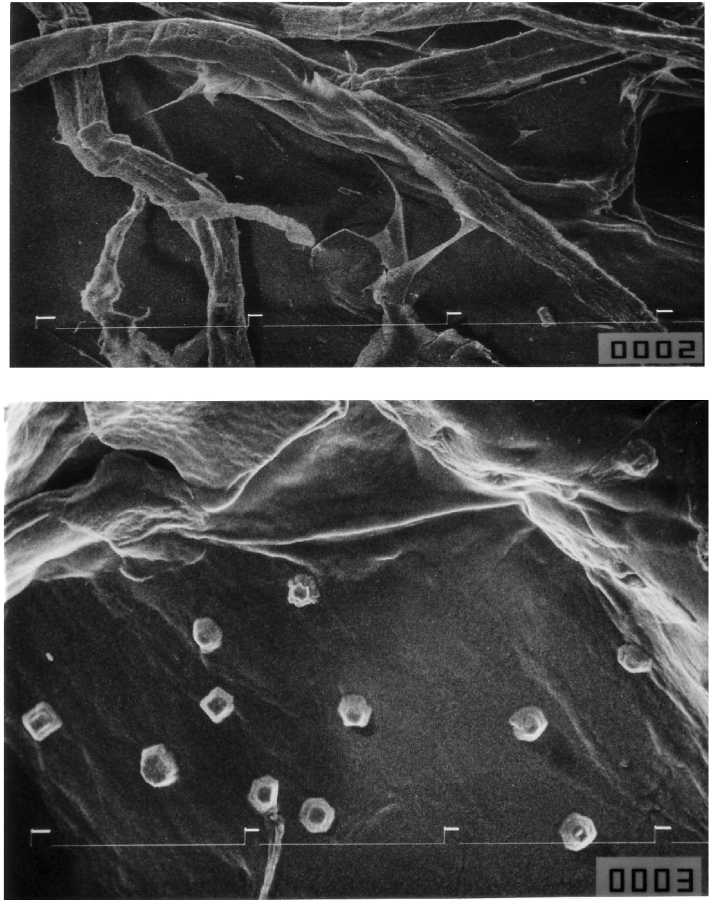


Fig. 1. SEM Chitosan –silica (a); Chitosan-silica-metal ion (b)

Kinetics

Figure 2 indicates the uptake of Pb(II) ions was increased as the contact time increases. The rapid removal of Pb(II) ion was noticed with the contact time variation from 30 to 240 mins, respectively. This is because at the early stage, more number of potentially active/vacant sites is available for adsorption. As the contact time increases maximum number of sites got adsorbed to the metal ions. Hence it is difficult for the metal ion to search for the very fewer remaining sites. Therefore rate of adsorption decreases in the later. Hence 60 min was found to be an equilibrium adsorption time.

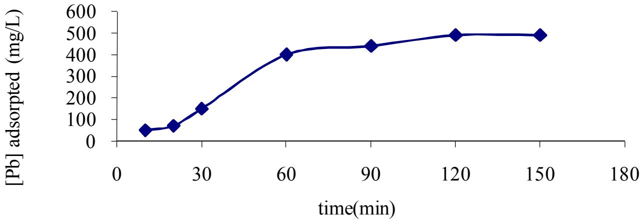


Fig 2. Effect interaction time on Pb(II) adsorption at chitosan silica

The analysis data with kinetic model showed in table 1. That compared to kinetic model of first order adsorption reaching equilibrium, this type of kinetic model has been proven to posses lower correlation coefficient so that adsorption Pb(II) metal ions tend followed kinetic model Kinetic pseudo-second order Ho.

Table 1 Adsorption rate constant and correlation coefficient for the adsorption of Pb(II) on chitosan-silica.

Adsorbent	kinetic model of first order adsorption reaching equilibrium.			Kinetic pseudo-second order Ho		
	$k_1.10^{-3}$ (min^{-1})	Q. $(mol/L)^{-1}$	R^2	H $(mg\ g^{-1}\ min^{-1})$	$k_2'.10^{-4}$ $(g\ mg^{-1} min^{-1})$	R^2
Chitosan-silica beads	5.1634	12.3109	0.7422	0.0631	8.0580	0.9192

Capacities and energy adsorption

The Langmuir and Freuchlich isotherm adsorption model with the mathematical expression given below was used to determine the capacity (b) and equilibrium constant (K) of adsorption, and energy (E) adsorption was then calculated

Langmuir isotherm model:

$$\frac{c}{m} = \frac{1}{bk} + \frac{b}{c} \qquad (1)$$

Freundlich isotherm model:

$$\log m = \log B + \frac{1}{n} \log C \qquad (2)$$

where C, the equilibrium concentration in solution; b, Langmuir's adsorption capacity; K, adsorption affinity; m metal adsorbed per g adsorbent at equilibrium; B, Freundlich's adsorption capacity and n, constants. Fig 2 and 3 showed that adsorption Pb(II) on chitosan silica has different R^2, from two models isotherm showed that this adsorption tend followed Langmuir isotherm model.

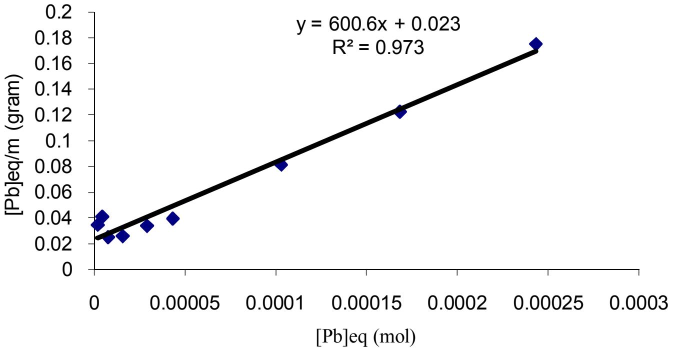


Fig 2. Linearitas Langmuir

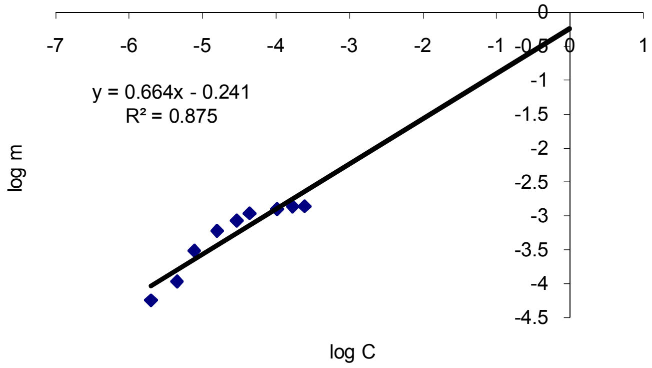


Fig 3. Linearitas Freunclich

The Langmuir isotherm is designed for monolayer adsorption of a species on a homogeneous surface with adsorption energy is the same for all active site regardless of the degree of coverage. Adsorption Pb(II) on chitosan silica. Adsorption capacity Pb(II) metal ion for the chitosan silica was 42.51 .10^{-4} mole/g, involving energies of adsorption in a 25.04 kJ/mole.

CONCLUSION AND SUGGESTION

This study has created a composite chitosan and silica for adsorbent Pb(II).SEM characterization results show that chitosan silica has the greatest pore size. The adsorption capacity of chitosan-silica is equal 42.51 .10^{-4} mole/g, involving energies of adsorption in a 25.04 kJ/mole with the adsorption rate following the pseudo second-order kinetics model.

REFERENCES

Amit, B., & Mika, S. (2009). Applications of chitin- and chitosan-derivatives for detoxification of water and wastewater—A short review. Advances in Colloid andInterface Science, 152, 26–38.

Bradbury, M. H., & Baeyens, B. (2002). Sorption of Eu on Na- and Camontmorillonites: Experimental investigations and modeling with cationexchange and surface complexation. Geochimica et Cosmochimica Acta, 66, 2325–2334.

Chang, M. Y., & Juang, R. S. (2004). Adsorption of tannic acid, humic acid and dyes from water using the composite of chitosan and activated clay. Journal of Colloid and Interface Science, 278, 18–25.

Chiou, M. S, Ho, P. Y., & Li, H. Y. (2004). Adsorption of anionic dyes in acid solutions using chemically cross-linked chitosan beads. Dyes and Pigments, 60, 69–84.

Hameed, N., & Guo, Q. P. (2009). Natural wool/cellulose acetate blends regenerated from the ionic liquid 1-butyl-3-methylimidazolium chloride. Carbohydrate Polymers, 78, 999–1004.

Huang, G. L., Zhang, H. Y., Jeffrey, X. S., & Tim, A. G. L. (2009). Adsorption of chromium(VI) from aqueous solutions using cross-linked magnetic chitosan beads. Industrial & Engineering Chemistry Research, 48, 2646–2651.

Kalyani, S., Ajitha, P. J., Srinivasa, R. P.,&Krishnaiah, A. (2005). Removal of copper and nickel from aqueous solutions using chitosan coated on perlite as biosorbent. Separation Science and Technology, 40, 1483–1495.

Kalyani, S., Veera, M. B., Siva, K. N., & Krishnaiah, A. (2009). Competitive adsorption of Cu(II), Co(II) and Ni(II) from their binary and tertiary aqueous solutions using chitosan-coated perlite beads as biosorbent. Journal of Hazardous Materials, 170, 680–689.

Varma, A. J., Deshpande, S. V.,&Kennedy, J. F. (2004). Metal complexation by chitosan and its derivatives: A review. Carbohydrate Polymers, 55, 77–93.

WanNgah, W. S., Ariff, N. F. M.,&Hanafiah, M. A. K. M. (2010). Preparation, characterization, and environmental application of crosslinked chitosan-coated bentonite for tartrazine adsorption from aqeous solutions. Water, Air and Soil Pollution, 206,225–236.

Won, S. L., Lee, H. C., Jeong, Y. G., Min, B. G., & Lee, S. C. (2009). Preparation and acid dye adsorption behavior of polyurethane/chitosan composite foams. Fibers and Polymers, 10, 636–642.

Zhu, H. Y., Jiang, R., & Xiao, L. (2010). Adsorption of an anionic dye by chitosan/kaolin/_-Fe2O3 composites. Applied Clay Science, 48, 522–526.

C-22

INTERVENTION EFFECT OF LIQUID SMOKE OF PYROLYSIS RESULT OF COCONUT SHELL ON PROFILE OF pH FILLET OF *LATES CALCARIFER*

Sofia Satriani Krisen [1], Bambang Setiaji[2], Wega Trisunaryanti[2], Harno Dwi Pranowo[2]

[1]*Department of Chemistry, Faculty of Matematics and Natural Sciences,*
Manado State University
[2]*Department of Chemistry, Faculty of Matematics and Natural Sciences,*
University of Gadjah Mada, Yogyakarta

Abstract

Non-fresh fish will have pH value (base) of meat greater than the fresh one.This is due to biochemical and microbial reactions taking place in post-mortem fish meat, thus producing base compounds such as ammonia, trimethylamine, and other volatile compounds.The present study is to disclose Intervention Effect of Liquid Smoke of Pyrolysis Result of Coconut Shell on the Profile of pH Fillet of *Lates Calcarifer*.

Concentration and optimum immersing duration of liquid smoke are determined by making optimum curve. By using concentration data and optimum immersing duration of liquid smoke, fillet *Lates Calcarifer* is immersed and drained for 2 hours at 40°C and then kept in freezer at 4°C until the sample will be analyzed every 2 days.

The result indicates that concentration and optimum immersing duration are 5% and 10 minutes, respectively and pH Fillet of *Lates Calcarifer* until the 10th keeping day does not bring about significant impact on the time.

Keywords: Liquid smoke, *Lates Calcarifer,* pH, concentration, soaking time

Introduction

Fish meat is source of protein for a large number of people, but extremely breakable foodstuffs if compared with other animal meats. In tropical region, for example, with high temperature, fish usually begins to spoil after being captured (Berkel *et al*., 2004). Such condition is called as chemical and biological transformation since main components of fish are broken in enzymatic manner, a condition in which after the fish dies oxygen supply in its tissue is discontinued as the result of disruption in blood circulation system, malfunction of mitochondria system, and periodic shortage of adenosine triphosphate through various ATPase activities. Following the running out of creatine phosphate, anaerobic glycolysis keeps on growing some ATPs with final product of accumulated lactic acid. The initial loss of freshness results from autolytic enzyme of endogen in muscle and followed by spoilage due to microbial activity, particularly the rapid growth of specific spoilage organism (SSO). Interaction between microbe and reaction of physiochemical metabolism accelerate degradation of fish quality such as amine formation, lipid oxidation, protein degradation, which contributes to bad odor, and texture being softened (Hernandez *et al.,* 2009).

Value of acidity degree (pH) is one of indicators in measuring the degree of fish freshness. In fish spoilage process, change in pH of fish meat autolysis process and bacterial attack (Fardiaz, 1992). To Erikson and Misimi (2008) anaerobic reaction occurring after fish mortality will benefit ATP and glycogen as sources of energy, making the amount of ATP keep on decreasing resulting in that pH of fish body decreases and muscular tissue is unable to ensure its flexibility (ductility).

pH is commonly used to measure fish deterioration and it is pH of muscular tissue to measure (Howgate, 2009). To Bhobe and Pai (1986), the increase in pH (*Metapeneusdohsnai*) during keeping period at lower temperature takes place due to amine formation through bacteria which reduces TMAO to become TMA, decomposition of tissue protein and deamination process. Kyrana and Lougovois (2002) discloses that the increased pH in fish meat takes place due to long period of keeping which is also correlated with rapid spoilage condition of fish.

The alive fish has 7.5 of meat pH. However, after mortality, glycogen is broken through glycolysis into pyruvic acid and then lactic acid, thus making the fish meat be more acidic. pH below 7.7 is considered to have good quality, preferably in range of 7.7 and 7.9 which is acceptable by consumers and pH above 7.9 be spoilage (Zamir *et. al*., 1998). To Bremner (2002) pH of fish meat when it is alive is 7.0 and after its death, it declines to be about 6.6 – 5.5 but depending on fish species.

To delay spoilage process, some attempts have been performed including keeping fresh fish by using ice, giving formalin or liquid smoke. The reason is that microbial growth may be delayed by reducing a_w and pH thereof, because low condition of pH in fish meat causes decomposition by bacteria to run slow. Liquid smoke of coconut shell as reported by Zuraida *et. al.* (2011) contains phenolic compounds such as phenol, 2-methoxylphenol (guaiacol), 3,4-dimethoxylphenols, and 2-methoxy-4-methylphenol. Dihydroxy benzoic acid, methoxybenzoic acid and 150 hydroxyl benzoic acid serves as acid components. To Bower *et al*.,(2009) phenol is one of components of smoke which acts as antioxidant, while other components are organic acid, alcohol, carbonyl, hydrocarbon, and nitrogen compound such as nitro-oxide. Phenolate is usually used in food industry as antioxidant and antibacterial (Esekhiagbe *et al.,* 2009).

A lot of researches on liquid smoke usage as preservative for fish have been carried out, but their outputs are just different.The present research is to study Intervention Effect of Liquid Smoke of Pyrolysis Result of Coconut Shell on the Profile of pH Fillet of *Lates Calcarifer*.

2.Procedure

2.1.Instruments / Materials:

pH meter analyzer 255 (Corning Scientific Co., Corning, NY), buffer pH 4, buffer pH 7, fillet *Lates calcarifer*, liquid smoke grade-2, blender, glassware, akuades.

2.2.Working Stage:

The concentration of liquid smoke is defined by making concentration variation, 0%; 5%; 10%; 15%; 20%; 25% and 30%, respectively.The data obtained is developed into optimum curve as to obtain optimum concentration data of liquid smoke.

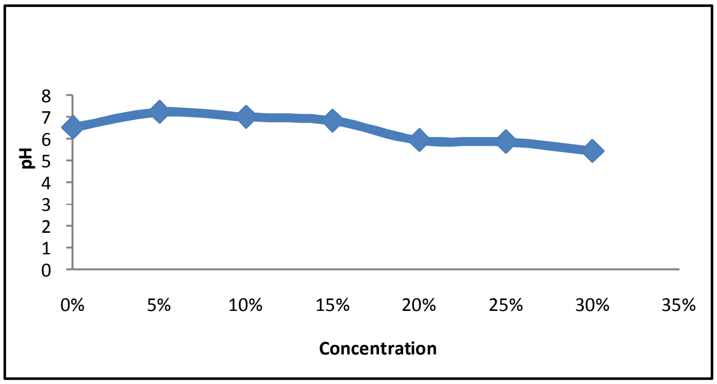
Immersion duration of liquid smoke is designated by making variation of immersion duration of 0 minute; 5 minutes; 10 minutes; 15 minutes; 20 minutes; 25 minutes and 30 minutes, respectively.The data obtained is developed into optimum curve as to data on optimum immersion duration of liquid smoke.

The degree of pH value is determined by using concentration data and optimum immersion duration of liquid smoke; fillet *LatesCalcifier* is immersed with liquid smoke and drained for 2 hours at 40°C and then put into Styrofoam, kept in freezer at 4°C until the sample will be analyzed every days.

3. Results and Discussion

3.1. Concentration and optimum immersion duration of liquid smoke.

The data of measurement result of pH of fillet *Lates Calcarifer* toward concentration variation of liquid smoke shows that the optimum concentration of liquid smoke at 5% of concentration has influenced pH of fillet *Lates Calcarifer*. Based on concentration variation of liquid smoke provided to Fillet *Lates Calcarifer*, 5% of concentration is optimum concentration of liquid smoke which can maintain the increase value of pH in fillet *LatesCalcarifer*.



Figur 1. Optimum concentration of liquid smoke

This is due to the fact that organic acid component on liquid smoke which provides acid contribution to Fillet *Lates Calcarifer* at 5% of concentration has been able to influence pH of fillet *Lates Calcarifer*. Similar to one reported by Tranggono, *et. al.,* (1996) smoke of coconut shell contains 7 main components: Phenol, 3-metyl-.2cyclopentadion, 2-methoxyphenol, 2-methoxy-4-methylphenol, 4-ethyl-2-methoxyphenol, 2.6-dimethoxy phenol and 2.5-dimethoxy benzyl alcohol.

Acids sourced from liquid smoke may influence aroma, pH and storage life of food. The combination between functional component of phenol and content of organic acid works synergistically in preventing and controlling microbial growth, and high content of acid may obstruct microbial growth because microbe can only grow at low content of acid (Pszczola,1995).

The duration of optimum immersion of liquid smoke in 10 minutes can withstand pH value of fillet *Lates Calcarifer* to 5.546, and increase again. With the increase time of immersion, there is a tendency in which pH value of Fillet *Lates Calcarifer* to decline again, because the longer it is immersed in acidic liquid smoke, the more acidic the fillet will be.

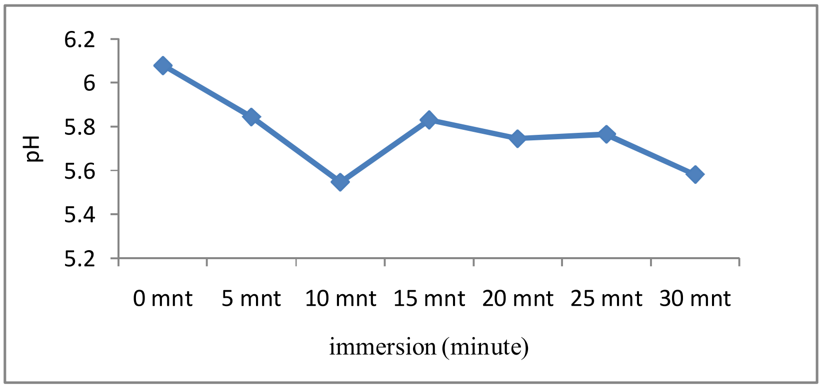


Figure 2. Optimum immersion duration of liquid smoke

3.2. Profile of pH of Sea Fish *Lates Calcarifer*

Value of acidity degree (pH) is one of indicators in measuring the degree of fish freshness. The data of analysis result of pH value of fillet *Lates Calcarifer* without allocation of liquid smoke in the 0th, 2nd, 4th, 6th, 8th, and 10th day is 6.39; 6.31; 6.09; 6.24; 7.09 and 8.13, respectively. And the pH value of fillet *Lates Calcarifer* with allocation of liquid smoke in the

0th, 2nd, 4th, 6th, 8th, and 10th day is 6.3992, 6.3162,6.4624, 6.0835, 6.3052, and 8.1215, respectively. The data indicates that the pH value in the beginning of storage decreases in the 6th day for fillet without liquid smoke, and thereafter, it increases again. Similarly, in fillet with liquid smoke, the pH value increases after the 8th day.

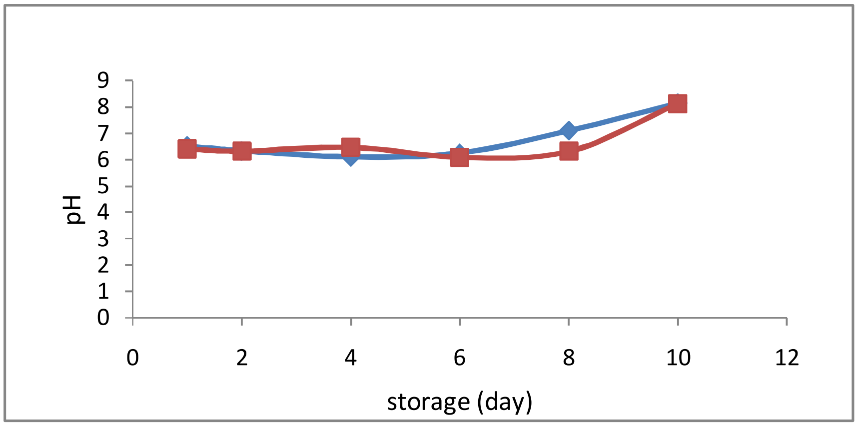


Figure 3. Profile of pH of Sea Fish *Lates Calcarifer*

In this study, the starting value of pH in fillet *Lates Calcarifer* is in range of pH value as found by (Periago *et. al*., 2005; Orban *et. al*., 2003) that is at pH 6.44 and 6.27, respectively. Ersoy *et. al.,*(2008) find starting pH value in fish meat is 6.23.

The increase in pH value in the 6th day for fillet without liquid smoke takes place two days earlier than that with liquid smoke, that is, in the 8th day. This is caused by not only considerable amount of accumulated lactic acid, but also effect of components contained in liquid smoke. To Nurjanah *et. al.,* (2007), the increase in pH value is caused by autolytic process in fish meat which brings about the occurrence of decomposition by enzyme into more simplified compounds. The enzymatic decomposition into simple compounds starts when pH value declines. The declined pH value will activate cathepsin enzim. The enzyme is capable of decomposing protein into more simplified compounds, thereby increasing pH value. The pH value in fish meat will continue to increase near to neutral value after rigor mortis phase ends. In line with the increased time of storage, there will be increase in pH value in initial post-rigor phase and it continues to increase in the last post-rigor phase (Nurjanah *et. al.,* 2007).

Eskin (1990) states that after the fish is dead, there will be biochemical transformation in body tissue which is marked by declination of pH resulting from accumulation of lactic acid, which is formed through glycogen decomposition process in fish meat, which is glycogen transformation into lactic acid in glycolysis process. To Erikson and Misimi (2008), anaerobic reaction taking place after the death of fish will benefit ATP and glycogen which has been formed during the life of fish as source of energy, resulting in that the amount of ATP keeps on decreasing. Accordingly, pH of fish body decreases and muscular tissue is unable to ensure its flexibility (ductility).

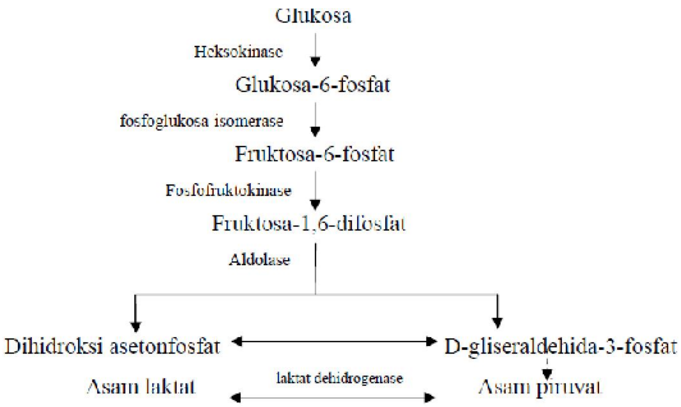


Figure 4. glycolysis process in fish meat

High content of glycogen may retard glycolysis process in fish meat, thus delaying the arrival of rigor mortis process. During rigor mortis process, the pH value in fish meat will suffer declination from 6.9-7.2, which is initial pH, to 6.2-6.6. High and low initial pH in fish will mainly depend on the amount of glycogen and strength of buffer in fish meat.The strength of buffer in fish meat is caused by protein, lactic acid, phosphoric acid, TMAO and volatile bases. The pH value in fish meat will keep on increasing closing to neutral value after the end rigor mortis phase (Farber, 1965).

Pacheco-Aguilar *et. al*., (2000) states that pH is one of determinant factors for life sustainability of microorganism growth during storage, processing, and distribution of fish. In the beginning of storage, the sample pH value will decrease to the lowest after the condition of rigor mortis, then it will increase until it has alkali property in the end of storage. The increase pH may be caused by accumulation of alkali compound such as ammonia and trimethylamine originated from microbial action during spoilage of fish muscles (Ruiz-Capillas and Moral, 2005; Özyurt, *et. al.,* 2009).

4. Conclusion

The grade-2 at 5% of concentration and 10 minutes of immersion duration may provide increase delay in pH of fillet *Lates Calcarifer* for 2 days if compared with that without liquid smoke. However, this does not give significant impact on the time.

References:

Berkel, B.M., Boogaard, B.V and Heijnen. 2004. *Preservation of Fish and Meat*. Agromisa Foundation, Wageningen, The Netherlands, 78-80.

Bhobe, A. M., and Pai, J.S. 1986. Study of the properties of frozen shrimps. *J. of Food Science*

Bower, C. K., Hietala, K.A., Oliveira, A.C.M., Wu, T.H. 2009. Stabilizing oils from smoked pink salmon (*Oncorhynchus gorbuscha*). *J. Food Sci*. 74, (3), 248-257.

Bremner, H. A., 2002, *Safety and Quality issues in Fish processing*. Wood head Publishing limited.

Eskin, N. A. M. 1990. *Biochemistry of Food Second Edition,* San Diego, Academic Press, Inc.

Erikson, U and Misimi, E. 2008. Atlantic salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis and storage. *J. of food science, 3, (2),* 50-59.

Ersoy, B., Aksan, E and Özeren, A. 2008. The effect of thawing methods on the quality of eels (*Anguilla anguilla*). *Food Chemistry*, 377–380

Farber, L. 1965. *Freshness test*. In:Fish As Food. Borgstrom, G (Editor). New York. Academic Press.

Fardiaz, S. 1992. Mikrobiologi Pangan I. Gramedia Pustaka Utama, Jakarta.

Hernandez, M. D., López, M. B., Alvarez, A., Ferrandini, E., Garcia, B.,Garrido M.D. 2009. Sensory, physical, chemical and microbiological changes in aquacultured meager (*Argyrosomus regius*) fillets during ice storage. *Food Chem,* 114, 237-245.

Howgate, P. 2009. *Traditional methods,* In: Fishery products quality, safety, and authenticity. Edited by: ehbein, H and Oehlenschläger, J.,Wiley-Blackwell. A John Wiley & Sons, Ltd, Publication, Chichester, U. K. 19 – 41.

Kyrana, V. R. and Lougovois, V. P. 2002. Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax)* stored in melting ice. *Int. J. of Food Science and Technology,* 37, (3), 319-328

Nurjanah., Tati, N., Fatmawati, Z. 2007. *Karakteristik mutu ikan bandeng (Chanos chanos) di Tambak Sambiroto Kabupaten Pati Jawa Tengah.* Seminar International Perikanan, Jakarta.

Orban, E., Nevigato, T., Di Lena, G., Casini, I., Marzetti, A. 2003. Differentiation in the lipid quality of wild and farmed sea bass (*Dicentrarchus labrax*) and gilthead bream *(Sparus aurata*). *J. Food Sci*., *68*, 128–132.

Ozyurt, G., Kuley, E., Ozkutuk, S., Ozogul, F. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry,114,* 505–510

Pacheco-Aguilar, R., Lugo-Sanchez, M. E., and Robles-Burgueno, M. R. 2000. Postmortem biochemical characteristic of Monterey sardine muscle stored at 0°C. *J. Food Sci.* 65, 40–47.

Periago, M. J., Ayala, M.D., Lopez-Albors, O., Abdel, I., Martinez, C., Garcia-Alcazar, A., Ros, G., and Gil, F. 2005. Muscle cellularity and flesh quality of wild and farmed sea bass. *Aquaculture*, 249, 175–188.

Pszczola, D.E. 1995. Tour highlights production and uses of smoke-based flavours. *Food Technology* , 49, 70–74

Ruiz-Capillas, C. and Moral, A. 2005. Sensory and biochemical aspects of quality of whole bigeye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres. *Food Chemistry*, 89, 347–354.

Tranggono, Suhardi, Setiaji, B.A.H. 1996. *Produksi Asap Cair dan Penggunaannya pada Pengolahan Beberapa Bahan Makanan Khas Indonesia.* Laporan Penelitian Akhir Riset Unggulan Terpadu III, Pusat Penelitian Ilmu dan Teknologi, Jakarta.

Zamir, R., Qasim, R and Ullah, A. 1998. Changes in physical and chemical constituents of Crab meat during storage at refrigerator temperature (7^0- 2^0 C). *Pakistan J. of. Pharm. Sciences,* 11, (1), 27-33.

Zuraida, I., Sukarno and Budijanto,S. 2011. Antibacterial activity of coconut shell liquid smoke (CS-LS) and its application on fish ball preservation. *Int. Food Research Journal* 18, 405 - 410

C-23

PHYTOCHEMICAL OF *KAEMPFERIA* PLANT AND BIOPROSPECTING FOR CANCER TREATMENT

Sri Atun

Chemistry education Faculty of Mathematical and Natural Science,
Yogyakarta State University,
Jl. Colombo No. 1 Yogyakarta, Indonesia, 55281
e-mail : Atun_1210@yahoo.com

ABSTRACT

Kaempferia genus is perennial member of the Zingiberaceae family and is cultivated in Indonesia and other parts of Southeast Asia. Number of studies has been conducted, providing information related to *Kaempferia* as antioxidant; antimutagenic; and chemopreventive agent. This paper reports some isolated compounds from this plant, biological activity, and bioprospecting for cancer treatment.

Keyword: Cancer treatment; *Kaempferia*; Zingiberaceae

INTRODUCTION

Kaempferia is a genus, belong to family of Zingiberaceae. This plant grows in Southeast Asia, India, Sri Lanka, Indonesia, and Southem China. *Kaempferia* genus sinonim with *Boesenbergia* genus by Baker. This plant has 8 different botanical names which are *Boesenbergia cochinchinensis* (Gagnep.) Loes., *Boesenbergia pandurata* (Roxb.) Schltr., *Curcuma rotunda* L., *Gastrochilus panduratus* (Roxb.) Ridl., *Gastrochilus rotundus* (L.) Alston, *Kaempferia cochinchinensis* Gagnep., *Kaempferia ovate* Roscoe, *Kaempferia galanga*, *Kaempferia rotunda*, and *Kaempferia pandurata* Roxb nonetheless it is currently known as *Boesenbergia rotunda* (L.)Mansf (Tan Eng-Chong, et. al, 2012). The plants grown naturally in damp, shaded parts of the lowland or on hill slopes, as scattered plants or thickets. Economically important species among the plant families, the Zingiberaceae, which are perennial rhizomatous herbs, contain volatile oil and other important compounds of enormous medicinal values (Singh C.B., 2013).

Phytochemical and biologycal activities of some species of *Kaempferia*

Phytochemical and biologycal some species of plants of the genus *Kaempferia* reported by many researchers, among others:

1. *Kaempferia galanga* (Kencur)

The *Kaempferia galanga* is a native of India, China, Taiwan, Cambodia, and other areas of southeast Asia. *K. galanga* or kencur in the local name in Indonesi is a flowering perennial plant that prefers a warm growing environment reminiscent of its tropical countries. *K. galanga* also prefers shady growing conditions and moist soil. The plant has small flowers that grow in clusters at the center of its three large, broad, dark green leaves. Flowers are most often white, pink, or lavender in color (Fig. 1). Since the rhizomes of this plant contain volatile oil and other important compounds of enormous medicinal values, they are very demanding to the traditional health care practitioner.The rhizome of this plant has been used traditionally for the treatment of many ailment and few biological activities have proven its importance. The rhizome is rich in essential oils and is being used for the treatment of indigestion, cold, pectorial and abdominal pains, headache, expectorant, diuretic, carminative, stomachic, coughs, pectoral affections, asthma and hypertension. The rhizoma used as an entheogeon and aphrodisiac in New Guinea, a flavor rice, and the root and leaves are put into curries as a flavoring South Asia like Indonesia, Malaysia, and Thailand. The rhizoma and edible fresh leaves represent a potential food values (Sing C.B., 2013).

Sumber: http://www.ibujempol.com

Figure.1. Rhizome and plant of *K. galanga*

Number of publications on the phytochemistry has to do with the essential oil and extracts from the rhizome and leaf of this plant. Some compounds can be identified from oil extract from rhizome of *K. galanga* include 3-carene, camphene, borneol, cineol, kaempferol, kaempferide, cinnamaldehyde, p-metho-xycinnamic acid, ethyl cinnamate and ethyl p-methoxycinnamate. 3-Caren-5-one, carvon, eucalyptol, and pentadecane, and flavonoids from the leaves (Shing C.B., 2013). Some compounds was identified from *K. galanga* can be seen in figure 2.

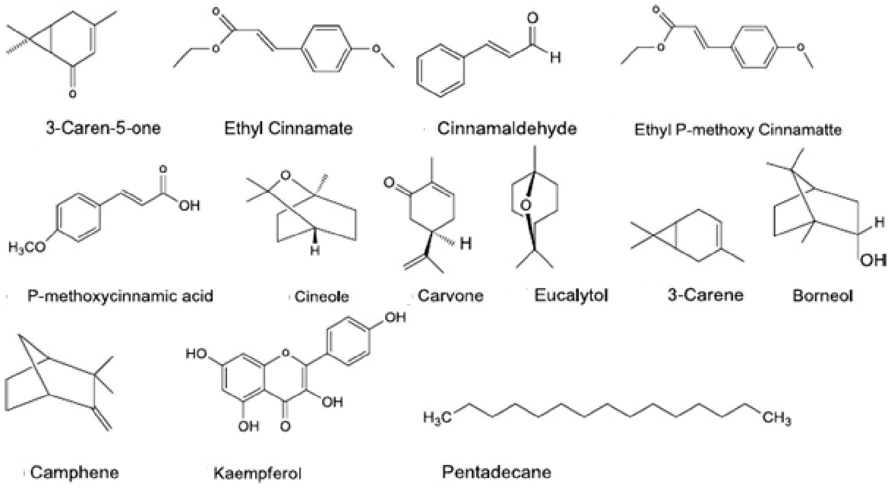


Figure 2. Some compounds was identified from *K. galanga*

Many researches reported biologycal activities of *K. galanga.* The rhizomes are stimulatory, expectorant, carminative, and diuretic. The methanol extract of the rhizome contains ethyl p-methoxy-trans-cinnamate, which is highly cytotoxic to Hela cells. Crude extracts of the rhizome of *K. galanga* with petroleum ether, acetone and methanol are screened for antimicrobial activities against two gram-positive and three gram-negative pathogenic bacteria i.e. *Bacillus sp., Pseudomonas sp., Escherichia coli, Salmonella s*p. and

Shigella sonnei (Singh C.B., 2013).

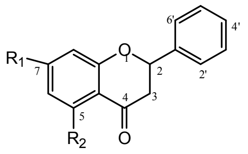
The aqueous extracts of *K. galanga* leaves show significant antinociceptive and anti-inflammatory effect in rats. The rhizome essential oils extracted from the rhizomes of *K. galanga* have shown considerable repellent and larvicidal activity against a number of mosquito species including *Aedes aegypti* . *K. galanga* extracts have weak anti-oxidant activity. Total phenolic content of ethanolic extracts of leaves and rhizomes is found to be 146 mg galic acid equivalent (GAE)/100 g and 57 mg GAE/100 g, respectively whereas the antioxidant activity of leaves and rhizome extracts is 77 mg ascorbic acid (AA)/100 g and 17 mg AA/100 g. This anti-oxidant activity is further reduced by drying using different thermal and non-thermal drying methods, however, this decrease is prevented if the plant is subjected to freez-drying. This antioxidant activity is mainly due to the total phenolic content and flavonoids, including luteolin and apigenin (Chan et al., 2009).

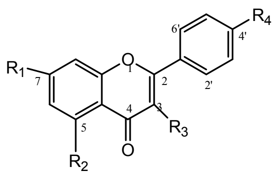
2. *Kaempferia pandurata* (*Boesenbergia pandurata)*

Boesenbergia pandurata (ROXB.) SCHLTR. [syn. *B. Rotunda* (L.) MANSF. KULTURPFL., sinonim with *Kaempferia pandurata* ROXB.], belonging to the family Zingiberaceae, is a perennial herb distributed in some tropical countries including Indonesia, Malaysia, Myanmar, and Thailand. The local name in Indonesia is "Temu kunci", this plant is a common edible ingredient in many Asian countries. It is normally cultivated at small home ranches and used as a condiment in food such as curry and soup due to its aromatic flavour, which promotes appetite. This herbal plant is also used as a traditional medicine to treat illnesses such as rheumatism, muscle pain, febrifuge, gout, gastrointestinal disorders, flatulence, carminative, stomach ache, dyspepsia, and peptic ulcer. In Indonesia, *B. rotunda* is typically used to prepare "jamu," a popular traditional tonic for women after childbirth as well as a beauty aid for teenage girls and to prevent leukorrhea (Tan Eng Chong, 2012). The rhizoma and the plant of *K. pandurata* in figure 3. The fresh rhizomes have a characteristic aroma and are used as a flavoring agent in Thai cuisine, it has been commonly used as self-medication by AIDS patients in southern Thailand. Previous investigations of the chemical constituents of *B. pandurata* reported the anti-HIV, antibacterial, anti-inflammatory, analgesic, antipyretic, antitumor, antioxidant, and insecticidal activities (Tan Eng Chong, 2012).

Figure 3. Plant and rhizoma of *K. pandurata* (Tan Eng Chong, 2012)

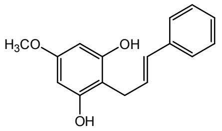
Many researches reported some isolated compounds from *K. pandurata* such as 5-hydroxy-7-methoxyflavanone (pinostrobin), 5,7-dimethoxyflavanone, 5-hydroxy-7-methoxy-flavone, 5-hydroxy-7,4-dimethoxyflavone, 5,7-Dimethoxyflavone, 5,7,4-Trimethoxy-flavone, 5,7,3,4-tetramethoxy-flavone, 5-Hydroxy-3,7-dimethoxyflavone, 5-Hydroxy-3,7,4-tri-methoxyflavone, 3,5,7-trimethoxy-flavone 5-hydroxy-3,7,3,4-tetramethoxy-flavone, 5,7-dihydroxyflavanone (pinocembrin), 2,6-di-hydroxy-4-methoxychalcone (pinostrobin chalcone), 2,4-dihydroxy-6-methoxy-chalcone (carda-monin), boesenbergin B, panduratin A, 4-hydroxy panduratin A (Tan Eng Chong, 2012). Some structure of the isolated compounds from *K. pandurata* can be seen at Figure.4.



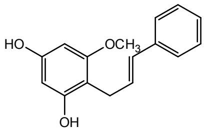


1. R1 =R2 = OH, 5,7-dihydroxyflavanone (pinocembrin)
2. R1 = R2 = OCH3, 5,7-dimethoxyflavanone
3. R1=OH; R2 =OCH3, 5-methoxy-7-hydroxyflavanone
4. R1=OCH3; R2=OH, 5-hydroxy-7-methoxyflavanone

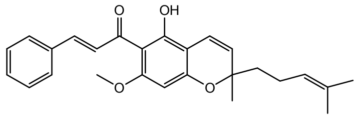
1. R1=R2=R4= OCH3; R4=H, 5,7,4'-trimethoxyflavone
2. R1=R2=OCH3; R3=R4=H, 5,7-dimethoxyflavone
3. R1=R2=R3=R4=OCH3, tetramethoxyflavon
4. R1=R3=OCH3; R2=OH; R4=H, 5-hydroxy-3,7-methoxyflavon
5. R1=R3=R4=OCH3; R2=OH, 5-hydroxy-3,7,4-



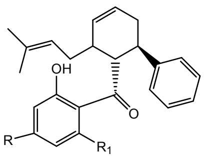
2,6-dihydroxy-4-methoxychalcone (pinostrobin chalcone)



2,4-dihydroxy-6-methoxy-chalcone (cardamonin)



Boesenbergin B



R = OH, panduratin A
R = OH, 4-hydroxy panduratin A

Figure 4. Structure isolated compounds from *K. pandurata*

Some essential oil from *K. pandurata* rhizoma identified such as geranyl formate, geranyl propionate, geraniol, neral, myrcene, isoborneol, β-pinene, neryl acetate, geranial, β-thujaplicin (E,E)-α-farnesene, borneol, tricyclene, terpinen-4-ol, terpinolene, myristicin, allo ocimene, α-thujene, (Z)-β-ocimene, sabinene, (E)-β-ocimene, (Z)-nerolidol, cis-linalool oxide, 3-carene, δ-elemene, (Z)-β-farnesene, γ-elemene, β-elemen (Jantan et al. (2001) cited by Tan Eng Chong, 2012). The polyphenols compounds can be identified such as quercetin, kaempferol, naringin, hesperidin, caffeic acid, p-coumaric acid, chlorogenic acid (Jing et al. (2009) cited by Tan Eng Chong, 2012). Many researcher reported of the chemical constituens of *B. pandurata* have activity as anti-HIV, antibacterial, anti-inflamasi, analgesic, antipyretic, antitumor, antioxidant. Some compounds such as panduratin isolated from *B. pandurata* showed cytotoxic against pancreatic PANC-1 cancer cell under nutrient deprived condition (Nwet Nwet Win, 2008).

4. Kaempferia rotunda

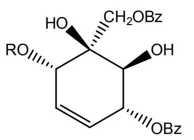
Kaempferia rotunda (Zingiberaceae), known as kunci pepet or kunir putih in Indonesia, has been traditionally used in as abdominal pain, sputum laxative, wounds and diarrhea colic disorder. The plant is used in the folk medicinal system of Bangladesh for treatment of high blood sugar levels as commonly observed in diabetic patients, as well as for treatment of pain. The plant is considered to be an important medicinal plant also in the ancient traditional medicinal system of India, namely, Ayurveda. The Ayurvedic drug, hallakam, containing tubers of the plant is considered as stomachic, antiinflammatory to wounds and bruises, and useful for treatment of mental disorders and insomnia (Sereena et al., 2011). The plant and rhizoma of *K.*

rotunda can be seen in figure 5.

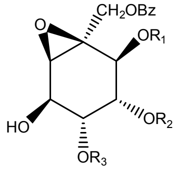
Figure 5. The plant and rhizoma of *K. rotunda*

Stevenson et. al (2007) reported nine new isolated compounds from methanol extracts of rhizomes of *K. rotunda* , that reportedly contained six polyoxygenated cyclohexane derivatives identified as (-)-6-acetylzeylenol (1), zeylenol (2), four acylated derivatives of 1-benzoyloxymethyl-1,6- epoxycyclohexan-2,3,4,5-tetrol (3-6), a Diels-Alder adduct of 3-benzoyl-1-benzoyloxy-methylcyclohexa-4,6dien-2,3-diol (7), crotepoxide (8), and 2-(benzoyloxymethylphenyl-(3,6-di-O-acetyl)-b-glucopyranoside (9). Some compounds demonstrated antifeedant activities against larvae of *Spodoptera littoralis*, the Egyptian cotton leafworm (Stevenson et al., 2007). Lotulung et.al (2008) reported two compounds has been identified from rhizomes of the plant, namely, 2'-hydroxy'-4,4',6'-trimethoxy-chalcone and (+) crotepoxide, the first possessing antioxidant activity. Three known flavanone can be isolated from chloroform extract from *K. rotunda*, namely 5-methoxy-7-hydroxy-flavanone, 5-hydroxy-7-metoksi-flavanon, and 5,7-dihydroxyflavanone (Sri Atun, 2013). Structure of the isolated compounds from *K. rotunda* can be seen at figure 6.

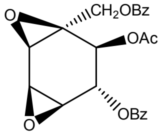
The antioxidant potential of the plant has been evaluated and flavonoids and phenolic derivatives found in the plant, which demonstrated inhibitory potential against lipid peroxidation, suggesting that the plant can be useful in diseases like myocardial infraction, diabetes mellitus, hepatic injury, atherosclerosis, rheumatoid arthritis, and cancer (Lotulung, 2008; Prassad, 2010). Cytotoxic test against the two compounds 5-hydroxy-7-metoksiflavanon and 5,7-dihydroxy-flavanones against MCF-7 cancer cells; HCT 116; Ca ski, and A549 showed that the compound 5,7-dihydroxyflavanone have cytotoxic activity more high. The fact showed hydroxyl group at position C-7 of this structure can increase the cytotoxic activity. Pinostrombin haven't a hydroxyl group at position C-7, but has a hydroxyl group at position C-5. Hydroxyl group at position C-5 is not free, and therefore can form hydrogen bonds with the carbonyl group (Sri Atun, 2013).



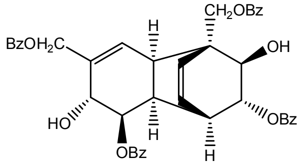
1.R = Ac, (-)-6-acetylzeylenol
2.R = H, zeylenol



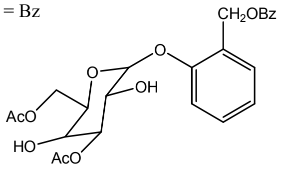
3. R1 = R3 =H, R2 = Bz,
4. R1 =R2 =H, R3 = Bz
5. R1 = Ac, R2 = Bz, R3 =H
6. R1 =Ac, R2 = H, R3 = Bz



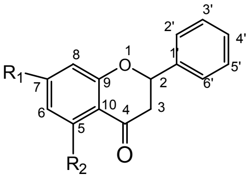
8. Crotepoxide



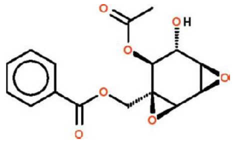
7. 3-Benzoyl-1-benzoyloxymethylcyclohexa-4,6dien-2,3-diol



9. 2-(Benzoyloxymethyl)phenyl-(3,6-di-O-acetyl)-b-glucopyranosida



$R_1 = OCH_3$; $R_2 = OH$, 5-hydroxy-7-methoxyflavanone
$R_1 = OH$; $R_2 = OCH3$, 5-methoxy-7-hydroxyflavanone
$R_1 = OH$; $R_2 = OH$, 5,7-dihydroxyflavanone



3-deacetylcrotepoxide

Figure 6. Isolated compounds from *K. rotunda*

Some flavanone compounds from *K. rotunda* also have activity as antimutagenic. Although the biochemical mechanisms underlying flavanone compounds activities are not yet clear, these results demonstrated that *K. rotunda* has a preventive effect against chromosome fragmentation *in vivo*, probably due to its free radical scavenging capability. Moreover, study showed that phenolic compounds have bioactivity as antioxidant, antimutagenic, and chemopreventive. Therefore, these results confirm and extend our knowledge on the ability of methanol extract and isolated flavanone compounds from *K. rotunda* to protect DNA, showing that both prevent chromosome damage after cyclophosphamid exposure in mice (Sri Atun, 2013). Prasad et. al (2010) investigated the effect of crotepoxide on TNF- and chemotherapy-induced apoptosis and on NF-κB signaling pathway activation. crotepoxide alone showed suppressed the proliferation of various types of tumor cells and potentiated TNF- and chemotherapeutic drugs-induced apoptosis. This correlated with the down-regulation of various gene products that mediate inflammation, cell proliferation, cell survival, invasion, and angiogenesis, all of which are regulated by NF-κB.

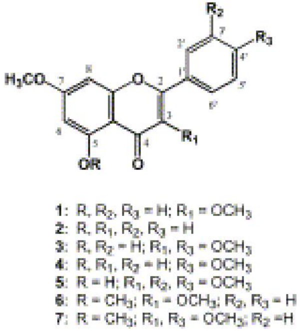
4. *Kaempferia parviflora*

Kaempferia parviflora (Thai Ginseng) is a herb that has some historical and medicinal usage for treating metabolic ailments and improving vitality in Thailand and limited to surrounding regions. It is also reported to be an aphrodisiac compound and physical enhancer. Leaves are green with a reddish underside. It has been used as a medicinal plant in Asia (Leardkamolkarn V, 2009). The plant *K. parviflora* and this rhizome at figure 7.

http://southeastgarden.com/kaempferia.html

Figure 7. The plant and rhizoma of *K. parviflora* (Black ginger)

Some isolated compound from *K. parviflora* reported by many researchers. They found polyphenolic flavonoids which have a wide range of biological activities, such as anti-inflammatory, anti-bacterial, anti-mutagenic, anti-oxidant, and anti-thrombotic The anti-inflammatory effect has been emphasized for a purified compound, 5,7-dimethoxylflavone. These compounds showed anti-bacterial and anti-fungal activities have been pointed (Tewtrakul S, 2009). Some isolated compounds from *K. parviflora* can be seen at figure 8.



1: $R, R_2, R_3 = H; R_1 = OCH_3$
2: $R, R_1, R_2, R_3 = H$
3: $R, R_2 = H; R_1, R_3 = OCH_3$
4: $R, R_1, R_2 = H; R_3 = OCH_3$
5: $R = H; R_1, R_2, R_3 = OCH_3$
6: $R = CH_3; R_1 = OCH_3; R_2, R_3 = H$
7: $R = CH_3; R_1, R_3 = OCH_3; R_2 = H$

Figure 8. Some isolated compounds from *K. parviflora*

Hossain A. et al (2012) reported several studies covered anti-proliferative and cytotoxic effects of the rhizome extracts of *Kaempferia parviflora*. The present study was evaluated the cytotoxicity of 3,5,7,4′-tetramethoxyflavone , 5,7,4′-trimethoxyflavone , and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone on human colorectal carcinoma (HCT-15) cells. All three compounds showed a dose-dependent inhibitory effect on HCT-15 cells. Significant numbers of apoptotic nuclei and condensed chromatin were also observed. These results strongly suggest that three compounds caused cell death via an apoptotic pathway. Therefore all these compounds have the potentiality to be the novel anti-cancer drugs.

CONCLUSION AND SUGGESTION

The current state of research of some species on *Kaempferia* genus clearly shows that the isolated bioactive compounds have high potential in treating many diseases. Chemical constiuents that have been reported for the some species of *Kaempferia* include cyclohexane oxide derivatives, chalcone derivatives, cinnamates, diterpenes, monoterpenes, and flavonoids. Some bioactive compounds showed high cytotoxic effects againt some cancer cell lines, so these plant have the bioprospecting for cancer treatment.

The genus *Kaempferia* is one of the Zingiberaceae family of plants that is widely available in Indonesia and contain a variety of bioactive compounds that are useful, it needs to be preserved and developed to produce quality drugs.

Refference

Chan, E.W.C.; Lim, Y.Y.; Wong, S.K.; Lim, K.K.; Tan, S.P.; Lianto, F.S. and Yong, M.Y. 2009, Effects of drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry*, vol. 113, no. 1, p. 166-172

Hossain Md. A, Wongsrikaew N, Yoo G.W., Han J., Shin C.G, 2012, Cytotoxic effects of polymethoxyflavones isolated from *Kaempferia parviflora* , *Journal of the Korean Society for Applied Biological Chemistry ,* Vol. 55, Issue 4, 471-476

Leardkamolkarn V, Sunida Tiamyuyen, Bung-orn Sripanidkul , 2009, Pharmacological Activity of *Kaempferia parviflora* Extract against Human Bile Duct Cancer Cell Lines, *Asian Pacific Journal of Cancer Prevention,* Vol 10.

Lotulung Puspa DN, Minarti, Kardono LBS, Kawanishi K, 2008, Antioxidant compounds from rhizomes of Kaempferia rotunda l, Pakistan J. Of. Biol. Sci, 11 (20), 2447-2450

Nwet Nwet W., Suresh A., Hiroyasu E., Yasuhiro T., and Shigetoshi K. *,* 2008, Panduratins D—I, Novel Secondary Metabolites from Rhizomes of *Boesenbergia pandurata, Chem. Pharm. Bull.* 56(4) 491—496

Prasad S., Vivek R. Yadav, Chitra Sundaram, Simone Reuter, Padmanabhan S. Hema, Mangalam S. Nair, Madan M. Chaturvedi, and Bharat B. Aggarwal, 2010, Crotepoxide Chemosensitizes Tumor Cells through Inhibition of Expression of Proliferation, Invasion, and Angiogenic Proteins Linked to Proinflammatory Pathway, *J Biol Chem*, 285(35): 26987–26997.

Singh C,B, Binita Chanu S, Bidya baby Th , Radhapiyari Devi W, Brojendro Singh S, Nongalleima, Lokendrajit. N, Kh, Swapana. N, and Singh L.W, 2013, Biological and Chemical properties of *Kaempferia galanga* L. Zingiberaceae plant , *NeBio*, Vol.4, No. 4, 35-41.

Sereena, K., Prakash Kumar, U., and Rema Shree, A.B., 2011. Histochemical and phytochemical markers for the authentication of Ayurvedic raw drug Hallakam (*Kaempferia rotunda*) and its marketed adulterant. International Journal of Pharmaceutical Sciences and Research, 2: 2952-2958.

Sri Atun, Retno A, Eddy S, Nurfina Az, 2013, Isolation and antimutagenic activity of some flavanone compounds from *Kaempferia rotunda, Int. J. of. Chem.and Anal. Sci.* 4 , 3-8

Stevenson P.C, Nigel C.V., Monique S.J.S, 2007, Polyoxygenated cyclohexane derivatives and other constituents from Kaempferia rotunda L., Phytochemistry, 68, 1579-1586

Tan Eng-Chong, Lee Yean-Kee, Chee Chin-Fei, Heh Choon-Han, Wong Sher-Ming, Christina Thio Li-Ping, Foo Gen-Teck, Norzulaani Khalid, Noorsaadah Abd Rahman, Saiful Anuar Karsani, Shatrah Othman, Rozana Othman, and Rohana Yusof, 2012, Boesenbergia rotunda : FromEthnomedicine to Drug Discovery, *Evidence-Based Complementary and Alternative Medicine* , Article ID 473637, 25 pages

Tewtrakul S., Subhadhirasakul S., Karalai C., Ponglimanont C, Cheenpracha S., 2009, Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata, Food Chemistry*, Vol. 115, Issue 2

C-24

STUDY OF ACID CATALYSIS FOR CONDENSATIONOF 4-HYDROXYBENZALDEHYDE WITH ACETONE

Sri Handayani

Chemistry Education, Faculty of Mathematics and Science, Yogyakarta State University

Abstract

In previous research, condensation reaction between 4-hydroxybenzaldehyde and acetone has been done with mol ratio 2:1 using hydrochloric acid as catalyst. Structure elucidation was conducted using FTIRspectrophotometer, 1D and 2DNMR proofed that the compound of 4-(hydroxy(4-hydroxyphenyl)methoxy)benzaldehyde was produced instead of 4,4′-dihydroxydibenzalacetone. It was predicted that intermolecular reaction of 4-hydroxybenzaldehyde compound through acid catalyzed hemiacetal formation involved within the mechanism.

Key words: 4-hydroxybenzaldehyde, hemiacetal, acid catalyst

INTRODUCTION

Introduction

Aldol condensation is intermolecular nucleopilic adition from enolate ion to carbonyl group, resulting β-hydroxy aldehyde. Condensation between aldehyde or acetone with carbonyl groups from different aldehyde or ketone was called as cross aldol condensation. Condensation reaction of aldehyde that has no Hα can be occurred as other aldehyde preserve Hα while as in its form the dimerization is not possible. A cross aldol condensation will be very useful if there is only one carbonyl compound with Hα, thus the reaction would not result mixed reaction product.

According to mechanism of the reaction, aldol condensation is classified in two reaction mechanism types namely enol and enolate mechanism. Enol mechanism occurs in an acidic condition, while the enolate is in a basic condition. Sodium hydroxide as basic catalyzed cross aldol condensation between benzaldehyde derivatives and acetone has been investigated to produce benzalacetone and its derivatives(Handayani & Arty, 2008).Synthesis of two hydroxydibenzalacetones with base catalyst through crossed aldol condensation also reported by Handayani, et al., (2010). Acid catalyst that often to be used in aldol condensation reaction is sulphuric and hydrochloric acid.

Benzalacetone, as a product of crossed aldol condensation still contained Hα in high level acidity make it is easyly to be break and become a new nucleophile. The new nucleophile from benzalacetone derivative could attacks other carbonyl through aldol condensation chain reaction to produce dibenzalacetone derivatives. Thus, double crossed aldol condensation reaction beetwen acetone and benzaldehyde in 1: 2 mol ratio will produce dibenzalacetone.

Previous research performed double crossed aldol condensation to produce analogue dibenzalacetone have been reported. Divanillilacetone as one of dibenzalacetone derivatives had been synthesize with hydrochloric acid catalyzed reaction (Sardjiman, 2000), Sulphuric acid catalyzed Claisen Scmidht condensation between benzaldehyde derivatives and cyclopentanone produced bis-benzylidenecyclopentanone derivatives (Pudjono et al., 2008). In acidic condition, cyclopentanone will forms enol structure and acts as nucleophile that could attacks benzaldehyde derivatives in condensation reaction.

Crossed aldol condensation between acetone and 2-hydroxybenzaldehyde in 1:2 mol ratio produce 2,2′-dihydroxydibenzalacetone in base catalyst, while acetone and 3-hydroxybenzaldehyde in the same mole ratio give 3,3′-dihydroxydibenzalacetone (Handayani et al., 2010). Theoretically, as acetone and 4-hydroxybenzaldehyde are reacted in the same mole ratio will give 4,4′-dihydroxydibenzalacetone as product. On the contrary, in present research, aldol condensation between acetone and 4-hydroxybenzalacetone in 1:2 mol ratio under acid catalyst had produced a new compound instead of 4,4′-dihydroxydibenzalacetone as dibenzalacetone derivative(Handayani, 2012).

The aim of this article is to study the reaction mechanism between 4-hydroxybenzaldehyde and acetone using hydrochloric acid as catalyst that give a new compound. 4-hydroxybenzaldehyde has carbonyl and hydroxyl group, where as acetone has carbonyl group and Hα. Based on the existence of nature groups there are four possible reaction that could be happen, namely aldol condensation, crossed aldol condensation, hemiacetal or hemiketal formation.

DISCUSSION

Aldol condensation reaction with acid or base catalyst, generally started with nucleophilic attack from enol or enolate to carbon of carbonyl group an aldehyde or a ketone. These reaction procedure was performed by dissolving 4-hydroxybenzaldehyde in 1:1 (v/v) ethanol : aquadest. Aldehyde was firstly added to the solution to prevent from acetone self aldol condensation. Acetone was added to the solution after 4-hydroxybenzaldehyde perfectly dissolved, then followed by addition of hydrochloric acid as catalyst. The reaction resulted redish purple solid and characterized by FTIR (Fig. 1) dan NMR spectroscopy.

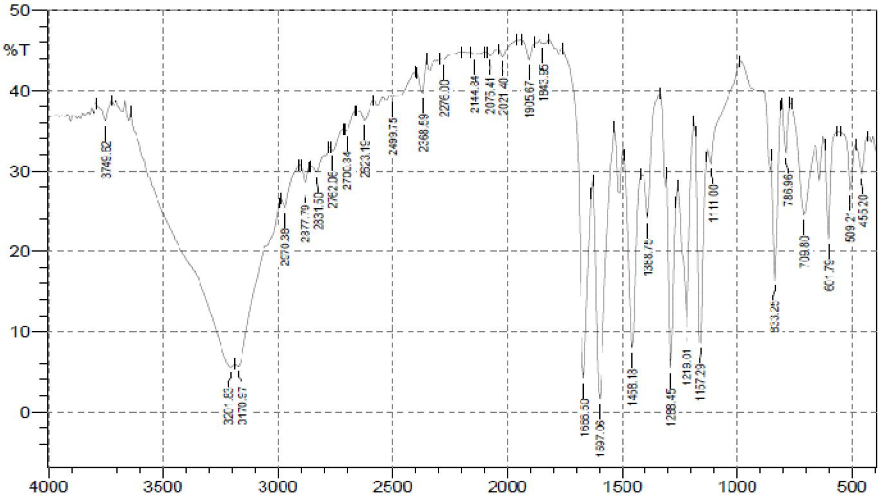


Fig 1. IR spectra of the product synthesis .

Spectroscopy data of the new compound from the reaction between acetone and 4-hydroxybenzaldehyde are : FTIR (KBr) υ = 3170 (=CH_{Str}), 1666.5 (C=O), 1597.06 and 1458.1 (C=C aromatic), 1111-1288.45 (COC) cm^{-1}. ^1H NMR (CD_3OD) : δ = 5.27 (1H, s, H2), 6.7 (2H, d, *J*=8.6 Hz, H3b and H5b), 6.9 (2H, d, *J*=8.6 Hz, H3a and H5a), 7.2 (2H, d, *J*=8.6 Hz, H2b and H6b), 7.7 (2H, d, *J*=8.6 Hz, H2a and H6a), 9.7 (1H, s, H1) ppm; ^{13}C NMR (CD_3OD): $\delta = 104$, 115, 116.9, 129, 133.5, 158.9, 165, 130.5, 130.3, 192 ppm (Handayani, 2012).

Identification of functional groups by using an infrared spectrophotometer showed the appearance of absorption at wave numbers of 3201.83 cm^{-1} of the OH bond, which is conjugated with the phenyl carbonyl at 1666.5 cm^{-1}, C=C aromatic at 1597.06 and 1458.1 cm^{-1} and COC bonds in 1111 - 1268.45 cm^{-1}. The IR spectra show the characteristic absorptions of the hydroxyl group at the position of the target compound cross aldol condensation reaction proceeds, but the characteristic peak of aldehyde also still exist in the region 2877 and 2870 cm^{-1}(Pavia et al., 2009).

Characterization number, position and kind of proton and carbon was done using spectropotometer NMR as showed in Fig. 2 and 3 and the data listed in Table 1. Thesinglet peak at 5.27 ppm regions connected to C at 104 ppm, and adjacent with C at 129 ppm. This peak is appeared in a farther upfield area compared to all other protons. The proton shift shows that it is attached to the aliphatic C (H-C). The other singlet peak appeared at the downfield area(9.7 ppm), connected to C at 192 ppm is a typical of the aldehyde group. These protons adjacent to C at 133.5 and 130.3 ppm of aromatic carbon. It is estimated that this proton is came from aldehyde group (CHO) attached to the aromatic ring.

In addition to the two singlet peaks, 4 peaks are doublets with coupling constants J are 8.6 Hz which indicates the aromatic protons. Integration of each doublet peak indicates that each peak has 2 equivalent protons, it is estimated that the synthesized compounds have two aromatic rings. HMBC spectra showed that the protons next to the aldehyde group is the peak at 7.7 ppm. Two proton peaks at 6.7 and 7.2 ppm adjacent to C 158 ppm indicating that the aromatic protons adjacent to the C-OH.

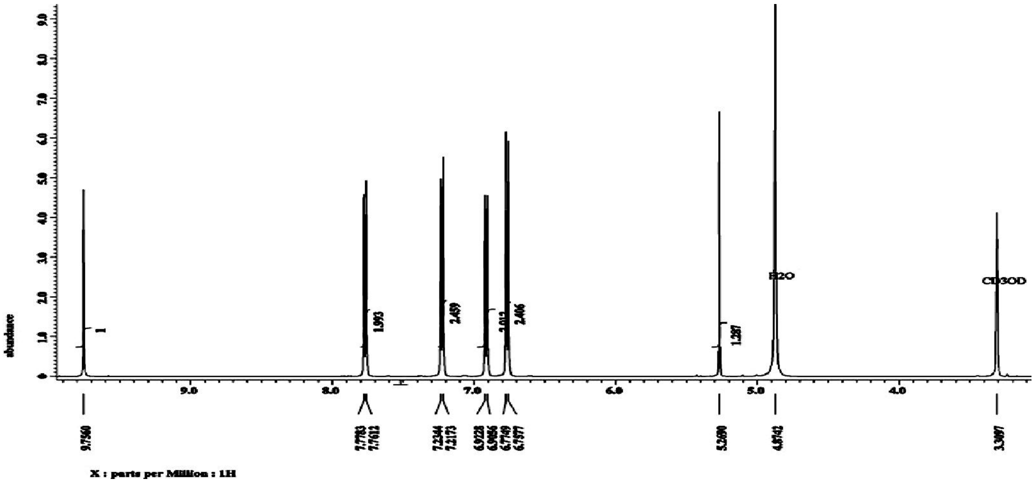


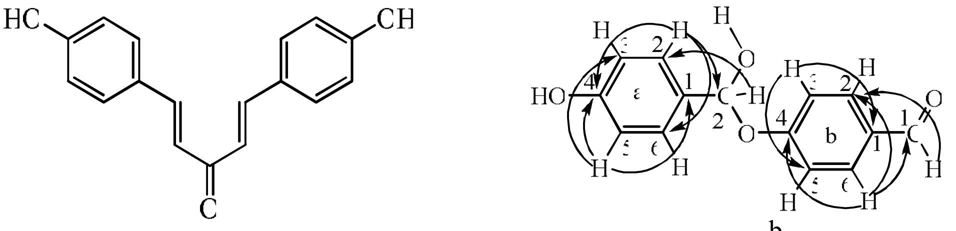
Fig 2. ^{1}H NMR spectra of the product synthesis (CD_3OD)

Fig 3. ^{13}C NMR spectra of the product synthesis (CD_3OD)

Table 1. NMR (^{1}H and ^{13}C) data of the product synthesis (CD_3OD)

No C	δH ppm	∑H, m, *J* (Hz)	δC ppm	HMBC (H→C)
1	9.7	-	192	C1a, C2a, C6a
1a	-	-	130.3	-
2a, 6a	7.7	2, d, 8.6	133.5	C2a, C6a, C4a, C1
3a, 5a	6.9	2, d, 8.6	116.9	C1a, C3a, C5a
4a	-	-	165	-
2	5.27	1, s	104	C2b, C6b
1b	-	-	130.5	-
2b, 6b	7.2	2, d, 8.6	129	C2, C2b, C6b, C1b
3b, 5b	6.7	2, d, 8.6	115	C4b, C3b, C5b, C1b
4b	-	-	159	-

NMR data in Table 1 shows no hydroxyl protons, because it is not always detected, but at 9.7 ppm appear aldehyde proton. In addition, there are only 8 aromatic protons with Jcoupling constant of 8.6 Hz. No protons α and β which haveJ coupling constant around 15-16 Hz of the target compound (1E,4E)-1,5-bis(4-hydroxyphenyl) penta-1,4-dien-3-one (Figure) which indicates the absence of trans alkenes. One proton singlet appeared at 5.2 ppm shows a protons of CH(-OH)(Pavia et al., 2009).



a.

b.

Figure 4.a. Structure of target compound (4,4′-dihydroxydibenzalacetone)
b. Structure of synthesized compound based on spectroscopy data.

Targetted structure of compound synthesis is presented in Fig. 4a while the estimated structure with HMBC correlations of new compound synthesized is presented in Fig 4b.^{1}H and ^{13}C NMR. Comparison between new compound synthesized and ChemDraw prediction are presented in Table 2.The data shows the similarity of chemical shift of compound synthesized.Based on these data it is concluded that the new compound synthesized is 4-(hydroxy(4-hydroxyphenyl)methoxy)benzaldehyde.

Synthesis of 4,4'-dihydroxydibenzalacetone is usually performed under acidic conditions. Acid catalyst was used to prevent proton abstraction of the hydroxy group by base catalyst. Hydroxy proton on the phenolic compound is more acidic than alcohol $Ka=10^{-10}-10^{-8}$) so easily dislodged by the presence of a base(Bruice, 2007). However, the use of acid catalyst did not give the expected result. The result provided that acetone and 4-hydroxybenzaldehyde did not give the target compound, i.e. 4,4′-dihydroxydibenzalacetone. Structure elucidation with FTIR and NMR 1D and 2D prove a new hemiacetal compound, i.e. 4-(hydroxy(4-hydroxyphenyl)methoxy)benzaldehyde. Unexpected hemiacetal formation has also been reported in the acetylation reaction of sterical hindered alcohols with vinyl acetate using lipase as catalyst (Högberg et al., 2000).

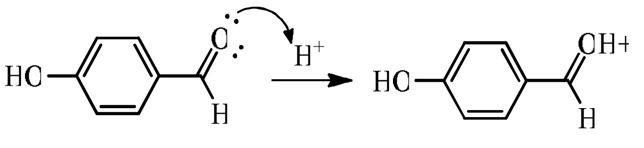
Table 2. ^{1}H and ^{13}C NMR Comparison between synthesized compound and ChemDraw prediction

No C	δ H (ppm)		δ C (ppm)	
	synthesized Compound	*Estimated Chembiodraw*	**synthesized** Compound	Estimated*chembiodraw*
1	9.7	9.88	192	191
1a	-	-	130.3	129.2
2a, 6a	7.7	7.88	133.5	132.3
3a, 5a	6.9	7.18	116.9	114.8
4a	-	-	165	166.4
2	5.27	6.71	104	107.1
1b	-	-	130.5	133.6
2b, 6b	7.2	7.19	129	127.4
3b, 5b	6.7	6.68	115	116.1
4b	-	-	159	157.4

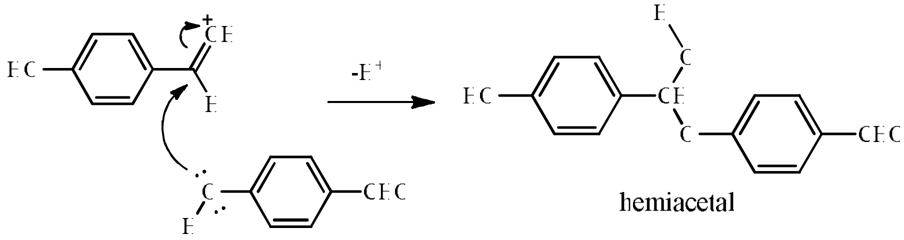
According to structure elucidation data, the resulting material was formed through hemiacetal formation reaction. Hemiacetal is a product that resulted from alcohol addition to an aldehyde with the presence of acid catalyst (Bruice, 2007). In this research, acetone was reacted with 4-hydroxybenzaldehyde–an aldehyde that have hydroxyl group–in acidic condition. Therefore, 4-hydroxybenzaldehyde could be acted as aldehyde or alcohol in hemiacetal formation. Acid catalyst played an important role to lead the reaction by protonating oxygen carbonyl so it more responsive to nucleophil attacks. Reaction of hemiacetal formation occurs between C carbonyl of 4-hydroxybenzaldehyde and OH group of other 4-hydroxybenzaldehyde as the nucleophil.

Reaction mechanism of suggested hemiacetal formation can be delivered on the following schemes:

1. Protonation of oxygen carbonyl by the acid (HCl)



2. Nucleophilic attack to carbon carbonyl



Cross aldol condensation between 4-hydroxybenzaldehyde and cyclopentanone using sulphuric acid catalyst has been reported by Pudjono et al. (2008). Reaction mechanism was through enol mechanism with cyclopentanone acted as the enol. Reaction was performed at reflux temperature with solvent of 2-butanol. Heat treatment was aimed to increase the reagent solubility which was expected to increase the number of collided particle, thus resulting higher yield and more effective reaction. This research have been performed at different reaction temperature and catalyst, which done at 10^{0}C with catalyst of hydrochloride acid. Acid strength of acid catalyst is different each other. It predicted that hydrochloride acid less able to protonate oxygen carbonyl of acetone to form enol. Acetone could undergoes tautomerisation keto and enol. Acetone tautomere in keto form more stable than enol form, because acetone energy is smaller than propen-2-ol (Figure 5). It means that acetone required less energy to reach equilibrium propen-2-ol. This condition caused acetone in enol form could not act as nucleophile in cross aldol condensation. Therefore, H acid was likely to protonate oxygen carbonyl 4-hidroxyibenzaldehyde leading to induction effect followed by hydroxyl group attack of other 4-hidroxysibenzaldehyde to form a hemiacetal.

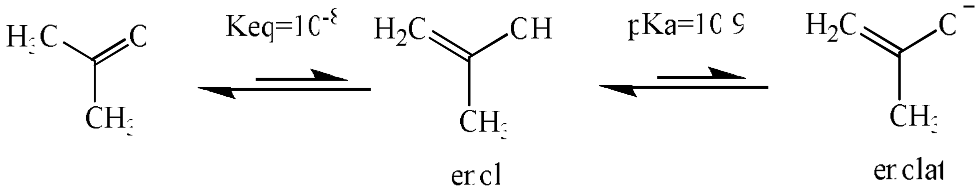


Fig 5. Tautomer keto-enol of acetone

In addition to induction effect, the oxygen carbonyl basicity of 4-hydroxybenzaldehyde compund was also confirmed by the presence of resonance effect (Fig. 6). The resonance in Fig. 6 showed that oxygen carbonyl of 4-hydroxybenzaldehyde carried negative charge. It is strongly suggested that oxygen carbonyl of 4-hydroxybenzaldehyde compound was more base than oxygen carbonyl of acetone, thus acid proton have higher possibility to protonate oxygen carbonyl of 4-hydroxybenzaldehyde.

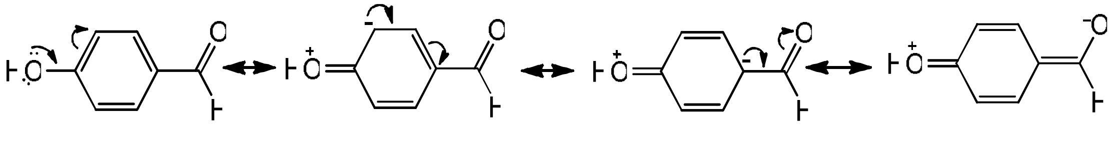


Fig 6. Resonance effect of 4-hydroxybenzaldehyde on oxygen carbonyl basicity

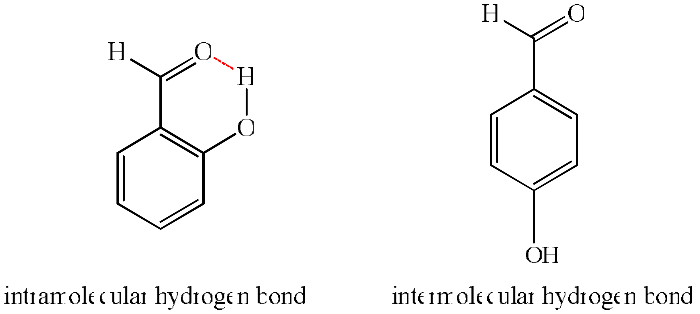


Fig 7. Intra and intermolecular hydrogen bond of ortho and para hydroxybenzaldehyde

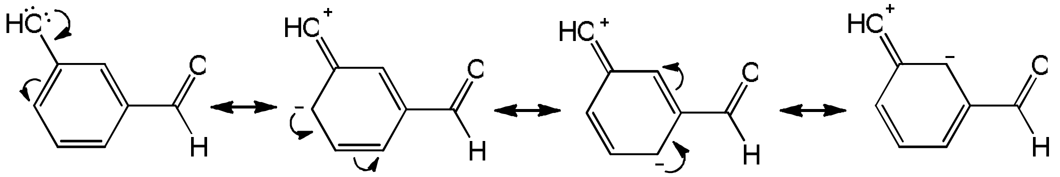


Fig8. Resonance effect of 3-hydroxybenzaldehyde on oxygen carbonyl basicity

Cross aldol condensation between 2-hydroxybenzaldehyde or 3-hydroxybenzaldehyde and acetone to form 2,2′-dihydroxybenzalacetone (IUPAC name:(1E,4E)-1,5-bis(2-hydroxyphenyl)penta-1,4-dien-3-one) and3,3′-dihydroxybenzalacetone (IUPAC name: (1E,4E)-1,5-bis(3-hydroxyphenyl)penta-1,4-dien-3-one) using base catalyst at 10^0C had been reported (Handayani et al., 2010). Both reactions are easier occur because 2-hydroxybenzaldehyde (ortho) could form intramolecular hydrogen bonding (Figure 7) so it reduces intermolecular attraction compared 3-hydroxybenzaldehyde (meta) or 4-hydroxybenzaldehyde (para). Therefore hydroxyl group at ortho or meta position of benzaldehyde was less reactive compared to hydroxyl group at para position and the hydroxyl group would not interfere the aldol condensation reaction. The resonance of 3-hydroxybenzaldehyde that having hydroxyl group at meta position was showed at Figure 10, which indicated that resonance effect of 3-hydroxybenzaldehyde does not influence the basicity of oxygen carbonyl. Based on the explanation above, it can be concluded that hydroxyl group at ortho and meta position does not influence to aldol condensation reaction.

CONCLUSION

Condensation reaction between 4-hydroxybenzaldehyde and acetone (mol ratio 2:1) using hydrochloride acid catalyst does not give the expected compound (i.e. 4,4′-dihydroxydibenzalacetone), but produced a new compound i.e. 4-(hydroxy(4-hydroxyphenyl)methoxy)benzaldehyde. Reaction mechanism of the synthesis was indicated through hemiacetal formation.

REFERENCES

Bruice P Y. (2007). *Organic Chemistry* (Fifth edit.). USA: Pearson Prentice Hall.

Handayani, S. (2012). *Synthesis of Benzalacetone Analogue Over NaOH-/ZrO2-montmorillonite as Catalyst and Its Antioxidant Activity Test.* Gadjah Mada University.

Handayani, S., & Arty, I. S. (2008). Synthesis of Hydroxyl Radical Scavengers from Benzalacetone and its Derivatives, *19*(2), 61–68.

Handayani, S., Matsjeh, S., Anwar, C. and Atun, S. (2010). Synthesis and activity test as antioxidant of two hydroxydibenzalacetones. In *PACCON2010* (pp. 686–688). Ubon Rachatani, Thailand.

Högberg, H.-E., Lindmark, M., Isaksson, D., Sjödin, K., Franssen, M. C. R., Jongejan, H., … de Groot, A. (2000). Formation of hemiacetal esters in lipase-catalysed reactions of vinyl esters with hindered secondary alcohols. *Tetrahedron Letters*, *41*(17), 3193–3196. doi:10.1016/S0040-4039(00)00328-2

Pavia Donald L., Lampman Gary M, Kriz George S, V. J. R. (2009). *Introduction to Spectroscopy* (fourth edi.). Washington: Brooks/Cole.

Pudjono, Sismindari and Widada, H. (2008). Synthesis of 2,5-bis-(4'-hydroxybenzylidene)cyclopentanone and 2,5-bis(4'-chlorobenzylidene) cyclopentanone compounds and Antiproliferative Test to Hela Cells. *Majalah Farmasi Indonesia*, *19*(1), 48–55.

Sardjiman. (2000). *Synthesis of some New series of Curcumin Analogues, Antioxidative, Antiinflamatory, Antibacterial Activities and Qualitative-Structure Activity Relationship*. Gadjah Mada University.

C-25

ISOLATION AND IDENTIFICATION SECONDARY METABOLITES COMPOUND ETHYL ACETATE : n-HEXANE (4 : 6) FRACTION OF GULMA SIAM LEAVES (*Chromolaena odorata* L.)

Sudding

Facultyof Mathematics and Science State Universiy of Makassar

Abstract

This study aims to isolate and identify the secondary metabolites compound ethyl acetate : n-hexane (4: 6) fraction from Gulma siam leaves (*Chromolaena odorata* L.), which is derived from Enrekang regency, South Sulawesi, Indonesia. The stages of research include extraction, fractionation, purification and identification. Fractionation is done by flash column chromatography; purification by recrystallization and identification using color test, melting point, solubility, KLT and spectroscopic methods. Obtained compound in the form of white crystal with a melting point about 144-145^{o}C, also react positively with Dragendorff and Wagner reagent that is marked by orange solution and brown precipitants which indicate the possibility of containing secondary metabolites compound that includes in alkaloid class. It is also supported by the presence of N-H absorption around 3329.14 nm; aliphatic C-H around 2916.37 nm; aromatic C-C around 1620.21 nm; C-N around 1357.89 nm and C-O around 1068.50 nm that is obtained on the *IR* spectrum.

Key words : Isolation, Gulma Siam , Identification, alkaloids, IR

INTRODUCTION

Chromolaena odorata plants or Gulma siam known in some areas with Kirinyu, Babanjaran, Darismin (Sunda), laruna, lahuna, kopasanda (Makassar) (Soerjani, et al., 1987 in Ward, 2006), spread almost all over the archipelago, both in the tropics and subtropics, reproduce very quickly. This plant is very rarely consumed by insects, including pets, tasted very bitter (Akoubundu 2010 in Riki, 2010).

Figure 2.1 Gulma siam Plant (*Chromolaena odorata*)

Classification of Gulma siam plants (Fox, 1990 in Ward, 2006):

Class	: Magnoliopsida
Sub Class	: Asteridae
Order	: Asterales

Family	: Asteraceae
Genus	: Chromolaena
Species	: *C. odorata* L.R.M. King and H. Robinson

This plant grows upright with a height of 2-6 m tall, trunk cylindrical shaped with color yellowish green with fine hairs, soft textured when young and the more mature the harder. Single flower colored mauve to whitish, in pile of bunches can reach 20-35 flowers (Abdullah, 2002 in nurhayati, 2006).

Gulma Siam plants (*C. odorata*) in some areas are used as a medicine of a new wound and as an ulcer medicine, becomes the first solution when getting wound to avoid infection. Several strands (to taste) *C. odorata* leaves are kneaded by hands until the extract is out then squeezed and applied or affixed the extract on the wound, it turned out to a very potent, and the wound dried. For patients with gastric inflammation (ulcer), simply by boiling the *C. odorata* plant, and drink its boiled water. Some people use it as a painkiller pain in the joints. Some countries in Asia, utilizing *C.odorata* as medicine, including Vietnam used in the treatment of wounds (new wounds, old wounds, burns) and other skin infections.

This plant contains variety of compounds that are antioxidants include tannins, flavonoids, alkaloids, steroids and triterpenoids (Yunilas, 2010), the possibility of the presence of those compounds that cause drug efficacy.

TOOLS, MATERIALS AND PROCEDURES

1. Tools

The tools used in the extraction step and identification include: evaporator, flash column, a regular funnel, Buchner funnel, Erlenmeyer flasks of various sizes, measuring cups, maceration vessel, capillary tube, spray bottles, tweezers,, UV light (wavelength 254 nm and 365 nm), hot plate, Pasteur pipette, scales, water bath, oven, chamber, vial bottle, stirring rod, FTIR spectrophotometer.

2. Materials

Gulma Siam leaves, methanol, n-hexan, acetone, ethyl acetate, acetone, kloroforom, stain apparition reagents Liebermann-Buchard, Dragendorff, and Wagner, 10% sulfuric acid, iron (III) chloride, Whatman filter paper, aluminum foil, tissue, silica gel 60 H Merck and silica gel G 60 (230-400 mesh) and aluminum KLT plate coated silica gel 60 G F254.

3. Procedure

a. Extraction

Fine powdered leaves of C. odorata macerated with methanol for 3 x 24 hours. The extract obtained was concentrated using a rotary evaporator to about a quarter of the initial volume (thick extract). The extract was then extracted with a non-polar solvent that is n-hexane to separate the non-polar compounds from the methanol extract, using separation funnel.

b. fractionation

n-hexane extract was then concentrated produce viscous extract with dark green color, then is thin layer Chromatography (TLC) with the mobile phase (eluent) that is varied they are; ethyl acetate: acetone; ethyl acetate: n-hexane; acetone: methanol to find the right mobile phase on vacuum liquid column chromatography (VLCC). On VLCC using stationary phase silica gel 60 H and the combined ethyl acetate: n-hexane, as the mobile phase in its polarity continuously improved, produced 20 fractions.

Fractionation results was TLC again with eluent ethyl acetate: n-hexane, and the fractions that have the same rf values combined, obtained with eluent ethyl acetate: n-hexane (4: 6) that were grouped into 3 combined fractions based on its chromotogram similarity that was A fraction (1-4 fraction) = 2.0 g, B fraction (5-9 fraction) = 1.4 g, and C fraction C (10-20 fraction) = 0.9 grams.

The C fraction is solid in a brownish green color weighing 0.9 g, tested with

Liebermann-Burchard reagent (terpenoids and steroids), FeCl3 (flavonoids), Dragendorff and Wagner (alkaloids). Then fractionated with flash column chromatography using silica gel G 60 H Merck as the stationary phase, and n-hexane 100%, ethyl acetate : n-hexane, and ethyl acetate 100% as the mobile phase.

Fractions obtained were analyzed with TLC with silica gel 60 GF254 as the stationary phase and eluent ethyl acetate : n-hexane as the mobile phase. The fractions that have the same rf values combined then evaporated to obtain a solid. Furthermore, the solubility test is done against some solvents such as n-hexane, chloroform, acetone, ethyl acetate and methanol.

c. Purification

The solid component obtained was washed with solvent n-hexane and then recrystallized using ethyl acetate solvent. The purity of the compounds obtained were determined by performing TLC systems three eluent they are chloroform : n-hexane (7: 3), ethyl acetate : n-hexane (9: 1), and acetone : n-hexane (9: 1) and then continued to the melting point test.

d. Identification

Identification of secondary metabolites compound from crystals obtained was done in two ways, they are:

1. Using reagent to determine the class of secondary metabolite compound that is Liebermann-Burchard for steroids and terpenoids, $FeCl_3$ for flavonoids, and Wagner and Dregendorf for alkaloids test.
2. Identification of functional groups with IR spectroscopy at the Laboratory of Chemistry FMIPA IPB.

RESULTS AND DISCUSSION

The extract obtained was fractionated by vacum liquid chromatography column (VLCC) using silica gel 60 H adsorbents as the stationary phase and 100 ml of n-hexane eluent that was enhanced its polarity with ethyl acetate as the mobile phase. The amount of eluent used in VLCC caused by on the first eluent its stain movement is still not good enough so the eluent used have to be increased its polarity. Fractionation with VLCC obtained 20 fractions as seen in Table 4.1, then chromatographed thin layer used eluent ethyl acetate : n-hexane (4: 6).

Based on the similarity stain on its chromatogram that is shown in Figure 4.1, The VLCC result fractions was combined till obtained 3 fractions combined as shown in Table 4.2.

Table 4.1 Observation Results of VLCC Fractionation

Fraction	eluent	Solution Color
	Ethyl acetate: n-hexane	
1	n-Heksan 100%	Yellow
2	1:19	Yellow
3	1:9	Yellow
4	1:9	Brown
5	2:8	Brown
6	2:8	Brown
7	2:8	Dark Green
8	3:7	Dark Green
9	3:7	Dark Green
10	4:6	Dark Green
11	4:6	Light Green
12	5:5	Light Green
13	5:5	Light Green
14	6:4	Greenish Yellow

15	6:4	Greenish Yellow
16	7:3	Greenish Yellow
17	7:3	Greenish Yellow
18	8:2	Greenish Yellow
19	9:1	Greenish Yellow
20	Ethyl acetate 100%	Greenish Yellow

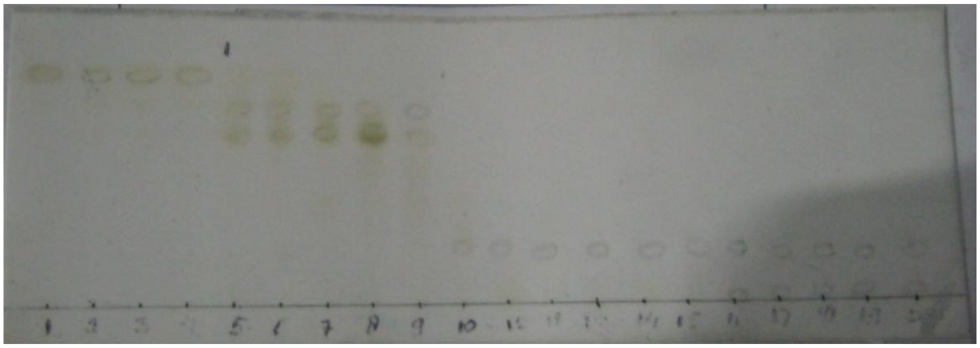


Figure 4.1 TLC chromatogram fractions VLCC eluent: ethylacetate: n-hexane (4:6), adsorbents: silica gel GF254, stain apparition: CeSO4 2%

Table 4.2 Results of Merger and Observation of VLCC fractions

Range of Fractions	Combined fractions	Mass (Gram)	Solids Color
1-4	A	2,0	Brownish Green
5-9	B	1,40	Brownish Yellow
10-20	C	0,90	Brownish Yellow

The C fraction shaped of solid brownish green color with weighing 0.9 g of was done a reagent test and than TLC.

FeCl3 reagent which showed negative results because no change of color.

Wagner and Dragendorf reagent gave a positive result against the alkaloid, is marked by a color change from brownish green to orange for Dragendrorf and the presence of a brown precipitate for Wagner. And positively to Liebermann Burchard that was marked by a change of color from brown to green.

The C fraction is not dissolved in the solvent n-hexane, but dissolved in polar solvents such as acetone, even dissolved completely in the solvent ethyl acetate, chloroform and methanol. This matter showed that the compound contained in fraction C is polar.

TLC test was then performed with eluent n-hexane: ethyl acetate and ethyl acetate: chloroform with a variety of comparisons, obtained eluent ethyl acetate: n-hexane 6: 4 by the apparition of stains CeSO4 2% after heating showed the appearance of stains with good separation pattern as in Figure 4.3. Based on that matter fraction C was fractionated further using eluent ethyl acetate: n-hexane 6: 4.

2. Results of Vacum Column Flash Chromatography Fractionation .

The combined of C fractions wighing 0.9 grams fractionated through flash column chromatography with silica gel G 60230-400 mesh as the stationary phase and eluent started from n-hexane 100%, ethyl acetate: n-hexane (6: 4), ethyl acetate 100% as the mobile phase resulted 33 fractions then continued with KLT analysis with developer eluent ethyl acetate: n-hexane (6: 4).

Table 4.3 Observation result of flash column chromatography.

Fractions	Eluent	Color
1	n-hexane 100%	Green
2 – 4	Ethyl acetate: n-hexane (6:4)	Blackish Green
5 – 9	6 : 4	Brownish Green
10 – 17	6 : 4	Yellowish Green
18 – 25	6 : 4	Yellowish Green
26 – 30	6 : 4	Clear Greenish
31 – 33	Ethyl acetate	Clear

Based on KLT analysis (Figure 4.4) from the 33 fractions, fractions that have the same chromatogram merged until it was obtained six fractions combined they are combined fractions Ca (fraction 1), Cb (fraction 2), Cc (fraction 3), Cd (fraction 4-7), Ce (fraction 8-19), Cf (fraction 20-33).

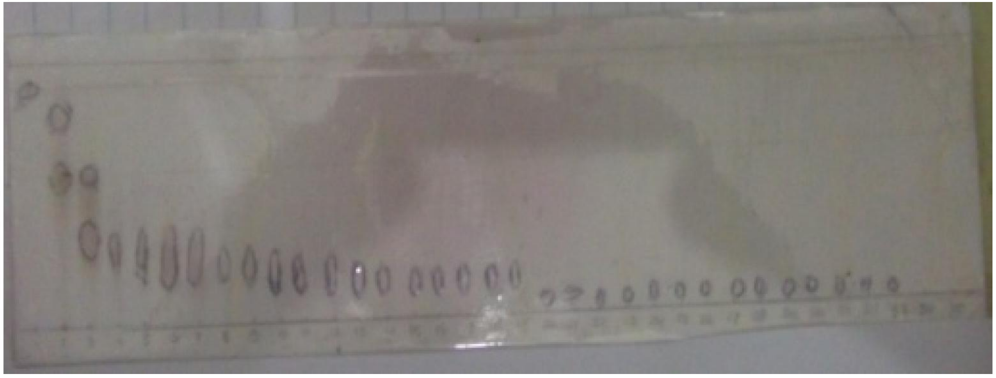


Figure 4.4 Chromatogram 33 fractions results from the chromatography flash column

Eluent : ethyl acetate: n-hexane (6: 4)
Adsorbents : silica gel GF234
Stains apparition : CeSO4

Fractions obtained were evaporated to one-fourth from its initial volume and then performed KLT analysis to combine fractions with the same Rf. From the result of Merging it was obtained 6 fractions they were Ca, Cb, Cc, Cd, Ce, Cf. After all of the fractions evaporated fraction that showed crystals was the fraction Cd.

3. Purification Result

The combined fraction 4-7 (Fraction Cd) showed the presence of crystals so potentially to be continued. After being evaporated the combined fraction Cd shaped crystalline amorphous with green color.

The amorphous-shaped crystals were then washed with n-hexane resulted white crystals with a weight of 30 mg then recrystallized with ethyl acetate resulted white crystals with a weight of 10 mg.

The C-d crystals were then purified with three eluents system test, that were chloroform: n-hexane (7: 3) with rf value of 0.325 (a), ethyl acetate: n-hexane (9: 1) with rf value 0.5 (b) and acetone: n-hexane (9: 1) with rf value of 0.825 (c), where the results showed a single kromotogram that can be seen in Figure 4.5.

4. Identification Results

The crystals obtained were identified including by:

a. Reagent test / color test with FeCl3 reagent, Liebarmann Burchard, Wagner, and Dragendroff. Cd crystals positive to Dragendorff reagent and Wagner. In the C-d crystal there was a color changed to orange on Dragendroff reagent and brown (there are brown sediment) on Wagner reagent as seen in Figure 4.6.

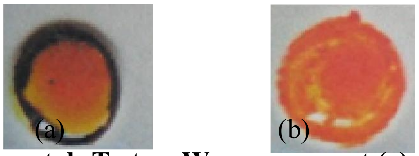


Figure 4.6 The result of C-d crystals Test on Wagner reagent (a) and Dragendorff reagent (b)

Untuk mengetahui kepolaran dari kristal C_d maka dilakukan uji kelarutan. Pada uji kelarutan kristal C_d tidak larut dalam n-heksan, larut dalam aseton, etil asetat, kloroform dan methanol. To know the polarity of th C-d crystal then solubility test was done. In the solubility test, C-d crystals were not soluble in n-hexane, soluble in acetone, ethyl acetate, chloroform and methanol.

b. On the melting point test it was obtained crystal Cd melting point at 144-145 oC. From this test, it can be seen the purity of a compound. If the compound has a melting point with a low trajectory then the compound can be categorized as a pure compound because if the trajectory is high then in that compound still contained impurities.

c. The results of IR spectroscopic measurements of C-d crystals.

Infrared spectroscopy (IR) can be used to identify a compound that has not been known because the spectrum produced is specific to the compound. Chromatograms IR spectroscopy results of C-d crystals can be seen in Figure 4.7.

Table.4.4 IR spectrum analysis results on the Gulma Siam leaves (*C. odorata*).

NO.	Wave number cm-1 on Gulma Siam leaves (*C. odorata*)	Cluster
1	3329,14	N-H
2	2916,37	C-H Aliphatic
3	1620,21	C=C Aromatic
4	1465,9	$-CH_2$
5	1357,89	C-N
6	1068,50	C-O

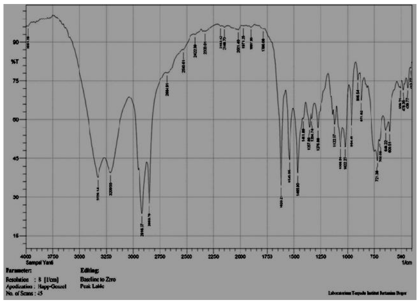


Figure. 4. 7. Infrared Spectrum of C-d Crystal

B. Discussion

1. Fractionation of flash column chromatography

The C Fraction (0.9 g) in the form of a brownish green solid of a VLCC result was a further analyzed fractions. Reagent FeCl3 test for C fraction showed negative results where there were no changes in color, which indicated that the fraction C contained no flavonoids.

For Wagner and Dragendroff reagent test gave a positive result on the alkaloid with color change from brown to orange at Dragendroff reagents and there was a brown precipitate on Wagner reagent. In Lieberman Burchard reagent showed positive results against steroid with a color change from brown to green, this is due by the time of merger fractions, some fractions that contained in C fraction were intersecting with B fraction. In the solubility test the C fraction was not soluble in n-hexane, slightly soluble in acetone and completely dissolved in ethyl acetate, chloroform, and methanol (Table 4.3), this was indicated that the compounds contained in fraction C is polar. This fraction has a pretty good TLC separation result. The C fraction with eluent ethyl acetate: n-hexane (4:6) first analyzed with TLC before flash column chromatographed using several comparisons of different eluent. TLC itself aims to find out the number of components compounds still contained in C fraction that can be seen from the number of stains that appear on the chromatogram.

Moreover, TLC also aims to find out the best eluent for flash column chromatography. From the TLC test gave result that the most good eluent for use on flash column chromatography was ethyl acetate : n-hexane eluent (6: 4).

2. Purification

The C-d fraction (green color crystals and amorphous shaped) were washed with n-hexane then recrystallized using ethyl acetate to obtain more pure results (white color crystals and amorphous shaped) with a weight of 10 mg and showed single chromatogram of three eluent system with varied polarity level from relatively less polar eluent to relatively polar eluent.

The rf value is greater in relatively polar eluent that are chloroform: n-hexane (7: 3) with rf values 0.325, ethyl acetate: n-hexane (9: 1) with rf values of 0.5 and acetone: n-hexane (9: 1) with rf value of 0.825. This was indicated that the compounds obtained are relatively polar compounds, in accordance with the solubility properties of a compound based on polar solvent with dissolved compounds that is if polar solvent enhanced then the solute compound rise in accordance with the increase of its solvent means that the compound is a polar compound.

Table4.5 The Results Identification Test of C-d Fraction

Test										
Solubility					Reagents				IR	
C6H6	Aceton	CHCl3	CH3C2H5	CH3OH	LB	DG	Wagner	$FeCl_3$	Absorption area (cm^{-1})	Functional Groups
-	+	+	+	+	-	+	+	-	3329,14	N-H
									2916,37	C-H Aliphatic
									1620,21	C=C Aromatic
									1465,9	$-CH_2$
									1357,89	C-N Amin
									1068,50	C-O
Alkaloids										

3. Identification

Identification result of C-d Crystal was positive on Wagner reagent that indicated a brown precipitate and also positive on Dragendroff reagent (Table 4.5) that gave color changed from clear to orange.

This was indicated that the crystal was alkaloid positive. The melting point was 144-145^{O}C that showed a sharp trajectory. For the solubility test of the C-d crystals was not soluble in n-hexane, but dissolved in ethyl acetate, chloroform, acetone and methanol (Table 4.5)

Table 4.5 Identification Results (solubility test, reagents and IR) Cd crystal

IR absorption spectra that widened in the area 3329.14 cm^{-1} indicated the presence of N-H group, strengthened by the presence of CN absorption in the area 1357.89 cm-1. Sharp absorption in the area 2916.37 cm-1 indicated the presence of aliphatic C-H group, strengthened by the presence of absorption in the area 1465.9 cm^{-1} indicated the presence of-CH2- bending. Whereas medium absorption was at the area 1620.21 cm^{-1} was suspected absorption from aromatic C=C group. For absorption in the area 1068.50 cm-1 was suspected absorption from C-O group.

Based on the interpretation of data obtained from infrared spectrum (IR), positive on the color test using reagent Dragendorf and Wagner, not soluble in n-hexane, but soluble in polar solvents such as ethyl acetate, chloroform, acetone and methanol as well as the literature search then it can be concluded that crystal Cd that was obtained suspected to be alkaloid class compound.

Bibliography

Achmad, S. A. 2006. *Hakekat Perkembangan Kimia Organik Bahan Alam Dari Tradisional Ke Moderen Dan Contoh Terkait Dengan Tumbuhan Lauraceae, Moraceae Dan Dipterocarpaceae Indonesia*. Makalah. ITB. Bandung.

Brock, J. 1986. *An Introduction to Aquaculture Disease*. Aquaculture Vol.10:3 -6.

Dalimartha, S. 2003. Obat tradisional. *Atlas Tumbuhan Obat Indonesia*; PDPERSI.co.id. Pusat Data Perhimpunan Rumah Sakit Seluruh Indonesia.

Harborne, J.B. 1987. *Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan.* Institut Tekhnologi Bandung. Bandung.

Markham, K. R. 1981. *Cara Mengidentifikasi Flavanoid*. Institut Tekhnologi. Bandung.

Riki, 2010. *Ekstraksi, Identifikasi Senyawa Metabolit Sekunder Dan Uji Bioaktivitas Ekstrak Metanol Daun Gulma Siam (Chromolaena Odorata L.R.M. King Dan H. Robinson) Terhadap Staphylococcus Aureus Dan Larva Artemia Salina Leach*. Skripsi tidak diterbitkan. Universitas Negeri Makassar.

Wardhani, N.D. 2006. *Aplikasi Mulsa Chromolaena odorata* (L.) Kings and Robinson *dan Cendawan Mikoriza arbuskula pada Tanah Latosol untuk Pertumbuhan dan Produksi Pueraria javanica.* Skripsi. Program studi nutrisi dan makanan ternak Fakultas peternakan Institut pertanian Bogor. Bogor.

Yunilas, 2010. Potensi Gulma Semak Bunga Putih (Chromolaena odorata) *Sebagai Pakan Ternak.* http://yuni-peternakan. Blogspot.com/2010/02/potensi-gulma-semak-bunga-putih.html. Diakses tanggal 23 Desember 2010.

C-26

REVIEW OF APPLICATIONS NANOPARTICLES OF TiO_2 AND ZnO IN SUNSCREEN

Sulistyani

Department of Chemistry, State University of Yogyakarta, Karang Malang 55281,
sulistyani@uny.ac.id

ABSTRACT

The use of sunscreen by the public, especially in Indonesia is quite popular. Health and beauty reasons underlying the popularity of the use of sunscreen products. This further fueled by increasing public awareness on the dangers of UV radiation of sunlight that the intensity at last decade more higher. It has been widely demonstrated that UV radiation of sunlight can cause sunburn (tanning effect to the skin), premature aging such as the appearance of dark spots and wrinkled skin, melanoma and non-melanoma skin, and cancer. Among the active particles are often used in sunscreen products as UV radiation filters are particles of TiO_2 and ZnO based nano size. Along with the development of nanotechnologies, sunscreen products are also diversified, often combined with cosmetics. This paper aims to disseminate to the public, especially in Indonesia about how the characteristics of nanoparticles TiO_2 and ZnO in sunscreens, the effectiveness of the particle as a barrier to UV radiation, and the dangers that can be caused by excessive use of the product.

Keywords: sunscreen, nanoparticle, TiO_2, ZnO, UV radiation.

A. Introduction

Nowadays, using of sunscreen by the public especially in Indonesia is quite popular. Most people use sunscreen products to maintain healthy skin and beauty. The presence of sunscreen on the skin layer is expected to anticipate the sun's heat so as not to burn the layer of skin, preventing skin cancer and premature aging (Steven-Wang, 2012). Initially, only a sunscreen cream that is used topically to the whole body, especially on body parts exposed to the sun. However, the development of technology gives diversified packaging of product sunscreen. In addition to the form of creams, sunscreens also exist in the form of a gel, lotion, mousse, spray, stick, and wipes. In fact, the development of nano technology allows the active ingredients of sunscreen combined with cosmetic so successful in the market. Based on data from the ongoing study, Friends of the Eart mention that the nanoparticles have been added in the individual care products such as deodorant, soap, toothpaste, shampoo, hair conditioner, anti wrinkle cream, moisturizer, foundation, powder, lipstick, blush on, eye shadow, nail polish, perfume and shaving lotion (Anuradha Patel *et al.* , 2011).

The dominant type of sunlight interacting with humans is ultraviolet light. Scientifically, ultraviolet light (UV) from the sun is divided into several types, i.e. UVA, UVB and UVC. The type of light is based on the UV wavelength region. UVA rays have a wavelength range of 320-400 nm, UVB rays have a wavelength range of 290-320 nm, and the UVC have a wavelength range of 100-290 nm. UVC is almost completely absorbed by the ozone layer (97-99%), whereas UVA and UVB rays partially able to reach the earth's surface and interact with human skin. During the day, the composition of the UV light is composed of 10% of UVB and 90%

UVA rays when the sun is perpendicular to the earth. When the sun inclines, the intensity of UVB rays will be reduced, while the intensity of UVA rays are relatively constant throughout the day (Nicholas *et al*., 1997).

From time to time, the intensity of the sun's UV rays that reach the earth's surface is constantly increasing. This phenomenon is attributed the depletion of the ozone layer and the greenhouse effect. Splendor of the use of ozone depleting substances (ODS) such as CFCs and HCFCs has led to radical particles are continuously decompose ozone into oxygen radicals and generate new particles (Fahey-Hegglin, 2010). CFCs and HCFCs are generally used to help power the spray on cosmetic appliances such as hair spray, mosquito spray, automotive maintenance equipment, house cleaners, spray paints, as well as air conditioning and refrigeration coolants. This condition is further aggravated by the greenhouse effect due to the high concentration of carbon dioxide in the earth. Increasing the amount of carbon dioxide in the lower atmosphere causing UV rays of the sun is trapped in the Earth's atmosphere so that the resulting radiation is also higher (IPCC, 2007).

Increasing UV radiation on earth has raise public awareness and concern on the dangers of radiation. Nearly all cancers are either melanoma or nonmelanoma skin caused by excessive sun exposure (Armstrong-Kricker, 1993; Armstrong *et al*., 1997). The International Agency for Research on Cancer (IARC) has identified that high-intensity UVB radiation can damage DNA cells directly, whereas UVA radiation trigger skin cancer when absorbed in melanin. Both UVA and UVB radiation trigger the production of reactive oxygen species (ROS). UVA radiation of sunlight is responsible for more than 90% of skin cancer cases, whereas UVB radiation is responsible for skin pigment brownish to burn, known as erythema (Grist *et al*., 1993). Malignant melanoma and basal cell cancer (BCC) is associated with intermittent sun exposure is mainly experienced by children and adolescents. Squamous cell cancer (SCC) due to accumulated radiation exposure is associated mainly by solar UVA radiation, whereas lip cancer caused by sun exposure throughout life. An estimated 93% and 50% of skin cancer lip cancer caused by UV radiation from the sun (Gallagher *et al*., 2010; Balk, 2011). The highest melanoma cases occur in Quessland, Australia that there are 56 new cases per year per 100,000 men and 53 women (Leiter-Garbe, 2008). In addition, high exposure of UV radiation stimulates the appearance of cataracts, decreased body immunity, permanent eye damage in the form of retinal disorders 'Age macular degeneration' and premature aging.

Sunscreen active ingredient is always progressing, both made from natural and synthetic chemistry. Initially, the organic materials are used as the sole active ingredients in sunscreens. However, susceptible to degradation when exposed to sunlight, then develop inorganic particles as active ingredient of sunscreen. Among the inorganic particles often used in sunscreens are titanium dioxide (TiO_2) and zinc oxide (ZnO). At the microscale, particle leads a white coating on the surface of the skin, so the particle size is reduced to the nanosize (Wolf, 2001). At present time, sunscreen containing nanosized of TiO_2 and ZnO have been widely circulated in the community. Nevertheless most people including Indonesia society still doubt about the effectiveness and the safety of nanoparticle-based sunscreens. Therefore, this article will discuss about the characteristics, effectiveness, and health risks in the use of nanoparticle-based sunscreens, especially nanoparticles of ZnO and TiO_2.

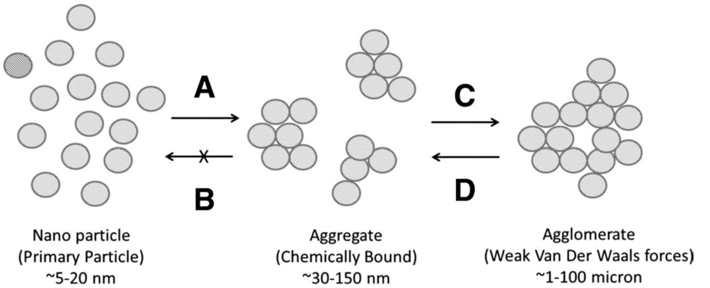
B. Discussion

Nanomaterial is a general term for both nanosize object in different shape and size (length, width or height). Nanotechnology is the manipulation of matter on a size between 1 to 100 nm, where one nanometer is equal to one millionth of a millimeter (National Nanotechnology Initiative, 2009). Changes of matter at the nanoscale is able to produce a much different material properties compared with larger particles. The nature of the changes include color, solubility, strength of materials, electrical conductivity, and magnetic behavior.

Nanoparticle materials have a larger surface area relative to volume. This makes the chemical nature of the substance to be more reactive than larger particles.

Physical Characteristics of Nanosized TiO_2 and ZnO

Sometimes, manufacturers do not put a label on the actual product nanoparticles using nanoparticle materials. That is because the characteristics of nanoparticles which easy to form clots. Based on its physical form, nanosized of TiO_2 and ZnO has three forms, namely primary particle, aggregate and agglomerate, as shown in Figure 1.



Source: Steven *et al*., 2011

Figure 1. Physical form of nanoparticle: primary particle, aggregate, and agglomerate.

The primary particle size of 5-20 nm is the smallest form of nano-crystalline particles. Primary particles tend to coalesce to form clumps (aggregates) due to the strong attractive force between the crystal particles. Size Aggregate particle is about 30-150 nm. Aggregates tend easily to form larger clumps and loose (agglomerate) by the effect of drying and heating during the production process. Agglomerate shape usually owned by TiO_2 and ZnO particles as a powder (Steven et al., 2011).

Potential Protection of Nanosized TiO_2 and ZnO in Sunscreen

Particles of TiO_2 and ZnO including inorganic active ingredients in sunscreens that have been developed to protect the skin of the harmful effects of UV rays. This is related to the characteristics and potential of those particles. Both minerals are not soluble in water and their performance are affected by particle size.

In sunscreen, there are three mechanisms of UV radiation attenuation, i.e. the absorption, scattering, and reflection. However, in general there are two ways of electromagnetic radiation interacts with matter, i.e. absorption and scattering. Comparison between scattered to radiation light absorbed depends on the particle size. The larger the particle size, the ratio between scattering and absorption greater. Conversely, the smaller the particle size the ratio between scattering and absorption are getting smaller. Therefore, in the case of nanosized ZnO and TiO_2 process uv rays absorption is more dominant occurs (Henry-Zoe, 2009).

Nanosized particle also affects the properties of the conductor material. The smaller the particle size causes the energy gap between the valence band and conductor band further away. It means that the nanoparticles are more semiconductor or even changed insulator. Thus, absorption of UV radiation required to raise an electron from the valence band to the conduction band requires a larger energy. Nanosized TiO_2 has the potential to protect the skin from the effects of UVA radiation, while nanosized TiO_2 has the potential to protect the skin from the effects of UVB radiation. So, the combination of sunscreen active ingredients consisting of nanosized TiO_2 and ZnO will provide protection to a broader spectrum (Nicholas *et al*., 1997).

In terms of stability, nanosized ZnO are much more stable than TiO_2 particles when exposed to sunlight. ZnO particles are not photoreactive so it does not generate harmful free radicals. While TiO_2 is more photocatalytic by releasing electrons or particles radicals when exposed to the radiation light. Therefore, the particle surface is usually wrapped inert material such as aluminum oxide or silicon oil. This coating is important because the photocatalytic properties of nanosized titanium dioxide harmful to skin fibroblasts, nucleic acids and cell-cell carcinoma of the human small intestine. In addition, it is also useful to improve the dispersion of nanoparticles in sunscreen formulas (Anuradha Patel *et al*., 2011).

Initially, microsize (powder) TiO_2 and ZnO particles used as a sunscreen active ingredient. Unfortunately, these microparticles have a shortage, i.e. less effective at absorbing UV light and lead to white residue on the skin surface. Furthermore, microsize (agglomerate) is reduced into nanosize (aggregate). Changes in particle size into the nano turns out to be transparent so that the white layer on the surface skin invisible either in the form of aggregate or agglomerate. In addition, nanosized particles make the surface become wider thereby increasing the absorption of the particles against UV radiation. Thus, smaller particle size higher the Sun Protection Factor (SPF) value significantly. SPF value indicates duration of sunscreen to protect the skin before burning. SPF can be defined as the rasio between the minimal dose that produces perceptible erythema on the skin (i.e., minimal erythema dose) /MED) in the presence or absence of 2 mg/cm^2 of sunscreen, using solar simulated radiation as a light source (United State Food and Drug Administration, 1999). The scientists recommend the use of sunscreen products that have SPF value of at least 15. Actually, SPF value of sunscreen only indicates protection against UVB radiation.

To obtain a wider absorbtion area (filter UVA and UVB), the composition of the formula should use a combination of nanoparticles with different sizes (Steven *et al*., 2011). Indicator of sunscreen protection against UVA radiation can be seen from the value of the critical wavelength (CW). CW is a wavelength region where the absorption of the UV light reaches at least 90%. The greater ability of sunscreen protection against UVA radiation, the greater the CW value. The label "broad spectrum" of sunscreen products are only allowed for products that CW value is larger than 370 nm (FDA, 2011). This is consistent with the results of research conducted by Costello *et al.* (2012) that broad spectrum sunscreens can reduce the damage of the dermal molecules so that preventing skin damage caused by the UV radiation.

Based on the characteristics of nanosized TiO_2 and ZnO which easy to agglomerate, sunscreen manufacturers must maintain the optimum particle size until the end of the production process. One way to do this is by adding a dispersing agent so that the components can be mixed in sunscreen evenly. The presence of dispersing agent in sunscreen formula was able to enhance the SPF value up to 5 times (Steven et al, 2011). Sunscreen usually also contain film-forming additives (such as acrylate, acrylamides, and copolymers) so that when sunscreen is applied to the skin will feel supple, uniform, and thoroughly wrap (Schwarzenbach-Huber, 2003; Hunter-Trevino, 2004).

Aggregate of nanosized TiO_2 and ZnO in sunscreen can be designed as a hollow sphere. It can improve the performance of the sunscreen products i.e. enhance the SPF value, reducing the potential for irritation of the skin, resulting in a softer texture, and more transparent when applied to the skin surface thereby increasing its aesthetic value (Jones, 2002; Miki Uematsu *et al.,*undate). Modified particle can also be done by doping TiO_2 crystal with manganese (Mn). Manganese ions in the crystal are placed in and on the surface of TiO_2 particles. This modified aim to overcome the reactivity of TiO_2 particles. The crystal modified successfully prevent the formation of free radicals, therefore TiO_2 coating is not necessary. Material doped Mn-TiO_2 particle improves retention percentage of organic ingredients and vitamins to make them more stable. Vitamins function as an anti-aging active ingredients, thus indirectly TiO_2 provide advantages in anti-aging skin products as effectively as its role as protector of UV light (Wakefield *et al*., 2006; Hewitt *et al*., 2008).

Potential Toxic Properties of Nanosized TiO_2 and ZnO in Sunscreen

The main issues of application of nanosized TiO_2 and ZnO as active ingredients in sunscreen is the potential toxic properties. The toxic properties associated with the ability of nanosized TiO_2 and ZnO to generate free radicals and reactive oxygen species (ROS) during exposure to UV radiation. Widely surfaces of particle at the nanosized will increase the reactivity of substances, particularly the formation of oxygen radicals. This is especially feared because of reactive oxygen species can damage DNA, can cause mutations, can damage proteins and fats causing permanent injury to cells and tissues. In fact, the widespread damage by ROS can cause changes in the genetic code of the entire body tissues. Although the body has a natural antioxidant defenses against ROS, but the system would be overwhelmed if the body's endogenous receive excessively oxidative substances.

Some things that need to be understood by the consumer sunscreen products including the following. First, nanoparticles of TiO_2 and ZnO in its application covered by a protective layer on its surface. The protective layer serves to protect the nanoparticles of contact with other materials including oxygen. Thus, nanoparticles are inert and the risk of free radical formation can be prevented. Components can be used as the protective layer such as silicon dioxide, stearic acid, and aluminum stearate (Lademann et al., 2000). Second, the skin has its own antioxidant mechanism consisting of molecules of enzyme and non-enzyme, to cope with ROS (Steven *et al*., 2011). Thus, ROS generated by nanosized TiO_2 and ZnO during UV exposure can be neutralized by the body's natural defense mechanism. Furthermore, modified TiO_2 crystals have been proved could stabilize the photocatalytic properties of TiO_2 particles (Wakefield *et al*., 2004).

In terms of solubility, ZnO particles are easily soluble form zinc ions compared to TiO_2 particles. Zinc ion is one of the minerals that the body needs to intake of about 10-15 mg/day (Henry-Zoe, 2009). It means that if there is zinc ions are absorbed into the body will be processed by the body. Thus the use of ZnO as active substance in sunscreen is relatively safe. Another important thing to be consider related the safety of use nanosized TiO_2 and ZnO are both nanoparticles have been widely used in various products for decades. TiO_2 is often used in various products such as toothpaste, lotions, skim milk, and cheese boxes, while the ZnO commonly used in baby powder, anti-dandruff shampoo, and various creams. However, the photocatalytic activity of nanoparticles must be continuously monitored so as not to damage the composite product system. Therefore it is necessary supervision and testing, especially when the merger of the matrix polymer with nano particles (Anuradha Patel, 2011).

Potential Penetration of Nanosized TiO_2 and ZnO into the Skin

The anxiety by the harmful effects of very small particle size (nano size) also appears. It happens because there are fears that nanoparticles will penetrate into the skin layers to the internal organs. Research on this subject have been carried out either by government, industry, and academia. The results showed that TiO_2 and ZnO relatively little penetrates the skin and is not visible to penetrate the stratum corneum in healthy human skin or wounded (Zvyagin, 2008; Sadrieh *et al*., 2010; Hallmans, *et al*., 1985; Agren, 1990; Agren *et al*., 1991). Analysis of possible penetration of nanoparticles on the burn skin due to exposure to radiation have been conducted (NA Monteiro-Riviere *et al*., 2011). The results showed that up to 48 hours of exposure no penetration ZnO occurs, while the TiO_2 penetration only occurs in the stratum corneum layer with the depth twice of the normal skin, but still tolerable, whereas the layer transdermal absorption does not occur. In general, the penetration of nanoparticles into the skin layer is minimal category.

When the nanoparticles are added to the cream or lotion, the primary particles tend to coalesce to form aggregates due to strong attractive forces between molecules. Aggregates formed in a lotion or cream is too large to cross the blood-skin barrier. Although some of the nanoparticles found in the pilosebaceous open and shallow part of the follicle, the possibility of nanoparticles enter the body tissues through of transfollicular route can be ignored. It is because the hair growth tends to push the particles to the surface of the skin. However, there is the possibility of nanoparticles accumulated in the stratum corneum, but the skin renewal process by the epidermis will prevent long-term accumulation and nanoparticle penetration into skin tissue sections (Steven *et al*., 2011).

Diversification Sunscreen Packaging

Packaging sunscreen products had been developed. In the beginning, most of the sunscreen packaging is cream. Neverthelesss the cream packaging is less favored because hands dirty and takes more time. This constraint is an important issue because majority of consumers products are children and adolescents. Then, sunscreen product packaging has varied into spray form. Currently, sunscreen in spray packaging have been widely sold in the market, either single phase oil-based (emulsion sprays) or single phase alcohol-based (aerosol sprays). Sunscreen in spray packaging is often expressed as "continuous spray sunscreen" or "no-rub sunscreen" because it can be applied in a sustainable manner and without the need to scrub by hand. The concept is very fine particles and the polymer added to the solvent phase to assist the formation of a layer (film) on the surface skin. This product is preferred because the results are very satisfactory, i.e. invisible on the skin, not grained, and does not feel greasy on the skin (Tadros *et al*., 2003).

Sunscreen in the wipe packaging is also available in the market. In this formula, a thin liquid emulsion containing active sunscreen ingredients impregnated into the wipe. This packaging is intended for use on small children to avoid the risk of spills or inhaled. In addition there is also a stick packaging for sunscreen. Stick packaging of sunscreen is generally used for the lips and face to be easily applied and avoid greasy effect on the hand (Henry-Zoe, 2009).

At present, the popular phenomenon is active ingredients of sunscreen combined in cosmetics. In terms of effectiveness, protection ability of sunscreen agents in cosmetic products may not be as effective as pure sunscreen. Nevertheless, the presence of multiple layers of sunscreen substances on the skin's surface will extend the performance of the sunscreen protection. In general, color cosmetic products are divided into several groups, i.e. facial foundation, facial powders, facial moisturizer, cosmetic eyelid, and lipstick. The presence of a number of pigment in the product formula will determine the level of product protection. For example, facial foundations that contain only small amounts of TiO_2 has an SPF value of about 2 because the radiation is still reach to the skin. Generally, facial foundation has an SPF value of about 6 which allows the product still produce invisibility effect (translucent). Moreover, the presence of organic UVA and UVB filter allows increased SPF value of the product to 15 (Floyd *et al*., 1997; Angelinetta *et al*., 1995).

In addition to enhancing the protection of the skin from UV radiation, the formulas of inorganic particles also increase the aesthetic value. For example, transparent white layer by nanosized TiO_2 and ZnO makes skin look brighter and feels smoother. In addition, the doped Mn-TiO_2 particles produce a light brown color (beige) in order to obtain natural color cosmetics such as skin color. Emulsion nanoparticles is also commonly used in products conditioner or lotion. Nano emulsion combined with traditional cosmetic ingredients such as water, oil, and surfactant, in a two-phase system which the size droplet about 50-100 nm dispersed in an external phase (aqueous). Tiny droplets that produce a transparent nano emulsion and smooth effect (Sonneville, 2004).

C. Conclusion and Suggestion

Application of nanoparticles of TiO_2 and ZnO as inorganic sunscreen active ingredient provides many advantages, of which raises no skin irritation, allergies, inert to the other constituent materials sunscreen, minimal penetration into the skin and has a broad spectrum of protection. In the production process of the sunscreen active ingredients, besides determine the optimum particle size is also important to keep the particle size until the end of the production process. This is related to the nature of the particles easily form aggregates or agglomerates. Advances in nanotechnology accompanied the development of nanoparticles of TiO_2 and ZnO as the active ingredient of sunscreen allows the creation of cosmetic products to be more effective and elegant. In addition to concentrating on the determination of the type and concentration of the active ingredient UV filter, it should be considered is compatible other ingredient in sunscreen formulas, such as dispersing agents, moisturizers, thickeners, stabilizers, and perfume. A suitable combination of such material will increase the performance of both the potential for protection from UV radiation and increased aesthetic value. However, the convenience and safety of the consumers becomes a major factor in calculating the efficacy of sunscreen ingredients.

The important thing people need to realize that sunscreen is only one attempt to overcome the harmful effects of solar radiation. Society should also anticipate the hazards of radiation in other ways that do not involve the use of chemicals continuously, for example by using protective clothing, a hat, or umbrella when exposed to sunlight. Thus, sunscreen especially made from nanoparticles of TiO_2 and ZnO are not used excessively and continuously so that the skin is able to regenerate and neutralize the toxic nature or the possibility of penetration into the skin and even blood vessels.

D. Reference

Agren MS., 1990, *Percutaneous Absorption of Zinc from Zinc Oxide Applied Topically to Intact Skin in Man*. Dermatologyca, 180(**1**): 36-39.

Agren MS., Krusell M., Franzen L., 1991, Release and Absorption of Zinc from Zinc Oxide and Zinc Sulfate in Open Wounds. Acta Derm Venereol, 71(**4**): 330-333.

Angelinetta C., Barzaghi G., *Influence of Oil Polarity on SPF in Liquid Crystal Emulsions with Ultrafine TiO_2 Pre-Dispersed in Oil and Cross-lingking Polymers*. Cosmet News (Italy), 100: 20-24.

Anuradha Patel, Parixit Prajapati, Rikisha Boghra, 2011, *Overview on Application of Nanoparticles In Cosmetics*, Ajpscr Vol. 1, Issue 2, 40-55.

Armstrong BK[1], Kricker A, English DR., 1997, *Sun Exposure and Skin Cancer*. Australas J Dermatol. Jun; 38 Suppl 1:S1-6.

Armstrong BK[1], Kricker A, 1993, *How Much Melanoma is Caused by Sun Exposure?*, Melanoma Res. Dec, 3(**6**): 395-401.

Balk SJ., 2011, *Ultraviolet Radiation: a Hazard to Children and Adolescents*. Pediatrics. Mar, 127(**3**): e791-817.

Costello, P., Thurstan, SA., Griffiths, CE., Bell, M., Brown, M., Langton, AK., Sherratt, MJ., and Watson, RE., 2012, *Flat Spectrum Sun Screen Protects against Ultraviolet Radiation Induced Damage to Key Extracellular Matrix Components in Vitro*. J Invest Dermatol. USA: Nature Publishing Group. p. S121-S121.

Fahey, DW. and Hegglin, MI., 2010, *Twenty Questions and Answers about the Ozone Layer: 2010 Update*, The Panel Review Meeting for the 2010 Ozone Assessment. Switzerland.

Floyd DT., Macpherson BA., Bungard A., *et al*., 1997, *Formulation of Sun Protection Emulsion with Enhanced SPF Response*, Cosmet Toilet, 112(**6**): 55-56.

Food and Drug Administration (FDA), 2011, *Labeling and Effectiveness Testing: Sunscreen Drug Products for Over-the-Counter Human Use*, http://www.gpo.gov/ fdsys/pkg/FR-2011-06-17/pdf/2011-14766.pdf, Accessed February 1, 2014.

Gallagher RP., Lee TK., Bajdik CD., Borugian M., 2010, *Ultraviolet Radiation*. Chronic Dis Can., 29 Suppl 1: 51-68.

Grist, Eleanor; Setlow, Richard B.; Thompson, Keith; Woodhead, Avril D., 1993, Wavelengths Effective in Induction of Malignant Melanoma, Proc. Natl. Acad. Sci. USA, July , vol. 90, pp. 6666-6670.

Halmans G., Lasek J., 1985, *The Effect of Tropical Zinc Absorption from Wounds on Growth and the Wound Healing Process in Zinc-Deficient Rats*, Scand J Plast Reconstr Surg, 19(**2**): 119-125.

Henry ML., Zoe Diana Draelon, 2009, *Clinical Guide to Sunscreens and Photoprotection*. New York: Informa Health Care USA Inc.

Hewitt JP., Stott J., Duggan A., 2008, *Using Inorganic Sunscreens to Enhance the Photostability of Organic Sunscreens: Dispersion Effects*, In: Proceeding of the 42nd Annual Conference of the Australian Society of Cosmetic Chemist, Mar 6-9; Gold Coast, Australia.

Hunter A., Trevino M., 2004, *Film-Formers Enhance Water Resistance and SPF in Sun Care Products*. *Cosmet Toilet,* 119(**7**): 51-56.

IPCC, 2007, *Climate Change 2007: The Physical Science Basis*, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, USA.

Jones CHE., 2002, *Hollow Sphere Technology for Sunscreen Formulation*, SOFW Journal, vol. 128 (4), pp. 36-40.

Lademann J., Weigmann H., Schaefer H., et al., 2000, *Investigation of the Stability of Coated Titanium Microparticles Used in Sunscreens*. Skin Pharmacol Appl Skin Physiol, 13(**5**): 258-264.

Leiter U., Garbe C., 2008, *Epidemiology of Melanoma and Nonmelanoma Skin Cancer--the Role of Sunlight*. Adv Exp Med Biol., 624: 89-103.

Miki **Uematsu et.al.,** ***Development of*** *Hollow Particle for UV Protection and its Application to Sunscreen Products*. *www.tukad.org.tr/tr/pdf/187%20Uematsu.pdf,* Accessed Februari 27, 2014.

NA. Monteiro-Riviere, K. Wiench, R. Landsiedel, S. Schulte, AO. Inman and JE. Riviere, 2011, *Safety Evaluation of Sunscreen Formulations Containing Titanium Dioxide and Zinc Oxide Nanoparticles in UVB Sunburned Skin: An in Vitro and in Vivo Study*. Toxicol. Sci., 123, 264-280.

National Nanotechnology Initiative, 2009, *Nanotechnology Facts*, Available at: http://www.nano.gov/html/facts/whatIsNano.html. Accessed October 7, 2009.

Nicholas, JL., Nadim, AS., Madhu, AP., 1997, *Sunscreen: Development, Evaluation and Regulatory Aspects*, New York: Mercel Dekker Inc..

Sadrieh, N. et al., 2010, *Lack of Significant Dermal Penetration of Titanium Dioxide from Sunscreen Formulations Containing Nano- and Submicron-Size* TiO_2 *Particles*. Toxicol. Sci., 115, 156-166.

Schwarzenbach R., Huber U., 2003, *Optimization of Sunscreen Efficacy*, In: Sun Protection. Ziolkowsky H (ed). Verlag Fur Chemische Industrie: Augsburg, Germany, pp. 131-137.

Sonneville-Aubrun O, Simonnet JT, L'Alloret F, 2004, *Nanoemulsions: a New Vehicle for Skincare Products*, Adv Colloid Interface Sci., 108/109: 145–149.

Steven Q., Wang, MD., 2012, *Challenge in Making an Effective Sunscreen*. The melanoma letter summer vol 30(**2**).

Steven Q., Wang, MD., and Ian R. Tooley, 2011*, Photoprotection in the Era of Nanotechnology*. Semin Cutan Med Surg, 30: 210-213, Elsevier.

Tadros T., Taelman MC., Leonard S., 2003, *Principles of Formulation of Sprayable Emulsions*. International Conference on Sun Protection: A Time of Change, London: Summit Events Ltd.

United State Food and Drug Administration, HHS. 1999, *Sunscreen Drug Products for Over-the-Counter Human uses Final Monograph*, Final rule, Fed Regist, 64(**98**): 27666-27693

Wakefield G., Lipscomb S., Holland G., et al., 2004, *The Effect of Manganese Doping on UV A Absorption and the Free Radical Generation of Micronised* TiO_2 *and Its Consequences for the Photostability of UVA Absorbing Organic Sunscreen Components*. Photochem Photobiol Sci., 3: 648-652.

Wakefield G, Stott J., 2006, *Photostabilisation of Organiv UV-Absorbing and Anti-Oxidant Cosmetic Component in Formulations Containing Micronized Manganese Doped Titanium Dioxide*, J Cosmet Sci., 57: 385-395.

Wolf, LK., 2011, *Scrutinizing Sunscreens*. *Chem. Eng. News*, *89*, 44-46.

Zvyagin, AV., et al., 2008, *Imaging of Zinc Oxide Nanoparticle Penetration in Human Skin in Vitro and in Vivo*, J. Biomed. Opt., 13, 064031.

C-27

A QM/MM SIMULATION METHOD APPLIED TO THE SOLUTION OF Zr^{4+} IN LIQUID AMMONIA

Suwardi[1,2],Harno D. Pranowo[2] dan Ria Armunanto[2]

[1]Department of Chemistry Education, Faculty of Mathematics and Natural Sciences,
Yogyakarta State University, Yogyakarta, Indonesia
[2]Austrian-Indonesian Centre for Computational Chemistry Laboratory
Gadjah Mada University, Yogyakarta, Indonesia

Abstract

The structural and dynamics properties of solution Zr (IV) in liquid ammonia has been investigated by the method of ab initio QM/MM molecular dynamics simulations at the Hartree-Fock level. In this method, region with a high interest in the ion solvation layer of Zr (IV) were investigated by ab initio quantum mechanical Born-Oppenheimer while the rest of the system is described as classical 2-body potential. Structural properties were indicated by the coordination number, radial distribution, and angle distribution, while the dynamic properties were characterized by the mean ligand residence time. The Solvation number of 5 was found through methods of QM/MM with the modified LANL2DZ ECP basis set for Zr(IV) ion and DZP for N and H, in contrast to 10 obtained through 2-body potential simulation. No ligand migration occurs between the first and second shell. The mean ligand residence time in the second solvation shell is 8.67 ps.

Key words: ab initio QM/MM MD method, 2-body potential, Zr (IV) ion, liquid ammonia, solvation

INTRODUCTION

The structure and dynamics of solvation of some metal ions has been investigated both experimentally and theoretically . This investigation is important because in the fact that the chemical processes occurring between substances in solution. Solvation structure of metal ions in solution can be determined by spectroscopic, scattering and simulation methods. Spectroscopic methods include Nuclear Magnetic Resonance spectroscopy (NMR), Extended X - ray Absorption Fine Structure Spectroscopy(EXAFS), Mössbauer, infrared (IR), and Raman scattering method include the X-ray, electron, and neutron diffraction. In addition to these two experimental methods, there are two methods of simulation that is often used is the Monte Carlo (MC) and Molecular Dynamics (MD) (Hofer et al . , 2006) .

Several studies of metal ion solvation in liquid ammonia has been done through MD and MC simulations. Examples of metal ions has been successfully investigated the Li^{+} (Kerdcharoen et al . , 1996) , Co^{2+} , Cu^{+}, and Cu^{2+} (Pranowo et al . , 1999) , Ag + , Au + , Co^{2+} (Armunanto , 2004) , and K + (Tongraar et al . , 1997).

By reaction of the tetrafluorides MF_4 (M = Zr, Hf) with dry liquid ammonia at –40 °C, the pentaammoniates $[M(NH_3)4F_4]{\cdot}NH_3$ (**1**, M = Zr; **2**, M= Hf) were obtained, and these compounds represent inorganic Zr and Hf compounds that simultaneously bear nitrogen and fluorine ligands. The Zr–N distance of 2.397 Å are known by X-ray diffraction method (Kraus, et.al., 2009).

Structure and dynamics of Zr^{4+} in water, liquid ammonia and ammonia solution is very interesting to study experimentally and theoretically for the role of ions in the nuclear industry, solution chemistry, and biochemistry. The use of zirconium and zirconium compounds currently as an artificial joint material and support material production of antibodies. Zirconium is also used in nuclear reactors as a structural material and containers (Messner, et.al, 2011).

Metallic zirconium in the aspect of the nuclear industry is very important but its role in biological has not been known, and only a few experimental studies and theoretical study of the structure of solvation ions in a state of the monomer so that the investigation of structure and dynamics of solvation of ions Zr^{4+} interesting to do through QM/MM dynamic molecular simulation. Structure and especially the dynamics of solvated transition metal ions are highly sensitive to the accuracy of simulation techniques, and it has been shown in several cases that only ab initio QM/MM simulations reach a sufficient level of accuracy for these data. In the present work, we carried out a QM/MM MD simulation for Zr^{4+} in liquid ammonia.

2. METHOD

2.1 Construction of potential functions

The simulation of molecular dynamics was done through some steps. The first step is determining a proper basis sets and calculation method used at interest region. The basis set of DZP for N and H atom were selected as proofed sucessfully in a previous investigations. The LANL2DZ ECP of Zr was modified in order to compatible with Zr(IV). The Hartree-Fock method was choosen because it usually gives information about structural solvation well and indicates results of calculation were not different with MP2 method.

In QM/MM dinamics molecular simulations, potential 2-body and 3-body must be composed accurate. In this case potential of Zr(IV)-NH3 and NH3-Zr(IV)-NH3 interaction were based on 7632 point of ab initio energy calculated using TURBOMOLE at Hartree-Fock level. The Interaction Energy of Zr(IV)-NH3 calculated as this formula:

$$\Delta E_{2bd} = E_{Zr(NH_3)^{4+}} - E_{Zr^{4+}} - E_{NH_3} \tag{1}$$

More than 7000 point energy are fitted by Levenberg-Marquardt algorithm to an analytical function of the following form (Durdagi, S., et. al, 2005) :

$$E_{fit}^{2bd} = \frac{q_{Zr(IV)}\, q_N}{r} + \frac{A_N}{r^5} + \frac{B_N}{r^9} + \frac{C_N}{r^{11}} + \frac{D_N}{r^{12}} + \sum_{i=1}^{3} \left(\frac{q_{Zr(IV)}\, q_H}{r_i} + \frac{A_H}{r^4} + \frac{B_H}{r^5} + \frac{C_H}{r^6} + \frac{D_H}{r^{12}} \right) \tag{2}$$

The fitting parameters of A, B, C, and D were listed in Table 1, while $q_{Zr(IV)}$, q_N, and q_H are the charges of Zr(IV), N, and H, respectively. The r and r_i are distances of Zr(IV)-N and Zr(IV)-H, respectively. The lowest of interaction energy for Zr(IV)-NH_3 was found to be -148.0177 kcal mol^{-1} at 2.3 Å, the root mean square deviation of the fitted function is 5. kcal mol^{-1}. The three-body ab initio energies were computed as follows:

$$\Delta E_{corr}^{3bd} = E_{L_1ML_2}^{ab} - (E_M^{ab} + E_{L_1}^{ab} + E_{L_2}^{ab}) - (E_{ML_1}^{2bd} + E_{ML_2}^{2bd} + E_{L_1L_2}^{2bd}) \tag{3}$$

where $E_{L_1ML_2}^{ab}$ is the SCF energy of the H_3N-Zr(IV)-NH_3 interaction, E_M^{ab}, $E_{L_1}^{ab}$, and $E_{L_2}^{ab}$ represent the ab initio energy of the Zr(IV) ion, ligand-NH_3(1) and ligand-NH_3(2). $E_{ML_1}^{2bd}$ and $E_{ML_2}^{2bd}$ account for the Zr-ligand-NH_3(1) and Zr-ligand-NH_3(2) interaction energies, respectively, obtained from the 2-body potential and $E_{L_1L_2}^{2bd}$ denotes the ligand-NH_3(1)-ligand-NH_3(2) interactions. (i.e., ammonia-ammonia interactions, calculated by the flexible four site ammonia model). The analytical three-body fitting function was set up as

$$E_{3bd} = A_1 e^{-A_2 r_{12}} e^{-A_2 r_{13}} e^{-A_3 r_{23}} (r_{cut} - r_{12})^2 (r_{cut} - r_{13})^2 \tag{4}$$

the ion-nitrogen distances for ammonia molecules are denoted by r_{12} and r_{13}, respectively whereas r_{23} is the nitrogen-nitrogen distances between two ammonia molecules. The r_{cut} is a cutoff limit set to 6.0 Å, where three-body terms become negligible.

Table 1. The final parameters of the analytical two-body potential functions and three-body correction function

2-body	A (kcal mol^{-1})	B (kcal mol^{-1})	C (kcal mol^{-1})	D (kcal mol^{-1})
Zr(IV)-N	-13416.0701810	627532.6722430	-2997676.689089	2652060.1088025
Zr(IV)-H	-3237.0978587	10444.5473273	-7650.8783546	799.5098014
3-body	A_1(kcal mol^{-1} Å^{-4})	A_2(Å^{-1})	A_3(Å^{-1})	
N-Zr(IV)-N	0.6430085	0.4313762	-0.2344404	

2.2. Protocol Simulation

The QM/MM MD simulation was performed in a canonical NVT ensemble, consisting of one Zr(IV) ion solvated by 215 ammonia molecules in a cubic box with a side length 20.826 Å was employed. The simulation temperature of 235.16 K was kept constant by the application of the Berendsen algorithm with a bath relaxation time of 0.1 ps. The density of the system was fixed to the density of pure ammonia at the simulation temperature (0.690 g/cm^3). The reaction field method was employed as a correction of the cutoff of long-range electrostatic interactions. The Newtonian equations of motion were integrated by a predictor corrector algorithm, and a time step of 0.2 fs was applied, which allows for explicit movement of hydrogens. A cut-off 10.413 Å was set except for N-H and H-H non-Coulombic interactions for which it was set to 6.0 and 5.0 Å . The reaction field method was used to account for long range electrostatic interactions (Armunanto, R., et.al., 2004).

Starting from a random configuration a classical 2-body potential molecular dynamics simulation was performed for 102 ps. With this equilibrated system the simulation was continued adding the 3-body potential as correction of 2-body potential for a total simulation time of 102 ps. Then the combined QM/MM treatment was invoked, in which the ion and its full first solvation shell were included into the QM region. To ensure the full inclusion of the first shell into the QM zone the radius of the QM sphere was set to 3.8 Å in accordance with the Zr–N RDF obtained from the classical simulation. After 2 ps of reequilibration, QM/MM simulation was continued for 400000 steps in resulting trajectory of 80 ps. The whole of QM/MM MD simulations were worked in Austrian-Indonesian Center for Computational Chemistry Laboratory, FMIPA UGM. The total force acting on a particle is calculated according to the expression

$$F_{\text{tot}} = F_{\text{MM}}^{\text{sys}} + (F_{\text{QM}}^{\text{QM}} - F_{\text{QM}}^{\text{MM}})\,S(r) \tag{5}$$

Where $F_{\text{MM}}^{\text{sys}}$ is the MM force of the whole system and $F_{\text{QM}}^{\text{QM}}$ and $F_{\text{QM}}^{\text{MM}}$ are QM and MM forces in the QM region while F_{tot} is the total force acting on a particle. The S(r) is a smoothing function that applied in a region of 0.2 Å to ensure smooth transitions of ammonia molecules between the MM and QM regions.

$$S(r) = 1, \quad for \;\; r \le r_1$$

$$S(r)=\frac{(r_0^2-r^2)^2\,(r_0^2+2r^2-3r_1^2)}{(r_0^2-r_1^2)^3},\ for\ r_1<r\le r_0$$
$$S(r)=0,\ for\ \ r>r_0 \qquad (6)$$

Free migration of ligands between QM and MM region is permitted in this approach (Hofer, T.S., et. Al., 2006)

2.3. Dynamical properties evaluation

The ammonia exchange between solvation shell is important for the reactivities of the ions. The rates of ammonia exchange was evaluated by mean residence time (MRT) analysis for the ammonia molecules in the second shell. The MRT of ammonia molecules in that shell was calculated by the direct method using a t^* value of 0.0 and 0.5 ps (Armunanto, R., et.al., 2004).

3. RESULT AND DISCUSSION

3.1 Structural properties

The RDFs of Zr-N and Zr-H and their integration number are displayed in Figure 1. The sharp peak located at 2.4 Å is observed for the first solvation shell, indicating a rigid structure of that shell. A coordination number of 5 is found in this shell, quite contrast to the flexible eight-coordinated hydrate complex, $Zr(H_2O)_8^{4+}$. The higher coordination number in this complex indicated Zr^{4+} has the higher affinity to H_2O compared to NH_3.

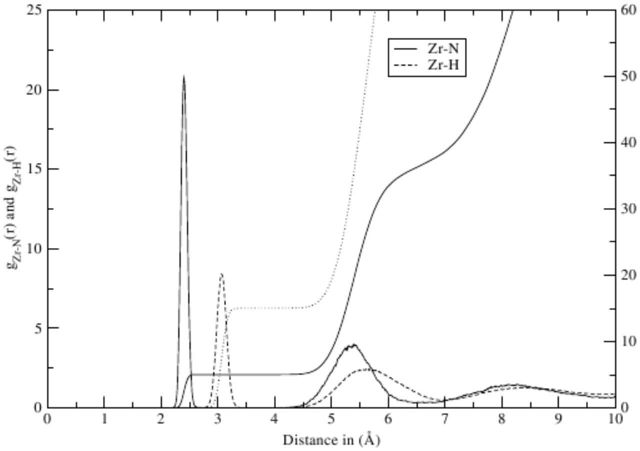


Figure 1. The Radial Distribution Functions of Zr-N and Zr-H and their corresponding running integration numbers.

The broad second peak is centered at 5.3 Å and extends from 4.3 to 6.5 Å indicating a much more flexible second coordination shell. The Zr–N distance of 2.4 Å is in good agreement with experimental investigation that resulted a Zr^{4+}-N distance is 2.397 Å (Kraus, F., et. al, 2009).

To clarify the number of ammonia molecules surrounding the Zr^{4+} ion, the Coordination number (CN) distributions for the first and second solvation shells obtained from the dynamics molecular simulation are depicted in Figure 3. A five-coordinated Zr^{4+} complex (100% occurrence) is observed for the first shell. In the second solvation shell, a large variation of the coordination number ranging from 9 to 21 is observed, with the highest occurrence percentage of 15. In the second shell, it was observed about 15 ammonia ligand that indicated almost all hydrogen in the first shell bind to nitrogens in the second shell form hydrogen bonds. In this respect, the presence of 5-coordinated complexes in liquid ammonia and also angle distribution (see Figure 4) supplies information that the first solvation shell of Zr^{4+} is trigonal bipyramidal $[Zr(NH_3)_5]^{4+}$ arrangement (see Figure 2.).

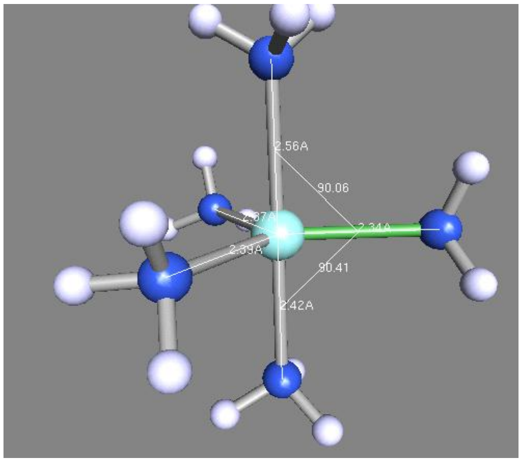


Figure. 2. Solvation structure of Zr^{4+} in liquid ammonia. Molecules form a trigonal bypiramidal arrangement.

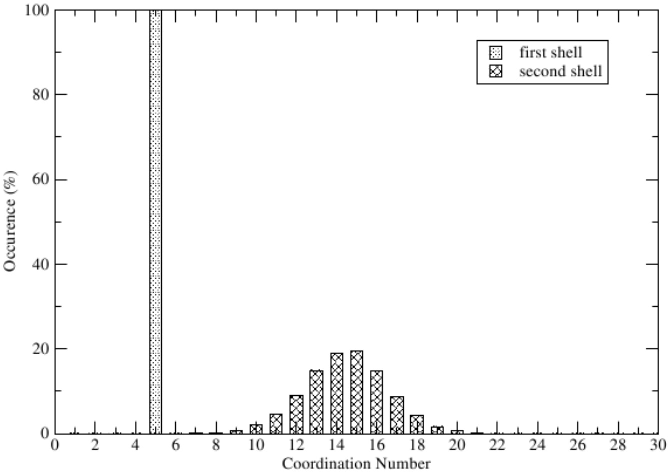


Figure. 3. Coordination number distributions of the first and second solvation shell of Zr(IV) in liquid ammonia obtained from a QM/MM MD simulation.

The distribution of the N–Zr–N angle within the first solvation shell was depicted in Figure 4. The ADF peaks at about 90, 120 and 170° was observed. The structural arrangement of the ammonia molecules within the first shell may be though as a trigonal bypiramidal arrangement.

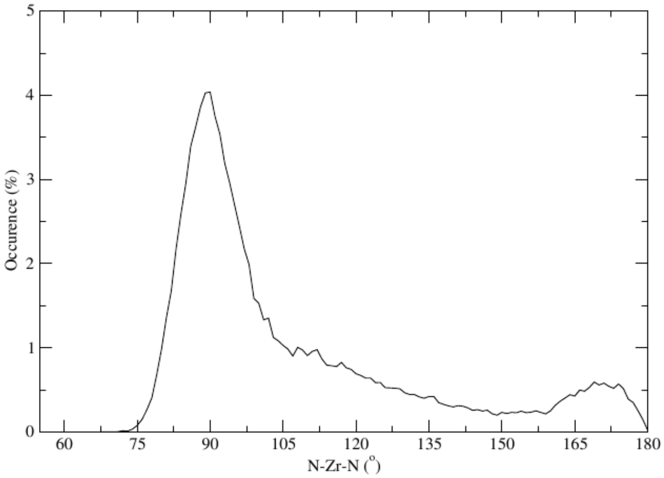


Figure. 4. Angular distribution function of the N–Zr–N angle in the first solvation shell of the Zr–NH_3 geometry obtained from a QM/MM MD simulation.

The ligand exchange of NH_3 can indicate reactivity of Zr^{4+} ion. In the case no migration ligand between the first solvation shell and second shell, however the exchange process occurred between the second shell and bulk as indicated in Figure 5. Because of that Zr^{4+} in liquid ammonia has the low reactivity.

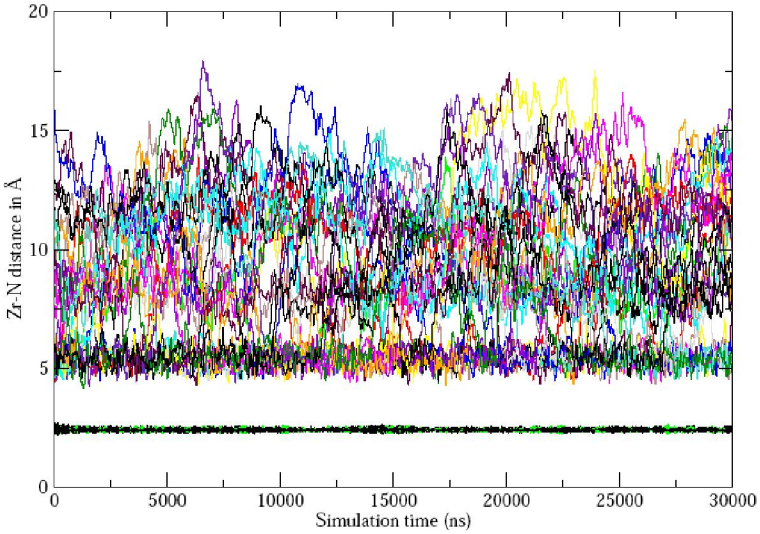


Figure. 5 The ligand exchanges of NH_3 between the second solvation shell and bulk. No ligand migration between the first and second shell.

The mean residences time of a ligand is defined as the average lifetime of a ammonia molecule in a given coordination shell. The mean residence times of ammonia molecules (τ) in solvation shells have been calculated according to Impey using $t^* = 0.5$ and 0.0 ps. For the QM/MM simulation, the mean residence time in the second solvation shell 8.67 ps (t^*=0.5) and 0.0437 ps (t^*=0.0 ps) for 30 ps simulations. The high MRT indicated the high stability of solvation structure in the first shell.

4. CONCLUSION AND SUGGESTION

The structure and dynamics of Zr^{4+} in liquid ammonia were investigated in much detail. The coordination number and Zr-N bond length was resulted in this simulation similar with experimental XRD data. The coordination number of 5 observed for the first solvation shell similar to an trigonal bypiramidal $Zr(NH_3)_5^{4+}$ complex. No first shell ligand-exchange processes occurred during the simulation time of 30 ps. In contrast, the mean residence time of ligands in the second coordination shell is large enough, indicating a high stability of the second solvation shell in the case of ammonia. The extended QM treatment in this simulations may improve the accuracy of the results or using a new method, that is QMCF method.

5. REFERENCES

Armunanto, R., Schwenk, C.F., Randolf, B.R., dan Rode, B.M., 2004, Structure and dynamics of Co^{2+} in liquid ammonia: ab initio QM/MM molecular dynamics simulation. Chemical Physics 305, 135.140

Armunanto, R., Schwenk, C.F., dan Rode, B.M., 2004, Gold(I) in Liquid Ammonia: Ab Initio QM/MM Molecular Dynamics Simulation, *J. AM. CHEM. SOC.*, 126, 9934-9935

Armunanto, R., Schwenk, C. F., Randolf, B.R., and Rode, B.M., 2004, Ag(I) ion in liquid ammonia, *hemical Physics Letters*, 388, 395-399.

Durdagi, S., Hofer, T.S., Randolf, B.R., Rode, B.M., 2005, Structural and dynamical properties of Bi^{3+} in water, *Chemical Physics Letters*, 406, 20–23

Hofer, T.S., Rode, B.M., dan Randolf, B.R., 2005, Structure and dynamics of solvated Ba(II) in dilute aqueous solution – an ab initio QM/MM MD approach, *Chemical Physics*, 312, 81–88.

Hofer, T. S., Scharnagl, H., Randolf, B. R., dan Rode, B. M., 2006, Structure and dynamics of La(III) in aqueous solution–An ab initio QM/MM MD approach, *Chemical physics*, 327, 31–42.

Kraus, F., Baer, S.A, and Fichtl, M.B., (2009), The Reactions of Silver, Zirconium, and Hafnium Fluorides with Liquid Ammonia: Syntheses and Crystal Structures of $Ag(NH_3)2F{\cdot}2NH_3$, $[M(NH_3)4F_4]{\cdot}NH_3$ (M = Zr, Hf), and $(N_2H_7)F$. *Eur. J. Inorg. Chem.* 2009, 441–447

Kerdcharoen, T., Liedl, K. R., dan Rode, B. M., 1996, A QM/MM simulation method applied to the solution of Li^+ in liquid ammonia, *Chemical physics*, *211*, 313–323.

Messner, C.B., Hofer, T.S., Randolf, B.R., dan Rode, B.M., 2011, Structure and dynamics of the Zr^{4+} ion in water, *Phys. Chem. Chem. Phys*., 13, 224–229

Pranowo, H.D., Mudasir, Kusumawardani, C., dan Purtadi, S., 2006, The structure of Co^{2+} in liquid ammonia: Monte Carlo simulation including three-body correction, *Chemical Physics*, 324, 573–578

Pranowo, H.D., dan Bernd M. Rode, 1999, Solvation of Cu^{2+} in Liquid Ammonia: Monte Carlo Simulation Including Three-Body Corrections, *J. Phys. Chem. A*, 103, 4298-4302

Pranowo, H.D., A. H. Bambang Setiaji, dan Bernd M. Rode, 1999, Cu^+ in Liquid Ammonia and in Water: Intermolecular Potential Function and Monte Carlo Simulation, *J. Phys. Chem. A*, 103, 11115-11120

Tongraar, A., Hannongbua, S., Rode, B.M., 1997, Molecular dynamics simulations of a potassium ion and an iodide ion in liquid ammonia, *Chemical Physics*, 219, 279-290

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COMPARATIVE STUDY OF METHODS IN THE SYNTHESIS OF MAGNETITE (Fe_3O_4)

Suyanta*), Eko Sri Kunarti, Muhamad Muzakir, Citra Pertiwi and Dian Pertiwi

Department of Chemistry UGM Yogyakarta
Contact person: suyantakimiaugm@yahoo.co.id

ABSTRACT

Comparative study of methods in the synthesis of magnetite between hydrolysis oxidative and co-precipitation method has been done. Hydrolysis oxidative method was done by added the solution of KOH (22.45 g) and KNO_3 (3.23 g) in water (120 ml) into solution of $FeSO_4\cdot7H_2O$ (40.00 g) in water (280 ml) in the N_2 atmosphere at 90 ^oC. Co-precipitation method was done by added dropwise the solution of NH_4OH (1 M) into 200 mL of $FeSO_4\cdot7H_2O$ and $FeCl_3\cdot6H_2O$ solutions (molar ratio 1:2) with total concentration of 6 M until pH 11.5. The solid phase formed in each method was then filtered, washed, and dried. The characterization of product by XRD and FTIR methods indicated that co-precipitation method produced higher in rendemen (81.25%) than that of hydrolisis oksidative method (71.26 %). In addition, the size of magnetite produced by co-precipitation method (15.7 nm) smaller than that of produced by co-precipitation method (27.8 nm).

Key words: magnetite, Hydrolysis oxidative, co-precipitation

Introduction

Magnetite, Fe_3O_4 is one of the important iron ores and ubiquitous in nature. It is a common constituent of igneous and metamorphic rocks. Due to its black colour, surface chemistry and strong magnetic property, it has found a great number of applications in industry (Wei and viadero, 2007; Mayo et al, 2007). Magnetite has attracted much interest in their applications in various fields, not only in the field of medical care, such as in drug delivery, magnetic hyperthermia and magnetic resonance imaging (Gupta et al., 2004; Yu et al., 2008; Kallumadil et al., 2009), but also in environmental protection (Oliveira et al., 2002; Wu et al., 2005). Particles of magnetite can be used to adsorbed contaminants from both of liquid and gas waste, then those adsorbent can be separated from medium by using some magnetic process with the result that can be reuse (Booker et al, 1991). There are several methods for synthesizing euhedral or irregularly shaped magnetite nanoparticles with sizes from 5 to 120 nm, including coprecipitation, hydrothermal and electrochemical synthesis, ultrasound irradiation, pyrolysis and hydrolysis oxidative (Corr et al. 2008; Liu et al. 2005). Each of those method has both of a weakness and superiority. In the present paper the authors reported the comparative study of methods in the synthesis of magnetite (Fe_3O_4), namely, co-precipitation of Fe(II) and Fe(III) in an alkali solution, and oxidation of ferrous hydroxide.

Research Method

a. Material

All of reagents used in this research are analitycal grade and used without further purification, it were purchased from E. Merck. The exceptions are aquadest that was produced by laboratory of analytical chemistry of Universitas Gadjah Mada and nitrogen that was purchased from PT Aneka Gas. Those reagents include $FeCl_3{\cdot}6H_2O$, $FeSO_4{\cdot}7H_2O$, HCl 37%, KOH, NH_4OH, and KNO_3.

b. Instrumentation

The instruments used in this research include FTIR spektrophotometer Shimadzu FTIR-8010PC, X-ray difractometer Shimadzu XD-3H, pH meter Orion 710A, analytic balance Mettler AE200, magnetic stirrer P/N 510-652, filter paper Whatman 42. Fourier transform infrared (FT-IR) spectra were recorded on Shimadzu Varian 4300 spectrophotometer in KBr pellets.

c. Procedure

Synthesis of magnetite by hydrolysis oxidative method. Synthesis of magnetite by co-precipitation method in this research refers to the method has been developed by Schwertmann and Cornell (1991) with some modification. $FeSO_4{\cdot}7H_2O$ (40 g) and 280 mL of aquadest were introduced into a four-neck bottom-rounded flask equipped with a thermometer, stirrer and continue N_2 bubbling through the solution. The function of continue N_2 bubbling was to get inert condition by remove O_2 from the solution and its atmosphere. The solution was then stirred and heated at 90°C, then slowly added dropwise. by 120 mL of solution containing KNO_3 (3.23 g) and KOH (22.45 g). The reactants were constantly stirred using a magnetic stirrer until the finishing of solution addition. The liquid precipitate was brought to a reaction temperature of 90°C and stirred for 1 h. The product was then cooled to room temperature. The precipitated magnetite was separated from the supernatant by filtration with Buchner funnel. To get free particles from kalium and nitrate compounds, the precipitate was then washed twice with distilled water. The solid was dried at 110°C in the oven for 2 hours, and weighted. Characterization of final product was carried out by X-ray diffraction and FT-IR methods.

Synthesis of magnetite by coprecipitation method. Synthesis of magnetite by co-precipitation method in this research refers to the method has been used by Vaclavikova et al (2003) with some modification. Solution of NH_4OH (0.1 M) was added dropwise into 200 mL of $FeSO_4{\cdot}7H_2O$ and $FeCl_3{\cdot}6H_2O$ solutions (molar ratio 1:2) with total concentration of 6 M. The reaction was carried out at room temperature and was vigorously stirred during the chemical reaction. When reach at 11.5 of pH, the mixture was stirred for another 10 minutes and then the precipitated magnetite was separated from the supernatant by filtration with Buchner funnel. The solid was washed 3 times with aquadest, dried at 110°C in the oven for 2 hours, and weighted. Characterization of final product was carried out by X-ray diffraction and FT-IR methods.

Result and Discussion

Materials produced by both of hydrolysis oxidative and co-precipitation methods were black powder with magnetic properties (Figure 1). Those physic properties agree with natures of magnetite. This statement was supported by some evidence from FT-IR spectra and X-ray difractogram as below.

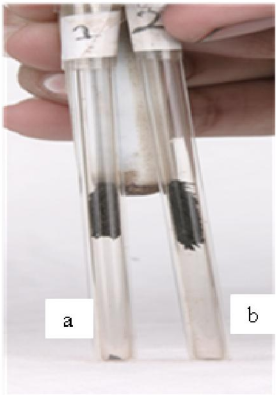


Figure 1 Materials produced by hydrolysis oxidative (a) and co-precipitation (b) methods were attracted by external magnetic field

The structural of the synthesized magnetite samples were obtained by X-ray powder diffraction (XRD) and FTIR spectroscopy. FTIR spectroscopy which is a useful technique to differentiate between different iron oxide crystals as well as between magnetite and maghemite phase (Tartaj, 2003; Cornell and Schwertmann, 2003) was carried out to characterize the synthesized magnetite. Figure 2 presented the Mid-Far infrared spectra of samples produced by hydrolysis oxidative and co-precipitation. There are two main bands corresponding to Fe_3O_4 can be observed, namely at 582 and 397 cm^{-1}. These are metal–oxygen bands that correspond to the intrinsic stretching frequencies of the tetrahedral and octahedral sites, respectively, of the inverse spinel cubic of Fe_3O_4 (Sen and Bruce 2009; Socrates 1994).

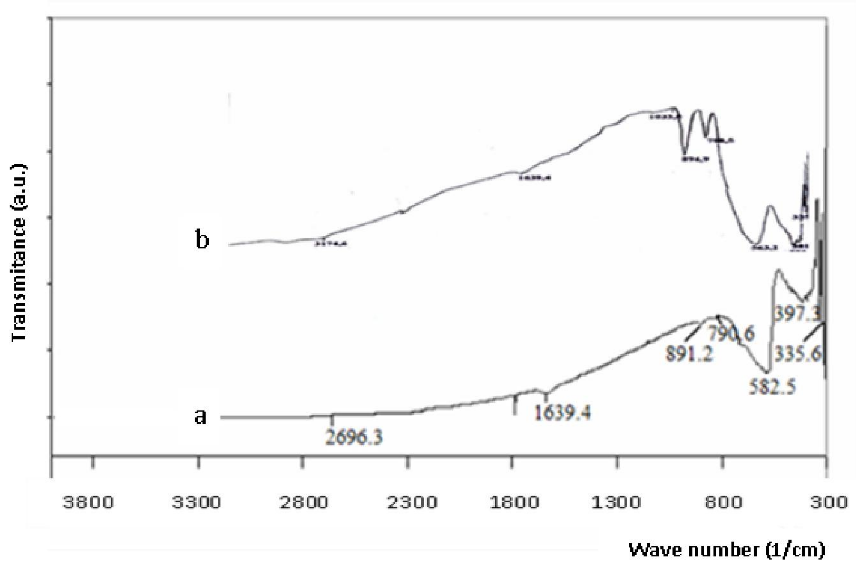


Figure 2 FT-IR spectra of samples produced by hydrolysis oxidative (a) and co-precipitation (b) methods

The number of bands and band positions of other iron oxide phases such as goethite, hematite, ferrihydrate and maghemite in Mid-Far IR region differ from what is observed in the FT-IR spectra of the sample synthesized by hydrolysis oxidative method, implying formation of magnetite as the dominant phase. In addition, the FT-IR spectra of the washed and dried sample shows no residual organic compound so the obtained product is pure enough.

The main difference between the spectrum of samples prepared by hydrolysis oxidative method and the spectrum of samples prepared by co-precipitation method is the bands appearing at 894.9 and at 798.5 cm^{-1}. These two bands were found in the spectrum of sample prepared by co-precipitation method (Figure 2b), but do not appear in the spectrum of sample prepared by hydrolysis oxidative method (Figure 2a). According to Parida and Das (1996), it can be attributed to the O–H bending that probably comes from goethite, γ-FeOOH.

The crystalline structure of the product was characterized by the XRD technique. The XRD spectrum can be matched to the series of Bragg reflections corresponding to the standard and phase pure spinel structure of Fe_3O_4 with a lattice constant a = 8.41 Å (JCPDS 02–1035) and no additional reflections were observed. The XRD pattern of sample produced by hydrolysis oxidative and co-precipitation methods are presented in Figure 3.

Both XRD patterns are characteristic of spinel phase crystallites where the peaks from left to right are assigned to 220, 311, 400, 422, 511 and 440 reflections (JCPDS 19-629). It was noted that although the goethite bands was detected at FT-IR spectra (for the sample synthesized by co-precipitation method), the peaks of goethite disappeared at the X-ray difractogram. It is indicated that the goethite formed still amorf. Figure 3(b) clearly showed the peak broadening. According to Suryanarayana and Norton (1998) the peak broadening is due to the smaller crystallite size of the material. Based on the reference above, we estimate that magnetite synthesized by coprecipitation has smaller in size than that of synthesized by hydrolysis oxidation method.

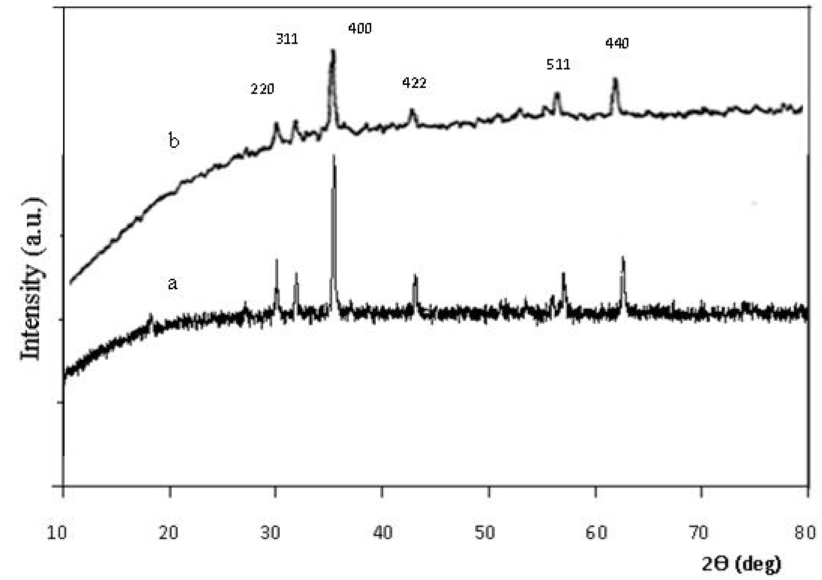


Figure 3 The XRD pattern of sample produced by methods of hydrolysis oxidative (a) and co-precipitation (b)

Quantitatively, the magnetite nanoparticles size can be estimated from the XRD pattern by using the Scherrer equation (Sun et al., 2004):

$$D = \frac{K\lambda}{B\cos\theta}$$

where λ is the X-ray wavelength (0.15406 nm), B is the full width at half maximum (FWHM), θ is the corresponding Bragg angle, and K is the shape parameter, which is 0.89 for magnetite and maghemite. Taking the highest intensity peak, namely the (311) plane, at 2θ = 35.4°, and the half maximum intensity width of the peak after accounting for instrument broadening, the calculated particle sizes are 67.8 nm for sample synthesized by hydrolysis oxidative method and 15.7 nm for sample synthesized by co-precipitation method.

Dufour (1997) stated that in the hydrolisis oxidative method the reaction begins with rapid precipitation of $Fe(OH)_2$ after addition of the kalium hydroxide to the iron(II) sulfate solution. It is believed that the magnetite formation then starts with oxidation of $Fe(OH)^+$ (the dissolved form of $Fe(OH)_2$) in water:

$$Fe(OH)_{2(s)} \rightarrow [Fe(OH)]^+_{(aq)} + OH^- \tag{1}$$

Subsequently, the oxidation takes place as follows:

$$2[Fe\,(OH)]^+_{(aq)} + \tfrac{1}{2}\,O_2 + H_2O \rightarrow [Fe_2(OH)_3]^{+3}_{(aq)} + OH^- \tag{2}$$

The intermediate entity $[Fe_2(OH)_3]^{+3}_{(aq)}$, can be combined with another $[Fe(OH)]^+_{(aq)}$ entity to form $Fe_3O(OH)_4{}^{2+}_{(aq)}$, which has the same Fe(II)/Fe(III) ratio as magnetite (equation 3).

$$[Fe_2(OH)_3]^{+3}_{(aq)} + [Fe(OH)]^+_{(aq)} + 2OH^- \rightarrow Fe_3O(OH)_4{}^{2+}_{(aq)} + H_2O \tag{3}$$

At high oxidation rate or low pH value, it will further oxidize to goethite (or other Fe(III) oxy-hydroxide), however, if the concentration of dissolved oxygen in water is low and the pH is high (such as in our experimental condition), slow oxidation takes place, and de-hydroxylation occurs prior to oxidation, so that the intermediate transfers to crystalline magnetite according to the following reaction:

$$Fe_3O(OH)_4{}^{2+}{}_{(aq)} + 2OH^- \rightarrow Fe_3O_{4\,(s)} + 3H_2O \tag{4}$$

Then the overall reaction is:

$$3Fe^{3+}_{(aq)} + 6OH^- + \tfrac{1}{2}\,O_2 \rightarrow Fe_3O_{4\,(s)} + 3H_2O \tag{5}$$

Based on the overall reaction, in the complete reaction 55.6 g of $FeSO_4 \cdot 7H_2O$ will produced 15.467 g of Fe_3O_4.. The magnetite produced in this research was 11.021 g, and the rendemen was 71.26%.

According to previous researcher (Bandhu et al, 2009, Zhao and Asuha, 2010; Valenzuela et al, 2009), mechanism of magnetite formation in the co-precipitation method using Fe^{2+} and Fe^{3+} ions reacting in alkaline conditions was:

$$Fe^{3+}{}_{(aq)} + 3OH^-{}_{(aq)} \rightarrow Fe(OH)_{3\,(s)} \tag{6}$$

$$Fe(OH)_{3\,(s)} \rightarrow FeOOH_{(s)} + H_2O \tag{7}$$

$$Fe^{2+}_{(aq)} + 2OH^-_{(aq)} \rightarrow Fe(OH)_{2\,(s)} \tag{8}$$

$$2FeOOH_{(s)} + Fe(OH)_{2\,(s)} \rightarrow Fe_3O_{4\,(s)} + 2H_2O \tag{9}$$

Giving an overall reaction:

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightarrow Fe_3O_{4\,(s)} + 4H_2O \tag{10}$$

Based on the overall reaction, in the complete reaction 55.6 g of $FeSO_4 \cdot 7H_2O$ and 54.1 g of $FeCl_3 \cdot 6H_2O$ will produced 23.2 g of Fe_3O_4.. The magnetite produced in this research was 18.85 g and the rendemen was 81.25%.

CONSCLUSION

Synthesis of magnetite by using co-precipitation method produced higher in rendemen (81.25 %) than that of hydrolisis oksidative method (71.26 %). In addition, the size of magnetite produced by co-precipitation method (15.7 nm) smaller than that of produced by co-precipitation method (67.8 nm).

REFERENCES

Anonim, Powder Difraction File, Joint Committee on Powder Difraction Standards (JCPDS). File No.19-629.

Bandhu, A., Mukherjee, S., Acharya, S., Modak, S., Brahma, S.K., Das, D., Chakrabarti, P.K., 2009, Dynamic magnetic behavior and Mössbauer effect measurements of magnetite nanoparticles prepared by a new technique in the co-precipitation method, *Solid State Commun*., 49, 1790–1794.

Booker, N.A., Keir, D., Preistly, A., Rithchie, C.D., Sudarmana, D.L., dan Woods, M.A., 1991, Sewage Clarification With Magnetite Particle, *Water Sci. Tech*., 123, 1703-1712.

Corr, S.A., Rakovich, Y.P., Gun'ko, Y.K., 2008, Multifunctional magnetic-fluorescent nanocomposites for biomedical application, *Nanoscale Res Lett* 3, 87–104.

Cornell, R.M. and Schwertmann, U., 2003, *The Iron Oxides, Structure, Properties, Reactions, Occurrences and Uses*, Weinheim ; Cambridge: Wiley-VCH, 2nd Edition.

Dufour, J., Marron, J. O., Negro, C., Latorre, R., Formoso, A., Lopez-Mateos, F., 1997, Mechanism and kinetic control of the oxyprecipitation of sulphuric liquors from steel pickling, *Chem. Eng. Journal*, 68, 173-187.

Gupta, A.K. and Wells, S., 2004, Surface-modified superparamagnetic nanoparticles for drug delivery: preparation, characterization, and cytotoxicity studies, *IEEE Transac. Nanobioscience*, 3, 66-73.

Kallumadil, M., Tada, M., Nakagawa, T., Abe, M., Southern, P. and Pankhurst, Q.A., 2008, Suitability of commercial colloids for magnetic hyperthermia, *J. Magn. Magn. Mater.,* 321, 1509-1513.

Liu, F., Cao, P., Zhang, H., Tian, J., Xiao, C., Shen, C., Li, J., Gao, H. 2005, Novel nanopyramid arrays of magnetite, *Adv. Mater.*,17, 1893–1897.

Mayo, J. T., Yavuz, C., Yean, S., Cong, L., Shipley, H., Yu, W., Falkner, J., Kan, A., Tomson, M., Colvin, V. L. , 2007, The effect of nanocrystalline magnetite size on arsenic removal, *Sci. and Tech. of Adv. Mater.*, 8, 71-75.

Oliveira, L.C.A., Rios, R.V.R.A., Fabris, J.D., Garg, V., Sapag, K. and Lago, R.M., 2002, Activated carbon/iron oxide magnetic composites for the adsorption of contaminants in water, *Carbon*, 40, 2177-2183.

Parida, K., and Das, J., 1996, Studies on Ferric Oxide Hydroxide: Structural Properties of Goethite Sample Prepared by Homogeneous Precipitation from $Fe(NO_3)_3$ Solution in the Presence of Sulfat Ion, *Jurnal of Colloid and Interface Sci*., 215, 586-598.

Sen, T., and Bruce, J.I., 2009, Mesoporous silica-magnetite nano-composites: fabrication, characterisation and applications in biosciences, *Micropor. Mesopor. Mat*., 120,246–251.

Schwertmann U. and Cornell R. M., 1991, *Iron oxide in the Laboratory-Preparation and Characterization*, VCH Publishers, Inc., New York.

Socrates, G., 1994, *Infrared characteristics group frequencies*, 2nd edn. Wiley, New York

Sun, Y.K., Ma, M., Zhang, Y., and Gu, N., 2004, Synthesis of nanometer-size maghemite particles from magnetite, *Colloids and Surf*., A 245,15-19.

Suryanarayana, C. and Norton, M.G., 1998, *X-Ray Diffraction*, A Practical Approach, New York and London, plenum press, 207-221.

Tartaj, P., Morales, M. P., Vientemillas-Verdaguer, S., Gonzalez-Carreno, T. and Serna, C. J., 2003, The preparation of magnetic nanoparticles for applications in biomedicine, *J. Phys. D. Appl. Phys*., 36, R182-R197.

Vaclavikova, M., Jagabsky, S., and Hredzak, S., 2003, *Magnetit Nanoscales Particles for Removal of Heavy Metal Ion*,http/drexel.edu/coe/research/ conferences/ NATO ASI 2003/ manuscripts/ 5.2.vaclavikova.pdf. Diakses tanggal 26 November 2005.

Valenzuela, R., Fuentes, M.C., Parra, C., Baeza, J., Duran, N., Sharma, S.K., Knobel, M., Freer, 2009, J., Influence of stirring velocity on the synthesis of magnetite nanopraticles (Fe_3O_4) by theco-precipitation method, *J. Alloys Compd.*, 488, 227–231.

Wei, X. and viadero Jr., R.C., 2007, Synthesis of magnetite nanoparticles with ferric iron recovered from acid mine drainage: Implications for environmental engineering, *Coll. and Surf. A: Physicochemical and Engineering Aspects*, 294, 280-286.

Wu, R., Qu, J. and Chen., Y., 2005, Magnetic powder MnO-Fe_2O_3 composite - a novel material for the removal of azo-dye from water, *Wat. Res.,* 39, 630-638.

Zhao, S. and Asuha, S., 2010, One-pot synthesis of magnetite nanopowder and their magnetic properties, *Powder Technol*., 197, 295–297.

C-29

CHEMICAL CONSTITUENTS OF INDONESIAN SILVER FERN (*Pityrogramma calomelanos*) AND THEIR CITOTOXICITY AGAINST MURINE LEUKEMIA P-388 CELLS

Suyatno, Nurul Hidajati, Khoriyah Umami, and Ika Purnama Sari

Department of Chemistry, Surabaya State University, Jl. Ketintang Surabaya, Indonesia (60231)
E-mail : suyatno_kimunesa@yahoo.com

ABSTRACT

Three known flavonoids, namely namely 2',6'-dihydroxy-4'-methoxydihydrochalcone, kaempferol, and quercetine had been isolated from the aerial part of the fern *Pityrogramma calomelanos.* Their structures were elucidated based on the spectroscopic evidence and by comparison with reported literature data. Flavonoid isolates showed cytotoxicity against the murine leukemia P-388 cells.

Keywords : *Fern, Pityrogramma calomelanos, flavonoid, cytotoxicity*

INTRODUCTION

Pityrogramma calomelanos was one of the ferns belonging the Polypodiaceae family widely distributed in tropical asia, especially Indonesia. It usually grew in open region, near streams, slope of mountain, and old wall (Steenish & Holttum 1982). This fern was used as the ornamental plant and phytoremediation land polluted arsenic (As), zink (Zn), lead (Pb), and mercury (Hg) (Lou, *et al*., 2007). Therefore, the chemical constituents of *P. calomelanos* and its bioactivity had not been reported.

In the course of our studies, three flavonoid namely 2',6'-dihydroxy-4'-metoxydihydrochalcone (**1**), kaempferol (**2**), and quercetine (**3**) had been isolated from the aerial part of *P. calomelanos..* In this paper, we reported the isolation and structure determination of those isolates and evaluation of their cytotoxicity against murine leukemia P-388 cells.

RESEARCH METHODS

General Experimental Procedures

Melting point was measured by Fisher John melting point apparatus and was uncorrected. UV spectra were recorded on Shimadzu Pharmaspec UV-1700 spectrophotometer. IR spectrum in KBr film was determined by Buck Scientific-500 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured by JEOL JNM ECA-500 spectrometer [operating at 500 MHz (^{1}H) and 125.7 MHz (^{13}C)] using tetra methyl silane (TMS) as the internal standart. Mass spectrum (MS) was recorded on Shimadzu QP-5000 spectrometer using ion mode EI. Kieselgel 60 GF-254 (Merck) and silica gel G 60 63-200 μm (Merck) were used for vacuum liquid chromatography (VLC) and flash chromatography (FC), respectively. Precoated silica gel 60 F-254 (Merck) 0.25 mm, 20 x 20 cm was used for thin layer chromatography (TLC) and spots were detected by spraying with the sulphuric acid solution 5% (v/v) in ethanol followed by heating. Cytotoxicity of flavonoid isolates against murine leukemia P-388 cells were evaluated using MTT [3-(4,5-

dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] assay (Cos, *et al*., 2001).

Plant Material

The aerial part of *P. calomelanos* was collected from Sawahan district, Nganjuk, East Java, Indonesia in March 2008. A voucher spesimen was deposited at the herbarium of the Purwodadi Botanical Garden, Indonesia.

Isolation

The aerial part dried powdered of *P. pityrogramma* (640 g) was exhaustively extracted successively with n-hexane (2 L x 3), ethyl acetate (2 L x 3), and acetone (2 L x 3) at room temperature. The n-hexane extract, ethyl acetate extract, and acetone extract were evaporated in vacuo, revealed 8.5 g (blackish green), 25.3 g (blackish green), and 10.5 g (blackish brown) residue, respectively.

A portion of ethyl acetate extract (8.0 g) was chromatographed by VLC and eluted with solvents of increasing polarity (n-hexane, n-hexane-ethyl acetate, ethyl acetate) yielded 120 fractions (15 mL each). Removal of the solvent under reduced pressure of the combined fractions of 50-75 gave the brown solid (1,2 g). It was yrecrystalized in benzene yielded a flavonoid 2',6'-dihydroxy-4'-metodihydrochalcone (1) (239 mg).

While a portion of acetone extract (5.0 g) was chromatographed by VLC and eluted with solvents of increasing polarity (chloroform, chloroform-methanol, methanol) yielded 127 fractions (15 mL each). Removal of the solvent under reduced pressure of the combined fractions of 60-100 gave the brown solid (1,2 g). It was rechromatographed by FC with chloroform-acetone (9:1) as eluen, obtained 60 fractions (10 mL each). The fractions 12-30 were collected, recrystalized in chloroform-methanol yielded a flavonoid 3, 5, 7, 4'-tetrahydroxy flavone (kaempferol) (2) (30 mg). While the combined fractions of 47-59 gave a flavonoid 3, 5, 7, 3', 4'-pentahydroxy flavone (quercetine) (3) (20 mg).

RESULTS OF RESEARCH AND DISCUSSION

Results of Research

Compound 1

Compound **1** was obtained as pale yellow crystal (benzene), mp. 169-171^{o}C, which gave positive test with $FeCl_3$ (greenish yellow) and Shinoda test (Mg-HCl) (yellow). It showed one spot on TLC using three eluents system with Rf of 0.86 (chloroform-ethyl acetate = 9 : 1), 0.44 (*n*-hexane-ethyl acetate = 4 : 1), and 0.31 (*n*-hexane-ethyl aceate = 9 : 1) as well as one peak on chromatogram of gas chromatography at Rt = 26.497 min. UV (MeOH) λ_{max} (log ε) : 285 (4.70), 336 (sh) (3.88) nm; (MeOH + NaOH): 295 (4.75), 361 (sh) (4.34) nm; (MeOH+$AlCl_3$): 306 (4.73), 371 (sh) (3.76) nm; (MeOH+$AlCl_3$+HCl): 306 (4.81), 368 (sh) (3.96) nm; (MeOH+NaOAc): 287 (4.66) nm; (MeOH+NaOAc+H_3BO_3): 286(4.67) nm. IR (KBr) $\bar{\nu}_{max}$: 3253 (OH), 3014 (aromatic C-H), 2969, 2969, 2862 (alkyl C-H), 1646 (chelated C=O), 1593, 1527 (aromatic C=C), 1435, 1384, 1216, 1074 cm^{-1}. ^{1}H-NMR (500 MHz,$CDCl_3$) δ (ppm) : 3.02 (2H, *t*, *J* = 7.95 Hz, H-β), 3.40 (2H, *t*, *J* = 7.3 Hz, H-α), 3.79 (3H, *s*, 4'-OCH_3), 5.93 (2H, *s*, H-3'and H-5'), 7.25 (5H, *m*, H-2,3,4,5,6). ^{13}C-NMR (125,8 MHz,$CDCl_3$) δ (ppm) : 30.7 (C-β), 45.8 (C-α), 55.7 (4'-OCH_3), 94.6 (C-3',5'), 104.9 (C-1'), 126.1 (C-4), 128.6 (C-2,6), 128.7 (C-3,5), 141.8 (C-1), 165.7 (C-2',4',6'), 204.7 (C=O). EIMS, m/z (rel. int., %): 272 (25), 255 (6), 177 (3), 167 (100, base peak), 140 (38), 136 (3), 124 (3), 111 (6), 104 (6), 91 (22), 77 (6), 69 (6), 51 (6), 39 (6).

Compound 2

Compound 2 was obtained as yellow needles crystal ($CHCl_3$-acetone), mp. 271-273^{o}C, which gave positive test (green) with $FeCl_3$ and Shinoda test (Mg-HCl) (orange). It showed one spot on TLC using three eluents system with Rf of 0.36 (chloroform-acetone = 3 : 1), 0.44 (chloroform-ethyl acetate = 1 : 1), and 0.73 (chloroform-methanol = 5 : 1). UV (MeOH) λ_{max} (log ε) : 267, 367 nm; (MeOH + NaOH): 275, 322 (sh), 405 nm; (MeOH+$AlCl_3$): 270, 353 (sh), 421 nm; (MeOH+$AlCl_3$+HCl): 269, 351 (sh), 421 nm; (MeOH+NaOAc): 276, 343 (sh), 426 nm; (MeOH+NaOAc+H_3BO_3): 270, 343 (sh), 423 nm. IR (KBr) ν_{max} : 3414 (OH), 3036 (aromatic C-H), 1659 (chelated C=O), 1613, 1567, 1510 (aromatic C=C), 1381, 1308, 1249, 1178 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm) : 6.17 (1H, *d*, *J* = 1.9 Hz, H-6), 6.38 (1H, *d*, *J* = 1,9 Hz, H-8), 6.89 (2H, *d*, *J* = 9,2 Hz, H-3’,5’), 8.07 (2H, *d*, *J* = 8,6 Hz, H-2’,6’). ^{13}C-NMR (125,8 MHz, CD_3OD) δ (ppm) : 94.5 (C-8), 99.3 (C-6), 104.6 (C-10), 116.4 (C-3’, C-5’), 123.8 (C-1’), 130.8 (C-2’, C-6’), 137.2 (C-3), 148.1 (C-2), 158.3 (C-5), 160.6 (C-4’), 162.6 (C-9), 165.7 (C-7), 177.4 (C-4).

Compound 3

Compound **3** was obtained as yellow needles crystal ($CHCl_3$-acetone), mp. more than 300 ^{o}C, which gave positive test (green) with $FeCl_3$ and Shinoda test (Mg-HCl) (orange). It showed one spot on TLC using three eluents system with Rf of 0.25 (chloroform-acetone = 3 : 1), 0.62 (chloroform-methanol = 5 : 1), and 0.23 (chloroform-methanol = 9 : 1). UV (MeOH) λ_{max} (log ε): 255, 372 nm; (MeOH + NaOH): 272, 325 (sh), 411 nm; (MeOH+$AlCl_3$): 270, 445 nm; (MeOH+$AlCl_3$+HCl): 264, 354 (sh), 429 nm; (MeOH+NaOAc): 272, 332 (sh), 451 nm; (MeOH+NaOAc+H_3BO_3): 272, 332 (sh), 451 nm. IR (KBr) ν_{max} : 3411 (OH), 3012 (aromatic C-H), 1641 (chelated C=O), 1510 (aromatic C=C), 1014, 883, 819 cm^{-1}. ^{1}H-NMR (500 MHz,CD_3OD) δ (ppm) : 6.18 (1H, *d*, *J* = 1.9 Hz, H-6), 6.39 (1H, *d*, *J* = 1.8 Hz, H-8), 6.88 (1H, *d*, *J* = 8.6 Hz, H-5’), 7.64 (1H, *dd*, *J* = 1.9 Hz and 8,0 Hz, H-6’), 7.74 (1H, *d*, *J* = 1,9 Hz, H-2’). ^{13}C-NMR (125.8 MHz, CD_3OD) δ (ppm) : 94.5 (C-8), 99.3 (C-6), 104.6 (C-10), 116.0 (C-2’), 116.3 (C-5’), 121.7 (C-6’), 123.6 (C-1’), 137.4 (C-3), 146.3 (C-4’), 148.9 (C-3’), 158.4 (C-2, 9), 162.6 (C-5), 165.8 (C-7), 178.9 (C-4).

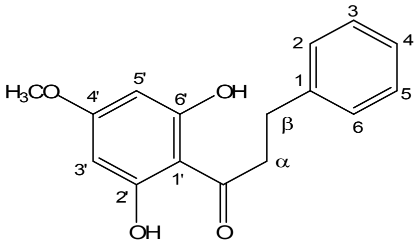
Discussion

Compound 1

Compound **1** showed the positive results on the test using $FeCl_3$ reagent (yellowish green) and Shinoda test (Mg + HC1) (yellow). It indicated that isolate was a flavonoid compund. The absorption bands of IR spectrum at 3267 (OH), 3023 (aromatic C-H), 2968, 2938 (alkyl C-H), 1647 (chelated C=O), 1597, 1529 (aromatic C=C) supported that isolate was a flavonoid.

The UV spectrum of 1 indicated absorbtion characteristic of dihydrochalcone-type compounds at 285 nm (band II) and 336 nm (sh) (band I) [7]. No bathochromic shift of band II on adding of NaOH and NaOAc reagents indicated that the isolates did not have a free OH group at C-4$'$. The bathochromic shift of band II on adding of $AlCl_3$ + HCl reagent supports the existence of an OH group free at C-6’. While the addition of NaOAc + H_3BO_3 did not cause the bathochromic shift of band II. This showed the absence of ortho-dihydroxy group at A ring in flavonoid isolate. The EIMS spectrum of **1** showed a molecular ion peak at m/z 272, corresponding a molecular formula $C_{16}H_{16}O_4$.

From the above results, compound 1 was identified as 2',6'-dihydroxy-4'-methoxy-dihydrochalcone.



Compound 2

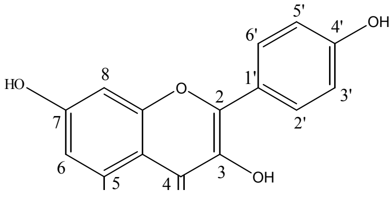
The positive results on the test using $FeCl_3$ reagent (yellowish green) and Shinoda test (Mg + HC1) (yellow) of compound 2 indicated that it was a flavonoid compound. The absorbtion maxima at 267 (band II) and 367 nm (band I) in the UV spectrum supported that 2 was a flavonol with a free 3-hydroxyl group (Markham 1982). The batochromic shift of band I on adding NaOH reagent (38 nm) and $AlCl_3$ + HCl reagent (54 nm) indicated the presence of a hydroxyl group at C-4' and C-5, respectively. The presence of a hydroxyl group at C-7 was exhibited by batochromic shift of band II (9 nm) on adding NaOAc reagent. No batochromic shift on adding NaOAc + H_3BO_3 reagent supported that 1 didn't have ortho-di hydroxyl group at A and B rings. The IR spectrum of 2 clearly disclosed absorbtion bands for OH group (3414 cm^{-1}), chelated carbonyl group (1659 cm^{-1}), and aromatic C=C (1613, 1567, 1510 cm^{-1}). The ^1H-NMR spectrum of 2 exhibited four doublet proton signals at δ_H 6.17, 6.38, 6.89 and 8.07 (Table 1). Two doublet proton signals at δ_H 6.17 (J =1.9 Hz) and 6.38 (J = 1.9 Hz) due to a pair of meta coupled protons H-6 and H-8 in the A-ring, respectively, supported the presence of a hydroxyl group at C-5 and C-7. While two doublet proton signals at δ_H 6.89 (J = 9.2 Hz, H-3',5') and 8.07 (J = 9.2, H-2',6') due to two pairs of ortho-cop;ed protons in the B-ring, confirmed the presence of a hydroxyl group at C-4'. The ^{13}C-NMR spectrum exhibited 15 carbon signals which corresponded to 1, containing five oxy aryl carbons [δ_C 148.1 (C-2), 158.3 (C-5), 160.6 (C-4'), 162.6 (C-9), and 165.7 (C-7)], one oxyolefine carbon [δ_C 137.2 (C-3)], and one carbonyl carbon [δ_C 177.4 (C-4)] (Table 1). The correlation spectroscopy (^1H-^1H COSY, HMQC, and HMBC) spectral data supported complete assignment of all proton-bearing carbon signals of 2.

Table 1. ^1H , ^{13}C, DQF-COSY, HMBC NMR data of 2 in CD_3OD and ^1H , ^{13}C NMR of kaempferol in CD_3OD (Suyatno, 2008)

Position of C	Compound **2**			Kaempferol	
	δ_H (mult, *J* dalam Hz)	δ_C	^1H-^{13}C HMBC	δ_H (mult, *J* dalam Hz)	δ_C
1	-	-	-	-	-
2	-	148.1	-	-	147.0
3	-	137.2	-	-	136.4
4	-	177.4	-	-	176.4
5	-	158.3	-	-	157.6
6	6.17 (*d*, 1.85)	99.3	C-7,C-8,C-9,C-10	6.2 (*d*, 2)	99.0
7	-	165.7	-	-	165.1
8	6.38 (*d*, 1.85)	94.5	C-4,C-5,C-6,C-7,C-10	6.5 (*d*, 2)	94.3
9	-	162.6	-	-	161.8
10	-	104.6	-	-	103.9
1'	-	123.8	-	-	123.0

2’	8.07 (*d*, 8.55)	130.8	C-2,C-3’,C-4’,C-5’,C-6’	8.1 (*dd,* 9.3)	130.3
3’	6.89 (*d*, 9.15)	116.4		7.0 (*dd,* 9.3)	116.2
4’	-	160.6	C-1’,C-4’,C-5’	-	160.2
5’	6.89 (*d*, 9.15)	116.4	-	7.0 (*dd,* 9.3)	116.2
6’	8.07 (*d*, 8.55)	130.8	C-1’,C-3’,C-4’ C-2,C-2’,C-3’,C-4’,C-5’	8.1 (*dd,* 9.3)	130.3

Futher supporting evidence of structure 1 for kaemferol came from comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data with those of reported data in literature (Markham & Geiger 1994, Li Bin & Luo Yongming 2003, Suyatno, 2008). From the above results, 2 was proposed for the structure of kaempferol (3,5,7,4’-tetrahydroxy flavone).



(2)

Compound 3

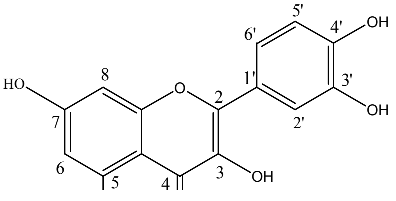
Compound 3 showed the positive results on the test using $FeCl_3$ reagent (yellowish green) and Shinoda test (Mg + HC1) (yellow). It indicated that compund 3 was a flavonoid compound. The absorbtion maxima at 255 (band II) and 372 nm (band I) in the UV spectrum supported that 3 was a flavonol with a free 3-hydroxyl group (Markham 1982). The batochromic shift of band I on adding NaOH reagent (39 nm) and $AlCl_3$ + HCl reagent (57 nm) indicated the presence of a hydroxyl group at C-4' and C-5, respectively. The presence of a hydroxyl group at C-7 was exhibited by batochromic shift of band II (17 nm) on adding NaOAc reagent. Batochromic shift on adding NaOAc + H_3BO_3 reagent (79 nm) supported that 3 had ortho-di hydroxyl group at A and B rings. The IR spectrum of 3 clearly disclosed absorbtion bands for OH group (3411 cm^{-1}), chelated carbonyl group (1641 cm^{-1}), and aromatic C=C (1510 cm^{-1}). The ^{1}H-NMR spectrum of 3 exhibited four doublet proton signals at δ_H 6.17, 6.38, 6.89 and 8.07 (Table 1). Two doublet proton signals at δ_H 6.18 (J =1.9 Hz) and 6.39 (J = 1.8 Hz) due to a pair of meta coupled protons H-6 and H-8 in the A-ring, respectively, supported the presence of a hydroxyl group at C-5 and C-7. While the presence two doublet proton signals at δ_H 6.88 (H-5’) and 7.74 ppm (H-2’) as well as a double douplet proton signal at δ_H 7.64 ppm (H-6’) supported the presence 3’,4’-dihydroxy group at B-ring. The existence ortho dihydroxy group caused H-2’ interacted with H-6’ at meta position with J = 1.9 Hz, H-5’ interacted with H-6’ at ortho position with J = 8,6 Hz, while H-6’ interacted with H-5’ at orhto position and H-2’ at meta position with J value of 1.9 Hz and 8 Hz, respectively. No singlet peak at δ_H 7 ppm caused by vinilic proton at C-3, corresponded to quercetine having hydroxyl group at C-3.

The ^{13}C-NMR spectrum exhibited 15 carbon signal which corresponded to quercetine containing six oxyaryl carbon [δ_C 158.4 (C-2), 162.6 (C-5), 148.9 (C-3’), 146.3 (C-4’), 158,4 (C-9), and 165.8 (C-7)], one oxy olefine carbon [δ_C 137.4 (C-3)] and one carbonyl carbon [δ_C 178.9 (C-4)] (Table 3). The correlation spectroscopy (^{1}H-^{1}H COSY, HMQC, and HMBC) spectral data supported complete assignment of all proton-bearing carbon signals of quercetine.

Table 2. ^{1}H , ^{13}C, HMBC NMR data of 3 in CD_3OD and ^{1}H data of quercetine in CD_3OD

Position of C	Compound **3**			Quercetine	
	δ_H (mult, *J* dalam Hz)	δ_C	^{1}H-^{13}C HMBC	δ_H (mult, *J* dalam Hz)	δ_C
1	-	-	-	-	-
2	-	158.4	-	-	156.9
3	-	137.4	-	-	134.6
4	-	178.9	-	-	178.2
5	-	162.6	-	-	161.8
6	6.18 (*d*, 1.85)	99.3	C-5,C-7,C-8,C-10	6.20 (*d*, 2.1)	99.3
7	-	165.8	-	-	165.1
8	6.39 (*d*, 1.89)	94.5	C-6,C-9,C-10,C-7	6.42 (*d*, 2.1)	94.1
9	-	158.4	-	-	157.7
10	-	104.6	-	-	104.4
1’	-	123.6	-	-	121.6
2’	7.74 (*d*, 1.85)	116.0	C-3’,C-4’,C-6’	7.69 (*d*, 2.1)	115.9
3’	-	146.3		-	145.7
4’	-	148.9	-	-	149.0
5’	6.88 (*d*, 8.6)	116.3	C-1’,C-3’,C-4’	6.90 (*d*, 8.5)	116.1
6’	7.64 (*dd*, 1.85 & 7.95)	121.7	C-2’,C-3’,C-4’,C-5’	7.55 (*dd*, 2.1 & 8.5)	121.2

Futher supporting evidence of structure 3 for quercetine came from comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data with those of reported data in literature (Markham & Geiger 1994). From the above results, 3 was proposed for the structure of quecetine (3,5,7,3’,4’-pentahydroxy flavone).



(**3**)

Cytotoxicity of isolate

Based on the cytotoxicity test, found that compound **1, 2,** and **3** showed cytotoxic activity against murine leukemia P-388 cells with IC_{50} values of 1.6, 14.1 and 6.4 µg /mL, respectively. Thus compund **1** and **3** strongly inhibited murine leukemia P-388 cells ($IC_{50} < 10$ µg/ mL, while compund **2** showed moderate activity against murine leukemia P-388 cells ($10 \leq IC_{50} < 100$ µg/ mL). Cytotoxicity of **1** on cancer cell lines have not been reported before, Therefore this is the first report of cytotoxicity of flavonoid above against murine leukemia P-388 cells.

CONCLUSIONS

Three flavonoid compounds namely 2',6'-dihydroxy-4'-methoxy-dihydrochalcone, kaempferol, and quercetine were separated from the fern *Pityrogramma calomelanos.* All isolated showed cytoxicity against murine leukemia P-388 cells.

ACKNOWLEDGMENTS

We thanks the Directorate General of Higher Education, Ministry of National Education, Indonesia, for financial support through National Strategic Research Grant 2009 and Mr. Wardaya from the Purwodadi Botanical Garden, Pasuruan, Indonesia, for help in collecting and identifying the plant material.

BIBLIOGRAPHY

Cos P, Calomme M, Sindambiwe J-B, Bruyne TD, Cimanga K, Pieters L, Vlietinck AJ, Berghe DV. 2001. Cytotoxicity and Lipid Peroxidation-Inhibiting Activity of Flavonoids. *Planta Med*. 67. 515-519.

Markham KR. 1982. *Techniques of Flavonoid Identification*. London: Academic Press.

Markham KR & Geiger H. 1994. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In *The Flavonoids. Advances in research since 1986*. Harborne JB (Ed). London: Chapman & Hall.

Li Bin & Luo Yongming. 2003. *Studies on chemical constituents of Camellia oleifera Abel*. http://www. chemistrymag.org//cji/2003/053020ne.htm. Accessed 5 June 2003.

Steenish V & Holttum RE. 1982. *Flora Malesiana.* London: Junk Publisher.

Stevgny C., Baily C., Quetin-Leclercq J. 2005. Cytotoxic and Antitumor Potentialities of aporphinoid Alkaloids. Current Medicinal Chemistry-Anti-Cancer-Agent. 5.173-182.

Sutoyo S, Indrayanto G & Zaini NC. 2007. Studies on chemical constituents of *Chingia sakayensis* (Zeiller) Holtt. *Natural Product Communications*, *2* (5): 579-580.

Suyatno. 2008. *Metabolit Sekunder dari Tumbuhan Paku Chingia Sakayensis (Zeiller) Holtt dan Aktivitas Sitotoksiknya terhadap Sel Murine Leukemia P-388 Secara in Vitro.* Disertasi. Universitas Airlangga.

C-30

CHITOSAN AND *N*-ALKYL CHITOSAN AS A HETEROGENEOUS BASE CATALYST IN THE TRANSESTERIFICATION REACTION OF USED COOKING OIL

Tatang Shabur Julianto and Restu Ayu Mumpuni

Department of Chemistry, Faculty of Mathematics of Natural Sciences
Islamic University of Indonesia
e-mail: tatang_shabur@staff.uii.ac.id

Abstract

It has conducted research on the use of chitosan, and N-alkyl chitosan (*N*-methyl chitosan, *N*-isopropyl chitosan) as a heterogeneous base catalyst in the transesterification reaction of used cooking oil.

Chitosan, *N*-methyl chitosan, and *N*-isopropyl chitosan synthesized by reacting chitosan with formaldehyde and acetone successively forming Schiff bases were then reduced using $NaBH_4$. Then chitosan, *N*-methyl chitosan, *N*-isopropyl chitosan are used as heterogeneous catalysts in the transesterification reaction of used cooking oil.

The results showed that inclusion of alkyl groups to the chitosan structure leads to increase in the level of alkalinity in which chitosan, *N*-methyl chitosan, and *N*-isopropyl chitosan respectively are 0.066 mol/g, 0.0068 mol/g, 0.0070 mol/g . It is also evident from the increase in the area of methyl palmitate and methyl oleate. The use of *N*-isopropyl chitosan gave the greatest methyl ester yield results is about 8.6%.

Keywords: chitosan, N-alkyl chitosan, methyl ester, transesterification, used cooking oil

INTRODUCTION

Biodiesel is a promising alternative fuel that can be derived from plant oils, animal fats or used oil through transesterification with alcohol. Biodiesel provides less pollution than petroleum fuels. In addition it can be used without diesel engine re-modification .

Biodiesel production methods commonly used today is through the process of transesterification of vegetable oils using alkaline catalyst. In this reaction, the alcohol in the form of methanol and ethanol, added to the triglyceride using homogeneous alkaline catalysts such as NaOH, KOH, $NaOCH_3$, or $KOCH_3$. This process runs quickly and efficiently at a relatively low temperature. Even so, the cost of biodiesel production is still expensive and becomes an important issue. The production costs can be reduced by means of cheap raw material selection, production proper place, and the efficiency of the process. For example , the current began to use used cooking oil and non-edible oils such as castor oil (*Jatropha curcas*) as the base material.

Homogeneous process actually has some advantages, but there are a number of missing values efficiency. In addition to biodiesel, a transesterification process in conditions produce byproducts such homogeneous catalysts, glycerol, and excess alcohol all of which require additional washing and drying step to obtain pure biodiesel product. In the homogeneous process, the base catalyst will be lost by leaching. This leads to reduced efficiency and increased production costs . One way to improve efficiency is using heterogeneous catalysts. In principle, the heterogeneous catalyst, the catalyst material can be taken back (not lost) and can be reused as a catalyst so that the process of making biodiesel becomes more simple. A number of studies have been done to take advantage of heterogeneous catalysts for transesterification process. One polymer that has potential as a heterogeneous base catalyst is chitosan (Gotch, 2008).

Chitosan is a polysaccharide made up of units of *N*-acetyl-D-glucosamine and D- glucosamine produced from the *N*-deacetylation of chitin natural polymers derived from the shells of marine animals, or fungi (Dunn, 1997). High reactivity of free amino groups makes chitosan has potential as a Lewis base. The longer the chain of chitosan, the more the content of free amino groups, the higher the alkalinity properties. Alkalinity is expected to replace the homogeneous alkaline catalysts used in transesterification processes such as NaOH and KOH. Additionally alkalinity chitosan amine group can be improved by incorporating the driving force electrons to the cluster amine group. One of the driving force of the electron is an alkyl group. Forces driving the electrons will increase the stability of the amine cation independent so alkalinity increased (Fessenden, 1994).

The Indonesian people are consuming particular foods using cooking oil as the processing of eating. The rest of the frying pan can no longer be used as food or as ingredients other. Usually the rest of the discarded frying pan without any benefits. This wasted material adverse impact on the environment if the capacity is large enough. Seeing a considerable amount of this kind of oil it would be worth if it be processed and used as fuel for diesel engines (Sandford, 1989; Shahidi, 1999; Sudhir, 2007).

RESEARCH METHODS

Chemicals and Analysis Instruments

All chemicals were obtained by Merck, Germany. Chitosan was kindly donated by LIPI Yogyakarta. Used cooking oil was collected from “pecel lele” food stalls around campuss of Islamic University of Indonesia.

Gas Chromatography-Mass and functional group analysis was performed using *Gas Chromatography* merk Agilent Technologies 6890 N, *Mass Spectrometer* merk Agilent Technologies 5975 B, and Spectrophotometer FTIR Thermo Nicolet Avatar 360.

Synthesis of N-methyl chitosan

Synthesized chitosan derivative refers to the study of Kim and Choi (2001) is made of chitosan solution of 1% w/v by dissolving 5 grams of chitosan into glacial acetic acid 1% v/v of 500 mL. Furthermore, 1.6683 mL of formaldehyde 10% was added to the chitosan solution at room temperature and stirred using a magnetic stirrer at 500 rpm. After one hour the pH of the solution was conditioned to 4.5 with NaOH 1M solution was added. Then this solution was added to the $NaBH_4$ solution of 10% w/v in 3.17 mL of water and the solution mixture was stirred for 1.5 hours. *N*-methyl chitosan produced subsequently precipitated by conditioning the pH 10. Material precipitate was washed with distilled water to neutralize the product. The precipitate was then dried at a temperature of 35^oC for several days in order to obtain dry *N*-methyl chitosan. Products were then characterized using FTIR spectrophotometer.

Synthesis of N-isopropyl chitosan

Synthesized chitosan derivative refers to the study of Kim and Choi (1997) is made of chitosan solution of 1% w/v by dissolving 5 grams of chitosan into glacial acetic acid 1% v/v of 500 mL. Furthermore, 4.411 mL of acetone 10% was added to the chitosan solution at room temperature and stirred using a magnetic stirrer at 500 rpm. After one hour the pH of the solution was conditioned to 4.5 with 1M NaOH solution was added. Then this solution was added to the $NaBH_4$ solution of 10% w/v in 3.17 mL of water and the solution mixture was stirred for 1.5 hours. *N*-isopropyl chitosan produced subsequently precipitated by conditioning the pH 10. Material precipitate was washed with distilled water to neutralize the product. The precipitate was then dried at a temperature of 35^oC for several days in order to obtain dry *N*-isopropyl chitosan. Products were then characterized using FTIR spectrophotometer.

Determination of Alkalinity of chitosan and *N*-alkyl chitosan

A total of 0.5 grams of chitosan dissolved in 60 mL of HCl 0.0782 N. Few drops of phenol pthalein indicators incorporated into the solution. The solution was titrated using 0.108 N NaOH and recorded volumes titrannya to know the volume of HCl remaining. Total volume of HCl that interact with chitosan and *N*-alkyl chitosan from the reduction of the initial volume of HCl with HCl remaining. Moles of HCl determine the level of alkalinity chitosan.

Treatment of used cooking oil

Used cooking oil was filtered with filter paper to remove solid particles ballpark. Then the second stage of cleaning is done by using adsorbents such as activated charcoal. As much as 100 grams of cooking oil put into different glass beaker is then heated to a temperature of 100^{o}C. After the temperature reached 100^{o}C adsorbents 5 grams put in a glass beaker containing used cooking oil. Further stirring for 80 minutes at 100°C with a speed of 500 rpm. The mixture was then filtered.

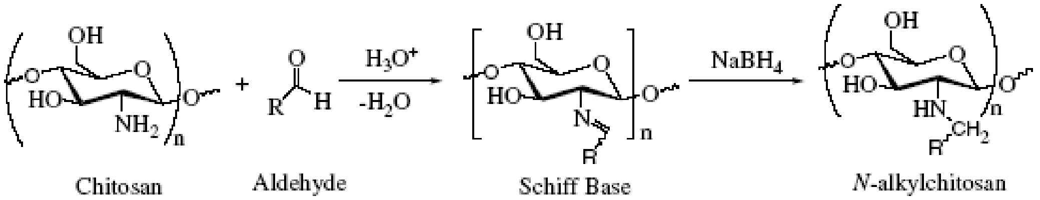
Transesterification reaction of used cooking oil

Transesterification reaction was carried out in a two-neck round bottom flask of 250 mL capacity that has been equipped with a hot plate, thermometer, magnetic stirrer, and cooling systems. One gram of chitosan incorporated into a two-neck round bottom flask and then coupled with the cooling system, a certain amount of oil and methanol added to the flask with a mole ratio of used cooking oil : methanol is 1:12 and refluxed for 2 hours at a temperature of 60^{o}C . In the solution of the many used cooking oil is 10 grams and as much as 5.67848 mL methanol. After 2 hours, the reflux was stopped and the mixture formed was poured into a test tube, the mixture was allowed some time at room temperature resulting in the separation solution. In order for the separation process completely formed then the mixture was centrifuged, and then will get 3 layers of the mixture is a row from top to bottom is the methyl ester, glycerol, and catalyst. Then methyl esters were taken, weighed and calculated yield of methyl ester produced. Transesterification process above is repeated for the other catalysts, *N*-methyl chitosan and *N*-isopropyl chitosan

RESULT AND DISCUSSION

Synthesis and characterization of chitosan and *N*-alkyl chitosan

Chitosan, *N*-methyl chitosan, and *N*-isopropyl chitosan synthesized by reacting chitosan with formaldehyde and acetone successively forming Schiff bases were then reduced using $NaBH_4$.



N-methyl chitosan produced by weighing 4.35 grams of pure chitosan weight used 5 grams. While the N-isopropyl chitosan products produced weighing 4.26 grams of pure chitosan weight of 5 grams.

N-methyl chitosan and *N*-isopropyl chitosan were then characterized using FTIR and FTIR results were compared with the data of chitosan. FTIR spectra of chitosan, *N*-methyl chitosan, and *N*-isopropyl chitosan is shown in Figure 1.

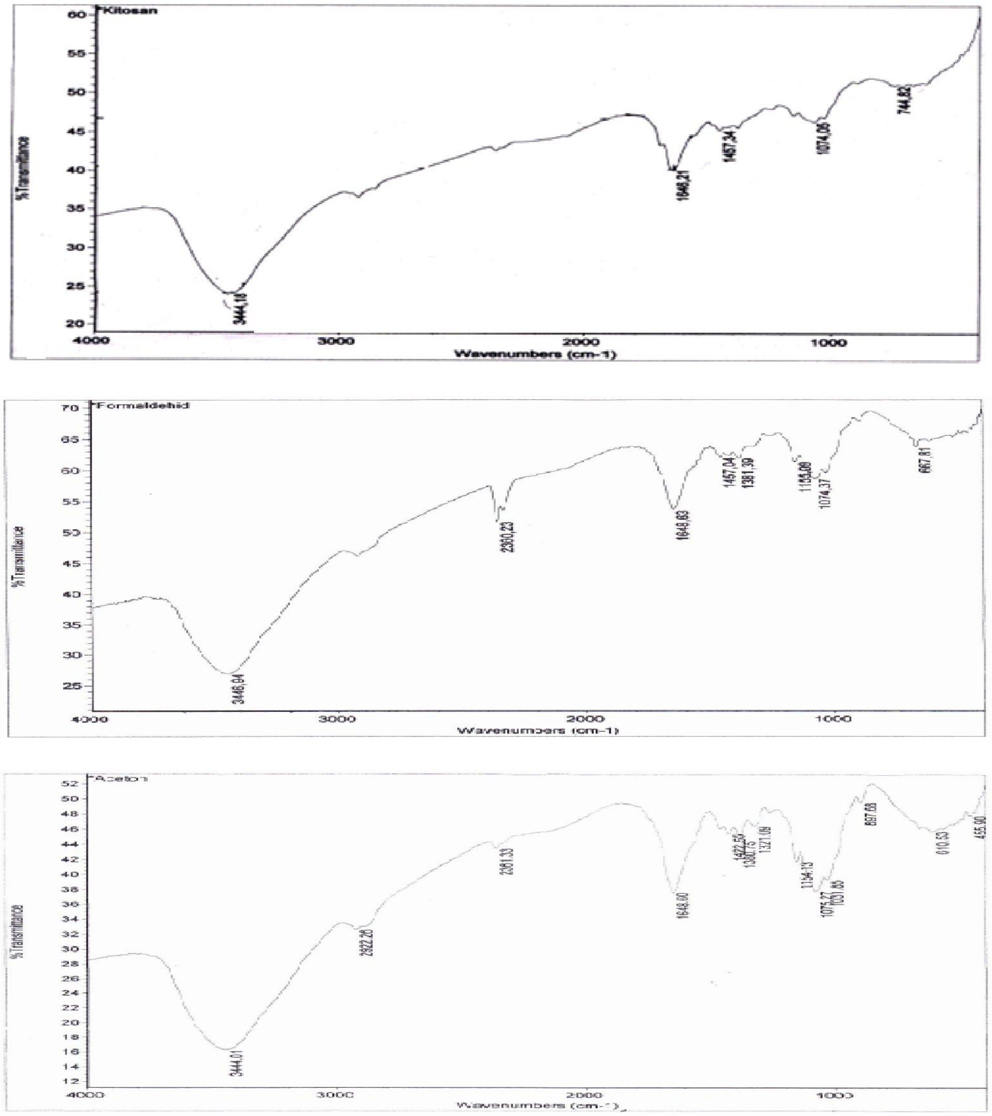


Figure 1. FTIR Spectra of (A) chitosan, (B) *N*-methyl chitosan, (C) *N*-isopropyl chitosan

FTIR spectra of chitosan in Figure 4(A) shows that 3444cm^{-1} is the absorption of hydrogen bonded OH groups. Absorption band at 1020 - 1155cm^{-1} shows the C-O vibration of chitosan in the ring and has several peaks due hydroxide on chitosan. Absorption band at 2919cm^{-1} is the uptake of alkanes and enhanced absorption at 1457cm^{-1} which indicates the type CH_2- alkanes. Uptake in the region 1580 - 1650 cm^{-1} (2 peak) showed uptake of primary amine NH_2. The difference between the uptake of chitosan and chitosan *N*-methyl group is located on NH absorption. NH_2 on chitosan uptake occurred in the 1580 - 1650cm^{-1} with 2 peaks , while the *N*-methyl chitosan uptake is only formed one peak which means the NH_2 group has been transformed into NH . Furthermore, to know that there has been a methyl group on chitosan (*N*-methyl chitosan) can be identified by the presence of absorption at 1381cm^{-1}. Uptake among NH with -CH_3 CN formed at 1250cm^{-1}. FTIR spectra of *N*-isopropyl chitosan in Figure 1(C) has an absorption at ~ 1380 - 1400cm^{-1} (2 peak) with a high intensity . This suggests that there has been isopropyl group in the *N*-isopropyl chitosan catalysts such.

FTIR spectra generated from it can be seen that there are some differences between the spectra of chitosan catchment area with *N*-methyl chitosan and *N*-isopropyl chitosan due to the modification of chitosan with increasing alkyl chain length. In the N-methyl chitosan chain is donated by methyl aldehyde group on formaldehyde interacts with the amine group on the chitosan. While the *N*-isopropyl chitosan, isopropyl group donated by a group of ketone from acetone.

Alkalinity of chitosan and N-alkyl chitosan

Determination of alkalinity of chitosan and N-alkyl chitosan showed that inclusion of alkyl groups to the chitosan structure leads to increase in the level of alkalinity in which chitosan, *N*-methyl chitosan, and *N*-isopropyl chitosan respectively are 0.066 mol/g, 0.0068 mol/g, 0.0070 mol/g.

Transesterification of used cooking oil

Transesterification reaction gave unsatisfaction result of methyl ester yield. Transesterification using chitosan catalyst only gave 2,0% of methyl ester. Catalyst *N*-methyl chitosan and *N*-isopropyl chitosan gave 3,1% and 8,6% methyl ester yield, respectively.

When compared with homogeneous catalysts, the results obtained are very low. According to Lee et al. (2009), the synthesis of methyl esters (biodiesel) laboratory scale is influenced by the type of catalyst (acid/alkaline), the mole ratio of methanol to oil, the content of impurities (usually free fatty acids and water) and the reaction temperature. The most important issues in the process of catalysis using heterogeneous catalysts is a slow reaction speed compared with the homogeneous catalyst.

These problems can be solved one by raising the reaction temperature (100-250^{o}C). According to Sakurai et al. (2000) chitosan has a thermal stability up to 203^{o}C so it is still possible if done further research on the effect of temperature on the reaction rate.

Methyl palmitate and methyl oleate are the main compounds of methyl esters. Area of chromatogram showed that methyl esters yield increased with the use of catalysts.

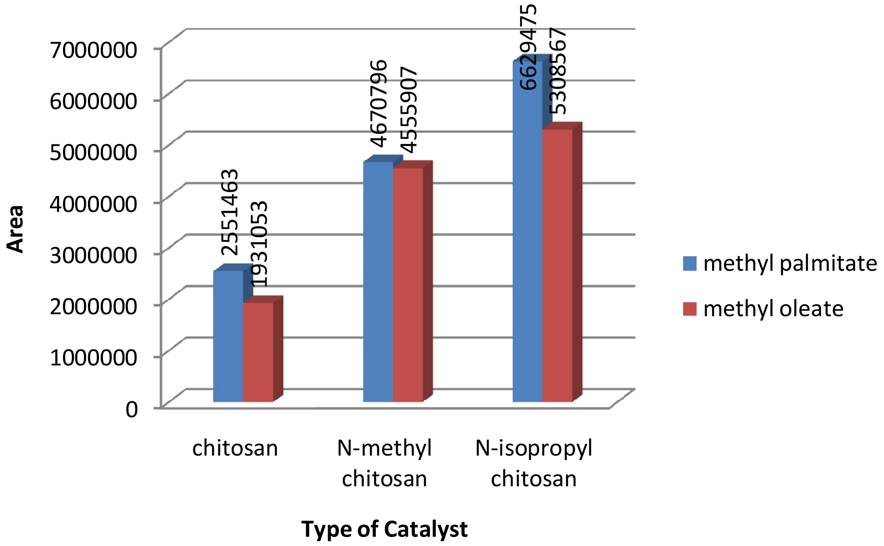


Figure 2. The methyl esters yield of methyl palmitate and methyl oleate with variation of chitosan and *N*-alkyl chitosan as catalyst of transesterification reaction

The results above showed the increasing of alkalinity enhanced the methyl ester yield of methyl palmitate and methyl oleate.

CONCLUSION AND SUGESTION

Inclusion of alkyl groups to the chitosan structure leads to increase in the level of alkalinity in which chitosan, *N*-methyl chitosan, and *N*-isopropyl chitosan. The increasing of alkalinity enhanced the methyl ester yield of methyl palmitate and methyl oleate. The problem of the very low methyl ester yield can be solved by raising the reaction temperatur up to 203°C base on the thermal stability of chitosan.

REFFERENCES

Gotch, Albert J.,† Reeder, Aaron J., and McCormick, A., **2008**, Study Of Heterogeneous Base Catalysts For Biodiesel Production, *Journal Of Undergraduate Chemistry Research*, 2008, 4 (58).

Dunn, E.T., Grandmaison, E.W., and Goosen, M.F.A., **1997**, *Application and Properties of Chitosan*, Technomic Pub., Basel, p. 3-30

Fessenden dan Fessenden, **1994**, *Kimia Organik*, jilid 2, edisi ke-3, Penerbit Erlangga, Jakarta

Kim, C.H., Cho, J.W., and Chun, H.J., **1997**, Synthesis of Chitosan Derivatives with Quaternary Ammonium Salt and Their Antibacterial Activity. *Polym Bull* **38**:387–393 in Entsar I Rabea, Mohamed EI Badawy, Tina M Rogge, Christian V Stevens, Walter Steurbaut, Monica H. Ofte1 and Guy Smagghel, **2006**, Enhancement of Fungicidal and Insecticidal Activity by Reductive Alkylation of Chitosan, *Pest Manag Sci* **62**, p. 890–897

Lee, D.W., Park, Y.M., Lee KY., **2009**, Heterogeneous Base Catalysts for Transesterification in Biodiesel Synthesis, *Catal. Surv. Asia*, 13. p.63–77

Sudhir, C.V., Sharma N.Y., and Mohanan, P., **2007**, Potential Of Waste Cooking Oils As Biodiesel Feed Stock, *Emirates Journal for Engineering Research*, 12 (3), p. 69-75

Sakurai, K.; Maegawa, T. T., and Takahashi, T., 2000, Glass transition temperature of chitosan and miscibility of chitosan/poly (n-vinyl pyrrolidone) blends. *Polymer*, V. 40, p. 19-56,

Sandford P., **1989**, *Chitosan: Comercial Uses and Potential Application*, in G. Skjak-Braek, T. Anthonsen, P. Sandford, *Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Application*., Elsevier, London

Shahidi, F., Arachchi J.V.K., and Jeon, Y.J., **1999**, *Food Application of Chitin and Chitosan, Trends in Food Science and Technology*, Springer Verlag, New York

Tanabe K., **1970**, *Solid Acids and Bases*, London: Academic Press in Elisabeth S. Goins, 1994, The acid/base surface characterization of sandstone, limestone, and marble and its effect upon the polymerization of tetraethoxysilane, *Institute of Archaeology,* 5, 19-28

C-31

STUDY ON GROWTH OF CARBON CRYSTAL FROM CHARCHOAL OBTAINED BY PYROLYSIS OF COCONUT SHELL

Meytij Jeanne Rampe[1], Bambang Setiaji[2], Wega Trisunaryanti[2], Triyono[2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Manado State University*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada.*
E-mail: meytij_rampe@yahoo.co.id

Abstract

Study on the growth of carbon crystal produced from coconut shell has been conducted. This research was aimed to study the effects of PVA as stimulant agent in the from of either powder or solution on structural growth of carbon crystal and its physic-chemical properties and the effect of temperature (1000-1500 ^{o}C) on the characters of the carbon. Several methods of characteristics were performed including FTIR to determine the functional groups of the carbon, XRD to identify the structure of carbon crystal, SEM-EDS to analyze the topography of surface and to analyze the component of material. The product characteristics of carbon material showed that there was a change on the physic-chemical properties of the raw material which has been sintered at 1000 ^{o}C, at which coke was produced in this process. Fabrication process at 1500 ^{o}C in the presence of PVA gave carbon material from charcoal amorphous to carbon with semi-crystalline structure.

Keywords: coconut shell charcoal, growth, carbon ctystal.

INTRODUCTION

The structure of chemical bonds that is formed contributes to the establishment of superior properties of carbon materials (Rampe *et al*., 2011; Buchman and Bryant, 1999). Carbon contains allotrope which has different types of chemical bonding structure, including graphite, diamond, black carbon, fullerence, carbon nano tubes (CNT). Graphite is a type of carbon material obtained from carbon atoms with sp2 orbital. One of these atoms forms a new bond with 3 carbon atoms. The microscopic material consists of graphite flat sheets from carbon atoms which are bonded, as the grapheme. The characteristics of the bond structure and dynamic interactions between the grapheme layers produce strong electrical conductivity properties (Buchman and Byant, 2002) which functions as a lubricant.

Carbon materials can be used in various applications, such as electrodes, catalyst supports, adsorbents and filters. Recent fact shows that it can be used as precursors for reaction which forms materials composite and structural ceramics (Lin and Yen, 2007). The advanced carbon materials such as synthetic carbon composites obtained from carbon fibers struggle to be accepted in wider practical use. Of well known carbon materials, the role of composite carbon cannot be overestimated either in modern industrial practice or in everyday life. It happens due to the unique physical and chemical characteristics of composite carbon which attract researchers to conduct certain research which explores those characteristics (Inagaki, 2000).

Carbon used in the industry is an example of carbon that can be heated at temperatures up to 1000 – 1300 ^{o}C, while graphite is a type of carbon which can be heated at temperatures higher than 2500 ^{o}C. Heating at higher temperature of 3000 ^{o}C will produce carbon that has an irregular structure which evolves to a more ordered graphite structure by removing the impurities of volatile material (Rampe, *et al*., 2012; Rampe *et al*., 2011; Elsayed *et al*., 2007).

This study used coconut shell charcoal as raw material which function as carbon to examine the growth of crystal in the development carbon composite. To obtain carbon from coconut shell charcoal, tar impurities and volatile material are removed. The removal of impurities is conducted by high temperature heating up to 600 – 1000 ^{o}C. This heating is also aimed to eliminate the compounds carried by the drainage of gas. During the heating process, all non-carbon materials must be removed to form a pure carbon as well as to arrange the structure (Wiratmoko and Halloran, 2009; Ebner *et al*., 2004). The removal of metals cam be carried out by soaking the carbon in a solution of HCl ((Fraga *et al*., 2002).

Heating process of coconut shell will produce a gradual change. In the first stages of the carbonization, the carbon samples become coke. The second stage is graphitization, in which coke is changed into graphite carbon whose irregular structures tend to grow (over the graphite whose structure is irregular) (Gupta *et al*., 2005; Miyazaki *et al*., 2005). The nature of carbon depends on its source (Kang *et al*., 2007), and on the method and conditions of synthesis (Lalena *et al*., 2008). The nature of carbon materials gives impact on their various uses, primarily as carbon electrodes and carbon material structure.

This study discusses the use of, coconut shell and polyvinyl alcohol (PVA) to produce carbon structure materials. The main purpose of this research is to study the crystal structure and chemical composition by using parameters such as concentration of polyvinyl alcohol and sintering temperature.

RESEARCH METHOD

A. Materials

This study used coconut charchoals as the raw material produced by PT. Tropica Nucifera Industry Bantul Yogyakarta, Indonesia. This charchoal is the source of carbon and polyvinyl alcohol. Further, Merck stimulant and argon gas were used as inert atmosphere to get air free.

Calcination was conducted using a tube-Thermolyne Furnace (Sybron) Type 21100 with a maximum temperature of 1200 ^{o}C. Calcinated charcoal as raw material was obtained through 100 mesh grinding and sifting. For carbon materials, Tarno compacted Grocki 312 model a maximum of 20 tons was used. Sintering was carried out by using a Carbolite furnace-Edwards Pirani 501 A6D 1600 ^{o}C maximum temperature with argon gas as the atmosphere, Scanning Electron Microscopy (SEM) JOEL JSM-6360 LA and Energy Dispersive Spectroscopy (EDS) System JOEL JED-2300 was used while the Goniometer model difractometer with Cu K_{α} (α = 1.54056 Å) radiation was used for X-ray Diffraction (XRD) analysis.

B. Methods

1. Preparation and Charcoal Calcination

Carbon powder 100 mesh sieves was calcined in an inert state though two stages. First, was the calcinations at temperatures of 600 ^{o}C for 3 hours with the existence of nitrogen gas then followed by the purification of charcoal powder which was extracted for 24 hours with 1 M hydrochloric acid at room temperature (Gupta *et al*., 2005). The sample was washed with distilled water until it reached constant pH and was dried in an oven at temperatures of 110 ^{o}C overnight (Fraga *et al*., 2002). Re-calcination was carried out for 3 hours at temperature of 750 ^{o}C with nitrogen.

2. The process of carbonization

The carbon powder was calcined with 2.5 to 7.5% wt. polyvinyl alcohol then suspended in distilled water at 353 K and stirred for one hour. Binder system was mixed with charcoal powder, forming a paste with the density in different systems. The next step was molding by using a cylindrical mold with a diameter of ~ 15 mm. Compaction was performed with one direction force by the means of Tarno Grocki with the thrust of 5 tons. In this stage, the process produced a sample of pellets (green compact). The samples were then dried at room temperature for one day before oven drying for 4 hours at 110 ^{o}C. The samples were then fed into a carbollite furnace for sintering at the temperature of 1000 ^{o}C. The rate of temperature was 10 K/min and reaction time was 3 hours. Then, these samples were cooled in the furnace at a rate of 8 K/min, with a flow of argon gas. This process produced dense coconut shell coke or carbon-carbon composite materials (Wiratmoko and Halloran, 2009; Miyazaki *et al*., 2005; Mothe and de Miranda, 2009).

3. Synthesis of carbon structures

The coke sample was heated for the 2nd time by sintering at 1200 ^{o}C, 1500 ^{o}C with a flow rate of 10 K/min, holding time of 3 hours, in the furnace Carbolite, with the furnace cooling rate of 8 K/min, under argon gas flow (Mendez and Santamaria, 2008).

RESULT AND DISCUSSION

XRD analysis

Figure 1 and 2 showed a typical X-ray diffraction (XRD) patent of testing charcoal, calcined carbon and coke carbon material that sintered at temperatures 1200 and 1500 ^{o}C. XRD diffractogram of charcoal, calcined carbon and coke carbon material gave value of interlayer spacing (d) (Å) as a graphite semi-crystalline structure. Yin *et al*. (2009) reported that the decrease of the (d) and the increase of the crystalline diameter and average stacking height of the aromatic carbon sheets (L_c) with increasing the temperature suggested the development of stacking structure, increased the size of crystallite as well as removal of defects and increased order in carbon material structure. The increase of L_c with increasing temperature resulted from crystalline growth in-plane and coalescence of crystallites. In this process, the structure of amorphous carbon structure was changed into semi-crystalline structure with a better degree of order, namely turbostatic structure (Rampe *et al*., 2010; Rampe *et al*., 2011). There was the change of internal structure by setting the position of equilibrium carbon atoms.

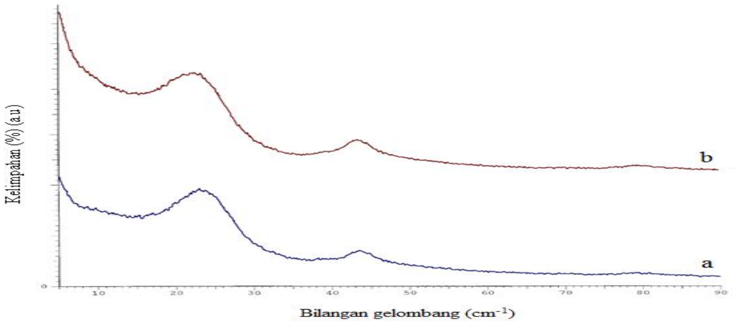


Figure 1. XRD pattern of carbon sintered at 1200 ^{o}C : (a) 5% wt. PVA, and (b) 7,5% wt. PVA

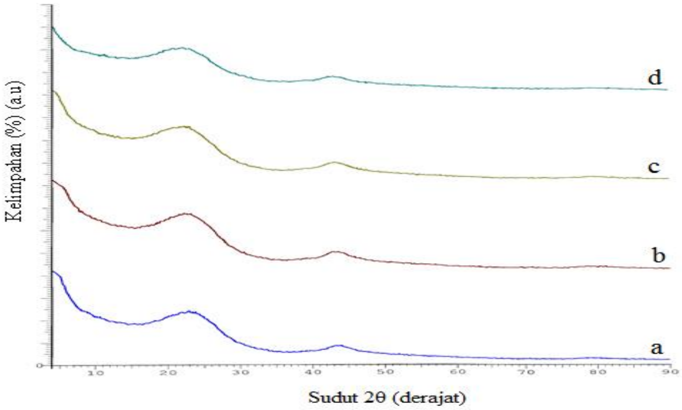


Figure 2. XRD pattern of carbon sintered 1500 : (a) coconut shell charcoal, (b) 2,5% wt. PVA, (c) 5 % wt. PVA, and (d) 7,5 % wt.PVA.

SEM-EDS analysis

Figure 3 and 4 showed the SEM photographs of carbon composite (C/PVA) sintered at 1500 °C, respectively. Figure 3 showed that coconut shell carbon was non-homogeneous particles distribution. Figure 4 showed a homogeneous and even surface morphology. The carbon structure from coconut shell cabon sintered at temperature of 1500 °C exhibited uniform particle size of micrometer dimensions and spherical particles shape (Lin and Yen, 2007). It was also shown in Figure 4 that the morphological features of carbon particles appeared to be more irregular, characteristics as a typical of carbon particles (Inagaki, 2000).

EDS analysis for carbon structure sintered at 1500 °C were shown in Figure 5,6 and 7, respectively. It was apparent that chemistry composition of carbon structure, carbon/PVA (2.5 and 5% wt.) (Figure 5 and 6) consisted of C element 97.16% wt. and C element was 97.35% wt. The carbon structure, carbon/PVA (7.5% wt.) (Figure 7) consisted of higher C element up to 97.70% wt. with average of C element was 97.40% wt.

Heat treatment ranging from 1000 to 1500 °C, as demonstrated in this study, caused the aromatic layers grow and coalesce each other (Inagaki, 2000). This is due to the evolution of volatile matter (Rampe *et al.*, 2011) and the breakage of cross-linking bonds such as CH_2 bridge.

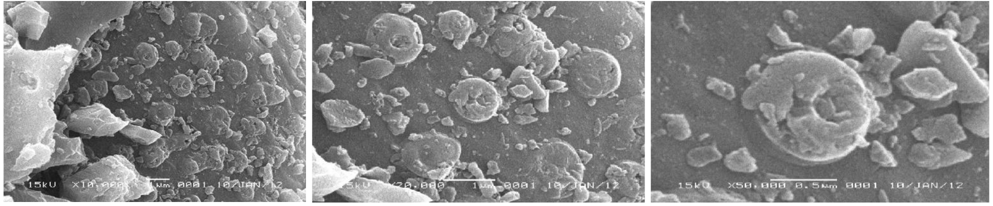


Figure 3. SEM micrographs of coconut shell carbon (5% wt. PVA) sintered 1500 °C

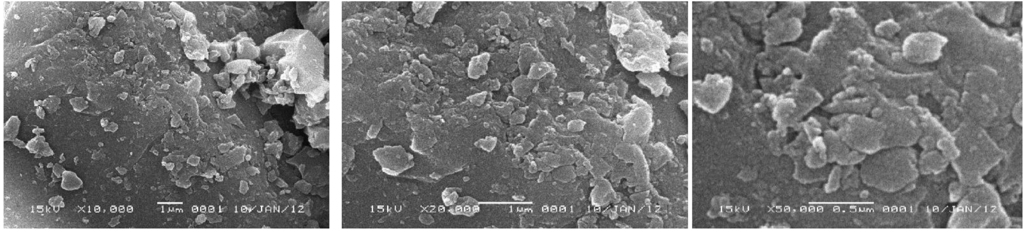
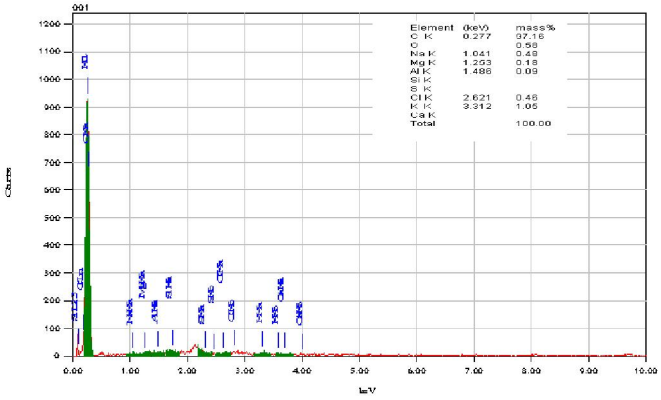
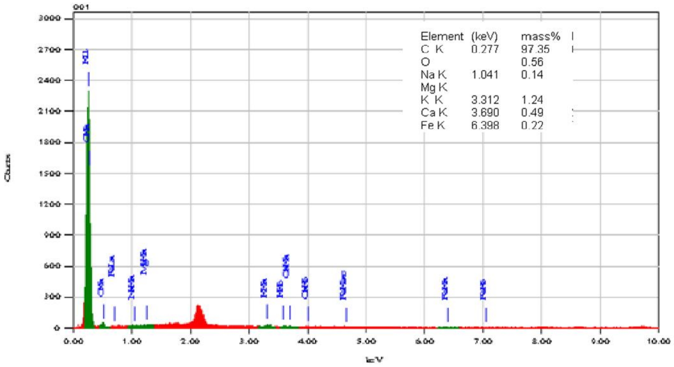


Figure 4. SEM micrographs of coconut shell carbon (7.5% wt. PVA) sintered 1500 °C



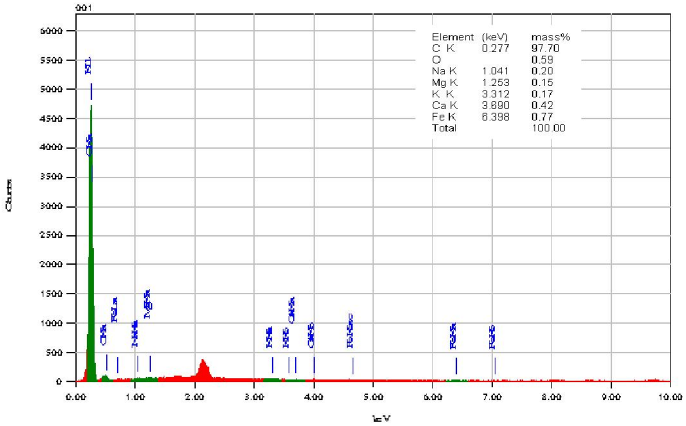
Element	(keV)	mass%
C K	0.277	97.16
O		0.58
Na K	1.041	0.48
Mg K	1.253	0.18
Al K	1.486	0.09
Si K		
S K		
Cl K	2.621	0.46
K K	3.312	1.05
Ca K		
Total		100.00

Figure 5. EDS analysis of the coconut shell carbon (2.5 % wt. PVA) sintered at 1500 ^{o}C.



Element	(keV)	mass%
C K	0.277	97.35
O		0.56
Na K	1.041	0.14
Mg K		
K K	3.312	1.24
Ca K	3.690	0.49
Fe K	6.398	0.22

Figure 6. EDS analysis of the coconut shell carbon (5 % wt. PVA) sintered at 1500 ^{o}C.



Element	(keV)	mass%
C K	0.277	97.70
O		0.59
Na K	1.041	0.20
Mg K	1.253	0.15
K K	3.312	0.17
Ca K	3.690	0.42
Fe K	6.398	0.77
Total		100.00

Figure 7. EDS analysis of the coconut shell carbon (7.5 % wt. PVA) sintered at 1500 ^{o}C.

CONCLUSION AND SUGGESTION

Carbon structure can be prepared from carbonized cocnut shell and polyvinyl alcohol (PVA). The results showed that the products were in uniform particle size of micrometer dimensions and spherical particles in shape. The product characteristics of carbon material showed that there was a change on the physic-chemical properties of the raw material which has been sintered at 1000 ^oC, at which coke was produced in this process. Fabrication process at 1500 ^oC in the presence of PVA gave carbon material from charcoal amorphous to carbon with semi-crystalline structure.

REFERENCES

Buchman, A dan Bryant, R.G., 1999, Molded Carbon-Carbon Composite Based on Microcomposite Technology, *App. Comp. Mat.,* 6, 309-326.

Ebner, F., Hofer, C dan Maurer, E.M., 2004, Conversion of Carbonaceous Material to Graphite Within the Grey Wache Zone of the Eastern Alps, *Int.J.Earth Sci* (Geo Rundsch), 93, 959-973.

Elsayed, M.A., Hall, P.J. dan Heslop, M.J., 2007, Preparation and Structure Characterization of Carbon Prepared from Resorcinol-Formaldehyde Resin by CO_2 Activation, *Adsorption*, 13, 299-306.

Fernandez, M.D., dan Fernandez, M.J., 2008, Thermal Degradation of Copolymers from Vinyl Acetate and Vinyl Alcohol, *J.Therm.Anal.Calorim*., 92, 829-837.

Fraga, M.A., Jordao, E., Mendes, M.J., Freitas, M.M.A., Faria, J.L., dan Figueredo, J.L.., 2002, Properties of Carbon-Supported Platinum Catalysts: Role of Carbon Surface Sites, *J. Catal*., 209, 355-364.

Gupta, S., Sahajwalla, V., Burgo, J., Chaubal, P. dan Youmans, T., 2005, Carbon Structure of Coke at High Temperatures and Its Influence on Coke Fines in Blast Furnace Dust, *Metall and Mat.Trans* B., 36B, 385-394.

Lalena, J.N., D.A Cleary, D.A., Carpenter, E.E., dan Dean, N.F., 2008, *Inorganic Materials Synthesis and Fabrication*, John Wiley & Sons, USA.

Mendez, S dan Santamaria, R., 2008, Structural Changes During Pitch-Based Carbon Granular Composites Carbonisatin, *J. Mater. Sci.*, 43, 906-921.

Miyazaki, K., Maskawa, N., ; Kobayashi, W., Kaku, M., Yasumaru, N. dan Kiuchi, J., 2005, Reflectivity in Femtosecond-Laser-Induced Structural Changes of Diamond-like Carbon Film, *Appl. Phys*., A80, 17-21.

Rampe, M.J., Setiaji, B., Trisunaryanti, W. dan Triyono, 2011, Fabrication and Characterization of Carbon Composite from Coconut Shell Carbon, *Indo.J.Chem*., 11:2, 124-130.

Rampe, M.J., Setiaji, B., Trisunaryanti, W. dan Triyono, 2012, The Production and Characterization of Carbon Composite from Coconut Shell Charcoal, Cord, 28:1, 43-53.

Rampe, M.J., Setiaji, B., Trisunaryanti, W. dan Triyono, 2011, The Characteristic of Polyvinyl Alcohol-Carbon from Coconut Shell Carbon, *Proceeding,* The International Conference on Bioscience and Biotechnology (ICBB) UIN Yogyakarta, October 11th – 12th 2011, P-73 – P-78.

Rampe, M.J., Setiaji, B., Trisunaryanti, W. dan Triyono, 2011, Effect of Carbonization Temperature on Physical Properties of Coconut Shell Carbon, *Proceedings Book*, The International Conference on Basic Science (ICBS) UB Malang, 555-559.

Rampe, M.J., Setiaji, B., Trisunaryanti, W. dan Triyono, 2011, The Infrared Absorption Spectral Changes of Coconut Shell with Polyvinyl Alcohol Stimulant, *Proceeding*, The 1st International Conference on Materials Engineering (ICME) FT UGM Yogyakarta, 153-158.

Rampe, M.J., Setiaji, B., Trisunaryanti, W. dan Triyono, 2010, The Effect of Temperature on the Crystal Growth of Coconut Shell Carbon, *Proceeding*, The third International Conference and Natural Sciences (ICMNS) ITB Bandung, 276-284.

Wiratmoko, A., dan Halloran, J.W., 2009, Fabricated Carbon from Minimally Processed Coke and Coal Tar Pitch as a Carbon-Sequestering Construction Material, *J. Mater. Sci.*, 44, 2097-2100.

Yin, Y., Zhang. J. and Sheng, C., 2009, Effect of Pyrolysis Temperature on the Char Micro-Structure and Reactivity of NO Reduction, *Korean J. Chem. Eng*, 26(3), 895-901.

C-32

PHENOLIC COMPOUNDS FROM CHLOROFORM EXTRACT OF *XYLOCARPUS MOLUCCENSIS* STEM BARK (MELIACEAE)

Tukiran, Nurul Hidayati, Nurul Aini, and Yunita Dwi Setyorahayu

Chemistry Department, Faculty of Mathematics and Natural Sciences
State University of Surabaya (UNESA)
Jl.Kampus Ketintang, Surabaya – 60231, e-mail: btukiran@yahoo.com

Abstract

The research aimed to know the chemical components that are contained into chloroform extract of *Xylocarpus moluccensis* stem bark. By using various chromatograpic techniques including vacuum liquid chromatography (VLC), gravitational column chromatography (GCC), and preparative-thin layer chromatography (P-TLC) always monitored by TLC, it had been succesfully isolated three phenolic compounds, i.e. 2-ethylhexyl 4-methoxycinnamate, myristicin, and scopoletin from the extract. Determination of molecular structures of them was performed by using spectroscopic evidence (UV-Vis, IR, and GC-MS). All of these compounds found are the first time from chloroform extract of the plant.

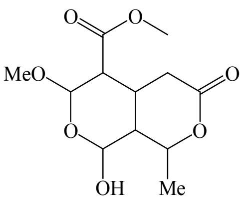
Keywords: 2-ethylhexyl 4-methoxycinnamate, scopoletin, myristicin, *Xylocarpus moluccensis*

Introduction

Based on literature study on chemical constituents of nyiri batu (*Xylocarpus moluccensis*) plant, it had been reported that the plant consists of some compounds from monoterpenoid i.e. xylomollin (**1**) (Bandaranayake, 2002; Balasubramanian, 2004) and limonoid, such as xyloccensin I (**2**) dan xyloccensin J (**3**). All of these compounds had also been isolated from the same genera, *Xylocarpus granatum* (Alvi *et al.,* 1991). Meanwhile, the other chemical consitituents that are consisted of the plant are acetonyldihydrochelerythrine (**4**) and *N*-methylflindersine (**5**) from stem, xylomollin (**1**) from fruit, xyloccensin I (**2**) and J (**3**) from seed, 7-oxogedunin (**6**), 2-hydroxyfissinolide (**7**), and 2-hydroxy-detigloyl-6-deoxyswietenin acetate (**8**) from seeds (Mulholland and Taylor, 1992), detigloyl-6-deoxyswietenin acetate (**9**), angustidienolide (**10**), 7-oxogedunin (**6**), and xyloccensin I (**2**) from timber (Mulholland and Taylor, 1992 and Taylor, 1993), detigloyl-6-deoxyswietenin acetate (**9**) (Mulholland and Taylor, 1992), 6β-hydroxystigmast-4-en-3-one (**11**), β-sitosterol (**12**), and stigmasterol **(13)** from woods (Bercich *et al.*, 1998), angustidienolide (**10**), 2,3,30-triacetatephragmalin (**14**), and 3,30-diacetatephragmalin (**15**) (Mulholland and Taylor, 1992). In view of structures, the major part of these fifteen compounds are monoterpenoids and high-modified triterpenoids (commonly called as limonoids) and the remain compounds are steroids (compounds **11**, **12**, and **13**). But, it has not been reported the findings of phenolic compounds from the plant yet untill now. Therefore, the goal of research is to do a research more focusing on the phenolic compounds.

Past investigations on the chemical constitutes of the chloroform extract of the plant stem bark, we had gained three compounds i.e. an esther derivative of 2-ethylhexyl 4-methoxycinnamate (**16**), β-sitosterol (**12**) and stigmasterol (**13**) (Tukiran *et al*., 2012). The last two compounds were also found from hexane extract of the same plant (Taufiqurrochman and

Tukiran, 2010). Just informed that these sixteenth compounds had been presented at Chemistry National Seminar on Nopember 16, 2013 at Yogyakarta State University (Tukiran *et al.,* 2013). As part of our continuing search for bioinsecticide of natural products from the plant, we now describe the isolation and structural elucidation of two phenolic compounds, i.e. myristicin (**17**) and scopoletin (**18**) from the chloroform extract of the plant.



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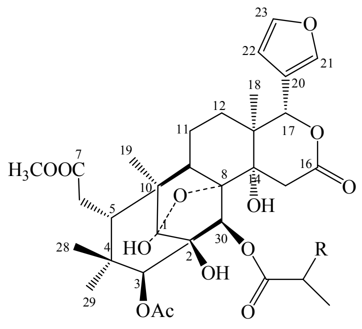

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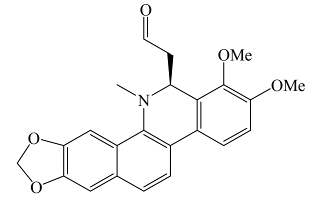

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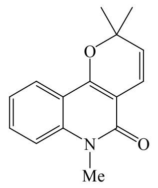

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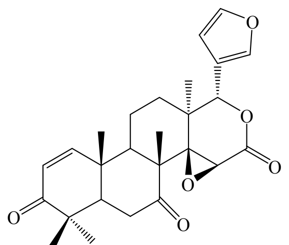

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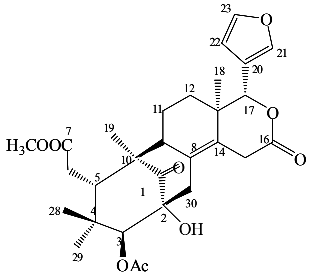

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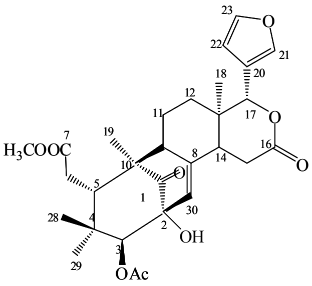

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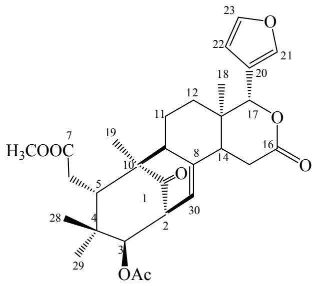

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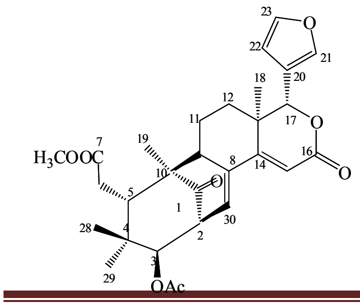
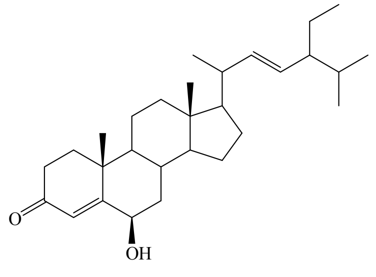
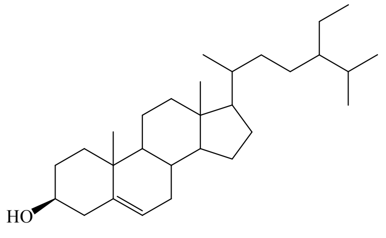
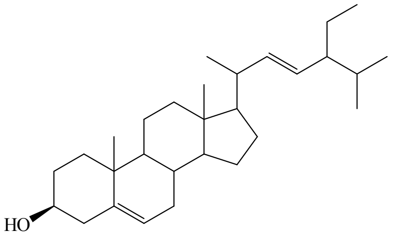






10 **11** **12**



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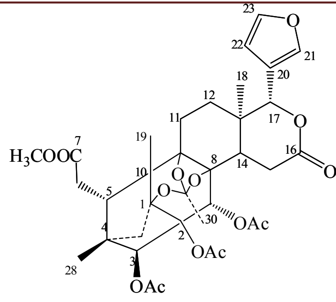

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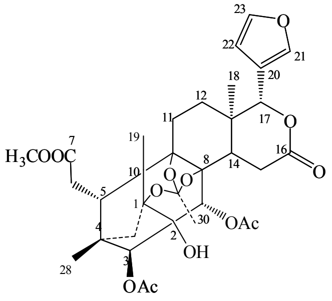

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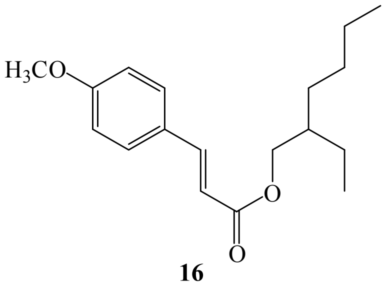

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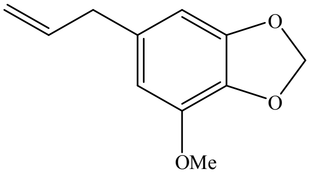

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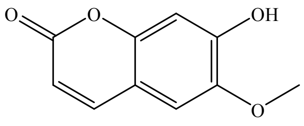

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Research Methods

Instruments

A number of instruments needed to identify and characterize an isolate included spectrophotometer IR type Buck Scientific M500, spectrophotometer UV-1700 SHIMADZU, spectrometer GC-MS type Shimadzu QP-2010S. The equipments used to do extraction and isolation are filter paper, Buchner funnel, Erlenmeyer flask, separating funnel, and *rotary vacuum evaporator* type Butchi Laboratechnic AG R-215. The equipment used to measure melting point of isolate is *Melting Point Apparatus Electrothermal*. Whereas, chromatographic techniques used for isolating phenolic compounds from chloroform extract included Vacuum Liqiud Chromatography (VLC) (using silica gel 60, 0.040-0.063 mm), Gravitational Column Chromatography (GCC) (silica gel 60, 0,063–0,200 mm and 0,200–0,500 mm or 70–230 mesh ASTM), and preparative-TLC (using silica gel 60 GF254 chromatoglass). TLC analyses were carried out on silica gel GF245 chromatoplates with the developing solvent systems. Each TLC analyses resulted from isolation process is always monitored by using UV lamp either on 254 nm or 366 nm.

Plant material

Xylocarpus moluccensis was collected in February 2013 from Sedati, Sidoarjo, East Java, Indonesia. The identification of the plant was performed by LIPI, Purwodadi, East Java. A voucher sample is kept in the Herbarium of LIPI.

Procedure

Extraction and Isolation

The dried stem bark (2.5 kg) of *Xylocarpus moluccensis* was macerated with methanol (20 L) at room temperatur three times. The solvent was removed under reduced pressure to yield 1678.7 g of a thick syrupy and brownish red extracts. After removal of the solvent by evaporation, the residue was suspended in methanol and defatted with chloroform yielded two layers. The chloroform layer (under layer) was evaporated to obtain chloroform extract (44 g).

A portion of chloroform extract (10 g) was then subjected to silica gel VLC using hexane-chloroform-methanol system (100 : 0 : 0 → 0 : 0 : 100) to yield 155 fractions. Of these, fractions 77-112 were combined on the basis of TLC to get 5.98 g (called as B1 fraction). The same manner, it was also applied to the other extract (10 g) using the same silica and eluent to yield 15 fractions. Of these, fractions 6-10 were also combined on the basis of TLC to yield 5.99 g (called as B2 fraction). Furthermore, fractions B1 and B2 were combined to obtain 11.97 g (called as B3). The combined fractions (B3) was then divided by two portions (B3a, 6.02 g and B3b, 5.95 g) in which each portion was subjected to VLC on silica gel using the same eluents as used above, to yield 16 and 13 fractions, respectively. Fractions 7-11 from the first portion (B3a) and fractions 4-7 from the second portion (B3a) were combined on the basis of TLC to get 9.36 g (called as B4).

The fraction B4 was then also performed to VLC on silica gel using one eluent system, i.e. hexane : chloroform : methanol (7 : 2 : 1) to yield 12 fractions. Fraction 1-5 and fraction 6-10 were combined to get 7.22 g (called as fraction B4a). This separation process was seemed to be unsuccesful. By observing the profile of TLC from 12 fractions resulted in VLC of fraction B4 above, it seemed that there are spots located at top with Rf 0.8 – 1.0. Therefore, it was necessary to add hexane to solve part that is soluble in hexane and occured precipitation. Of these, the fractions was then filtered to yield precipitation that is soluble in chloroform. After the chloroform soluble part was evaporated, it was obtained browniesh red extract (5.35 g). Furthermore, the extract was divided into three portions (B4a1 (1.5 g), B4a2 (1.5 g), and B4a3 (2.35 g)). Each portion was chromatographed through GCC using eluent system hexane: chloroform : methanol (3 : 6 : 1) to get 50 fractions, respectively. On the basis of TLC, from 50 fractions of each portion that give the same value of Rf can be grouped into 7 fractions [A1 (1-8), A2 (9), A3 (10), A4, (11), A5 (12), A6 (13-14), and A7 (15-50)]. When observed on the fraction A4, A5, and A6 from each portion, it seemed that all fractions gave simple chromatogram profile. Therefore, preparative-TLC using eluent system (hexane:chloroform:methanol=3:6:1) was carried out toward each fraction of A4, A5, and A6 for respect portions, to yield 6, 6, and 4 band fractions, i.e. A4 (a1-a6), A5 (a1-a6), and A6 (a1-a4). Finally, the fraction A5a3 was then purified using P-TLC with the same eluents and followed by evaporation at room temperature yielded a pure enough isolate as yellow crystal (19.8 mg) with m.p. 178-179 ^{o}C. with m.p. 178-179 ^{o}C. The crystal was characterized by UV-Vis, IR, and GC-MS spectroscopies and determined its structure to give compound **17.**

Using the same separation process including VLC, GCC, and P-TLC always monitored by TLC toward fraction A6a2, it gave a pure enough isolate as white crystal with m.p. 164-165 ^{o}C. Furthermore, the isolate was characterized by the same spectrocsopies as mentioned above, to gain compound **18.** scopoletin).

Result of The Research and Discussion

The chloroform extract of *Xylocarpus moluccensis* that was partitionated using the various eluent system and repeated chromatographic purification yielded two compounds,i.e. compounds **17** and **18**.

Compound 17: was obtained as yellow needle crystals (19.8 mg), m.p. 178-179 ^{o}C. The UV-Vis spectrum of compound **17** showed maximum absorption at 261 nm indicating phenolic compound. The IR spectrum exhibited the following absorption frequencies (KBr, ν̃max.): 2961, 2852, 1615, 1542, 1466, 1367, 1262, 1096, and 802 cm^{-1}. The IR spectrum of compound

17 showed absorption bands at 2961 and 2869 cm^{-1} indicating C-H stretching, 1615, 1542, and 1466 cm^{-1} indicating benzene ring. For a while, absorption bands at 1367 cm^{-1} showed methyl group, 1262 and 1096 cm^{-1} indicated –O-aryl and –O-alkyl, respectively. The last absorption band at 802 cm^{-1} showed substituted benzene. EI-MS exhibited a molecular ion peak at *m/z* 192 (M+) which is compatible with the molecular formula C11H12O3. The EI-MS of compound **17**: 39, 53, 65, 77, 91, 103, 119, 131, 147, 160, 177, and 192 were all consistent with skeletal pattern of **Myristicin** series.

Compound **18:** was yielded as white crystal (12 mg), m.p. 164-165 °C. The UV-Vis spectrum of compound **18** exhibited maximum absorption at 240, 295, and 341 nm indicating phenolic compound. The IR spectrum of it showed the following absorption frequencies (KBr, $\tilde{v}$max): 3339, 2925, 2852, 1705, 1609, 1567, 1509, 1376, 1291, 1141, 1019, 922, 863, and 821 cm^{-1}. The IR spectrum of compound **18** showed absorption bands at 3339 cm^{-1} indicating hydroxyl group, whereas absorption bands at 2925 and 2852 cm^{-1} indicated C-H stretching. The IR spectrum with peaks at 1705 cm^{-1} indicating δ-lactone carbonyl group, and then at 1609, 1567, and 1509 cm^{-1} indicating benzene ring support that compound is a coumarin. For a while, absorption bands at 1376 cm^{-1} showed methyl group, 1291, 1141, and 1019 cm^{-1} indicated –O-aryl, O-esther and –O-alkyl, respectively. The rest absorption band at 863 and 821 cm^{-1} showed substituted benzene. EI-MS exhibited a molecular ion peak at *m/z* 192 (M+) which is compatible with the molecular formula C10H8O4. The EI-MS of compound **18**: 39, 51, 69, 79, 92, 107, 121, 135, 149, 164, 177, and 192 were all consistent with skeletal pattern of **scopoletin** series.

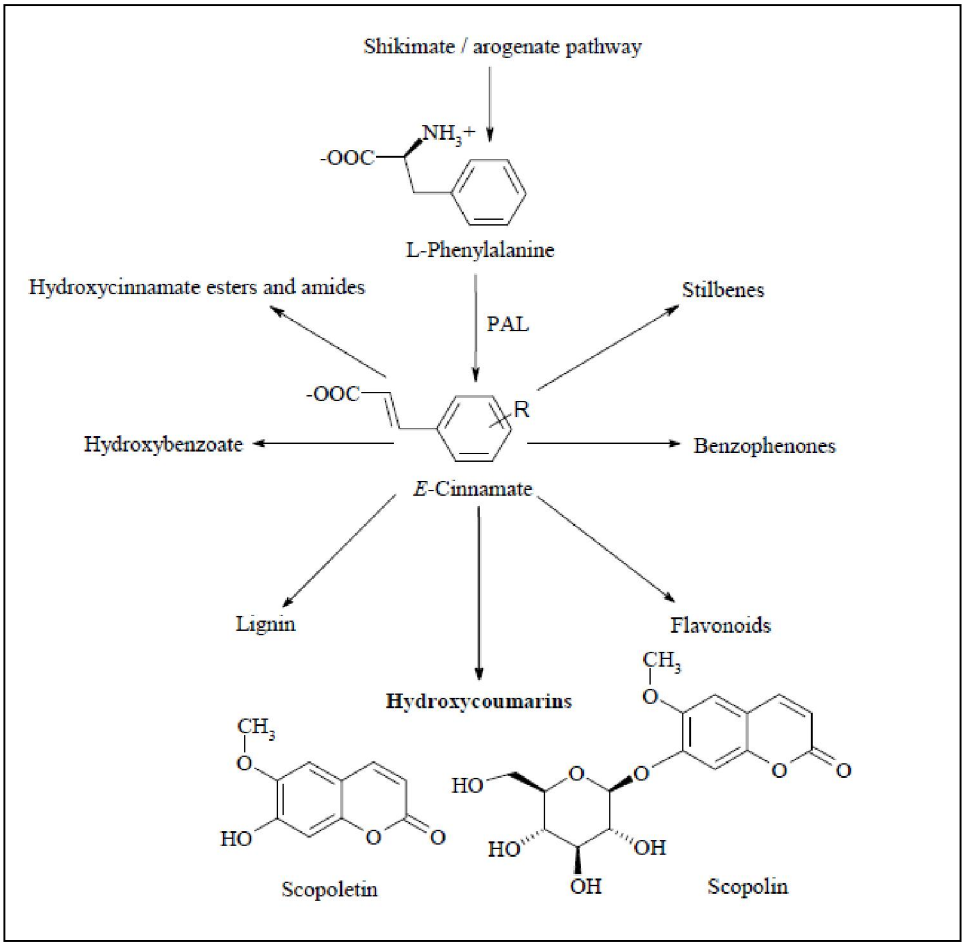
Discussion Contains Explanation Supported with References

Chromatographic analyses of the chloroform extract of the stem bark of *Xylocarpus moluccensis* afforded three phenolic compounds, namely 2-ethylhexyl 4-methoxycinnamate (**16**, Tukiran *et al.,* 2012 and 2013), Myristicin (**17**), and a simple coumarin, scopoletin (**18**). As known that three phenolic compounds that was obtained is phenylpropanoids derivates because of having C6-C3 in their basic skeleton.

Based on literature study as presented above, these findings of three phenolic compounds is the first report on the occurrence of phenylpropanoids in the stem bark of this plant. Scopoletin (**18**) also occur in genera of the family Rubiaceae (Siddiqui *et al*., 2007) such as *Morinda citrifolia* as well as other families, e.g. Euphorbiaceae (Bayoumi *et al*., 2008) such as *Euphorbia heteradena* (Oksus *et al*., 2002).

Kai *et al.* (2006 in Bayoumi *et al*., 2008) recently reported the occurrence of high levels of the coumarins scopoletin found in the wildtype roots 180-fold higher than in aerial parts. Furthermore, it was also reported that scopoletin can be suggested biosynthezed through precursor of cinnamate as shown the following picture. Because of 2-ethylhexyl 4-methoxycinnamate is a hydroxycinnamate ester, it can also be suggested to be formed from the precursor. Because Myristicin also include phenypropanoids, of course it was suggested that the compound can be biosynthezed from the cinnamate.

On the other hand, myristicin, or methoxysafrole, is a phenylpropene that is the principal aromatic constituent of the volatile oil of nutmeg, the dried ripe seed of *Myristica fragrans (Essam and Maytham, 2012)*. Myristicin is also found in several members of the carrot family (*Umbelliferae*) (Helena and Thuvander, 1997).



Picture. Biosynthetic Pathways of Phenylpropanoids

Conclusion

Three phenolic compounds, i.e. 2-ethylhexyl 4-methoxycinnamate, myristicin, and scopoletin were successfully isolated for the first time from the chloroform extract of *Xylocarpus moluccensis* stem bark. All of these compounds are phenypropanoids that can be suggested that they can be formed from precursor, cinnamate.

Acknowledgement

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Bibliography

Alvi, **Khisal** A., Crews Phil., Aalbersberg Bill & Prasad Regina. 1991. **Limonoids from The** Fijian Medicinal Plant Dabi (*Xylocarpus*). *Tetrahedron*. 47(43), 8943-8948. http://www.sciencedirect.com/science?_ob=ArticleURL_udi=B6THR42G5VF94W&_user=10&_coverDate=11%2F04%2F1991&_alid=868093796&_rdoc=30&_fmt=high&_orig=mlkt&_cdi=5289&_sort=v&_st=17&_docanchor=&view=c&_ct=129&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=87850394589d3c74ef314af957a3d83e (diakses pada tanggal 17 Pebruari 2009).

Balasubramanian, T. and S. Ajmal Khan, 2004. Chemotaxonomic Markers In Mangroves. *Seshaiyana,* Vol.12 No.2. http://www.casmbenvis.nic.in/ pdf/Seshaiyana12-2.pdf *(diakses pada tanggal 16* Pebruari *2009)*.

Bandaranayake, W.M., 2002. Bioactivities, Bioactive Compounds and Chemical Constituents of Mangrove Plants, *Wetlands Ecology and Management* 10: 421–452.

Bayoumi, S. A. L., Rowan, M. G., Blagbrough, I. S. and Beeching, J. R. 2008. Biosynthesis of scopoletin and scopolin in cassava roots during post-harvest physiological deterioration: The E-Zisomerisation stage. *Phytochemistry*, 69 (17), 2928-2936.

Cui, Jianxin., Zhiwei Deng., Jun Li., Hongzheng Fu., Peter Proksch & Wenhan Lin, 2005. Phragmalin-type limonoids from the mangrove plant *Xylocarpus granatum*. *Phytochemistry*. 66(19), pp. http://servente.area.ge.cnr.it/sds/DbToC/include/file_fr.php? id=2472155&wh=nm (diakses pada tanggal 16 Pebruari 2009).

Essam F. Al-Jumaily and Maytham H. A. Al-Amiry, 2012. Extraction and Purification of Terpenes from Nutmeg (*myristica fragrans), Journal of Al-Nahrain University*. 15 (3), 151-160.

Helena Hallström* and Ann Thuvander, 1997. Toxicological evaluation of myristicin. *Natural Toxins*, 5(5), 186-192.

Hisham D.M.Nazrul, J.M. Lip, J. Arif Zaidi and A. Normah. 2010. Main Non-Polar Chemical Constituent from *Morinda citrifolia* Fruits, *J. Trop. Agric. and Fd. Sc.* 38(1), 97–102.

Hu, Wei-Min and Jun Wu, 2010. Protoxylogranatin B, a Key Biosynthetic Intermediate from *Xylocarpusgranatum*: Suggesting an *Oxidative Cleavage* Biogenetic Pathway to Limonoid, *The Open Natural Products Journal,* 3, 1-5.

Kokpol , Udom., Warinthorn Chavasiri., Santi Tip-pyang., Gaysorn Veerachato., Fenglin Zhao., Jim Simpson & Rex T. Weavers, 1996. A limonoid from *Xylocarpus granatum*. *Phytochemistry*. 41(3), 903-905. http://servente.area.ge.cnr.it/sds/DbToC/include/file_fr.php?id= 2472155&wh=nm (diakses pada tanggal 16 Pebruari 2009).

Chou FY, Hostettmann K, Kubo I, Nakanishi K., and Taniguchi M., 1977. Isolation of An Insect Antifeedant N-Methylflindersine and several benz(C) Phenanthridine Alkaloids from East African Plants; A Comment on Chelerythrine. *Heterocycles* 7, 969-977.

Mulholland, DA, and Taylor, DAH, 1992. Limonoids from Australian Members of the Meliaceae. *Phytochemistry* (Oxford), 31, 4163-4166.

Ng AS. and Fallis AG. 1979. 7-Alpha Acetoxy Dihydronomilin and Mexicanolide Limonoids from *Xylocarpus granatum*. *Canadian Journal of Chemistry,* 57, 3088-3089.

Okorie DA. and Taylor DAH, 1970. Limonoids From *Xylocarpus granatum*. *Journal of The Chemical Society* Section C Organic Chemistry, 211-213.

Oksus, S., Ayhan Ulubelen, Asl Barla, And Wolfgang Voelter. 2002. Terpenoids and Aromatic Compounds From *Euphorbia heteradena, Turk J Chem.,* 26, 457-463.

Roy, Amit and Shailendra Saraf, 2006. Limonoids: Overview of Significant Bioactive

Triterpenes Distributed in Plants Kingdom, *Biol. Pharm. Bull*. 29(2), 191—201.

Siddiqui B.S., Fouzia A. Sattar, Fayaz Ahmad, and Sabira Begum. 2007. Isolation and Structural Elucidation of Chemical Constituents from The Fruits of Morinda citrifolia Linn., *Arch Pharm Res*., 30(8), 919-923.

Taufiqurrochman, M. dan Tukiran, 2010, "Uji Bioaktivitas Insektisida Senyawa Hasil Isolasi Dari Kulit Batang Tumbuhan Nyiri Batu (Xylocarpus moluccensis (Lamk) M. Roem) (Meliaceae)", Presentasi Oral, Seminar Nasional Sains 2010 di Prodi Pendidikan Sains Pascasarjana, Universitas Negeri Surabaya, 16 Januari 2010.

Taylor, David A. H. **1983**. Limonoid Extractives From*Xylocarpus moluccensis*. *Phytochemistry*.Volume 22, Issue 5, Pages 1297-1299. Http://Servente.Area.Ge.Cnr.It/Sds/Dbtoc/Include/File_Fr.Php?Id=2472155&Wh=Nm (diakses pada tanggal 16 Pebruari 2009).

Tukiran, Septiani Setyo Cahyasari, and Kuniyoshi Shimizu, 2012. An Ester of 4-Methoxy Cynnamic Acid Isolated From *Xylocarpus moluccencis* (Lamk) M. Roem (MELIACEAE), *Indo. J. Chem.,* 12(2), 184 – 188.

Tukiran, Nurul Hidayati, and Dwi Adi Sunarto, 2013, Fitokimia Tumbuhan Nyiri Batu (*Xylocarpus Moluccensis*): Suatu Studi Awal., Prosiding Seminar Nasional Kimia 2013, Jurusan Pendidikan Kimia, FMIPA, UNY, Yogyakarta, 16 Nopember 2013, 139-145.

Wu, Jun, Si Zhang, Qiang Xiao, Qingxin Li, Jianshe Huang, Zhihui Xiao, and Lijuan Long, 2003. Xyloccensin M and N, Two New B, D-*seco* Limonoids from *Xylocarpus granatum,* Z. Naturforsch. 58b, 1216 – 1219.

Wu, Jun, Si Zhang., Qiang Xiao., Qingxin Li., Jianshe Huang., Lijuan Long & Liangmin Huang, 2004. Xyloccensin L, a novel limonoid from *Xylocarpus granatum*. *Tetrahedron Letters*. 45(3), pp. http://servente.area.ge.cnr.it/sds/DbToC/include/file_fr.php?id=2472155& wh=nm (diakses pada tanggal 19 Pebruari 2009).

Wu, Jun, Qiang Xiao., Si Zhang., Xiang Li., Zhihui Xiao., Haixin Ding & Qingxin Li, 2005. Xyloccensins Q–V, six new 8,9,30-phragmalin ortho ester antifeedants from the Chinese mangrove *Xylocarpus granatum*. Tetrahedron. 61(35), 8382-8389. http://servente.area.ge.cnr.it/sds/DbToC/include/file_fr.php?id=2472155&wh=nm (diakses pada tanggal 16 Pebruari 2009).

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PREPARATION OF CHITIN FROM SHRIMP SHELLS BY PAPAIN LATEX (*CARICA PAPAYA*)

Yuli Rohyami[*], Reni Banowati Istiningrum, Ida Sulistyaningrum

Department of Chemical Analyst
Faculty Mathematics and Natural Science Islamic University of Indonesia
Yogyakarta Indonesia
**Corresponding author, email : rohyami@uii.ac.id*

ABSTRACT

Papain is an enzyme containing cysteine proteases that have catalytic properties. This enzyme is thermostable, active in the pH range 3.0 to 9.0 and can be obtained from papaya latex (*Carica papaya*). Activity of these enzymes can be utilized in the process of chitin from shrimp shells deproteination. Papaya latex is tapped and then mixed with a solution of 0.1% v / v ($NaHSO_3$: NaCl, 1:1). The mixture was stirred as he added 100 mL of phosphate buffer pH 7. The mixture was left for 1 hours at 4°C and then centrifuged at 1500 rpm for 20 minutes. Supernatants were stored at 4°C was obtained.

Deproteination of chitin by 2 to 6% papain in phosphate buffer solution at pH 7 for 3 to 5 hours at 50ºC. Demineralized with lactic acid for 3 hours 40°C. Decolorization of chitin using chloroform, methanol and water (1: 2: 4, v / v). Chitin obtained was filtered and washed using distilled water and then dried in an oven at a temperature for 12 hours at 65^oC

The results showed that the chitin produced has better physicochemical properties than the chemically chitin preparation. The characterization results showed that the degree of deacetylation of chitin was 13.5 to 25.2^o. The results also showed that the concentration of the enzyme papain was increased on deproteination of chitin.

Key word : papain, chitin, deproteination

INTRODUCTION

Chitin is a linear polysaccharide compounds containing N-acetyl-D-glucosamine. Chitin is a biopolymer that is non-toxic, biodegradable, and biocompatible. Compound chitin and its derivatives have been widely used in various applications in medicine, pharmacy, biotechnology, agriculture, environment, water treatment, and paper industry, textile, plastics and batteries (Cheba, 2011). Chitin is citocompatible and have bioactivity (Kumirska, et al., 2011). Bioactivity is influenced by physicochemical properties consisting degree of deacetylation, molecular weight, solubility, crystallinity, viscosity, biodegradability and biocompatibility.

Chitin can be obtained from crustaceans, insects, mollusca and fungi with deproteinasi reaction kimiawimelalui extraction with alkaline and acid demineralization. The use of strong alkali such as NaOH in deproteination process and the using for acid demineralization could be expected to impair the properties of chitin fisikokimiawi. Demineralization with hydrochloric acid in the process can destroy physicochemical properties of chitin (Das and Ganesh (2010).

One of the methods that has been developed chitin extraction is the extraction of biologically by using microorganisms or enzymes. Isolation of chitin by biological processes have advantages over chemical processes (Arbia, et al., 2013). Extraction is carried out using bacterial proteolytic or enzyme protease that act in the reaction of chitin deproteination. The reaction produces lactic acid is useful in demineralization.

The structure of chitin obtained more homogeneous, biodegradability, biocompatibility, and non-toxic (Arbia, et al., 2013). Chemical process be expected to affect the molecular mass and the degree of deacetylation of chitin, as well as allowing the formation of organic salts of deacetylation and depolymerization reactions of chitin. Biological extraction has been carried out using various protease enzyme producing bacteria, such as *Aspergillus niger* and *Lactobacillus plantarum* (Das and Ganesh, 2010; Khorrami, et al., 2012), *Erwinia chrysanthemi* (Giyose, et al., 2010), *Bacillus subtilis, Lactobacillus paracasei, pentosaseus Pediococcus, Pseudomonas aeruginosa,* and *Penicillium chrysogenum* (Troger and Nirajan, 2010).

Deproteination of chitin can also be done with the protease enzyme. Alkalase including protease enzyme which can release up to 64% protein and produce a degree of deacetylation of chitin with up to 77.67% (Mizani and Aminlari, 2007). Preparation of chitin was performed through protein hydrolysis which takes place optimally at temperatures 40^{o}C for 1 hour at pH 8.

Protease enzymes which allegedly has the same ability to accelerate the reaction of papain hydrolysis of the protein is obtained from the sap of papaya fruit (*Carica papaya*). Papain is a type of enzyme protease-containing group sulfihidril (Monti, et al., 2000; Yuniwati et al, 2008) found in cysteine (Dubey, et al., 2007). The group can actively participate in the process of hydrolysis of the protein so that it can be used in the reaction suspected deproteination chitin.

The ability of papain in the hydrolysis of protein in stage deproteination of chitin important to be studied. This method is one of the extraction of chitin which is expected to produce chitin extract having a more homogeneous structure and has better physicochemical properties. The use of protease enzymes from papaya latex in deproteination of chitin is one of the studies conducted in order to reduce the use of harmful chemicals and are quite expensive. Protease enzyme from papaya latex can be obtained easily and more environmentally friendly. Therefore, this study is important to study the potential of papain in the preparation of chitin.

RESEARCH METHOD

Materials

Sodium hydrogen sulfite, sodium chloride, potassium dihydrogen phosphate potassium hydrogen phosphate, lactic acid, sodium hydroxide, methanol, chloroform, and hydrochloric acid of reagent grade, were all purchased from Merck, Germany. Natural chitin was obtained by isolating it from shrimp shells and papain was obtained from papaya latex. Other materials used are distilled water, filter paper Wathman 42, universal pH paper and standard buffer solution of 4.00 and 7.00.

Preparation of Papain Latex from Papaya (*Carica papaya*)

Papain latex from papaya (*Carica papaya*) was obtained from the fruit sap at 5:00-08:00 pm. Preparation of papain latex refers to procedures performed by (Geantaresa and Supriyanti, 2010). Papaya latex that has been was obtained is mixed with a solution of 0.1% v / v ($NaHSO3$: NaCl, 1:1). The mixture was stirred until smooth and then added 100 mL of phosphate buffer pH 7. Mixture is left to stand for 1 hour at 4 °C and then centrifuged at 1500 rpm for 20 minutes. The resulting supernatant was stored at 4 °C.

Preparation of Chitin Using Papain Enzyme

Extracted from the shells of shrimp chitin obtained from shrimp suppliers in Yogyakarta. Shrimp shells are washed with clean water and dried at 65^{o}C for 48 hours. Shrimp shells was milled to obtain a powder with a size of approximately 100 mesh.

Chitin Extraction carried out with deproteination using an enzyme papain. Twenty-five grams of shell powder put into a erlenmeyer flask then added 50 mL of 2 to 6% solution of crude papain in phosphate buffer solution at pH 7. Reaction was carried out at 50 ^{o} C for 3 hours while stirring with a magnetic stirrer. Heating was continued until a temperature of 80 º C for 30 minutes. The results was filtered and was washed with distilled water and neutralized. The results obtained were dried in an oven at a temperature of 65^{o}C for 6 hours.

The results of deproteination was demineralized with 100 mL lactic acid at 40^{o}C (Das and Ganesh, 2010). Demineralization carried out for 3 hours while stirring with a magnetic stirrer. The results was filtered and was washed with distilled water. Depigmentation of chitin has been obtained by using 50 mL of a mixture of chloroform, methanol and water (1: 2: 4, v / v) (Arbia, et al., 2013). Chitin obtained was filtered and was washed using distilled water and then dried at 65^{o}C for 6 hours.

Characterization of Chitin

Characterization of chitin carried out with infrared spectrophotometer . Infrared spectra of chitin were measured from KBr pellets by a Shimadzu FT-IR/8201 PC spectrophotometer. The degree of deacetylation of chitin was determined using infrared spectral data with the wave number range 4000 to 400 cm^{-1}. The degree of deacetylation was calculated using the method of Baxter baseline by comparing the absorbance at 1655 cm^{-1} and 3450 cm^{-1} (Brugnerotto, 2001 and Junaidi, et al., 2009).

RESULT AND DISCUSSION

Characterization of Chitin

Potential as enzyme papain protease in the release of proteins in chitin binding. Figure 1 represents the results of the characterization of infrared spectra of chitin preparation results with papain 2, 4, and 6%. The result of deproteination using 2 % of papain showed -OH stretching vibration of aliphatic groups bonded to each monomer unit of chitin in the wave number 3448.72 cm^{-1},-NH stretching vibration of the acetamide group at 1573.91 cm^{-1}, -C stretching vibration =O of acetamide group at 1627.92 cm^{-1}, -CN-stretching vibration of the acetamide group at 1319.31 cm^{-1}, -CH-stretching vibration of -CH_3 at 1381.03 cm^{-1} and vibration -COC- from glucosamine at 1072.43 and 1026.13 cm^{-1}.

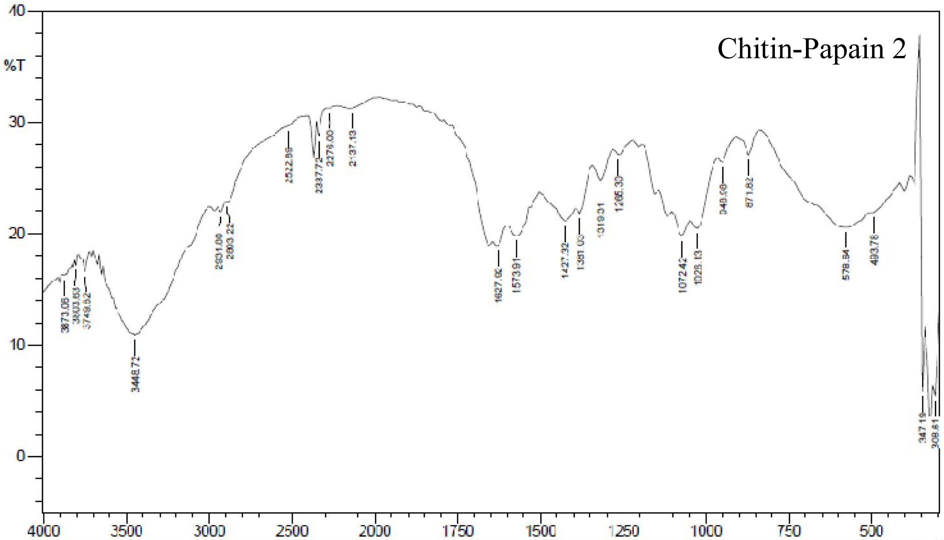
Similar spectra are also shown at 4% of papain. Figure 1. showed -OH stretching vibration of aliphatic groups bonded to each monomer unit of chitin in the wave number 3448.72 cm^{-1},-NH stretching vibration of the acetamide group at 1566.20 cm^{-1}, -C stretching vibration =O of acetamide group at 1635.64 cm^{-1} , -CN-stretching vibration of the acetamide group at 1319.31 cm^{-1},-CH-stretching vibration of -CH_3 at 1381.03 cm^{-1} and vibration -COC- from glucosamine at 1072.42 and 1026.13 cm^{-1}.

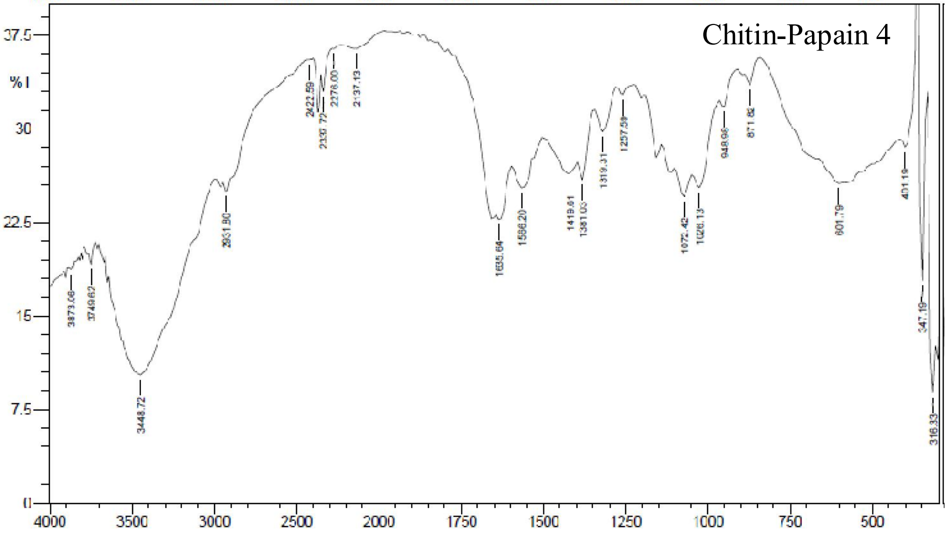
The same spectra are also shown in the results with 6 % of papain. Chitin that deproteination using 6 % of papain showed -OH stretching vibration of aliphatic groups bonded to each monomer unit of chitin in the wave number 3448.72 cm^{-1},-NH stretching vibration of the acetamide group at 1566.20 cm^{-1},-C stretching vibration =O of acetamide group at 1635.64 cm^{-1}, -CN-stretching vibration of the acetamide group at 1319.31 cm^{-1}, -CH-stretching vibration of -CH_3 at 1381.03 cm^{-1} and vibration -COC- from glucosamine at 1072.42 and 1026.13 cm^{-1}.

This study showed similar spectra between chitin from chemically and enzymatically processes. Papain have potential to hydrolyze proteins bound to chitin, as shown in Figure 2. Figure 2. Provide information that chitin produced in the enzymatic reaction spectra have a structure similar to a chemical process.Figure 2. showed -OH stretching vibration of aliphatic groups bonded to each monomer unit of chitin in the wave number 3348.72 cm^{-1}, -NH stretching vibration of the acetamide group at 1558.43 cm^{-1}, -C stretching vibration =O of acetamide group at 1651.07 cm^{-1},-CN-stretching vibration of the acetamide group at 1319.31 cm^{-1}, -CH-stretching vibration of -CH_3 at 1381.03 cm^{-1} and vibration -COC- from glucosamine at 1072.43 and 1026.13 cm^{-1}. The infrared spectra are similar to those studied by Palpandi et al.

(2009)

The presence of protease enzyme which containing group sulfihidril (Monti, et al., 2000; Yuniwati et al, 2008; Dubey, et al., 2007) was potentially for deproteination of chitin. It were actively participate in the process of hydrolysis of the protein so that it can be used in the reaction suspected deproteination chitin.





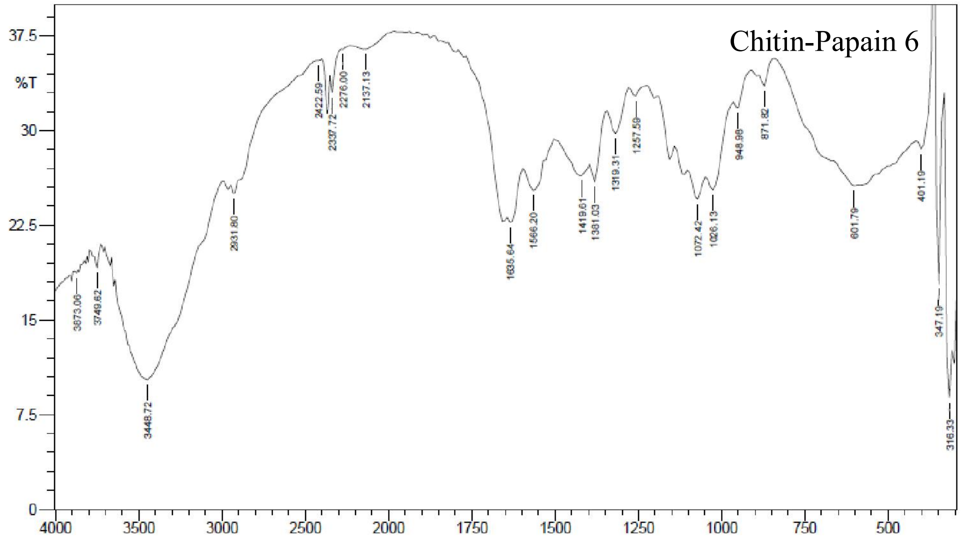


Figure 1. Chitin from enzymatically processes

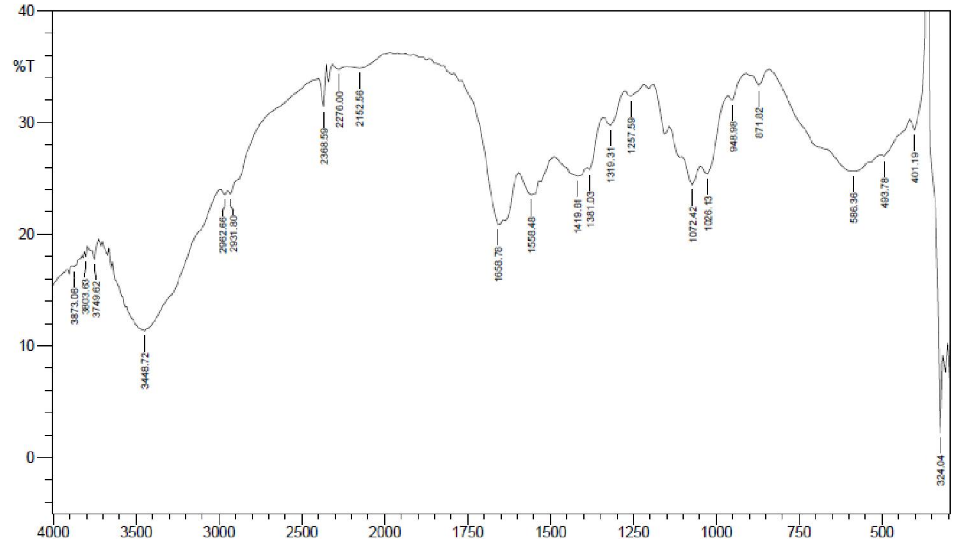


Figure 2. Chitin from chemically processes

Concentration Effect of Papain on the degree of deacetylation

Table 1. was showed the degree of deacetylation chitin from enzymatically processes by papain. The degree of deacetylation chitin was calculated using the method of Baxter baseline by comparing the absorbance at 1655 cm^{-1} and 3450 cm^{-1} (Brugnerotto, 2001 and Junaidi, et al., 2009). Chitin which extracted enzymatically with papain have degree of deacetylation 13.5 to 25.2^{o}. The results also showed that the concentration of the enzyme papain was increased on deproteination of chitin.

This study was showed the nature physicochemical of chitin better than chemically processes (Arbia, et al., 2013). The degree of deacetylation determined by the ability of papain to hydrolyze proteins. Concentration effect of papain on the magnitude of the degree of deacetylation. In the enzymatic process requires a comparison of the concentration of the enzyme and the substrate so as to obtain optimum results.

Table 1. The degree of deacetylation of chitin

Kitin	Concentration of papain	Degree of deacetylation (DA)
Chitin-NaOH		17,2 %
Chitin-Papain 2	2 %	13,5 %
Chitin-Papain 4	4 %	25,2 %
Chitin-Papain 6	6 %	20,9 %

CONCLUSION

The results showed that the chitin produced has better physicochemical properties than the chemically chitin preparation. The characterization results showed that the degree of deacetylation of chitin was 13.5 to 25.2^{o}. The results also showed that the concentration of the enzyme papain was increased on deproteination of chitin.

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BIBLIOGRAPHY

Arbia, W., Arbia, L., Adour, L., Amrane, A. (2013). Chitin Extraction from Crustacean Shells Using Biological Methods – A Review, *Food Technol. Biotechnol.* 51, 12–25

Brugnerotto, J., Lizardi, J., Goycoolea, F.M., Monal, A.,W., Aresa, D. J. (2001). An Infrared Investigation in Relation with Chitin and Chitosan Characterization. *Polymer,* 42, 3569-3580

Cheba, B.A. (2011) Chitin and Chitosan : Marine Biopolymers with Unique Properties and Versatile Applications. *Global Journal of Biotechnology & Biochemistry* 6, 149-153

Das, S., Ganesh, E.A. (2010). Extraction of Chitin from Trash Crabs (Podophthalmus vigil) by an Eccentric Method. *Current Research Journal of Biological Sciences* 2, 72-75

Dubey, V.K., Pande, M., Singh, B.K., Jagannadham, M.V. (2007). Papain-like Proteases: Applications of Their Inhibitors. *African Journal of Biotechnology* 6, 1077-1086

Geantaresa dan Supriyanti (2010). Pemanfaatan Ekstrak Kasar Papain sebagai Koagulan pada Pembuatan Keju Cottage Menggunakan Bakteri. *Jurnal Sain dan Teknologi Kimia,* 1, 38 – 43

Giyose, N.Y., Mazomba, N. T. and Mabinya, L.V. (2010). Evaluation of Proteases Produced by Erwinia chrysanthemi for the Deproteinization of Crustacean Waste in a Chitin Production Process, *African Journal of Biotechnology* 9, 707-711

Khorrami, M., Najafpour, G.D., Younesi, H., Hosseinpoura, M. N. (2012). Production of Chitin and Chitosan from Shrimp Shell in Batch Culture of *Lactobacillus plantarum. Chem. Biochem. Eng. Q.* 26, 217–223

Kumirska, J., Weinhold, M.X., Thoming, J., Stepnowski, P. (2011). Biomedical Activity of Chitin/Chitosan Based Materials Influence of Physicochemical Properties Apart from Molecular Weight and Degree of N-Acetylation. *Polymers* 3, 1875-1901

Mizani, A.M. and Aminlari, B.M. (2007). A New Process for Deproteinzation of Chitin from Shrimp Head Waste. Proceedings of European Congress of Chemical Engineering (ECCE-6) Copenhagen, 16-20 September 2007

Monti, R., Basilio, C.A., Trevisan, H.C., Contiero, J. (2000). Purification of Papain from Fresh Latex of *Carica papaya. Brazilian Archives of Biology and Technology*, 43, 501-507

Palpandi, C., Shanmugam, V., Shanmugam, A. (2009). Extraction of Chitin and Chitosan from Shell and Operculum of Mangrove Gastropod Nerita (Dostia) crepidularia Lamarck. *International Journal of Medicine and Medical Sciences,* 1, 198-205

Troger, C., Nirajan, K. (2010). Sustainable Chitin Extraction and Chitosan Modification for Application in the Food Industry. Internacional Conference on Food Innovation, Universidad Politecnica de Valencia, 25-29 October 2010.

Yuniwati, M., Yusran, Ramadany. (2008). Utilization Enzymes Papain as a coagulant in cheese making. National Seminar on Science and Technology Applications 2008, IST AKPRIND Yogyakarta, 127-133

C-34

CHARACTERIZATION OF Cu(II) COMPLEXES OF 4-METHYLBENZENESULFONYLHYDRAZONE AND THE POTENTIAL AS REAGENT FOR PHENOLIC COMPOUND DETECTION

Yusnita Juahir[1], Norlaili Abu Bakar[1], Wan Rusmawati Wan Mahamod[1], Saripah Salbiah Syed Abdul Azziz[1], Rozita Yahaya[1], Wong Chee Fah[2]

[1]*Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak.*
[2]*Department of Biology, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak.*

Abstract

Sulfonamide analogues such as sulfonylhydrazones, exhibited various applications. The present study involves synthesis a series of 4-methylbenzenesulfonylhydrazone derived from the condensation reaction of 4-methylbenzenesulfonylhydrazine with the derivative of salicylaldehyde. The ligands were coordinated to Cu(II) by the reaction of metal acetates. The ligands and metal complexes were characterized by analysis of CHN, IR, NMR and UV/Vis. The involvement of azomethine nitrogen and phenolic oxygen atom upon the coordination of Cu(II) complexes of SALMeBSH was determined in their IR spectra. Additionally, the bands of NH and SO_2 remain more or less at the same position indicating that these groups did not take part in the coordination. The appearance of a single broad absorption band in the spectra UV-Vis of Cu(II) at 735 nm respectively which was assigned to d-d transitions indicating the complexes were in square planar. The potential of Cu-$(5BrSALMeBSH)_2$ complex as reagent for phenolic compound detection was also investigated.

Key words: *Cu(II) complexes, 4-methylbenzenesulfonylhydrazone, phenolic compound detection*

INTRODUCTION

The basic strength of the C=N group in Schiff base ligands is its ability to coordinate with many different metals such as copper, nickel, iron and zinc and stabilized them in various oxidation states by simple co-ordination of the lone pair to a metal ion. Besides the nitrogen donor atom, considerable attention also has been paid to the chemistry of the metal complexes of Schiff bases containing other donor atoms; oxygen and sulfur act as good chelating agent which can be designed to yield mono-, bi- or dimer complexes. The intensively coloured metal complexes were produced and some of which have been shown to exhibit interesting physical and chemical properties [1].

In the beginning, research on the Schiff base complexes was focused on the characteristics of the chelation of ligands with metal complexes. Magnetic susceptibility, absorption spectra, elemental analysis, molecular weight determination, conductivity, thermal analysis of many Schiff base complexes has been reported. Nowadays, the research field dealing with Schiff base metal complexes has been expanded enormously, and embraces very wide and diversified subjects comprising vast areas of organometallic compounds [2].

Benzenesulfonylhydrazines and their derivatives are capable of being neutral or charged moieties. Investigations to assess the possible use of metal chelates in the stabilization of unusual high oxidation states has been described previously. Interest in such studies, especially on hydrazone compounds also has arisen, owing to their potential application in view of their ability to form metal chelates and the appearance of novel structural features in co-ordination chemistry [3].

Hydroquinone, chlorophenol, nitrophenol and cresol are present in a number of consumer products such as antioxidant in food and cosmetic [4]. The serious effects of the harmful substance usually increase as both the level and length of exposure increase. Repeated exposure to low levels of phenol in drinking water has been linked with diarrhea and mouth sores in humans and eating very large amounts of phenol has resulted in death [5]. Phenol will stay in the air, soil, and water for much longer times if a large amount of it is released at one time, or if a steady amount is released over a long time. Therefore, a rapid and effective method should be investigated for the determination of phenolic derivatives compound.

Calorimetric, spectrophometer and HPLC are used for the determination of phenolic compounds as standard methods. However, the high cost and slow turnaround times of conventional photometric and chromatographic methods indicate a need for more sophisticated analytical techniques [6]. There are many chemical reagent used in the determination of phenol that have been reported [7,8]. Nevertheless there is limited research in the literature on phenolic compound detection using metal complexes. To the best our knowledge, there is no report on the potential of benzenesulfonylhydrozone complexes as reagent in the phenolic compound detection. This fact was the basis for developing reagent by using Cu(II) complexes of benzenesulfonylhydrozone in the detection of phenolic compound. In the beginning study, Cu(5Br-SALMeBSH)$_2$ is the only complex that has been investigated.

RESEARCH METHOD

Materials and measurements

4-methylbenzenesulfonohydrazine, 5-bromo-2-hydroxybenzaldehyde, 5-chloro-2-hydroxy benzaldehyde, 3-bromo-5-chloro-2-hydroxybenzaldehyde, 3,5-dibromo-2-hydroxy benzaldehyde, 3,5-dichloro-2-hydroxybenzaldehyde, dimethylformamide, phenol, 4-chlorophenol, 4-nitrophenol and hydroquinone were purchased from Aldrich-Sigma or Acros Organics. Ethanol was distilled prior to use. All chemicals were of analytical grades and used without any further purification. The IR spectra were recorded on a Perkin-Elmer RX1 FTIR spectrometer in the range of 400-4000 cm^{-1} at room temperature. Samples were prepared as KBr pellets. The electronic spectra were measured by means of a Shimadzu 1601 spectrophotometer in the region 200-1100 nm. The ^{1}H and ^{13}C spectra were recorded using a Lambda JEOL 500 MHz FT-NMR spectrometer.

General method for preparation of ligands

5Br-SALMeBSH was obtained by adding 4-methylbenzenesulfonohydrazine (0.30g, 1.61 mmol) dropwise into an acidified ethanolic solution of 5-bromo-2-hydroxybenzaldehyde (0.32 g, 1.61 mmol). The mixture was refluxed for about 3 h. The resulting colorless solution was evaporated in vacuo to give brown crystalline percipitates which was then filtered off, washed with cold ethanol and then dried over silica gel. 5Cl-SALMeBSH, 3Br5Cl-SALMeBSH, 3,5-Cl$_2$SALMeBSH and 3,5-Br$_2$SALMeBSH were obtained following the same procedure as for 5BrSALMeBSH, by adding the relevant 2-hydroxybenzaldehyde compounds. The percentage yields obtained are in the range 70-81%.

5Br-SALMeBSH

Melting point: 201 °C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Ni$: C, 45.66; H, 3.26; N, 7.93 %. Found: C, 45.70; H, 3.24; N, 7.78%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3131; $\nu_{C=N}$ 1618; $\nu(C_{Ph}$ -O) 1288; $\nu_{as}(SO_2)$ 1324; $\nu_s(SO_2)$ 1167. UV–Vis [λ_{max} (nm) (DMSO)]: 331 ($n \rightarrow \pi^*$). ^{1}H NMR: (DMSO-d6, ppm) δ: 11.59 (1H, s, OH); 10.45 (1H, s, NH); 8.14 (1H, s, N=CH); 6.88-7.76 (m, ArH); 3.35 (3H, s, CH_3); ^{13}C NMR: (DMSO-d6, ppm) δ 155.18 (C-OH); 143.68 (N=CH); 118.09-143.37 (Ar-C); 21.05 (CH_3).

5Cl-SALMeBSH

Melting point: 193 °C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Ni$: C, 51.81; H, 3.65; N, 8.54%. Found: C, 51.92; H, 3.71; N, 8.65%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3120; $\nu_{C=N}$ 1620; $\nu(C_{Ph}$ -O) 1260; $\nu_{as}(SO_2)$ 1324; $\nu_s(SO_2)$ 1170. UV–Vis [λ_{max} (nm) (DMSO)]: 331 ($n \rightarrow \pi^*$).^{1}H NMR: (DMSO-d6, ppm) δ: 11.58 (1H, s, OH); 10.45 (1H, s, NH); 8.10 (1H, s, N=CH); 6.838-7.74 (m, ArH); 3.35 (3H, s, CH_3); ^{13}C NMR: (DMSO-d6, ppm) δ 155.58 (C-OH); 143.17 (N=CH); 110.64-143.27 (Ar-C); 21.05 (CH_3).

3Br5Cl-SALMeBSH

Melting point: 175 °C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Ni$: C, 41.73; H, 2.96; N, 7.13 %. Found: C, 41.75; H, 2.98; N, 7.23 %. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3216; $\nu_{C=N}$ 1599; $\nu(C_{Ph}$ -O) 1284; $\nu_{as}(SO_2)$ 1328; $\nu_s(SO_2)$ 1162. UV–Vis [λ_{max} (nm) (DMSO)]: 332 ($n \rightarrow \pi^*$). ^{1}H NMR: (DMSO-d6, ppm) δ: 12.06 (1H, s, OH); 11.09 (1H, s, NH); 8.11 (1H, s, N=CH); 7.45-7.75 (m, ArH); 3.44 (3H, s, CH_3); ^{13}C NMR: (DMSO-d6, ppm) δ 152.30 (C-OH); 146.34 (N=CH); 111.00-144.14 (Ar-C); 21.06 (CH_3).

3,5-Cl_2SALMeBSH

Melting point: 164 °C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Ni$: C, 46.89; H, 3.34; N, 8.12%. Found: C, 46.93; H, 3.35; N, 8.16 %. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3178; $\nu_{C=N}$ 1599; $\nu(C_{Ph}$ -O) 1285; $\nu_{as}(SO_2)$ 1317.; $\nu_s(SO_2)$ 1163. UV–Vis [λ_{max} (nm) (DMSO)]: 327 ($n \rightarrow \pi^*$). ^{1}H NMR: (DMSO-d6, ppm) δ: 12.00 (1H, s, OH); 10.98 (1H, s, NH); 8.13 (1H, s, N=CH); 7.49-7.75 (m, ArH); 3.35 (3H, s, CH_3); ^{13}C NMR: (DMSO-d6, ppm) δ 151.24 (C-OH); 145.48 (N=CH); 121.64-144.05 (Ar-C); 21.07 (CH_3).

3,5-Br_2SALMeBSH

Melting point: 182 °C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Ni$: C, 37.57; H, 2.64; N, 6.23%. Found: C, 37.60; H, 2.68; N, 6.27 %. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3197; $\nu_{C=N}$ 1599; $\nu(C_{Ph}$ -O) 1284; $\nu_{as}(SO_2)$ 1321; $\nu_s(SO_2)$ 1160. UV–Vis [λ_{max} (nm) (DMSO)]: 346 ($n \rightarrow \pi^*$). ^{1}H NMR: (DMSO-d6, ppm) δ: 12.05 (1H, s, OH); 11.13 (1H, s, NH); 8.10 (1H, s, N=CH); 7.66-7.79 (m, ArH); 3.33 (3H, s, CH_3); ^{13}C NMR: (DMSO-d6, ppm) δ 152.7 (C-OH); 146.24 (N=CH); 111.00-144.10 (Ar-C); 21.05 (CH_3).

General method for preparation of ligands Cu(II) complexes

Each SALMeBSH schiff base (0.30 mmol) was dissolved in ethanol (50 mL) and three drops of triethylamine were added. An ethanolic solution containing hydrated copper(II) acetate or nickel(II) acetate (0.15 mmol) was then added and the mixture was heated to reflux for 5 h. On cooling to room temperature, the resultant brown or green precipitation were filtered off, washed with cold ethanol and dried under vacuum. The percentage yields obtained are in the range of 52-65%. The proposed structure of the complexes is depicted in Figure 1.

Cu(5Br-SALMeBSH)$_2$

Melting point: 259°C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Ni$: C, 41.98; H, 2.83; N, 6.97%. Found: C, 42.07; H, 3.03; N, 7.01%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3122; 3435; $\nu_{C=N}$ 1607; ν(C$_{Ph}$ -O) 1305; ν_{as}(SO$_2$) 1326; ν_s(SO$_2$) 1166; UV–Vis [λ_{max} (nm) (DMSO)]: 315 (n→π*); 390 (LMCT), 735 (d–d transition).

Cu(5Cl-SALMeBSH)$_2$

Melting point: 247°C. Anal. Calc. For $C_{23}H_{29}N_5O_6S_2Ni$: C, 47.29; H, 3.34; N, 7.78%. Found: C, 47.34; H, 3.40; N, 7.89%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3123; 3435; $\nu_{C=N}$ 1613; ν(C$_{Ph}$ -O) 1309; ν_{as}(SO$_2$) 1309; ν_s(SO$_2$) 1166; UV–Vis [λ_{max} (nm) (DMSO)]: 310 (n→π*); 395 (LMCT), 735 (d–d transition).

Cu(3Br5Cl-SALMeBSH)$_2$

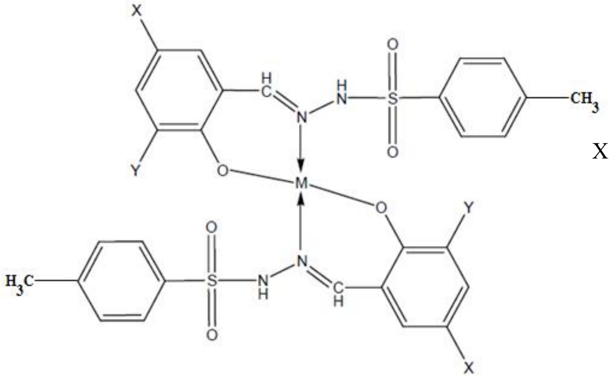
Melting point: 274°C. Anal. Calc. For $C_{23}H_{29}N_5O_8S_2Ni$: C, 38.68; H, 2.53; N, 6.43%. Found: C, 38.73; H, 2.55; N, 6.45%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3158; 3435; $\nu_{C=N}$ 1601; ν(C$_{Ph}$ -O) 1314; ν_{as}(SO$_2$) 1335; ν_s(SO$_2$) 1170; UV–Vis [λ_{max} (nm) (DMSO)]: 315 (n→π*); 390 (LMCT), 735 (d–d transition).

Cu(3,5-Cl$_2$SALMeBSH)$_2$

Melting point: 275°C. Anal. Calc. For $C_{21}H_{23}N_5O_6S_2Zn$: C, 43.09; H, 2.81; N, 7.17%. Found: C, 43.15; H, 2.85; N, 7.19%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3194; 3435; $\nu_{C=N}$ 1604; ν(C$_{Ph}$ -O) 1316; ν_{as}(SO$_2$) 1317; ν_s(SO$_2$) 1170; UV–Vis [λ_{max} (nm) (DMSO)]: 315 (n→π*); 390 (LMCT), 735 (d–d transition).

Cu(3,5-Br$_2$SALMeBSH)$_2$

Melting point: 270°C. Anal. Calc. For $C_{25}H_{35}N_5O_6S_2Zn$: C, 35.08; H, 2.29; N, 5.75%. Found: C, 35.14; H, 2.32; N, 5.85%. IR [ν_{max} (cm^{-1}) (KBr)]: ν_{NH} 3158; 3435; $\nu_{C=N}$ 1601; ν(C$_{Ph}$ -O) 1312; ν_{as}(SO$_2$) 1335; ν_s(SO$_2$) 1170; UV–Vis [λ_{max} (nm) (DMSO)]: 314 (n→π*); 390 (LMCT), 735 (d–d transition).



X = H, Br, Cl and Y= H, Br, Cl
M = Cu^{2+}

Figure 1 The proposed structure of Cu(II) complex of SALMeBSH

Methods for Detection of Phenol by Using Cu(5Br-SALMeBSH)$_2$

Cu(5Br-SALMeBSH)$_2$ solution with concentration of 6.67 mg/L was prepared by mixing 0.05 mL of 400 mg/L of Cu(5Br-SALMeBSH)$_2$ stock solution with 2.95 mL of 200 mg/L of phenol stock solution in the cuvette. The total volume for the solution is 3 mL. The absorbance spectrum was measured by using ultraviolet visible spectrophotometer Cary 60. The absorbance intensity obtained at 342 nm was considered as the response of reagent before and after reacted with phenol. The relative absorbance was calculated by using the Equation 1.

$$\mathrm{A_R} = \mathrm{A_F} - \mathrm{A_O} \text{ ———————— (Equation 1)}$$

Where

$\mathrm{A_R}$ = Relative absorbance intensity of Cu(5Br-SALMeBSH)$_2$

$\mathrm{A_F}$ = Absorbance intensity of Cu(5Br-SALMeBSH)$_2$ after reacted with phenol

$\mathrm{A_O}$ = Absorbance intensity of Cu(5Br-SALMeBSH)$_2$ before reacted with phenol

Experimental Parameters

The effect of reagent, phenol concentration and phenol derivatives are the experimental parameters that were investigated. The concentration of Cu(5Br-SALMeBSH)$_2$ in the range of 1-9 mg/L was examined to obtain the optimum of reagent concentration. Meanwhile the effect of phenol concentration against Cu(5Br-SALMeBSH)$_2$ was also conducted by using the concentration of phenol in the range of 5−200 mg/L. The different derivatives of phenolic compound were also investigated by using the reaction between Cu(5Br-SALMeBSH)$_2$ with 4-chlorophenol (CP), 4-nitrophenol (NP) and hydroquinone (HQ). The whole experimental parameter was carried out in triplicates.

RESULT AND DISCUSSION

General characterization

Infrared spectra

The shift of C=N band in the Cu(II) complexes to lower frequency from the free ligands indicates the involvement of azomethine nitrogen in the complexation [9]. This shift has been explained as a weakening of CN bond resulting from loss of electron density from nitrogen to the metal atom [10]. However it was only significant in the Cu(II) complexes of 5BrSALMeBSH and 5ClSALMeBSH, but not in the series of 3Br,5ClSALMeBSH, 3,5-Cl$_2$SALMeBSH and 3,5-Br$_2$SALMeBSH metal complexes. Cu(II) complexes undergo a shift toward higher frequency of C-O (phenolic) band indicating the participation of phenolic oxygen atom of aldehyde moiety in coordination to the Cu(II) metal ion [11]. Additionally, the bands of NH and SO$_2$ remain more or less at the same position indicating that these groups did not take part in the coordination [10]. The appearance of band at 560-585 cm^{-1} and 452-466 regions in the complexes spectra can be assigned to ν(M-O) and ν(M-N) respectively. This further supports the coordination of the ligand to their metal ions [9].

Absorption spectra

The electronic spectral data of the Cu(II) complexes showed the absorption bands in the range of 310-315 nm, corresponding to the $n \rightarrow \pi^*$ transitions. A new intense bands appeared significantly in the high-energy region at 390-395 nm and was attributed to the charge transfer O(phenolate) ↔/Cu(II) LMCT or MLCT. The presence of charge transfer transition band in the electronic spectra of these complexes further supports the bonding of the ligands to the Cu(II) ion [12]. The coordination was also confirmed by the appearance of a single broad absorption band in the electronic spectra of Cu(II) complexes at 735 nm and was assigned to the d-d transitions. The result was consistent for CuN$_2$O$_2$ d-d transition which could appear in the 770-500 nm (13,000-20,000 cm^{-1}) region as proposed in Figure 1. The d-d absorption band could be attributed to $^2B_{1g} \leftarrow {}^2A_{1g}$ [13,14].

Detection of Phenol by Using Cu(5Br-SALMeBSH)$_2$

A series of experiments were performed in order to investigate the response of Cu(5Br-SALMeBSH) in the presence of phenolic compound. Phenol was used as a substrate to study the potential of reagent interms of experimental parameter such as the effect of Cu(5Br-SALMeBSH)$_2$ concentration, phenol concentration and various type of phenol derivatives. The absorption spectra of Cu(5Br-SALMeBSH) is observed at wavelength of 342 nm in the absence of phenol compound. The addition of reagent into the phenol compound did not give any colour changes. Therefore there is no new peak can be observed in the presence of 80 mg/L phenol at the wavelength of 300-800 nm. However the response of reagent can be monitored. The UV spectra shows the absorbance intensity decreases in the presence of 6.67 mg/L Cu(5Br-SALMeBSH)$_2$ reagent and 80 mg/L phenol.

The result occurred could be due to the redox reaction between Cu(5Br-SALMeBSH) and phenol substrate. Phenol substrate was oxidized to phenolate ion giving the intramolecular electron transfer from deprotonated phenol (phenolate) to copper(II) to generate a copper(I)-phenoxyl radical which has been proposed by Fiehn [7]. However, it is not yet possible to discuss the actual mechanism and correlate the decrease absorption of 342 nm in the presence of phenol due to the limited data. The finding result is different with a study that has been reported using Gibbs reagent in phenolic detection [15]. The reaction yields a compound that turns to blue colour and the increase in the absorption intensity was observed with maximum absorption at 670 nm.

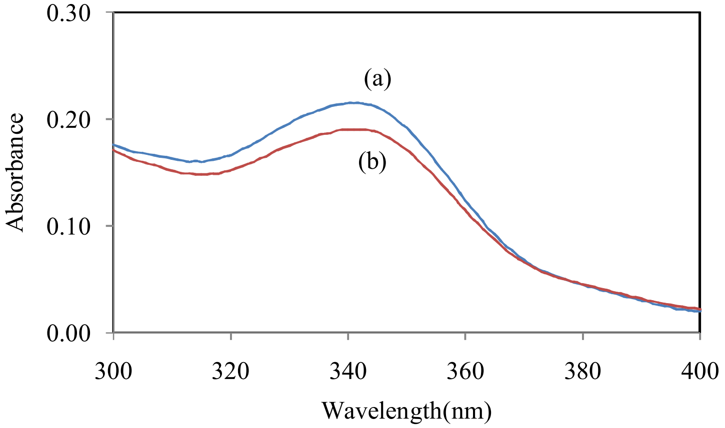


Figure 2 The Absorbance spectrum of Cu(5BrSALMeBSH)$_2$ (a) before and (b) after reacted with phenol

The effect of Cu(5Br-SALMeBSH)$_2$ concentration in the range of 1-9 mg/L is shown in Figure 3. The increased of relative absorbance is observed with the increase of Cu(5Br-SALMeBSH)$_2$ concentration. The highest relative absorbance was observed when 6.67 mg/L Cu(5Br-SALMeBSH)$_2$ is used in the reaction with phenol. The relative absorbance decreased when the reagent is used more than 6.67 mg/L. Therefore, a concentration of 6.67 mg/L of Cu(5Br-SALMeBSH)$_2$ was employed for all subsequent studies.

This result is in agreement with previous study using immobilized tyrosinase enzyme in a chitosan film for the detection of phenol. Tyrosinase is a copper-containing monooxygenase enzyme that catalyzes conversion of phenolic substrates to quinone and then was reacted with 3-methyl-2-benzothiazolinone hydrazone (MBTH). The study showed that MBTH response gradually increase when the phenol concentration used was fixed and reached a maximum level 0.2 mM [8].

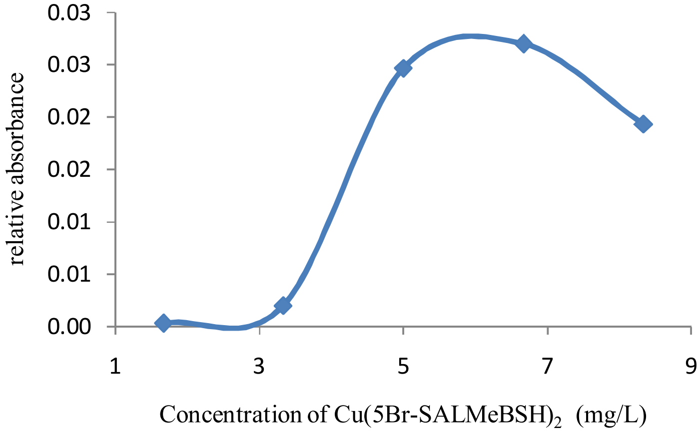


Figure 3 Effect of $Cu(5Br\text{-}SALMeBSH)_2$ concentration on the absorption of UV-visible spectrophotometer.

The dynamic range study was done by varying the concentration of phenol. Figure 4 shows that the relative absorbance of $Cu(5Br\text{-}SALMeBSH)_2$ is propotional to the phenol concentration. It shows that the higher concentration of phenol is used, the more reaction occurs. However the addition of phenol concentation more than 80 mg/L was no longer increased the relative absorbance of $Cu(5Br\text{-}SALMeBSH)_2$ at wavelength of 342 nm. Therefore the favourable range for $Cu(5Br\text{-}SALMeBSH)_2$ to detect phenol was until 80 mg/L.

The effect of various type of phenol derivatives against the relative absorbance of $Cu(5Br\text{-}SALMeBSH)_2$ is shown in Figure 5. The reagent shows the following order of sensitivity; phenol>hydroquinone>4-chlorophenol. Meanwhile the reagent of $Cu(5Br\text{-}SALMeBSH)_2$ did not show any response for nitrophenol. In comparison to the result, a progress of optical determination for phenolic compounds and their derivatives effect using aminoantipyrine (4-AAP) has been reported [16]. They found phenols determined by 4-APP method are phenolic compound in which there is no substituent in para position to the hydroxyl group except halogen and hydroxyl group. When the para position is blocked by nitro group, no colour reaction takes place.

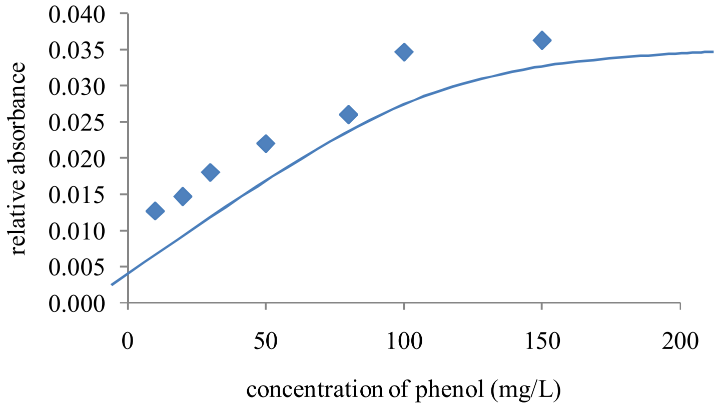


Figure 4 Effect of various concentration of phenol towards relative absorbance of $Cu(5Br\text{-}SALMeBSH)_2$

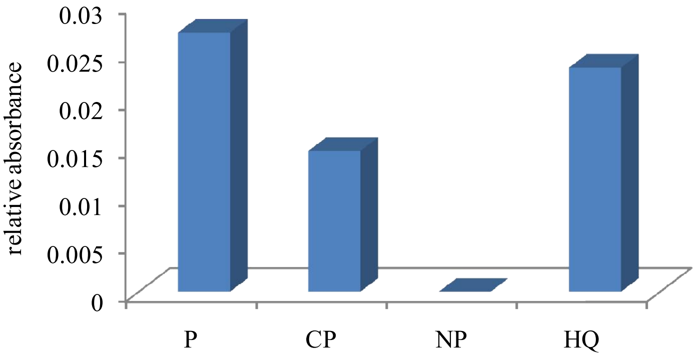


Figure 5 Effect of various types of phenol derivatives against relative absorbance of $Cu(5Br\text{-}SALMeBSH)_2$

CONCLUSION AND SUGGESTION

Cu(II) complexes of 4-methylbenzenesulfonylhydrazone(SALMeBSH) are chelated by azomethine nitrogen and phenolic oxygen atoms. The potential of $Cu(5Br\text{-}SALMeBSH)_2$ used as reagent in phenolic detection is giving response at 342 nm of UV spectra wavelength. The relative absorbance of $Cu(5Br\text{-}SALMeBSH)_2$ is decreased in the presence of phenolic compound. $Cu(5Br\text{-}SALMeBSH)_2$ however, is not the only Cu(II) complex to consider to explain the potential of complex that can be used as reagent in phenolic detection. Thus, the investigation of other Cu(II) complexes is also required.

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REFERENCES

1. Omar, M.M., Mohamed, G.G., Ibrahim, A.A. *Spectrochim. Acta*, *Part A* 73 (2009) 358.
2. Yamada, S. *Coord. Chem. Rev*. 190-192 (1999) 537.
3. Alyar, S., Özmen Ö., Karacan, N., Şentürk, O.Ş., Udachin, K.A. *J. Mol. Struct.* 889 (2008) 144.
4. Peñalver, A., Pocurull, E., Borrull, F., Marcé, R.M. *J. Chromatogr. A* 953 (2002) 79.
5. Keith, L.H., Telliard, W.A. *Environ. Sci. Technol*. 13 (1979) 416.
6. Sadegi, S., Doosti, S. *Sensors and Actuators B* 135 (2008) 139.
7. Fiehn, O., Jekel, M. *J. Chromatogr. A* 769 (1997) 189.
8. Abdullah, J., Ahmad, M., Karuppiah, N., Heng, L. Y., Sidek, H. *Sensors and Actuators B* 114 (2006) 604
9. Abdel-Latif, S.A, Hassib, H.B., Issa, Y.M. *Spectrochim. Acta, Part A* 67 (2007) 950
10. Sert, S., Şentürk, O.S., Özdemir, Ü., Karacan, N., Ugur, F. *J. Coord. Chem*. 57(2004)183
11. Özdemir, Ü.Ö., Olgun. G.,. *Spectrochim. Acta*, *Part A* 70 (2008) 641
12. Golcu, A., Tumer, M., Demirelli, H., Wheatley, R.A. *Inorg. Chim. Acta* 358 (2005) 1785.
13. Georgieva, I., Trndafilova, N. and Bauer, G. *Spectrochim. Acta, Part A* 63 (2006) 403.
14. El-Behery, M., El-Twigry, H. *Spectrochim. Acta, Part A* 66 (2007) 28.
15. Mat Arip, M.N., Ahmad, M., Heng, L.Y. *Chemistry Central Journal* 7(2013)122.
16. Cun-guang, Y. *Journal of Environmental Sciences* 10(1998)76.

CE-1

A BRIEF REVIEW OF THE COMPLEXITIES OF TEACHING AND LEARNING CHEMICAL EQUILIBRIUM WITH SPECIFIC REFERENCE TO MALAYSIA

[1]A.L. Chandrasegaran, [1]David F. Treagust, [2]Mauro Mocerino, [1]Mihye Won & [3]Mageswary Karpudewan,

[1]Science and Mathematics Education Centre, Curtin University, Perth, Australia; [2]Chemistry Department, Curtin University, Perth, Australia; [3]School of Education, Universiti Sains Penang, Malaysia

Abstract

The purpose of this study was to review the extant literature on chemical equilibrium research in high school chemistry. The review involved understanding of the nature of chemical equilibrium, particularly about chemical reactions not going to completion, the reversibility of chemical reactions and the idea of *dynamic equilibrium*. Associated with these understandings was the derivation of the Equilibrium Law and the significance of the equilibrium constant followed by the use of Le Chatelier's Principle including the limitations of this principle. The review then focused on the common alternative conceptions associated with the chemical equilibrium concept. The study next considered these features in the Malaysian context. For this purpose, the researchers formulated an instructional program relevant to the Malaysian Higher School Certificate curriculum that was implemented over 11 hours with 56 high-achieving students in Year 12 from a private secondary school. To evaluate students' understanding of chemical equilibrium concepts after instruction the *Chemical Equilibrium Conceptual Test-1* (*CECT-1*) was administered after instruction. The test consisted of 10 two-tier multiple-choice items that were adapted from previously developed questionnaires. The results indicated very limited understanding of the relevant concepts. The total scores in the *CECT-1* ranged from 0 to 9 (out of a maximum score of 10) with a mean score of 5.04. Less than 50% of students correctly answered five of the 10 items. The findings suggest the need for teachers to address students' preconceptions about chemical equilibrium concepts and use appropriate strategies to enable students to acquire scientifically acceptable understandings.

Keywords: Chemical equilibrium; dynamic equilibrium; Le Chatelier's Principle; reversibility of chemical reactions

Introduction

The topic of chemical equilibrium has been widely researched in several countries (Cheung, 2009; Özmen, 2008; Piquette & Heikkinen, 2005; Quilez-Pardo & Solaz-Portoles, 1995; Tyson, Treagust & Bucat, 1999). Chemical equilibrium is one of the key concepts in chemistry (van Driel & Gräber, 2002) that is important for the understanding of several other concepts in chemistry like, redox reactions, phase changes, weak and strong acids and solubility. This study summarises a brief review of studies that have been conducted on the teaching and learning of chemical equilibrium and will provide valuable guidelines to teachers when planning their instruction on chemical equilibrium by focusing on the complex issues associated with understanding the related concepts.

Literature review

Traditionally, chemical equilibrium has been taught using a kinetic approach that is based on the assumption that at equilibrium, the two opposing reactions occur at the same rate. For the equilibrium system aB + bB ↔ cC + dD, $K_{eq} = k_f / k_r = [C]^c.[D]^d / [A]^a.[B]^b$, (where k_f and k_r are rate constants for the two opposing reactions). However, this derivation is incorrect (too simplistic) because for the reaction aA + bB → products, it is not always correct that $r = k.[A]^a.[B]^b$ (van Driel & Gräber, 2002). More correctly, it is common to introduce chemical equilibrium qualitatively based on the idea of *dynamic equilibrium* using the Equilibrium Law, $K_{eq} = [C]^c.[D]^d / [A]^a.[B]^b$ (Tyson, Treagust & Bucat, 1999). The Equilibrium Law is derived not from kinetic information, but as an empirical law for ideal equilibrium systems (very dilute solutions and gases at low pressures).

Students generally encounter problems understanding chemical equilibrium concepts. When they first learn about chemical reactions, they associate reactions with observable macroscopic changes like colour changes, heat changes, gas evolution and precipitate formation. Their observations confirm that reactions proceed to completion in one direction. However, when they learn about chemical equilibrium, they find that many chemical reactions are reversible, and may not go to completion, i.e., all the reactants and products are present in the equilibrium system. In addition, students are expected to know that all macroscopic properties are constant in an equilibrium system, yet due to the dynamic nature of chemical equilibrium, two opposite reactions are taking place at equal rates so that there is no observable effect. Hence, students are expected to revise their understanding about chemical reactions.

Several studies over the past three decades or so are documented in the extant research literature that suggest that high school students (e.g. Gussarksy & Gorodetsky, 1990; Hackling & Garnett, 1985), university students (e.g., Thomas & Schwenz, 1998) as well as teachers (Banerjee, 1991) have difficulty understanding chemical equilibrium concepts.

As a result of the difficulties that students experience with chemical equilibrium concepts, they display several alternative conceptions such as (1) at equilibrium no reaction is taking place (Griffiths, 1994) indicating failure to understand the dynamic nature of chemical equilibrium, (2) equilibrium is like oscillations of a pendulum (Van Driel et al., 1998), (3) both sides ('left' and 'right') of a chemical equilibrium system can act independently of one another (Wheeler & Kass, 1978), (4) inability to distinguish between *rate* and *extent* of a chemical reaction (Johnstone, MacDonald & Webb, 1977), (5) there is a simple arithmetic relation between concentrations of reactants and products at equilibrium (Hackling & Garnett, 1985; Huddle & Pillay, 1996), (6) the concentrations of the reactants are equal to the concentrations of the products (Hackling & Garnett, 1985; Huddle & Pillay, 1996), and (7) when a reactant is added to an equilibrium system, the rate of the forward reaction increases with time until equilibrium is established (Hackling & Garnett, 1985).

Several strategies have been suggested in the research literature to help students overcome these alternative conceptions. In one example, in a study involving grade 10 students (15-16 year-olds), van Driel, De Voss, Verloop and Dekkers (1998) successfully used conceptual change strategies to challenge the students' conceptions about chemical equilibrium by creating cognitive dissonance about their understandings. As a result, the students were able to accept the "incompleteness of a chemical conversion as an empirical fact" (p. 389). By doing so, they introduced the idea of *dynamic equilibrium* to account for the reversibility of chemical reactions and for the reactions not going to completion.

To further help students with understanding the concept of *dynamic equilibrium*, several instructional strategies involving the use of simulations and analogies are discussed in the research literature (van Driel & Gräber, 2002).

Concerns have also been raised about the inadequacy of Le Chatelier's Principle (LCP) (Quilez-Pardo, 1995), often stated as "*When a stress is applied to a system, the system responds in a way to try to relieve the stress*" (van Driel & Gräber, 2002; p. 279). Students tend to learn the principle by heart and often apply it without understanding (Furió, Catalayud, Barcenas & Padilla, 2000). Students have the tendency to apply LCP outside the area of validity of the principle. Examples include the addition to or removal of solids from heterogeneous equilibrium systems, and the addition of non-reacting substances like water and an inert gas to equilibrium systems (Tyson et al., 1999; Voska & Heikkinen, 2000). Also, when the temperature of an equilibrium system was changed, students believed that the effect could be predicted without knowing whether the reaction was exothermic or endothermic (Voska & Heikkinen, 2000). Furthermore, the principle breaks down by not being specific about the conditions under which a gaseous system is in equilibrium (Lacy, 2005), like for example, whether the equilibrium is at constant temperature or pressure. Especially in gaseous systems, it is important that the conditions of equilibrium are unequivocally stated.

The simplistic definition of LCP that is used by students is likely to cause serious errors in its application to equilibrium systems. The over-simplification in the use of LCP is illustrated in particular when there is a change in the number of moles in a gaseous reaction like the dissociation of dinitrogen tetroxide, $N_2O_4(g) \leftrightarrow 2NO_2(g)$. Assume for example, that the system is in equilibrium in a syringe with a movable piston and an inert gas say argon is added to the mixture at constant temperature and pressure. Using LCP, some teachers and textbooks suggest a blanket explanation that the equilibrium is not disturbed because argon does not take part in either reaction. However, when the reaction quotient (Q) expression for the equilibrium is considered, the total volume of the system appears in the denominator. When the inert gas argon is added, the total volume increases and Q becomes less than K_{eq}. Therefore, the equilibrium moves to the right producing more NO_2. In another example, involving the ammonia equilibrium, Cheung (2009) has shown how the application of LCP breaks down for gaseous chemical equilibrium systems in which there is a change in the number of moles.

As noted by Cheung, Ma and Young (2009), purely relying on LCP to make predictions of the effects of disturbing an equilibrium system often leads to erroneous predictions. Earlier, a multi-faceted approach suggested by Tyson et al. (1999) involved using in addition to LCP, the equilibrium law and using collision theory to analyse the forward and reverse reactions. However we have not identified any research on teaching using this approach.

Rationale of the study in the Malaysian context and research question

Chemical equilibrium is widely included in several secondary school chemistry and tertiary curricula (Cheung, Ma & Yang, 2009; Kousathana & Tsaparlis, 2002; Özmen, 2008; Tyson, et al., 1999; Voska & Heikkinen, 2000). In Malaysia, the topic is introduced in Years 11-12 and includes understanding of reversible reactions, dynamic equilibrium, equilibrium law, equilibrium constant, heterogeneous equilibria, effects of changing concentrations of reactants & products, addition of inert gas, addition of catalyst, and Le Chatelier's Principle. In view of the extensive difficulties faced by students that have been documented in the research literature as previously discussed, this study was conducted with a sample of Malaysian students' to elucidate their understanding of chemical equilibrium concepts and thereby to highlight the complexities associated with the teaching and learning of chemical equilibrium concepts.

Based on this rationale, the research question that was posed was: What is the understanding of a sample of Year 12 high-achieving students about chemical equilibrium? The findings of this study will benefit chemistry teachers by helping them plan more effective instruction to facilitate more appropriate understandings of the complex issues associated with the teaching and learning of chemical equilibrium concepts.

Methodology

Research design

A mixed methods design (Cohen, Manion & Morrison, 2011) using a treatment group was used in this study. As the purpose of the study was merely to evaluate students' understandings of chemical equilibrium concepts and not to evaluate the effectiveness of the instructional program, it was decided not to include a control group.

Research sample

The last author liaised with the chemistry teacher from a private secondary school who had agreed to participate in the study. Two classes consisting of 56 Year 12 students were involved in the study. The same teacher taught both classes, thus ensuring consistency in instruction in both classes.

Instructional program

The instructional program was developed by the authors based on several propositional content knowledge statements (see Figure 1) related to the chemistry syllabus. Lessons were conducted in eight lessons over 11 hours during a period of four weeks.

Research instrument

The *Chemical Equilibrium Conceptual Test-1* (*CECT-1*) that consisted of 10 two-tier multiple-choice items was adapted from previous studies (Cheung, 2009; Özmen, 2008). A summary of the items is provided in Figure 2, and an example of an item is given in Figure 3. The complete instrument may be obtained from the first author. Eight of the items (except Items 7 and 10) were adapted from Özmen (2008). All these eight items were not specific about the conditions under which the chemical equilibrium systems were established or the responses in one or both tiers were not sufficiently clear. Consequently, these items were improved to avoid the shortcomings that are mentioned in the literature review. Conditions like equilibrium systems in a closed container or at constant temperature or pressure were not indicated in the original items. Item 7 about the addition of a catalyst to the $2CO(g) + O_2(g) \leftrightarrow 2CO_2(g)$ equilibrium system was developed by the authors. Item 10 about the effect of adding an inert gas to the gaseous equilibrium system $CO(g) + 2H_2(g) \leftrightarrow CH_3OH(g)$, was adapted from Cheung (2009). However, his item was a multiple-choice item. So, the authors developed the second tier of reasons. The instrument had a Cronbach's alpha reliability of 0.63 that was above the threshold value of 0.5 for two-tier multiple-choice items (Nunally & Bernstein, 1994).

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1. Beginning with the reactants, as a reaction mixture approaches equilibrium, the concentrations of the reactants decrease while those of the products increase.
2. Beginning with the reactants, as a reaction mixture approaches equilibrium, the rate of the forward reaction decreases and the rate of the reverse reaction increases.
3. When equilibrium has been established, the concentrations of all the reactants and products remain constant.
4. Once equilibrium has been established, the forward and reverse reactions continue to occur (i.e. the equilibrium system is a dynamic one, not static), at the same rate.
5. At equilibrium, the concentrations of the reactants and products are related by the equilibrium law, where K_c and K_p are the equilibrium constants based on concentrations and partial pressures, respectively. For the hypothetical system $2A(g) + B(g) \leftrightarrow 3C(g)$,

   $$K_c = \frac{[C]^3}{[A]^2\,[B]} \quad \text{and} \quad K_p = \frac{(p_C)^3}{(p_A)^2\,(p_B)}$$
6. A large value of K_c indicates that the concentration of the products is relatively greater than that of the reactants, and vice versa.
7. According to Le Chatelier's Principle, when some change is made to an equilibrium system, the system reacts to partially counteract the imposed change and re-establish equilibrium.

8. When a *catalyst* is added to an equilibrium system, the rates of the forward and the reverse reactions increase to the same extent.
9. The concentrations of the reactants and the products remain unchanged when a catalyst is added to an equilibrium system.
10. In the presence of a catalyst, the value of the equilibrium constant, K_c, remains the same as in the initial equilibrium system.
11. When an inert gas like helium is introduced at constant volume, the total pressure of the system increases, but does not change the partial pressures (or the concentrations) of the reactants. Therefore, it has no effect on the system.
12. In a heterogeneous equilibrium system the equilibrium constant, K_c, does not include substances in the solid state.
13. There is no effect on the addition or removal of some solid reactant or product to a heterogeneous equilibrium system; LCP is not applicable in this case.

Figure 1 Major propositional content knowledge statements defining instruction on chemical equilibrium

No.	Description of items
1.	Establishing equilibrium: $CO(g) + 3H_2(g) \leftrightarrow CH_4(g) + H_2O(g)$
2.	Establishing equilibrium: $PCl_5(g) \leftrightarrow PCl_3(g) + Cl_2(g)$
3.	LCP-changing concentration in aqueous solution: $2CrO_4^{2-}(aq) + 2H^+(aq) \leftrightarrow Cr_2O_7^{2-}(aq) + H_2O(l)$
4.	LCP-changing temperature in gaseous system: $N_2(g) + 3H_2(g) \leftrightarrow 2NH_3(g)$, $\Delta H = -92.4\ kJmol^{-1}$
5.	Equilibrium constant, K_{eq}: $4NH_3(g) + 5O_2(g) \leftrightarrow 4NO(g) + 6H_2O(g)$, $\Delta H = -905.6\ kJmol^{-1}$
6.	Equilibrium constant, K_{eq}: $H_2(g) + I_2(g) \leftrightarrow 2HI(g)$
7.	Addition of a catalyst: $2SO_2(g) + O_2(g) \leftrightarrow 2SO_3(g)$, $\Delta H = -197.78\ kJmol^{-1}$
8.	Addition of a catalyst: $2CO(g) + O_2(g) \leftrightarrow 2CO_2(g)$, $\Delta H = -556.0\ kJmol^{-1}$
9.	Heterogeneous equilibrium: $CaCO_3(s) \leftrightarrow CaO(s) + CO_2(g)$
10.	Addition of inert gas: $CO(g) + 2H_2(g) \leftrightarrow CH_3OH(g)$

Figure 2 Summary of items in *CECT-1*

Item 3: Suppose that 0.30 mol PCl_5 is placed in a **closed reaction vessel** of volume 1000mL and allowed to reach equilibrium with its decomposition products, phosphorus trichloride and chlorine at 250° C, according to the equation below:

$$PCl_5(g) \quad \leftrightarrow \quad PCl_3(g) + Cl_2(g).$$

What can we say about the concentrations of PCl_3 gas and Cl_2 gas at equilibrium?

A Equal to 0.15 $molL^{-1}$
*B Lower than 0.30 $molL^{-1}$
C Equal to 0.30 $molL^{-1}$

The reason for my answer is:

1 The concentrations of all species in the reaction mixture are equal at equilibrium.
2 All of the phosphorus pentachloride decomposes into the products.
3 Half of the phosphorus pentachloride decomposes into products.
*4 Some of the phosphorus pentachloride decomposes into products.

(* correct response)

Figure 3 Example of an item in *CECT-1*

Data analyses techniques

The data obtained from the *CECT-1* were analysed using SPSS software package (version 21). The responses to the first tier of the items were first recorded as A, B, C, etc. while the combined tiers responses were recorded as A1, A2, .., B1, B2, ..C1, C2, … etc. The combined tier responses were then coded 1 if correct and 0 if wrong. These codes were then used in the analyses. The maximum score was therefore 10.

Results and discussion

CECT-1 responses

The overall correct response to each of the items was not very encouraging (see Table 1). Less than 50% of students provided correct responses to four of the 10 items. The total score achieved by the students ranged from 0 to 9 (out of a maximum score of 10) with a mean score of 5.04. Figure 4 summarises the distribution of the total scores achieved by the students in the *CECT-1*.

Table 1 Frequency of correct responses to both tiers of each item in the *CECT-1* (N = 56) (% in parentheses)

Item no.	Combined tiers frequency	Item no.	Combined tiers frequency
1	50 (89.3)	6	33 (58.9)
2	31 (55.4)	7	34 (60.7)
3	7 (12.5)	8	36 (64.3)
4	39 (69.6)	9	14 (25.0)
5	23 (41.1)	10	15 (26.8)

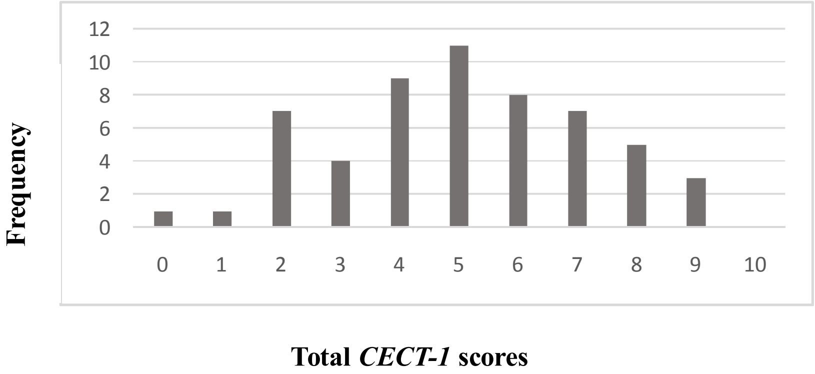

Figure 4 Distribution of Total *CECT-1* Scores (N = 56)

Conclusion and recommendations

The review of the research literature that was conducted as part of this study highlights several deficiencies in students' understanding about chemical equilibrium concepts. It is important that teachers themselves are aware of the misunderstandings that students tend to develop and therefore are able to take measures to develop scientifically acceptable understandings about chemical equilibrium concepts.

This research study has shown that similar to the studies that have been discussed in the literature review, the Year 12 Malaysian students possessed limited understanding about several chemical equilibrium concepts like, the reversibility of chemical reactions leading to establishing a state of equilibrium, the use of LCP in predicting changes in an equilibrium system resulting from perturbations to the system, and the effects of a catalyst or an inert gas on an equilibrium system. When using LCP in particular, it is important to be aware of the conditions under which the system concerned is being disturbed.

One major source of concern is in the inappropriate interpretation of LCP when referring to equilibrium systems involving the addition of an inert gas or when adding or removing a solid from a heterogeneous equilibrium system. Limited understanding about the influence of solids in equilibrium systems was reflected in Item 9 in the *CECT-1* involving the decomposition of solid $CaCO_3$ in a closed container; as many as 46.4% of students inappropriately applied LCP and suggested that addition of more solid $CaCO_3$ to the equilibrium system resulted in the production of more CaO and CO_2.

It is important therefore, that high school students have a firm foundation about the basic concepts of chemical equilibrium in preparation for more advanced study at university. Educators in preservice teaching programs need first to be made aware of students' misconceptions so that they are able to take appropriate measures to convey the correct ideas about chemical equilibrium concepts to their student teachers. At the same time, well-coordinated professional development courses need to be considered for inservice teachers.

References

Banerjee, A. C. (1991). Misconceptions of students and teachers in chemical equilibrium. *International Journal of Science Education, 13,* 487-494.

Cheung, D. (2009). The adverse effects of Le Chatelier's principle on teacher understanding of chemical equilibrium. *Journal of Chemical Education, 86*(4), 514-518.

Cheung, D., Ma, H.-J., & Yang, J. (2009). Teachers' misconceptions about the effects of addition of more reactants or products on chemical equilibrium. *International Journal of Science and Mathematics Education, 7*(6), 1111-1133.

Cohen L., Manion L, and Morrison K., (2011), *Research methods in education* (7th ed.). Oxford, UK: Routledge.

Furió, C., Catalayud, M. L., Barcenas, S. L., & Padilla, O. M. (2000). Functional fixedness and functional reduction as common sense reasonings in chemical equilibrium and in geometry and polarity of molecules. *Science Education, 84*, 545-565.

Griffiths, A. K. (1994). A critical analysis and synthesis of research on students' chemistry misconceptions. In H. J. Schmidt (Ed.), *Problem solving and misconceptions in chemistry and physics* (pp. 70-99). Proceedings of the 1994 International Seminar, University of Dortmund, Germany. Dortmund: ICASE.

Gussarsky, E., & Gorodetsky, M. (1990). On the concept 'chemical equilibrium': The associative framework. *Journal of Research in Science Teaching, 27*, 197-204.

Hackling, M. W., & Garnett, P. J. (1985). Misconceptions of chemical equilibrium. *European Journal of Science Education, 7*, 205-214.

Huddle, P. A., & Pillay, A. E. (1996). An in-depth study of misconceptions in stoichiometry and chemical equilibrium at a South African university. *Journal of Research in Science Teaching, 33*, 65-78.

Johnstone, A. H., MacDonald, J. J., & Webb, G. (1977). Chemical equilibrium and its conceptual difficulties. *Education in Chemistry, 14*, 169-171.

Kousathana, M., & Tsaparlis, G. (2002). Students' errors in solving numerical chemical equilibrium problems. *Chemistry Education Research & Practice, 3*(1), 5-17.

Lacy, J. E. (2005). Equilibria that shift left upon addition of more reactant. *Journal of Chemical Education, 82*(8), 1192-1193.

Nunally, J. C. & Bernstein, I. H. (1994). *Psychometric theory* (3rd. ed.). New York: McGraw-Hill.

Özmen, H. (2008). Determination of students' alternative conceptions about chemical equilibrium: A review of research and the case of Turkey. *Chemistry Education Research & Practice, 9*(3), 225-223.

Piquette, J. S., & Heikkinen, H. W. (2005). Strategies reported used by instructors to address student alternative conceptions in chemical equilibrium. *Journal of Research in Science Teaching, 42*(10), 1112-1134.

Quílez-Pardo, J. (1995). Students' and teachers' misapplications of Le Chatelier's Principle: Implications for the teaching of chemical equilibrium. *Journal of Research in Science Teaching, 32*(9), 939-957.

Quilez-Pardo, J., & Solaz-Portoles, J. (1995). Students' and teachers' misapplications of Le Chatelier's Principle: Implications for the teaching of chemical equilibrium. *Journal of Research in Science Teaching,* 32(9), 939-957.

Thomas, P. L., & Schwenz, R. W. (1998). College physical chemistry students' conceptions of equilibrium and fundamental thermodynamics. *Journal of Research in Science Teaching, 35*, 1151-1160.

Tyson, L., Treagust, D. F., & Bucat, R. (1999). The complexity of teaching and learning chemical equilibrium. *Journal of Chemical Education, 76*(4), 554-558.

Van Driel, J. H., & Gräber, W. (2002). The teaching and learning of chemical equilibrium. In J. K. Gilbert, O. D. Jong, R. Justi, D. F. Treagust & J. H. van Driel (Eds.). *Chemical education: Towards research-based practice* (pp. 271-292). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Van Driel, J. H., De Vos, W., Verloop, N., & Dekkers, H. (1998). Developing secondary students' conceptions of chemical reactions: The introduction of chemical equilibrium. *International Journal of Science Education, 20,* 4, 379-392.

Voska, K. W., & Heikinnen, H. W. (2000). Identification and analysis of student conceptions used to solve chemical equilibrium problems. *Journal of Research in Science Teaching, 37*(2), 160-176.

Wheeler, A. E., & Kass, H. (1978). Student misconceptions in chemical equilibrium. *Science Education, 62*, 223-232.

CE-2

THE 1^{ST} YEAR CHEMISTRY UNDERGRADUATE STUDENTS' UNDERSTANDING IN NAMING SIMPLE COMPOUNDS

Habiddin

Chemistry Department, State University of Malang. Jl. Semarang No. 5 Malang
Email: Habiddin_wuni@um.ac.id

ABSTRACT

The aim of this study was to describe the understanding of freshmen majoring in chemistry in naming simple compounds whether simple ionic compounds or covalent compounds. Data were collected from38 students of State University of Malang using a test conducted after they took Basic Chemistry I class. The test was designed in short answer test type in which they had to name the ionic and covalent compounds from their chemical formulas, and vice versa. The result showed that the students' ability in naming several ionic and covalent simple compounds is greatly poor. Naming ionic compounds with polyatomic ions was considered much more difficult for several students. All in all, naming the binary ionic compound that contained metal that formed more than one type of positive ion and thus formed more than one type of ionic compound with a given anion was considered as the most difficult one. It meant that the basic chemistry class that had been took by the 1st year students did not increase their understanding significantly in naming simple compounds. The implication of this study wasthat the teaching strategy in Basic Chemistry I lecture must be optimized in order to increase students' understanding on this topic well.

Keywords: The 1st year chemistry students understanding, naming simple compounds

INTRODUCTION

In considering the characteristic of naming simple compounds that is commonly considered as a factual knowledge in chemistry, the research in this topic including ionic and covalent compounds is far more limited than other topics. Some researchers in science education especially in chemistry education is much more interested in other topics such as the particulate nature of matter, energy, solution and others that are considered contain more conceptual aspects. Harrison &Treagust (2002) emphasized the importance of the particulate nature of matter to be understood by students because this understanding can be used to explain the properties of matter' and the structure of the particles. Indeed, a vast array of biological, chemical and physical phenomena can only be explained by understanding the changes in the arrangement and motions of atoms and molecules. In addition, some researchers such as Treagust, Mocerino, Taber and the others have many publications in microscopic, symbolic and macroscopic levels of chemistry concepts. Meanwhile, Barke, Al Hazari&Yitbarek (2009) collected their research results in identification of students' misconception on Substances and Properties, Particle Concept of Matter, Structure–Property Relationships, Chemical Equilibrium, Acid–Base Reactions, Redox Reactions, Complex Reactions, and Energy. They found the large number of students' misconception on some chemical concepts.

Previous Research in Chemistry Undergraduate Students Understanding

Despite the number of research results focused on the undergraduate students conceptions and understandings in chemistry is not as much as that of on high school students, several publications related to this aspect have been revealed. Sozbilir&Bennet (2007) stated that Interest in children's understanding of science ideas hasits origins in the classic studies of Jean Piaget. Much of the research into students' understanding of chemical ideas has focused on school-age pupils, with less emphasis on undergraduates. The fact showed that many university students even the prospective teachers got difficulties in understanding some fundamental concepts in chemistry as indicated in several research below. Sozbilir&Bennet (2007) found that the majority of the students could not use thermodynamic principles to explain the change in entropy of a system. The students' thinking was found to be poor and limited at a microscopic level. Meanwhile, Carson & Watson (1999) investigated the conceptions held about chemical thermodynamics by first year chemistry undergraduate students. They concluded that students' lack of understanding about work (and lack of any knowledge of pV work) means that it was inevitable that they would have had a limited knowledge of enthalpy at the beginning of the course.

In a more specific purposes, Cartrette&Mayo (2011) investigated the organic chemistry students' understanding and application of acid/base theories in organic chemistry contexts. They knew that most students maintain declarative knowledge rooted in general chemistry training related to acids/bases, but they can't apply it in problem solving tasks. This assertion held true for most participants, whether chemistry majors or pre-professional majors. Further, flaws in student conceptual understanding of acid/base chemistry principles were revealed.Meanwhile, Orgill& Sutherland (2008) identified the Undergraduate Chemistry Students' Perceptions of and Misconceptions about Buffers and Buffer Problems. They conclude that both upper and lower-level chemistry students struggle with understanding the concept of buffers and with solving corresponding buffer problems. While it might be reasonable to expect general chemistry students to struggle with this abstract concept, it is surprising that upper-level students in analytical chemistry and biochemistry continue to struggle even though buffers are taught in many chemistry classes in an undergraduate program.

Boudreaux & Campbell (2012) investigated the students understanding of liquid-vapor phase equilibrium who have enrolled in introductory physics and chemistry courses. Their responses showed thateven after instruction on the relevant material, many students fail to recognize that for one-component systems in which a liquid and its vapor coexist in equilibrium, the pressure is controlled solely by the temperature. Kelly, etc (2010) examined how 21 college-level general chemistry students, who had received instruction that emphasized the symbolic level of ionic equations, explained their submicroscopic-level understanding of precipitation reactions. Students' explanations expressed through drawings and semistructured interviews revealed the nature of the misconceptions that they held. In addition, the research results related to the undergraduate students difficulties in understanding several fundamental concepts in chemistry are widely published by many researchers.

Even more surprising than the first year students' understandings, even the prospective chemistry teachers showed their difficulties in several basic concepts as investigated by several researchers below. Boz (2009) identified thirty-eight prospective chemistry teachers and found that most of them were found to have problems in understanding the neutralization concept, the distinction between strength and concentration of acids and linking the acids and bases topic to daily life. Another study conducted by Sozbilir, etc (2010) involved 67 prospective chemistry teachers at Kazim Karabekir Education Faculty of Ataturk University in Turkey during 2005-2006 academic year and found that the prospective chemistry teachers' got difficulties in determining the differences between the concepts of chemical thermodynamics and kinetics. The analysis of results showed six major misconceptions about the difference between the concepts of chemical thermodynamics and kinetics indicating that the prospective chemistry teachers attempted to interpret the kinetics of several phenomena by using thermodynamics data.

To sum up, based on these research results it can be concluded that students at all grade levels encounter conceptual difficulties even with fundamental chemistry concepts, and they often develop conceptions which differ from those held by the scientific community. Some of these conceptions derive from individuals' direct or indirect observation of, and spontaneous everyday interaction with, thenatural world around them (Driver, Squires, Rushworth, & Wood-Robinson, in Buket & Emine, 2012). Other sources of these conceptions might be textbook misrepresentations, misleading everyday language, and even the act of teaching itself due to inappropriate instructional materials or teachers' own alternative conceptions (Adbo & Taber; Duit & Treagust; Lin, Cheng, & Lawrenz in Buket & Emine, 2012).

Previous Research in Chemical Nomenclature

As stated previously, research results in students understanding in chemical nomenclature especially for simple compounds included ionic and covalent compounds are much more limited. Nevertheless several studies in this topic have been published. A surprising result was found by Habiddin (2011) who has collected several data from the incoming chemistry undergraduate students State University of Malang, class of 2011. He investigated several preconceptions of students in some fundamental concepts in chemistry, one of them is students' abilities in naming simple ionic and covalent compound and vice versa. Many students could not name the simple ionic and covalent compounds from their chemical formulas and vice versa. For example, they name Aluminum(III) hydroxide for $Al(OH)_3$ and write iron(II) oxide and iron oxide for Fe_2O_3.

Some researchers realize the difficulty faced by students in remaining the rules in naming simple compound. Therefore, Morris (2011) used a Card Game to Help Students Learn Chemical Formulas. He stated that for beginning chemistry students, the basic tasks of writing chemical formulas and naming covalent and ionic compounds often pose difficulties and are only sufficiently grasped after extensive practice with homework sets. Therefore, an enjoyable card game that can replace or, at least, complement nomenclature homework sets is described. "Go Chemistry" is similar to the "Go Fish" card game in that students earn points by using cards to correctly form the formulas of covalent and ionic compounds and by subsequently providing the names of these compounds. By playing the game, students practice identifying an element or ion from its chemical symbol, categorizing elements as either metals or nonmetals, determining whether elements will combine to form ionic or covalent compounds, combining the cards in the correct ratio for electrical neutrality for ionic compounds or valency for covalent compounds, and applying the nomenclature rules. The difficulty of the game can be easily adjusted to match the course objectives and the knowledge level of the students.

Another research conducted by Kavak (2012). He suggested thatlearning the symbolic language of chemistry is a difficult task that can be frustrating for students. Therefore, he designed a game, ChemOkey, thatcan help students learn the names and symbols of common ions and their compounds in a fun environment. ChemOkey, a game similar to Rummikub, is played with a set of 106 plastic or wooden tiles. The object of ChemOkey is to create the formulas and names of ionic compounds from tiles on which the names and formulas of common cations and anions are written. With ChemOkey, students can learn the symbols and names of common ions and acquire a level of familiarity with the electroneutrality principle and the names and formulas of ionic compounds.

Understanding Rules in Naming Simple Compounds

In the beginning development of chemistry, there were no systematic rules in naming chemical compounds. Some substances were named based on the name of person who was found them such as Gmelin Compound, Masgnus Salt, Vauquelin Salt and others. The other compounds were named based on their colors such as Prussian Blue. Today, a huge number of chemical compounds have been found, so that a giving name based on the person or the color of certain compounds will bring a big difficulty in recognizing the chemical compounds. To facilitate in recognizing the name and the chemical formula of chemical compounds, IUPAC give the systematic way for naming compounds in which thename tells something about the composition of the compound. The rules are accepted worldwide,facilitating communication among chemists and providing a useful way of labelingan overwhelming variety of substances.

The summarize rules in naming simple compounds are given below. Binary ionic compounds (type I) contain a positive ion (cation) always written first in the formula and a negative ion (anion). In naming these compounds, the following rules apply. A monatomic (meaning "one-atom") cation takes its name from the name of the element. For example, Na^+ is called sodium in the names of compounds containing this ion. A monatomic anion is named by taking the root of the element name and adding-ide. Thus the Cl^- ion is called chloride. Besides, there are many metals (type II) that form more than one type of positive ion and thus form more than one type of ionic compound with a given anion. For example, the compound $FeCl_2$ contains Fe^{2+} ions, and the compound $FeCl_3$ contains Fe^{3+} ions. In a case such as this, the charge on the metal ion must be specified. The systematic names for these two iron compounds are iron(II) chloride and iron(III) chloride, respectively, where the Roman numeral indicates the charge of the cation.The other type of simple compounds is ionic compounds with polyatomic ions such as ammonium nitrate, NH_4NO_3, contains the polyatomic ions $NH_4^+$and NO_3^-. Polyatomicions are assigned special names that must be memorized to name the compoundscontaining them. Lastly, Binary covalent compounds (Type III) are generally formed between two nonmetals. Although these compounds do not contain ions, they are named very similarly to binary ionic compounds.

The Importance of Recognizing Rules in Naming Simple Compounds

Basically, understanding the rules in naming simple ionic and covalent compoundswill facilitate the students understanding at the next concepts. On the other hand, the students' difficulty in recognizing these rules will obstacle their understanding at the next concepts too. For example, in unpublished research Habiddin (2013) found that most students could not solve this problem "If you were to react 10 g of potassium chlorate with excess red phosphorus (P_4), what mass of tetraphosphorusdecaoxide (P_4O_{10}) would be produced?". The main reason for this problem is that they could not write the chemical formula of potassium chlorate properly and write the wrong chemical equation. As a result, they showed an inappropriate result although they understand how to solve a stoichiometric problems in chemistry as well. This result showed that understanding the rule in naming simple compounds is so important in supporting the students' success in their next lecture. Mastering these rules now will prove beneficial almost immediately as we proceed with our study of chemistry.

Goal and Benefit of This Research

This study explored what the first year chemistry undergraduate students of State University of Malang understand and how they implement the rules in naming simple compounds, ionic and covalent compounds. Therefore, the following research question was addressed in this study. "What do chemistry undergraduates students of State University of Malang understand about the rule in naming simple compounds and implement the rule in naming the simple compounds from their chemical formulas and write the chemical formulas of simple compounds from their names?". Thereby, the primary aims of this research are to identify the 1st chemistry undergraduate students' preconception in naming simple compound and to suggest the appropriate teaching learning method of this topic in general chemistry class. These research result provide an applicable solution for the Basic Chemistry lecturers in learning in this topic.

METHOD

Data were collected from 38 students' class 2013 at Chemistry Education Department, Mathematics and Science Faculty UM. The data were collected by a diagnostic test with short answer type. The test was divided into two parts. The first part contains several names of simple compounds in which they have to write the chemical formulas of these compounds correctly. Another parts contains several chemical formulas of simple compounds in which they have to name these compounds correctly.The data were collected after these students have had took the basic chemistry I. the The descriptive research design was used for this study.

RESULT AND DISCUSSION

Research Question Part 1

This part contains several names of simple compounds whether ionic or covalent compounds. The students was asked to write the chemical formulas of these compounds correctly. The problems sample and students' responses were listed in the Table 1 below.

Table 1. Students' responses in naming simple compounds

No.	Chemical Formula	Students Response: Original/English	Number Answered (%)
1.	H_2	Dihidrogen/ dihydrogen	28,95
		Hidrogendioksida/ hydrogen dioxide	2,63
		Hidroksida/ hydroxide	2,63
2.	$AlBr_3$	Aluminiumtribromida/ aluminum tribromide	7,89
		Alumunium (III) bromida/ aluminum (III) bromide	34,21
		Alumuniumbromintrioksida/ aluminum bromine trioxide	2,63
3.	SO_2	Sulfit/ sulfite	10,52
		Monosulfurdioksida/ monosulfur dioxide	2,63
		Belerang (II) oksida/ sulfur (II) oxide	2,63
		Sulfur oksida/ sulfur oxide	5,26
		Sulfide/ sulfide	2,63
		Oksigendioksida/ oxygen dioxide	5,26
		Sulfat/ sulfate	2,63
		Sulfur (IV) oksida/ sulfur (IV) oxide	2,63
		Sulfatdioksida/ sulfate dioxide	2,63
		Disulfioksida	2,63
4.	Na_2S	Dinatriumsulfida/ disodium sulfide	26,32
		Dinatriumsulfat/ disodium sulfate	10,52
		Dinatrium sulfur/ disodium sulfur	5,26
		Natriumsulfat/ sodium sulfate	2,63
		Natriumdisulfida/ sodium disulfide	2,63
		Natrium(II) belerang/ sodium(II) sulfur	2,63
5.	CuCl	Tembagaklorida/ copper chloride	44,73
		Tembaga(II) klorida/ copper (II) chloride	2,63
		Cusiumklorida	2,63

At the question number 1, students were asked to name H_2, one type of covalent simple compound (type III). About 60% of respondents/students gave the correct answer while the other gave the wrong one. The students' who gave the wrong answer were divided into 3 kinds of answers. Two kinds of students answer, **hidrogendioksida and hidroksida**, written by 2,63% of respondents for each kind of answer. Although the number of students given this answer was relatively small, this answer is surprising. The mentioning of **oksida**and **hidroksida**that are associated with the presence of oxygen is inacceptable because there is no oxygen in the formula. This phenomenon showed that the respondents absolutely have no idea in this aspect. The high number of students with about 28,95% wrote**dihidrogen** for this formula. It can be assumed that they implemented on of rules in naming binary covalent compounds, prefixes such as mono, di, tri, tetra, etc are used to denote the numbers of atoms present. Because hydrogen is one of elements that normally occur as diatomic molecules like oxygen, nitrogen, and the halogens (H_2, O_2, N_2, F_2, Cl_2, Br_2, and I_2), the naming of **dihidrogen** for H_2 is not recommended.

In the next number, the students' were asked to name $AlBr_3$ that contains aluminum metal, a kind of metal which only form one type of cation (Al^{3+}). Students' response showed that differentiation between the metals which can only form one type of cation and the other that can form more than one type of cations is still a confusing aspect. it is strengthen with the high number of students' who answered **alumunium (III) bromida**for this formula with 34,21%. This difficulty is supported with the students' responses at the last three numbers. The vast majority of students' named **tembagaklorida**for CuCl while the other wrote **tembaga (II) klorida.** The later kind of answer has a mistake not only in recognizing the charge of Cu but also in giving the space between cu and its oxidation number. The similar case occurred in their responses for Na_2S.

Meanwhile, students' responses for the number 3 are much more surprising for several reasons. Because $SO_2$is a binary covalent compound, the name **Belerang (II) oksida** and **sulfur (IV) oksida**are really surprising. Another reason is that this compound is so familiar for the chemistry students as well as CO_2. The extreme point in this number is the number of students' who named **sulfit** for this compound with more than 10% of respondents meanwhile **sulfat** was wrote as an answer for this formula by 2,63%. It seems that they failed in remaining the rule for some oxyanions occur in series of ions that contain the same central atom and have the same charge, but contain different numbers of oxygen atoms.

Research Question Part 2

This part contains several chemical formulas of simple compounds whether ionic or covalent compounds. The students was asked to name these compounds correctly. The problems sample and students' responses were listed in the Table 2 below.

Table 2. Students' responses in writing the chemical formulas of simple compounds

No.	Chemical Formula	Students Response	Number Answered (%)
1.	Besi(III) sulfat/ Iron(III) sulfate	$Fe_2SO_4^-$	2,63
		$Fe_2SO_4^{2-}$	2,63
		Fe_2SO_3	7,89
		$Fe_2SO_3^-$	2,63
		$Fe_2(SO_4)_2$	5,26
		$Fe_3(SO_4)_2$	2,63
		CuS_3	2,63
		Fe_3SO_4	5,26
		Fe_2S_3	5,26
		Fe_2SO_4	2,63
		$Fe(SO_4)_3$	5,26
		Fe_3S	2,63
		$Fe_3(SO_4)_3$	2,63
		$Fe_3(SO_4)_3^{2-}$	2,63
		FeS_3	2,63
		$Fe(SO_3)_3$	2,63
2.	Dinitrogenpentaoksida/ Dinitrogen pentoxide	N_2O_4	10,52
		$N_2O_4^{2-}$	2,63

3.	Magnesium florida/ MagnesiumFluoride	MgF^+	2,63
		MgFr	2,63
		MgF	18,42
		Mg_2F	2,63
		MgFl	2,63
		Mg_2F	2,63
		Mg_2F^-	2,63
		Mg_2O_4	2,63
4.	Mangan(IV) oksida/ Manganese(IV) oxide	Mn_2O_4	23,68
		MnO_4	10,52
		MnO_4^-	2,63
		MnO_4^{2-}	2,63
		Mn_4O	10,52
		MnO_2^{2-}	2,63
		Mn_2O	2,63
		Mn_4O_2	2,63
		$Mn(O_2)_4$	2,63
5.	Natriumhipoklorit/ sodium hypochlorite	$NaCO_3^-$	5,26
		Na	2,63
		$NaClO^-$	7,89
		$NaHCO_3$	2,63
		$NaPO_4$	2,63
		NaCl	10,52
		NaHClO	2,63
		$NaClO_2$	2,63
		$Na(Cl_5)^-$	2,63
		NaHo	2,63
		$NaHCl_2$	2,63
		$N_2O_5^-$	2,63
		$NaClO_3$	5,26
		$NaClO_4$	2,63
		$NaCl_3$	2,63

At the question number 1, the students, asked to write the chemical formula of **Besi(III) sulfat** correctly. As showed in Table 2, there are so many kinds of students' responses with almost equal number for each kind. The highest one is **Fe_2SO_3** with 7,89% while **Fe_3SO_4**and **Fe_2S_3** were wrote by 5,26% of students' for each answer. Those answers indicate the difficulty faced by students' as showed in the number 2,3,4 and 5 at Table 1.

In the next number, students were asked to write the chemical formula of **dinitrogenpentaoksida** correctly. This compound is a binary covalent compound as same as SO_2 in the part 1. The highest incorrect number in this number is 10,52% for N_2O_4. In this number, the difficulty faced by students' is related to the use of prefix mono, di, tri, tetra, etc. Several students' recognized the prefix penta as 4.

In the last number, students were asked to write the chemical formula of **Natriumhipoklorit**, an ionic compound that contains polyatomic ion. Like the atoms in a molecule, the atoms that make up a polyatomic ion are held together by covalent chemical bonds. The primary barrier for the students' to solve this problem is that polyatomic ions are assigned special names that must be memorized to name the compounds containing them. In addition, **hipoklorit** associated with **klorit**, **klorat** and **perklorat** that must be memorized by them. Even for the **sulfit** and **sulfat**, they could not memorize well. The most surprising answer for this problem is **NaCl**that was choose by 10,52% respondent and considered as the peak. NaCl is so familiar for all chemistry leaners. Therefore this answer is really inacceptable and intolerable for chemistry undergraduate students'.

The students' responses as stated in Table 1 and Table 2 showed thationic compound that contains polyatomic ions is considered much more difficult as showed from the students' responses for number **natriumhipoklorit**. The total number of students' who could not answer this problem correctly is 57,86%. In the mean time naming simple/binary compound (type II) that contains metal that form more than one cationsis considered as the most difficult one. The total number of students' who gave the wrong answer reached about 60.50% and more as showed in number 4 Table 2. This number is not calculated yet for some of students' who did not give an answer for this type of problem. They confused in differentiating whether the metal can only forms one cation or that can forms more than one cation even in differentiating between ionic compounds and covalent compounds.This phenomenon is strengthen with the many kind of students answer for **Na_2S**, **SO_2, besi(III) sulfat** and **natriumhipoklorit**. Besides, they commonly implemented the rule for binary ionic compounds for covalent compounds and vice versa. Another point that can be interpreted from this study is writing the chemical formula from their names is more difficult for students than naming the simple compounds from their chemical formulas. The total number of students' responses in the Table 1 and Table 2 show it.

Implication for Teaching

The results of this study suggest that many students in the first year chemistry undergraduate class have difficulties in understanding the IUPAC nomenclature of simple compounds whether ionic or covalent compounds.After taking basic chemistry class, students' abilities in naming simple compounds did not increase their understanding well. This result showed the similar result in students understanding with the incoming chemistry undergraduate students' as found by Habiddin (2011). The findings of this study may provide some clues about the quality of student learning in typical basic chemistry classes.

This study suggested that students' mastery in naming simple compounds must be assured first before they go to learn the next topics. The understanding in this topic will facilitate their understanding for the next lectures, on the other hand their difficulties in this topic will obstacle their next classes. The consequence of this difficulty has been proved by Habiddin (2013), in which the students' could not solve the stoichiometric problem because of their lack abilities in this topic. The results indicate that a substantial review of teaching strategies at the university level especially in basic chemistry class is essential. The strategies which have been implemented by Morris (2011) and Kavak (2012) can be used especially with the more innovative way. In addition, it is suggested for the lecturers of basic chemistry to give an attention in this aspect.

CONCLUSSION AND SUGGESTION

To sum up everything stated above, the first year chemistry undergraduate students' have difficulties in naming several ionic and covalent simple compounds. Naming ionic compounds with polyatomic ions was considered much more difficult for several students. The possible reason for this difficulty is how to memorize the special names of several polyatomic ions. Meanwhile naming the binary ionic compound that contained metal that formed more than one type of positive ion and thus formed more than one type of ionic compound with a given anion was considered as the most difficult one. They confused in differentiating whether the metal can only forms one cation or that can forms more than one cation even in differentiating between ionic compounds and covalent compounds. It meant that the basic chemistry class that had been took by the 1st year students did not increase their understanding significantly in naming simple compounds. The implication of this study was that the teaching strategy in Basic Chemistry I lecture must be optimized in order to increase students' understanding on this topic well.

BIBLIOGRAPHY

Barke, H.D., Al Hazari & Yitbarek, S. 2009. *Misconceptions in Chemistry, Addressing Perceptions in Chemical Education*. Berlin: Springer-Verlag Berlin Heidelberg

Boudreaux, A&Campbell, C. 2012. Student Understanding of Liquid-Vapor Phase Equilibrium. *Journal of Chemical Education*. 89(6): 707-714

Boz, Y. 2009. Turkish Prospective Chemistry Teachers' Alternative Conceptions about Acids and Bases. *School Science and Mathematics*. 109(4): 212-222. http://eric.ed.gov

Buket, Y.G., & Emine, A. 2012. Use of Multiple Representations in Developing Preservice Chemistry Teachers' Understanding of the Structure of Matter. *International Journal of Environmental & Science Education.* 8(1): 109-130

Carson , E.M. & Watson, J.M. 1999. Undergraduate Students' Understanding of Enthalpy Change. *University Chemistry Education.*3(2*)*: 46 - 51

Cartrette, D. P.&Mayo, P.M. 2011. Students' Understanding of Acids/Bases in Organic Chemistry Contexts. *Chemistry Education Research and Practice*, 12(1): 29-39. http://eric.ed.gov

Habiddin. 2011. Identification of Incoming Chemistry Undergraduate Students PreconceptionsIn Order To Determine The Learning Strategy of General Chemistry Lecture. *Paper, Presented at The national Seminar of Lesson Study 4, Mathematics and Science Faculty State University of Malang. November 12th 2011*

Habiddin. 2013. *The First Year Chemistry Undergraduate Students Ability in Solving the Stoichiometric Problems in Chemistry*. Unpublished research

Harrison, A.G. & Treagust, D.F. 2002. The Particulate Nature of Matter:Challenges In Understanding TheSubmicroscopic World. In J.K. Gilbert,. O. De Jong, R. Justi, D.F. Treagust, & J.Van Driel (Eds), *Chemical Education: Towards Research-Based Practice* (189–212). Dordrecht Kluwer

Kavak, N. 2012. ChemOkey: A Game to Reinforce Nomenclature. *Journal of Chemical Education*, 89(8):1047-1049

Kelly, R.M., Barrera, J.H., Mohamed, S.C. 2010. An Analysis of Undergraduate General Chemistry Students' Misconceptions of the Submicroscopic Level of Precipitation Reactions. *Journal of Chemical Education*, 87(1): 113-118. http://eric.ed.gov

Morris, T.A. 2011. Go Chemistry: A Card Game to Help Students Learn Chemical Formulas. *Journal of Chemical Education.* 88(10):1397-1399. http://eric.ed.gov/?q=nomenclature&ff1=subTeaching+Methods&id=EJ946454

Orgill, M.K. &Sutherland, A. 2008. Undergraduate Chemistry Students' Perceptions of and Misconceptions about Buffers and Buffer Problems. *Chemistry Education Research and Practice*, 9(2):131-143. http://eric.ed.gov

Sozbilir, M. & Bennet, J.M. 2007. A Study of Turkish Chemistry Undergraduates'Understandings of Entropy. *Journal of Chemical Education*, 84(7): 1204 - 1208

Sozbilir, M., Pinarbasi, T., &Canpolat, N. 2010. Prospective Chemistry Teachers' Conceptions of Chemical Thermodynamics and Kinetics. *EURASIA Journal of Mathematics, Science & Technology Education*, 6(2): 111-120

CE-3

THE USE OF HYBRID MULTIMODAL LEARNING ON CHEMISTRY AT SENIOR HIGH SCHOOL TO IMPROVE STUDENTS' MOTIVATION

Hesty Parbuntari[1] and Jaslin Ikhsan[2]

The Department of Chemistry Education, Faculty of Mathematics and Science
Yogyakarta State University

Abstract

Integration of delivery systems of learning such as model, method and media in teaching-learning in this research was called multimodal learning. This research investigated the effect of multimodal learning to students' motivation.

Multimodal learning in this research was cooperative learning delivered through hybrid, a combination between face-to-face and online using learning management system (LMS) Edmodo, and completed by some multimedia. Samples of this research were students of Grade XI-Science of SMAN 1 Purworejo in the academic year of 2013/2014. The samples were from two groups, experiment and control. Data of students' motivation from both groups were collected using a questionnaire and analyzed using independent sample t-test to study the difference of students' motivation between the groups, and using paired t-test to investigate the improvement of students' motivation in each group.

The results showed the improvement of students' motivation from experiment group was higher significantly than that from control one (sig/ p = 0,000). The motivation of student from experiment group improved significantly due to multimodal learning (sif/p = 0,000), but that from control group did not improved significantly (0,054).

Key words : multimodal, motivation, media

INTRODUCTION

Using the combination of modal and media in the right composition is one of solution for teacher in teaching-learning process, especially for difficult subject, such chemistry (Charles Fadel, 2008: 14). Chemistry matter consists of facts, concepts, rules, laws, principles, theories, and questions (C. Middlecamp and E. Kean, 1985: 9). It pushes teacher to be more creative in setting the teaching-learning, so the class will be more interesting and meaningful. It is caused each modal and media have their own strengths and limitations.

Integration of varience teaching strategies is known as multimodal learning (Dominic W. Massaro, 2012: 1). In the implementation of multimodal learning, teacher do not only focus on a learning media or modal but also use any media that help them and suit for the learning matter. Even, teacher also can use any academic online community such Edmodo.

Everyone can not be separated from technology in this era. Online community is the favorite one. The student's dependence of technology can help teacher to set the teaching–learning by using academic online community such Edmodo. Edmodo is an online platform that mix social media with electronic class management.

Multimodal learning in this research was cooperative learning delivered through hybrid, a combination between face-to-face and online using learning management system (LMS) edmodo, and completed by some multimedia. The setting of multimodal learning can improve student motivation because the strategy is suited with the matter. Student will be more interesedt when learning condition does not only focus on a strategy. The increase of the interest will improve student motivation.

This research studied the improvement of students' motivation from the group that implement hybrid multimodal learning compared to that from the group that does not implement hybrid multimodal learning and improvement of students' motivation in each group before and after treatment. The advantage of this research to inform hybrid multimodal learning design in chemistry and to introduce teacher for long distance learning that can be one of learning alternatives.

RESEARCH METHOD

A. The Research Design

This research is an experiment research that studied the influence of hybrid multimodal learning on chemistry to students' motivation.

B. The Research Variable

There are three variables in this research. They are independent variable, hybrid multimodal learning on chemistry and dependent variable, the learning motivation in chemistry teaching-learning.

C. Population And Sample

The population of research is grade eleventh of science class student of SMAN 1 Purworejo in 2013/2014 academic year. Samples were chosen by purposive sampling.

D. Instrumentation and Data Collection

The instrument that used in this research are lesson plan, questionnaire, and interview sheets. The lesson plan was written for two groups seperately, experiment group with lesson plan that was designed to implement hybrid multimodal learning and control group whose students studied without hybrid multimodal learning. Questionnaire is the instrument to measure the improvement of students' motivation. The questionnaire is given before and after treatment. Interview sheets is another instrument to measure the improvement of students' motivation. The interview is used to get the data of motivation in more detail. All of instrument were measured for validity.

E. Data analysis

Data were analyzed by SPSS 16.0 to investigate the effect of hybrid multimodal learning on students' motivation. Before the analysis was done, the normality and homogenity of data were measured. The hypothesis test consist of two test. They are paired sample t test to analyze the improvement of students' motivation in each groups and independent sample t test to analyze the improvement of students' motivation between the groups.

RESULT AND DISCUSSION

A. Result of Data Analysis

Before the hyphotesis of this research was tested, the data in this research should be normal and homogenous. The normality test was done by SPPS 16.0. All data showed the significancy more than 0,05. It means that all of data were in normal distribution. The homogenity test was done for prior motivation with significancy more than 0,05. It means that the population is homogenous.

Based on independent samples t test, the p value of experiment result is 0,000, less than 0,005. It means that there is significant difference of students' motivation from each group, experiment and control. Paired samples t test showed the improvement of students' motivation in each group. The test showed that there was significant improvement of students' motivation in experiment class, the p value of experiment results is 0,000. But, that for control class did not improve with the p value was 0,054, more than 0,05.

B. Design of Learning

Teaching-learning of experiment group was done for six meeting. Before treatment, students was given the questionnaire of motivation. Teaching-learning was always done in group because the learning model which is cooperative learning of group investigation type. Each group should be heterogeneous. Investigation group was proposed to create the student that can well working in group, discussion, sharing, and help each other when solving problems.

Trianto (2009: 80), cooperative learning of group investigation type is consisted of six steps. They are topic selection, procedure explanation, investigation, analytical synthesis, presentation, and evaluation. Teaching-learning in experiment group was done for that six steps but there are some modification, i.e. combination between face to face and online by Edmodo and the startegy when group presentation their investigation result, and evaluation (Fig. 1)

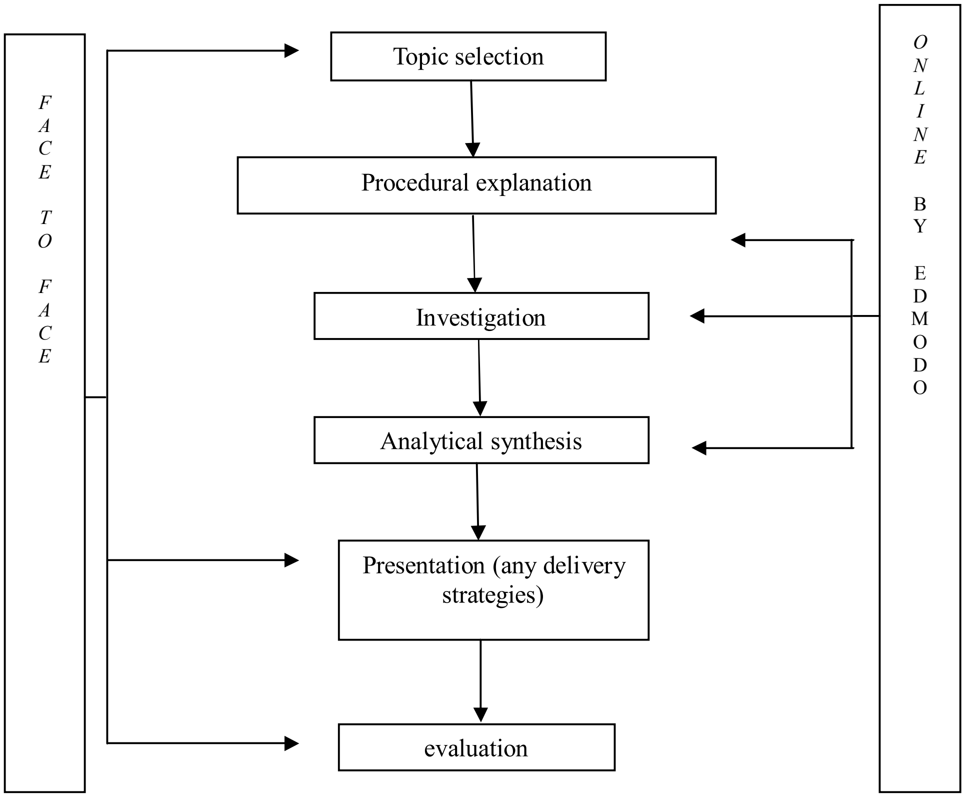

Fig. 1, The teaching-learning steps in experiment group

In the topic selection, teacher chose a topic and gave it to each groups. The topic was informed a week before teaching-learning. Each group was given different topic. Next, first group will receive the instructions from teacher, related to the topic and media needed for the investigation. This process was done in Edmodo. After getting the instruction, the group can start their investigation, analysis, and synthesis of related topic (Fig. 2)

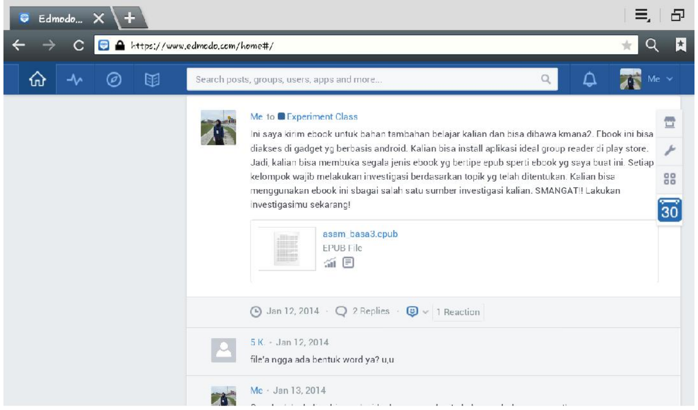

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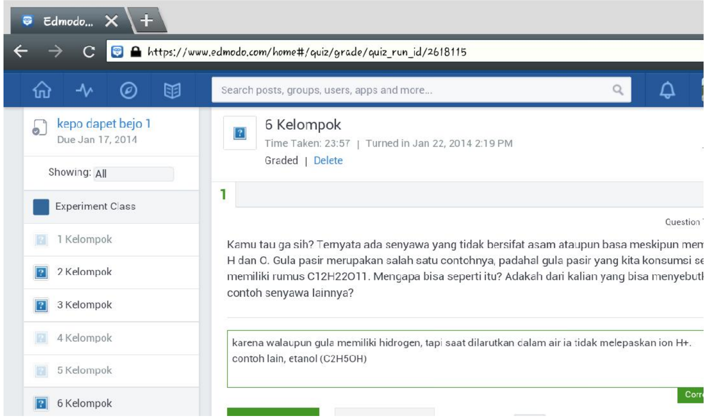
Fig. 2. Posting through edmodo in hybrid learning of experiment group

Teaching-learning which combine face to face and online learning, with various strategies of presentations in this research is one of application of hybrid multimodal learning. In online learning, the group also can ask any problems related to the topic. Next steps, presentation of group investigation results and evaluation was done in the class by face to face learning. They presented their investigation with different strategies, such as demonstration, experimentation, or presentation. In this step, questions and discussions among the groups was opened. Teacher facilitated the discussion and highlighted the concepts during the discussion. Then, the last step was evaluation in which all students concluded overall teaching-learning results. In the last step of face to face, teacher informed the topic for next discussion. Teacher also gave any quiz through Edmodo and the best answer was given points that will accumulated in last meeting (Fig. 3). The best group with the highest points was given rewards.



6 Kelompok

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Kamu tau ga sih? Ternyata ada senyawa yang tidak bersifat asam ataupun basa meskipun men
H dan O. Gula pasir merupakan salah satu contohnya, padahal gula pasir yang kita konsumsi se
memiliki rumus C12H22O11. Mengapa bisa seperti itu? Adakah dari kalian yang bisa menyebut
contoh senyawa lainnya?

karena walaupun gula memiliki hidrogen, tapi saat dilarutkan dalam air ia tidak melepaskan ion H+.
contoh lain, etanol (C2H5OH)

Fig. 3. The answer of students through Edmodo

The questionnaire of motivation was given to student in experiment and control group in the last meeting. Based on the questionnaire result, hybrid multimodal learning can bring democratic learning. It was shown by the fact that teacher called students as responsible person, mature person with well decision, and good person in solving problems. Based on Nana Sudjana

(2002: 42), democratic learning improved learning motivation and increased learning achievement compared with inflexible teaching-learning. It was also showed by the questionnaire of motivation result, the total score of prior motivation is 3298 and the final motivation score is 3445 for experiment group. It means that there was big improvement of motivation of experiment group. The analytical data also showed for the same result.

C. Learning Media

Not only investigated delivery startegies, this research alsostudied the use of learning media. They were Ebook and Lectora. The media were used to help student to investigate as one of alternative learning sources. Ebook is created with *Sigl (.lnk)* program. Ebook can be played by using *Reader Program* such *Ideal Group Reader* (Fig. 4). So, student can access anytime by using their smart phone that supported with that *epub Reader*.

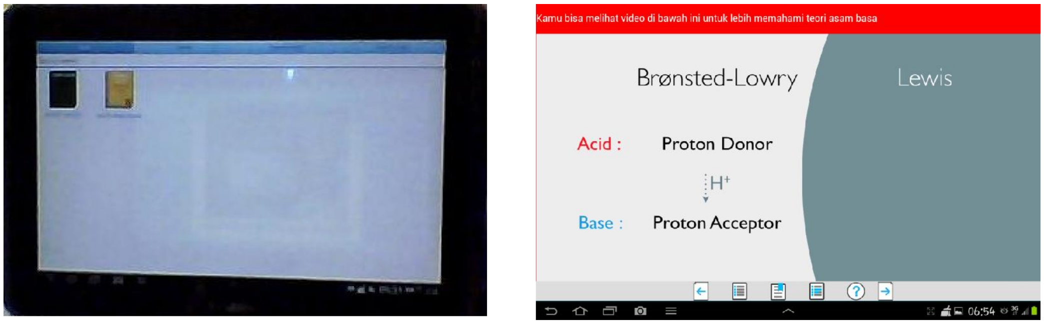

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Fig. 4. Screen shot of *Ideal Group Reader* and *Ebook* in the Android tablet

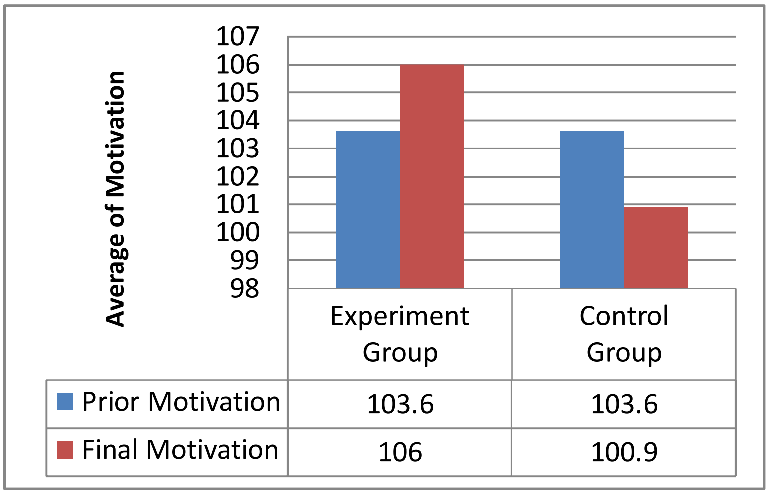
Lectora was one application used to develop media in this research by which students presented their investigation results (Fig. 5.)

Fig. 5. Lectora appearance

Both media had been validated by chemistry teachers that reviewed for the content of media and by students from chemistry education of UNY that reviewed for the layout of media.

D. The Effect of Hybrid Learning of Students' Motivation

Chemistry learning motivation was measured by using questionnaire. The motivation of students in each group was analyzed by using paired sample t tests, and showed that there was significant difference of motivation of students from experiment group before and after implementation of hybrid multimodal learning. But in the control group, the motivation of students did not increase. The results of interview also showed similar results. Most of students in experiment group stated that their motivation improved during the teaching-learning by using hybrid multimodal learning. Based on Dodon Yendri research reslut (2010: 6), teaching-learning process that combined between face to face and online teaching-learning was one of teaching-learning alternatives to reach the optimum teaching-learning objective. The independent t test result shows the significant difference of improvement of motivation between groups (Fig. 6).



	Experiment Group	Control Group
🟦 Prior Motivation	103.6	103.6
🟥 Final Motivation	106	100.9

Fig. 6. The mean score of students' motivation from experiment and control groups

Some factors that influence the improvement of students' motivation are

1. The cooperative multimodal learning of group investigation based on web can increase the student interest and the student's curiousity (Lori Kupczynski, et. al. : 2012, 82-90). The curiousity arise after the student is given the investigation topic in Edmodo. Beside that, teacher also give some quizes and if they can solve the problem right and fast, they will get more stars/ points.
2. The cooperative multimodal learning of group investigation based on web give the same chances to make a good investigation. Micheal Sankey, Dawn Birc, and Michael Gardiner research result (2012 : 852-863) said that student involvement in multimodal learning based on web environment give bigger time and chance in understanding the matter than face to face learning.
3. The cooperative multimodal learning of group investigation based on web give the chance to show their presentation and explanation ability in front of class (Micheal Sankey, Dawn Birc, and Michael Gardiner research result, 2012 : 852-863).
4. The cooperative multimodal learning of group investigation based on web create individual or group fair competition. Fair competition will make condusive learning condition (J. Shindler, 2010: 193 – 209). Students seriously study to get the best result, so the motivation improves.

CONCLUSION AND SUGGESTION

A. Conclusion

1. There was significant difference of improvement student's motivation between the group that used hybrid multimodal learning and non hybrid multimodal learning.

2. There was significant difference of student's motivation in group that used hybrid multimodal learning.
3. There was not significant difference of student's motivation between the group that used non hybrid multimodal learning.

B. Suggestion

1. For the next research, teacher should inform students about the time of discussion daily.. Teacher may manage and schedule the time that are effective for online discussion in Edmodo.
2. For the next research, media should be validated empirically to show how effective that media are.
3. For the next research, media should be more various, innovative, and interesting, , so students will be more interested in using them.

REFERENCES

Charles Fadel. (2008). *Multimodal Learning Through Media: What the Reseach Says*. California: Cisco System Inc, p. 14.

C. Middlecamp and E. Kean. (1985). *Panduan Belajar Kimia Dasar*. Jakarta: PT. Gramedia, p. 9.

J. Shindler. (2001). Improving Classroom Climate and Student Achievement by Assessing Student Participation, *Journal of Research in Science Teaching*. 28(2): 193-209.

Dominic W. Massaro. (2012). Multimodal Learning. *Encyclopedia of the Sciences of Learning*. California University, p. 1.

Lori Kupczynski, et. al. (2012). Cooperative Learning In Distance Learning: A Mixed Method Study, *International journal of instruction*. 5(2): 82-90.

Michael Sankey, Dawn Birch, and Micheal Gardiner. (2012). Engaging students through multimodal learning environments: The journey continues. *Proceedings ascilie Sydney*. p. 852–863.

CE-4

THE USE OF ICT-BASED MEDIA IN WEB-BASED COLLABORATIVE ASSISTANCE OF HYBRID LEARNING ON CHEMICAL KINETIC TO IMPROVE STUDENTS' ACADEMIC PERFORMANCE

Jaslin Ikhsan

Department of Chemistry Education, Faculty of Mathematics and Natural Science
Yogyakarta State University (UNY)
Karangmalang, Yogyakarta 55281 INDONESIA
Phone: +6281328608258, email:jikhsan@uny.ac.id, jaslinikhsan@gmail.com

Abstract

This research studied the implementation of web-based assistance using opened source Learning Management System (LMS), completed by other learning media on Chemistry, such as e-book and video at Chemistry Education Department, UNY, and its effect to students' achievement, learning independence and discipline.

Delivery system of learning Chemical Kinetic was carried out by hybrid learning, the. combination between face-to-face and online learning. Online learning was provided as enrichment and assistant supports. Not only are students able to join in online learning, but also parents are. The samples of this research were students attending the lecture of Chemical Kinetics at UNY. Due to the use of web-based assistance, the improvement of students' achievement, and students' responses towards their learning independence and discipline were measured.

This research showed that Chemistry learning media including video, e-book that accompanying the LMS were good in quality based on the review of students and Chemistry lecturers of UNY. Students strongly agreed with the advantages of LMS for hybrid learning of Chemical Kinetic, and they did not face significant constraint with internet connection whether at home or campus. Students strongly agreed that this hybrid learning can improve their learning independence and discipline. Whereas, parents supported the use of hybrid learning of Chemical Kinetic and stated that they were able to accompany and to guide students effectively in learning. Moreover, this research showed that by the use of hybrid learning on Chemical Kinetic, students' cognitive achievement increased significantly.

Keywords*:* ICT,hybrid learning, learning independence, learning discipline; chemical kinetic.

INTRODUCTION

The advancement of technology has changed paradigm of communication and information. It can affect significantly various sectors of human lives, including education. Information and Communication Technology (ICT) in education can enhance the quality of education (Cabrol and Severin, 2009) with advanced teaching methods, improved learning output and outcomes (Aristovnik, 2012) and enable reform or better management of education systems. ICT are presently driving, moving in many cases from print to the variety of media and to the virtual environments carried through the web, computer-mediated conferencing, and CD-ROM.

ICT and internet network play an important role in education. It enables to deliver efficiently learning resources anywhere at any time without limitation of time and geographic position (Jaslin dan Hafid, 2012). Some well-known universities in Indonesia have launched learning methods and delivery systems through internet networks to enlarge access of education. They are called distance learning, web-based education, and e-learning. The delivery systems basically use internet network. Moreover, some overseas universities implement m-learning and ubiquitous-learning, such as in Korea (Park, 2011).

Due to the fast improvement of ICT role in education, educational entities that do not apply ICT for teaching and learning can be left far behind other universities in this global era. Therefore, this is very crucial time for lecturers and students in UNY Indonesia to begin to optimize internet facilities provided by campus as one of potential learning resources to reach educational mission of UNY.

Indonesian government, via the Ministry of Education and Culture has encouraged all educational institutions to integrate ICT into education as stated on the Strategic Plan 2010 – 2014, and based on the government regulation no. 17/2010 j.o. 66/2010 about Management and Organization of Education. The use of multiple mode in learning was based on the regulation of Minister of Education and Culture no. 24/2012 about the Management of Open and Distance Learning (ODL) in Higher Education Institutions. Previously, upgrading Indonesian Elementary school Teachers Qualification also implemented ICT-based ODL through the program called Hybrid Learning for Elementary School Teachers (Hylite), which was based on the regulation of Minister of National Education no. 58/2008. All those regulations in general encourage Indonesian educational institutions to use ICT in education in order to increase dramatically the access of education for Indonesians even for the ones living in boarder and remote areas. ODL as an important part in hybrid learning have become increasingly common in higher education (Rooney *et al.*, 2006). In 2005, about 62% of the 2- and 4-year higher education institutions offered distance education courses (Rooney *et al.*, 2006), up from 56% in 2001 (Waits & Greene, 2003).

The use of internet networks in Indonesia becomes very important for most students. It can be seen from the use of online social networks that are very popular amongst the students of UNY. Unfortunately, few lecturers and students in the Department of Chemistry Education of UNY use the networks for learning purposes whether through official LMS of UNY BeSmart or opened sources LMSs. In fact, web-based learning can be implemented easily in UNY due to the availability of representative infrastructures such as wi-fi connection and computer laboratories. In this point of view, the use of web-based learning assistance and enrichment should be conducted in UNY. This is not only to increase the quality of the output, but also to effectively use the finance that has been paid for the connection and infrastructures.

This study is about the implementation of web-based collaborative assistance through an opened source LMS edmodo as a hybrid learning model on Chemical Kinetic. There are two important terms in this study, e.i. collaborative assistance and hybrid learning. Collaborative assistance is online support given to students by other students, and guidance by parents. So learning was guided not only by lecturer, but also by parents and peers. The collaborative assistance was given to provide more flexible learning in which each student can learn as his/her own learning multiple intelligent, style, and speed. While, hybrid learning is the mixture of regular face-to-face lecture and online learning using edmodo. Hybrid learning that is also known as blended learning by some researchers can affect attitude of students, as reported by Yushau (2006). He found that blended learning affected significantly students' computer and mathematics attitudes.

Edmodo was chosen as the LMS because of its user-friendly and its simple performance of fb-like, so that students get used to accessing it. In spite of its simplicity, edmodo has reasonable features that are eligible for running and managing lecture. Several features of edmodo are (1) planner section facilitating lecture plan, (2) wall to write notes or announcements with variously formatted file attachments, (3) poll, (4) assignment to post scheduled assignments and to get feedback from students, and (5) quiz to assess students' competence and achievement. Grouping can also be facilitated by edmodo using smaller group feature. All learning processes of students were documented and saved into folder and library, and can be easily monitored by students themselves, parents and lecturer.

Without ignorance of regular face-to-face learning and based on the wide opportunity for students to improve their achievement which is assisted through ICT tool, this study focuses to examine the improvement of students' cognitive achievement on Chemical Kinetic and students responses toward the use of edmodo, a secure social networking that has not yet widely utilized in students environment, dealing with students learning independence and discipline.

The research was to answer the questions:

1. How is the iconic model of hybrid learning with the use of multimedia for web-based assistance at Chemistry Education Department, UNY ?
2. What is the advantage and disadvantage of the use of web-based assistance in hybrid learning of Chemical Kinetic at Chemistry Education Department, UNY?
3. Can the use of web-based assistance in hybrid learning of Chemical Kinetic at Chemistry Education Department, UNY improve students' cognitive achievement?
4. What are the responses of students toward the effect of the use of web-based assistance in hybrid learning of Chemical Kinetic to their learning independence and discipline?

RESEARCH METHOD

Learning design was illustrated by Fig. 1, and was conducted by classroom action.

S	X O v X O v X O v X O	IE

Fig. 1. the Design of Hybrid Learning Implementation on Chemical Kinetic at UNY

X : face-to-face learning in class
O :Mediated learning/ online discussion using media throughedmodo or web conferencing tools
V : Online assignment through edmodo
IE: Impact Evaluation

Multimedia were reviewed first both by lecturers as the expert of content, multimedia, and instruction, and by students as the users. Eight lecturers and 25 students involved in the multimedia reviews. Most lecturers were from the Department of Chemistry Education of UNY, and the students were from International group of Chemistry Education Department of UNY. Data were collected from the process of 10 cycles of hybrid learning. The data were about students' responses toward the advantage and disadvantage of edmodo for Chemical Kinetic lecture, and the effect of hybrid learning using edmodo onto learning independence and discipline, besides the quantitative data of pre-test and post-test.

Data of multimedia reviews were collected using a set of Likert scale questionnaire, with 5 options of answers; very dissatisfied, dissatisfied, neutral, satisfied, and very dissatisfied. The data were analyzed descriptively to determine the quality of multimedia. Multimedia can be used as the enrichment materials and independent learning resources when both lecturers and students feel satisfied with the multimedia.

Pre- and post-students' achievement were measured by use of sets of test instruments. The data were then analyzed using Wilcoxon Signed Ranks test of SPSS to know the improvement of students due to the use of ICT-based media in web-based collaborative assistance of hybrid learning. Three students did not join to the pre-test, so only 22 cases were for students' achievement.

While the data about responses of students were also collected by Likert scale questionnaire with five agreements for positive statements that were applied as strongly disagree = 1, disagree = 2, not sure = 3, agree = 4, and strongly agree = 5, and so gathered qualitative data from Likert scale were converted to become quantitative data, which were then analyzed and reconverted into five categories to conclude students' response (Table 1) as described by Eko (2009).

Table 1.Determination of response category

Range	Mean of Response
$Xi + 1,8\ SBi < \bar{X}$	Strongly Agree (SA)
$Xi + 0,6\ SBi < \bar{X} \leq Xi + 1,8\ Sbi$	Agree (A)
$Xi - 0,6\ SBi < \bar{X} \leq Xi + 0,6\ Sbi$	Doubt (NS)
$Xi - 1,8\ SBi < \bar{X} \leq Xi - 0,6\ Sbi$	Disagree (DA)
$\bar{X} \leq Xi - 1,8\ SBi$	Strongly Disagree (SDA)

Where:
$\bar{X}$: mean
Xi : ideal mean = ½ x (biggest score + least score)
SBi : ideal deviation standard = $^1/_6$ x (biggest score - least score)

RESULT AND DISCUSSION

There are three main components on the development of instructional design. They are the contents, interactivity, and delivery system as illustrated on Fig. 2.

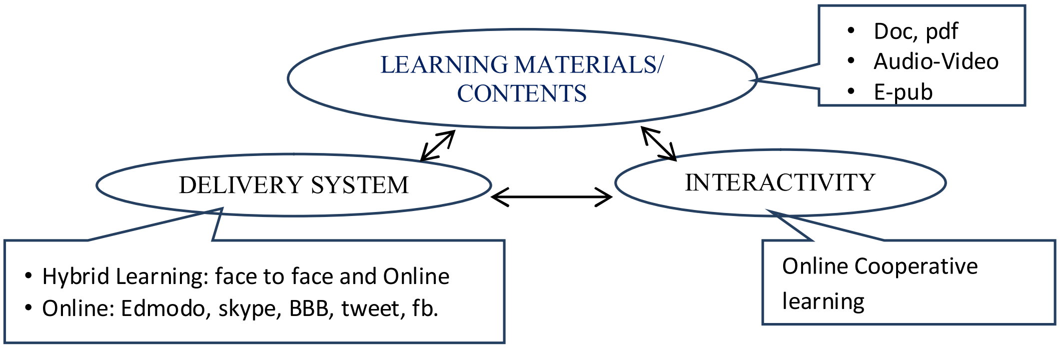


Fig. 2. Main components on development of instructional design

Learning materials whether in the format of multimedia or other digital resources must be valid. Therefore, the media were validated first before being used. The multimedia as resources of learning developed in this research were in the formats of mobile based digital e-book (epub) and video. Each of e-pub and video file were validated by 6 Chemistry lecturers. The validation aspects included multimedia and contents. Multimedia reviews were also conducted by 2 lecturers from outside of Chemistry department, in this case they are two lecturers from Mathematics and Physics departments.

Delivery system was defined as the system to deliver learning object materials, on which hybrid learning was chosen. Hybrid learning is the mixture of face-to-face and online using LMS edmodo, in which the proportion of face-to-face and online learning was 1 to 1 as illustrated on Fig. 1.

Interactivity was facilitated by cooperative learning model through online with edmodo and face-to-face. The online interactivity was also supported by chatting, web conferencing using Skype or BigBlueButton (demo.bigbluebutton.org). Main constraints implementing web conferencing were limited bandwidth to which students cannot access well the conferences.

Multimedia Reviews by Lecturers

Videos whose duration was about 15 minutes and e-pub (android-based digital book) were reviewed by lecturers. The results of reviews were given by Fig. 3. Based on lecturer reviews both videos and e-pub in general were satisfying to be used as independent learning media and resources.

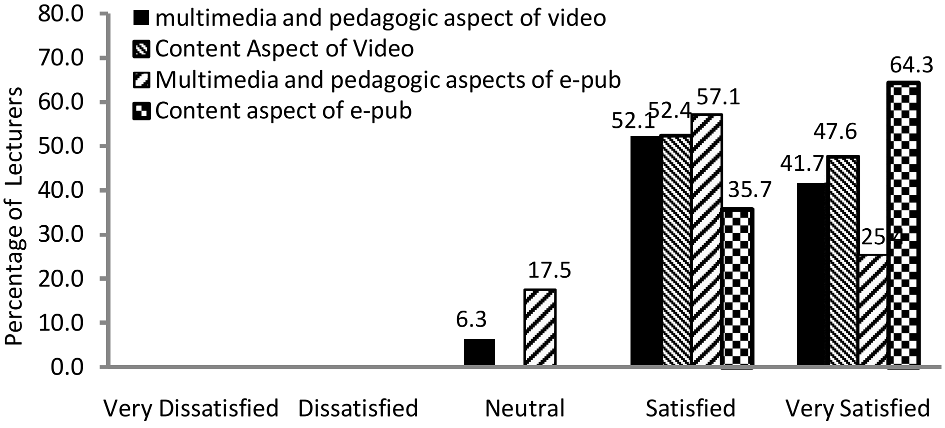


Fig. 3. Lecturers Reviews for Videos and e-pub

Students' Responses to the Multimedia

Responses of students toward the multimedia were also very positive. More than 60% of students stated that video and e-pub on Chemical Kinetic were satisfying and had very good quality (Fig. 4). As the reviews by lecturers, the responses of students were also on multimedia and contents aspects.

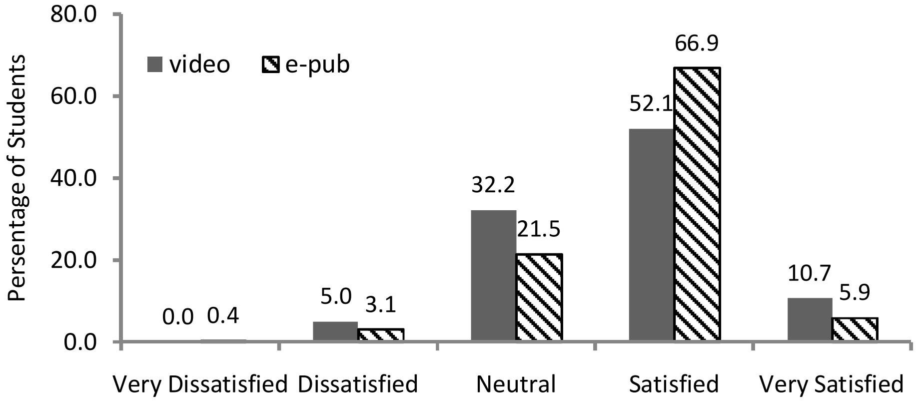


Fig. 4. Students' Responses toward the videos and e-pub

Students' Responses to the LMS edmodo and Hybrid Learning

Data of students' responses toward the advantage of edmodo as an LMS for hybrid learning on Chemical Kinetic, and toward learning independence and discipline are depicted on following Fig. 5.

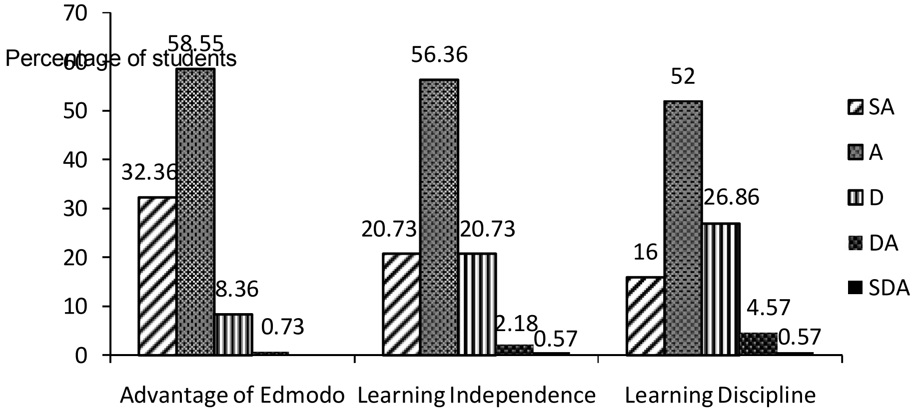


Fig. 5. Percentage of students' responses

Students' Responses to the Advantage and Disadvantages of edmodo as an LMS for Hybrid Learning

Since LMS edmodo was not widely used in the Faculty of Mathematics and Science of UNY, its advantage as a right LMS should be reviewed. Determination of response category using equations in Table 1 for the advantage of edmodo shows that students' responses were very positive. They strongly agreed with the use of edmodo as an LMS for hybrid learning on Chemical Kinetic because edmodo had some advantages. As reported previously (Warschauer 1996) that students can take much advantages from the use of IT for learning. ICT also enables to sharpen students' critical thinking and deepen students' understanding. The integration of ICT into learning in a class becomes very crucial because ICT in learning can be media of collaboration, communication and publication and be useful as learning resources. ICT can facilitate collaborative learning in which students build their own concepts and share experiences (Chou and Chen, 2008).

Advantage of edmodo to which students gave responses included the features, security, types of learning materials included, lecture plan, evaluation and feedback, and parent joining. Most students strongly agreed (52%) and agreed (48%) that edmodo was user-friendly like Facebook. Since most students in Indonesia got used to accessing Facebook as reported by Donny (2012) that Indonesia had 40.6 million Facebook users, the use of edmodo as an LMS would be familiar and easy for students. Based on the response, students strongly agreed (40%) and agreed (60%) that edmodo was suitable to be a good LMS because it provided learning functions as described by Berking and Gallagher (2010) that general functions normally provided by LMS were structure, security, registration, delivery, interaction, assessment, tracking, reporting, record keeping, facilitating reuse, personalization, integration, and administration.

Security was also facilitated by edmodo because only students and parents given access code by lecturer can join. When all students and respective parents joined the group, the class can be closed by locking the group code. Stranger joining the group can be removed and the code should be reset. Based on the fact, most students (64%) agreed that only registered students can join the class and edmodo was very secure for learning.

The materials available in edmodo were kept in library, and can be downloaded and accessed at any time. Various formats of the materials can be stored, such as video, power point, exel, MS word, and pdf files that result in easiness of lecturer and students to upload and download the materials of learning. As stated by Berking and Gallagher (2010) that integration should be the function that provided by an LMS. Learning materials uploaded to edmodo were very useful as enrichment materials for students. Since the access can be done anywhere at any time, the use of materials can be also very flexible.

The availability of Planner on edmodo provides the easiness of students to check the schedule of next learning session that enable students to prepare better. Therefore, students responded that they know lecture plan of learning. Better planning in learning would result in better achievement of students. Related to evaluation and feedback, students can have better reflection from feedback given by lecturer toward assignment answer. Students could also ask freely to lecturer and check trends of his/her achievement from all remarks given by lecturer.

Edmodo facilitated parents to join the class. Students reviewed that parents participation was very positive aspect for their learning. Therefore, students strongly agreed (36%) and agreed (44%) and expected that their parents could join and attend the Class, even though not all parents finally can actively attend the class due to some constraints. Only five of parents joined to online class. All of them supported the use of edmodo as online assistance for students because they can check students' participation and scores from the assignments and examinations. Unfortunately, most parents did not actively participate in the class. It can be said that only once on average they logged in by use their own account, the others they just looked at when students logged in.

Students' responses toward the disadvantages of the use of edmodo were very weak. Based on the analysis as listed in Table 1, students disagreed that the use of edmodo was difficult and that edmodo was not useful for Chemical Kinetic learning. Accessing edmodo was fun, easy with limited constraints from the point of view of internet infrastructure, the features of edmodo, and parent participation. Students stated that they did not face difficulties to access internet because more than 70% of them had internet access at home. Meanwhile, at campus they had 24-hour-internet-coverage a day. As reported by Donny (2012) from ICT Watch Indonesia that Indonesia has 150 Internet Service Provider (ISP), 35 Network Access Provider (NAP), and 5 broadband 3G operator. To interconnect the data traffic within ISPs in Indonesia, there are about 10 nodes of Indonesian Internet eXchange (IIX) managed APJII (Indonesian ISP Association, APJII.or.id). Donny (2012) also stated that from population of Indonesia about 240 million people, now has 55 million Internet users, not including 33 million Internet mobile users who use a smartphone, tablet or modem dongle. There are at least 100 million active mobile numbers used for broadband/ 3G data access. For social media, Indonesia has 29.4 million users of Twitter. This figure puts Indonesia as the most active twitter country in the world, and Jakarta is the most active twitter city in the world. Indonesia also has 40.6 million Facebook users and about 3.3 million bloggers, with 33 local blogger communities.

Based on the fact, it can be concluded that ICT infrastructure in Indonesia, especially in some big cities, like Yogyakarta, was relatively sophisticated either in quantity or quality to support internet or web-based learning or hybrid learning. Some Indonesian communities also got used to using social networks. Consequently, edmodo which was also called facebook for education was not be hard to be used as an LMS in UNY. Therefore, lecturing and learning with assistance of internet connection and edmodo was easily conducted.

Since most students are in native ICT ages and at digital era, they were quite challenged and happy to use ICT as media of learning; 88% of them stated so. However, few of them stated that uploading the answer of assignment through edmodo took a long time as shown by the number of respondents, 12% agreed and 20% were not sure. The easiness on assignment download and upload provided convenience of learning that could be efficient and enhance students learning achievement. Based on the responses, 20% of students stated that most parents could not join to the lecture because of IT skill constraint despite edmodo facilitated parents participation on learning.

Students also stated in opened statement that negative effect on the use of edmodo in this case was cheating amongst some students by copying friend's answers to fulfill assignments. Lecturer can avoid this by comparing documents of students answer, but unfortunately, it took a long time and attention.

Students' Responses to the Effect of Collaborative Hybrid Learning to Students' Learning Independence and Discipline

After face-to-face lecture, students were given assignments through edmodo, and this is so called one cycle of hybrid learning. In this point of view, students may spend more time for learning and follow the regulation of credit semester principles that students need structural and independent assignments besides face-to-face lecture on each credit of subject matter. All activities on edmodo were recorded safely and can be downloaded. By edmodo, teacher can manage learning very well, including planning, lecturing, and assessing that affected students learning independence and discipline. Based on the analysis as was in the Table 1 and was depicted in Fig. 8, students strongly agreed (20.73% of 25 students strongly agreed and 56.36% agreed) that collaborative hybrid learning using edmodo could affect students' learning independence. They also agreed (16% of them strongly agreed and 52% agreed) that those could affect their learning discipline.

Edmodo enabled students to share freely their knowledge and had discussion via online connection. By online discussion students were able to learn with different speed. It also facilitated students to express their arguments via written reports, and that were very good for the ones who did not prefer to express arguments by oral discussion. Therefore, such written discussion provided high flexibility of learning for students. Online discussion on edmodo was always under supervision of lecturer, because the discussion was always on lecturer wall and edmodo did not provide direct post from one student to another. Final result of discussion was then shared in a face-to-face presentation session.

So after long discussion for the assignment through edmodo, representative of the group had strong confidence to present group discussion results. It was quite amazing because the tough constraints in Indonesian education is the eagerness of students to express their arguments or ideas in discussion, and this is a kind of bad learning culture in general. Through written discussion on edmodo, however, students even braved to ask or remind lecturer from mistaken based on their prior knowledge. As reported by Bowker (2010) in his questions-centered pedagogy that the brave of questioning was very important and positive in learning because the questions themselves were the answers, that could improve the quality of learning.

Edmodo facilitated collaborative learning because discussion through edmodo could be knowledge sharing amongst the students. Better students shared their ideas and knowledge to the others, conversely weak students can raise questions for discussion and solution. Positive interdependence, interaction, social and leadership skills, and trust building were developed through this learning. The collaborative become stronger because learning can be observed and guided by parents. Parents could act as observers who were thoughtful care and responsible for the success of learning. Collaborative discussion amongst the students was mainly about the materials of learning, but parents were more likely to collaborate on technical, administrative and psychological aspects.

Edmodo provided high learning flexibility because Planner on edmodo told students about next week activities. As consequences, students can read and prepare well, download materials, and search relevant topics making students had better preparation for next face-to-face lecture. Learning flexibility could also enhance learning independence (Lin, 2008).

The Effect of Collaborative Hybrid Learning to students' achievement

Some researchers defined hybrid learning were the same as blended learning. It is learning with the mixture between face-to-face and computer-mediated online learning (Buzzetto-More and Sweat-Guy, 2006). There is no exact commitment of the definition of hybrid learning yet, and it is still on discussion. Partridge (2011) highlighted the definition of hybrid learning as follows:

- A learning program in which more than one delivery modes are used, with the objectives of optimizing learning outcome and cost.
- A combination learning model of web-based technology
- A combination learning approach of constructivism, behaviourism, and cognitivism
- A combination of various instructional technology with face-to-face

The proportion of face-to-face and online component in the combination was very various, and has yet to be implemented with obvious learning regulation. It depended on the characteristics of subjects and lots of condition as reported by Ranganathan *et al.* (2007), who implemented hybrid learning on six universities in USA. Hybrid learning of Hylite program to upgrade qualification of Indonesian elementary school teachers in 2007–2010, used the proportion of ICT based online learning component with the range of 30-79%, while allowing 30% of delivery to be face-to-face (Paulina *et al.*, 2007). The proportion of web-based learning in Hybrid learning of Chemical Kinetic in this research was about 50%. After face-to-face lecture, students were given assignments or discussion topic that was supposed to discuss via online through edmodo.

Hybrid learning was very effective and efficient by which students can have better understanding. The more frequency to access hybrid learning, the higher of the understanding is. Students can also be challenged to meet their learning progress. As reported by Echo (2011) that most students prefer to have extra hybrid learning after having experiences of learning with hybrid.

Collaborative learning is an umbrella term of a variety of learning approaches requiring intellectual efforts by group of students, or between lecturer and students together. In collaborative learning, students usually work in a 3 or 4-member-group (Gokhale, 1995), who collaborate mutually to understand learning materials or to develop a product. The activities of students in their group of collaborative are various, but the core of collaborative learning is students centered learning in which students build their own concepts, but is not solely knowledge transferred from lecturer or listening or note-taking enforcing students to be very passive. Giving talks methods of teaching when delivering learning materials should not appear in collaborative learning. Lecturer should act as a professional instructional facilitator, motivator, and designer.

The characteristics of collaborative learning are (Felder and Brent, 2007):

- Positive interdependence: group members should rely on one another to meet the group goal
- Individual accountability: each student in a group must share the work for the materials of learning
- Face-to-face interaction: working group must facilitate face-to-face interaction so each student can contribute on feedback for the others and have discussion to encourage each other.
- Appropriate use of collaborative skills: students can build their social skill such as leadership, trust-building, conflict management etc. through collaborative works.
- Group processing: group members should commit to group goal, access the progress, and make sure that they can reach the goal.

The data analysis using Wilcoxon Signed Rank test showed that web-based collaborative assistance through edmodo can increase students' academic achievement (p: 0.042). This is in line with the statement of Yatim Riyanto (2010) that cooperative learning is learning model to improve both students' academic skill and social skill. Agus dan Andi (2010) reported their research that test score obtained in cooperative learning was much better than the score achieved by the best student in that group who learnt in regular individual learning. A research on the use of cooperative learning on Biology of secondary school students in Kenya reported the same results (Muraya *et. al.*, 2011). Even, cooperative learning was good for students with disabilities. They could get better academic achievement when they learnt by cooperative learning that incorporate individual accountability and groups rewards (McMaster and Fuchs, 2002).

CONCLUSION AND SUGGESTION

Based on the responses of students, it can be concluded that:

- The model of hybrid learning with the use media in web-based learning was initiated by giving 50% proportion of online learning, and the media that were developed were satisfying. Therefore, the media were suitable for independent learning resource and enrichment materials in the web-based assistance.
- Edmodo was very good with several advantages and lack of disadvantages to be used as an opened source LMS on web-based assistance in hybrid learning of Chemical Kinetic in UNY Yogyakarta Indonesia.
- The use of web-based assistance in hybrid learning of Chemical Kinetic at Chemistry Education Department, UNY can improve significantly students' cognitive achievement.
- Students strongly agreed the use of web-based assistance in hybrid learning of Chemical Kinetic can improve students' learning independence and discipline?

From the results, it may be good ideas to initiate the use of hybrid learning for studies in universities in Indonesia due to the availability and advancement of internet connection in higher education institutions in Indonesia. The proportion and design of hybrid learning may be extended as the characteristics of the topic of discussions.

REFERENCES

Agus J.,Andi S. (2010). *Pengaruh Pembelajaran Pemecahan Masalah Secara Kelompok Kooperatif Terhadap Kemampuan Pemecahan Masalah*. Jurnal Pendidikan Matematika dan Sains, Volume 15 No. 1: 13-17.

Aristovnik, A. (2012). *The Impact of ICT on Educational Performance and its Efficiency in Selected EU and OECD Countries: a Nonparametric Analysis*, MPRA Paper No. 39805, http://mpra.ub.uni-muenchen.de/39805/

Berking, P., Gallagher, S. (2013).*Choosing a Learning Management System*, Serco Services, Inc., Retrieved on August 10, 2013, http://www.adlnet.gov/wp-content/uploads/2013/05/Choosing_an_LMS.pdf

Bowker, M. H. (2010). *Teaching Students to Ask Questions Instead of Answering Them*, The NEA Higher Education Journal, retrieved on July 30, 2013 from http://www.nea.org/assets/img/PubThoughtAndAction/Bowkershort.pdf

Buzzetto-More, N. A., Sweat-Guy, R. (2006).*Hybrid Learning Defined (*Adapted From: Incorporating the Hybrid Learning Model into Minority Education at a Historically Black University), Journal of Information Technology Education, Volume 5.

Cabrol, M., Severin, E. (2009).*ICT To Improve Quality In Education — A Conceptual Framework And Indicators In The Use Of Information Communication Technology For Education (ICT4E)* (in Assessing the Effects of ICT in education: Indicators, criteria and benchmarks for international comparisons, Edited by Friedrich Scheuermann and Francesc Pedró), ISBN 978-92-79-13112-7, Luxembourg: Publications Office of the European Union, pp. 83 -104.

Chou, P.-N., Chen, H.-H. (2008). *Engagement in Online Collaborative Learning: A Case Study Using a Web 2.0 Tool*, MERLOT Journal of Online Learning and Teaching, No.4(4).

Donny B.U. (2012). *Internet Freedom at Indonesia*, Retrieved on August 10, 2013 from http://bestbits.net/wp-uploads/2012/10/internet-freedom-indonesia.pdf

Echo, (2011).*The Student View of Blended Learning*, retrieved on June 19 2013, from http://www.ecsu.edu/academics/offices/distanceeducation/docs/studentViewBlendedLearning.pdf, ECHO, 2011.

Eko P. W. (2009). *Evaluasi Program Pembelajaran.* Yogyakarta: Pustaka Pelajar

Felder, R., Brent, R. (2007).*Cooperative Learning* (in *Active Learning: Models from the Analytical Sciences*, ACS Symposium Series 970, P.A. Mabrouk, ed., Chapter 4, pp. 34–53. Washington, DC: American Chemical Society.

Gokhale, A. A. (1995).*Collaborative Learning Enhances Critical Thinking*, Journal of Technology Education Vol. 7 No. 1.

Jaslin I., Hafid S. H. (2012).*Strategi Penyebaran Konten Pendidikan Berbasis Digital Video Broadcasting over Satellite*, Seminar Nasional ISPI, Pasca Sarjana UNY, Yogyakarta

Lin, Q. (2008).*Student Views of Hybrid Learning: A One-Year Exploratory Study*,Journal of Computing in Teacher Education, Volume 25 / Number 2 Winter 2008–2009.

McMaster, K. N. and Fuchs, D. (2002). *Effects of Cooperative Learning on the Academic Achievement of Students with Learning Disabilities: an Update of Tateyama-Sniezek's Review*, Learning Disabilities Research & Practices 17(2).

Muraya, D. Ng., Kimamo, G. (2011).*Effects of Cooperative Learning Approach on Biology Mean Achievement Scores of Secondary School Students' in Machakos District, Kenya*, Educational Research and Reviews, Retrieved on August 10, 2013*http://academicjournals.org/ERR2,*

Park, M.-G.(2011)*, Integrated Service Platform of Ubiquitous Learning and Ubiquitous Work Systems*, Proceeding of International Conference on e-Learning (I-CeL), ITB Bandung

Partridge, H., Ponting, D. McCay, M. (2011).*Good Practice Report: Blended Learning*, Australian Teaching and Learning Council, ISBN: 978-1-921856 – 81-5, Sydney

Paulina P., Rahayu D. R., Esti P. (2007).*HYLITE Program: An ICT-based ODL for Indonesian Teachers Education*, Proceeding of the 11th UNESCO APEID Conference on Revitalizing Higher Education, Dec. 12-14, 2007, Bangkok

Ranganathan, S., Negash, S., Wilcox, M. V. (2007). *Hybrid Learning: Balancing Face-to-Face and Online Class Sessions*, Proceedings of the 2007 Southern Association for Information Systems Conference.

Rooney, P., Hussar, W., Planty, M., Choy, S., Hampden-Thompson, G., Provasnik, S. (2006). *The Condition of Education 2006* (NCES 2006-071). Washington, DC: National Center for Education Statistics. Retrieved on July 19, 2013, from http://nces.ed.gov/pubs2006/2006071.pdf

Waits, T., Greene, B. (2003). *Distance Education at Degree-Granting Postsecondary Institutions: 2000-2001 (nces 2003-017)*. Washington, DC: National Center for Education Statistics. Retrieved on July 19, 2006, from http://nces.ed.gov/pubs2003/2003017.pdf

Warschauer, M. (1996). *Computer-assisted language learning: An introduction*. Tokyo: Logos International

Yatim R. (2010). *Paradigma Baru Pembelajaran*: *Sebagai Referensi bagi Pendidik dalam Implementasi Pembelajaran yang Efektif dan Berkualitas*. Jakarta:Kencana Prenada Media Group.

Yushau, B., (2006). *The Effects of Blended E-Learning on Mathematics and Computer Attitudes***,** The Montana Mathematics Enthusiast, ISSN 1551-3440, Vol. 3, no.2.

CE-5

DEVELOPMENT OF ONLINE LEARNING USING MOODLE VERSION 2.5.3 (CASE STUDY AT SECONDARY SCHOOLS, JAKARTA)

Maria Paristiowati and Amanda Franciska

Chemistry Department, State University of Jakarta
Email: maria.paristiowati@unj.ac.id

Abstract

Paper reports the study of designing and developing Colloid's online learning using Moodle version 2.5.3 at Secondary Schools, Jakarta. Design and developmentalresearchmethodology are conducted based on Richey and Klein model. Developmental process consists of several steps: (1) identyfying problems;(2) design and development research methodology;(3) participant selecting;(4) collecting data;(5) interpreting data; and (6) future development. Participants of the study are (1) researchers as designers, developers, and instructors; (2) teachers as instructors; (3) the experts as evaluators; and (4) students as learners. Data collection implemented several instruments, such as: (1) instrument of needs analysis; (2) feasibility test instruments, and (3) trial tests instruments. The results show the use of computers and internet is limited for searching references and learning materials. The online learning in Chemistry subjects in schools is not yet available. Teachers have not been able to create the online learning materials. On feasibility and trial test, the results show good performance on subject matter, clarity of information, and design.Therefore, the online learning can be implemented in chemistry learning.

Keyword: Design and Development Research, Online Learning, Moodle version 2.5.3, Colloid

A. Introduction

Internet is one of the application of information communication and technology (ICT) which is currently being developed rapidly. The report of MarkPlus Insight (2013) show that Internet users in Indonesia increase from 24.2 million people in 2012 to 31.7 million in 2013. Utilization of ICT for education in Indonesia based on the Regulation of the Minister of National Education Number 22 of 2006 and Regulation of the Minister of National Education Number 41 of 2007 which is said the curriculum should encourage students for using ICT to find information about the material (Kemdiknas, 2006), (Kemdiknas, 2007). So, internet can be used as a media to develop online learning.

The needs analysis have been done at 81 Senior High School Jakarta, 54 Senior High School Jakarta, and 2 Senior High School Jakarta. The results of the needs analysis show that 73 students grade XII and 7 chemistry teachers have used the internet and computers. It is the fact that 90.4% students and 100% teachers use Internet more than two times a week. 79.5% students and 66.7% teachers use computer more than twice a week. All students and teachers state that the schoolshaven't got any chemistry online learning system.

Online learning can be accessed through mobile phones, smartphones, and tablet PC. It allows students to access the learning materials independently and repeatedly. However students may understand better and make them more active to find out information about the subject matter. The result of Yehudit and Miri's research (2003) said that learning by website can improve student learning outcomes and show high progress in understanding chemistry.

Online learning can be developed by one of the application of LMS (*Learning Management System*), Moodle, which is open source. Moodle has more advantages than other LMS. The features which are built based on the concept of pedagogy and technology (Tambovceva& Artis, 2013). Moodle version 2.5.3 is more stable and more compatible with the new features than old Moodle version.

Colloid contains a lot of applications in daily life, so it will be easier for learning if the material is presented in form of visualization and interactive. Website which is developed by Moodle version 2.5.3 can support visualization and interactive because it combines text, images, and videos which will be presented interestingly. Websites which have good quality visual can motivate students to learn chemistry (Margareta & Rahela, 2007).

Based on the above which describes about the use of internet and computers, the results of the needs analysis of students and teachers, the advantages of online learning, the advantages of Moodle version 2.5.3, and the characteristics of colloidal material, the research will develop an online learning based on Moodle version 2.5.3 for high school students grade XI at 81 Senior High School Jakarta. It is expected to increase students's understanding and interest to learn Colloid.

B. Research Methodology

The research methodology which is use is design and development research refers to Richey and Klein. The steps of research consist of: 1) Identyfying Problem, 2) Design and Development Research, 3) Selecting Participant, 4) Collecting Data, 5) Intepreting Data, and 6)Future Development (Richey &Klein, 2007). The aim of research is to design and develop Colloid's online learning based on Moodle version 2.5.3.

C. Result and Discussion

1. *Identyfying Problem*

The aim of this step is to identify problems in chemistry learning system that has been doing in schools by distributing questionnaires to students and teachers. The result are all of students often usecomputer or laptop and 79.5% of students use them more than twice a week. All students are familiar with internet and 90.4% of them use it more than twice a week. Most of them access internet via computers, laptops, mobile phones, and smartphones. Internet is accessed at home, school, and other places that have internet facilities. They access internet for several purposes, including searching for information, doing homework, interacting in social media, watching video, and browsing learning materials.

Whole teachers ever use a computer or laptop and 66.7% of them use it more than twice a week. They know the internet and use it more than twice a week. Internet is used to find information, browse learning materials and chemistry reference, enter evaluation score, open email, and interact in social media. Websites that are used to search chemical reference are Royal Society of Chemistry, Chem-is-try.org, Wikipedia, and Cambridge Area Guide. Teachers use internet via smartphones, mobile phones, computers, and laptops.

The result identifies the usage of computers and the internet has been growing rapidly among students and teachers. Using computers and Internet is limited for searching reference and learning materials. Chemistry online learning system subject is not available. Teachers isn't able to create an online learning.

2. *Design and Development Research*

The type of design and development research is product development. The steps consists of a needs analysis, product planning, and product arranging.The need analysis step identifies content and background of learning online. The result show thatthe teachers and the students are desired attractive appearance with bright colors, using language which is understood easily, present material clearly, present applications, and video.

Step of product arranging is made via online, the website address is http://elearningchemistry.com. It is started by analysis characteristics of material, plan design, content, and presentation technique of online learning, and select supporting software. Software which is used are Moodle 2.5.3 are supported Microsoft Word, Photoscape, Adobe Photoshop, and Corel Video Studio Pro X6. They are used to create quizzes, pictures and video.

Telur yang menggumpal ketika menggoreng telur dan agar-agar yang menggumpal jika dipanaskan merupakan beberapa contoh sifat-sifat koloid

Subbab ini akan menjelaskan perbedaan larutan, koloid, dan suspensi, serta jenis-jenis koloid berdasarkan fase terdispersi dan pendispersinya

Subbab ini akan menjelaskan sifat-sifat koloid, koloid liofil dan koloid liofob, serta koloid pelindung.

Subbab ini akan menjelaskan konsep pembuatan koloid yang disertai dengan contoh-contoh pembuatan koloid

Figure 1. Home Display

There are three parts which are presented in website, Home (front view), Login Page, and Course (learning activity). Course consist three sections, system and the properties of colloids, making colloid, and chemoenterpreneurship. The features in each section are introduction, subject matter (pdf), video, forum, chat, games, and quiz.

Unduhlah file pdf ini, bacalah dan pahami dengan sungguh-sungguh agar kalian dapat memahami pembuatan koloid

Bukalah link video ini agar Kalian lebih memahami proses pembuatan *colloidal oatmeal*

Forum ini disediakan sebagai sarana untuk saling berbagi ilmu atau bagi Kalian yang merasa kesulitan dalam mempelajari subbab Pembuatan Koloid dapat di*share* di forum ini

Aktivitas sejak Jumat, 19 Juli 2013, 20:40

laporan lengkap aktifitas terbaru...

Tidak ada yang baru sejak Anda terakhir login

Figure 2. Course Display

3. *Selecting Participant*

Selecting participants refers to the type of product design and development, there are: a) Researchers as designers, developers, and instructors, b)Teachers as instructors, c) Experts media, materials, and language as evaluators, d) Students as learners.

4. *Collecting data*

The step of collecting data is used several instruments, there are needs analysis instruments, feasibility instrument, and trial test instrument. Needs analysis to determine the condition of learning chemistry in school and online learning content and display are desired by students and teachers. Feasibility test instrument to evaluate the feasibility of online learning. Trial test instrument to determine the opinion of students and teachers of chemistry online learning has been developed. The trial test is conducted to 20 students of class XII at 81 Secondary School Jakarta and 4 Chemistry teacher.

The results of feasiblity test and trial test are processed by rating scale score of 1 to 4. In addition, inter-rater reliability test is to determine the inter-rater consistency in evaluating learning online using ICC (intraclass correlation coefficient) test (Djaali & Pudji, 2008). In the following below is a feasibility test and trial test data to students and teachers.

a. Feasibility Test

1) Material Experts

Table 1. Intepretation Data of Feasibility Test by Material Experts

No	Indicator	Step 1	Interpretation	Step 2	Interpretation
a	Conformity of material description to the curriculum	95,7%	excellent	95,7%	excellent
b	Accuracy of material	90,3%	good	94,3%	excellent
c	Quiz and games	90,0%	good	97,5%	excellent
d	Conformity of the material with the development of science and everyday life	90,0%	good	93,3%	excellent
e	Clarity of material	89,2%	good	89,2%	good
f.	Presentation techniques	82,1%	good	84,2%	good

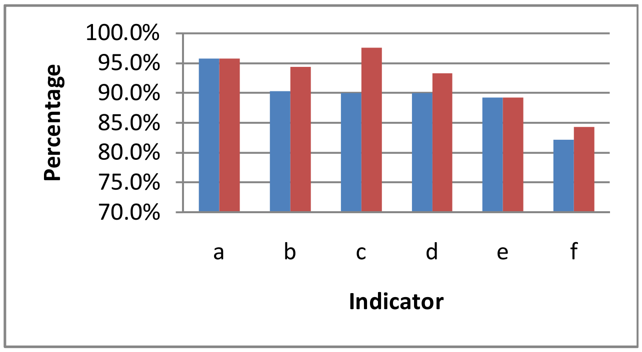


Figure 3. Percentage Data of Feasibility Test by Material Experts

2) Media Experts

Table 2. Intepretation Data of Feasibility Test by Media Experts

No	Indicator	Step 1	Interpretation	Step 2	Interpretation
a	Navigation key	74,2%	good	83,8%	good
b	Design view	73,5%	good	83,5%	good
c	Use of letters	71,7%	good	83,8%	good
d	Use of images and video	74,3%	good	80,7%	good

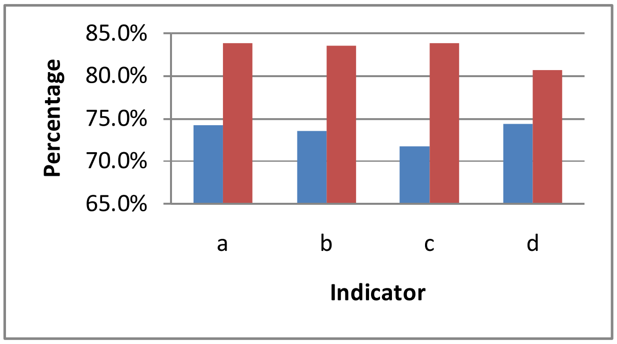


Figure 4. Data Percentage of Feasibility Test by Media Experts

3) Language Experts

Table 3. Intepretation Data of Feasibility Test by Language Experts

No	Indicator	Step 1	Interpretation	Step 2	Interpretation
a	Accordance to the developmental level of students	85,8	good	87,1%	good
b	Communicative	89,2	good	89,2%	good
c	Use of letters and punctuation correctly	83,2	good	87,7%	good
d	Use of the term correctly	86,7	good	86,7%	good
e	Use of the sentences correctly	81,3	good	85,0%	good
f.	The precision of writing a bibliography and source image	76,3	good	88,8%	good
h	Wholeness and unity of ideas	87,5	good	87,5%	good

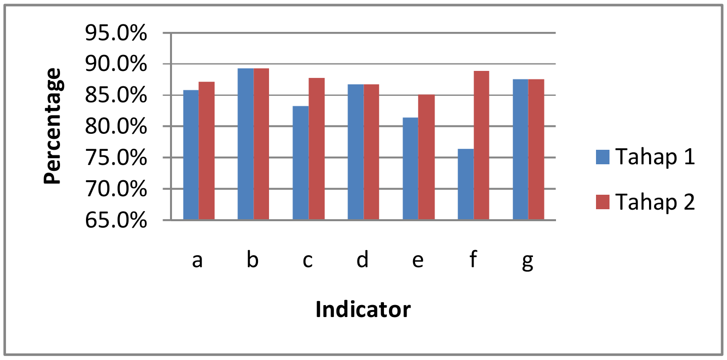


Figure 5. Percentage Data of Feasibility Test by Language Experts

b. Trial Test

1) Students

Table 4. Intepretation Data of Trial Test for Students

No	Indikator	∑	∑max	%	Interpretation
1	Clarity of information on teaching materials	29	32	90,6%	good
2	Compliance with the curriculum	31	32	96,9%	excellent
3	Quiz	15	16	93,8%	excellent
4	Use of language	15	16	93,8%	excellent
5	Design view	27	32	84,4%	excellent
6	Use of letter	45	48	93,8%	excellent
7	Image, animation and video	58	64	90,6%	excellent
8	Navigation key	13	16	81,3%	good
9	Websiteas supportonline learning	14	16	87,5%	good

2) Teachers

Table 5. Data Intepretation of Trial Test To Teachers

No	Indicator	∑	∑max	%	Interpretation
1	Clarity of information on teaching materials	29	32	90,6%	good
2	Compliance with the curriculum	31	32	96,9%	excellent
3	Quiz	15	16	93,8%	excellent
4	Use of language	15	16	93,8%	excellent
5	Design view	27	32	84,4%	excellent
6	Use of letter	45	48	93,8%	excellent

7	Image, animation and video	58	64	90,6%	excellent
8	Navigation key	13	16	81,3%	good
9	Websiteas supportonline learning	14	16	87,5%	good

5. *Intepreting Data*

a. The results of feasibility test by material experts

Based on table 1 and figure 1, all indicators have good and excellent intepretation although there is a different range of values and an enhanchement from step 1 to2. Some improvement whichare recommended by material, there are: "colloidal particles of $Fe(OH)_3$ positive charged in water.The surface will adsorb Fe^{3+}, which is supposed to adsorb H^+ and "water purification with water treatment plant should be replaced by natural materialsuch asmoringa seeds ".

Value of inter-rater reliability is 0.63, means good rater consistency. Based on the results of feasibility test and inter-rater reliability show materials are well developed, so it can be tested to students and teachers.

b. The results of feasibility test by material experts

Based on table 2 and figure 2, found that indicators navigation, display design, the usage of letters, images and video show good interpretation increase percentage from step 1 to2. Some corrections whichare recommended by media experts, the letters on section title should use upright font and videos that are presented should be equipped with explanation.

Value of inter-rater reliability is 0.64, it means good rater consistency. Based on the results of feasibility test and inter-rater reliability show media is well developed, then it can be tested to students and teachers.

c. The results of feasibility test by language experts

Based on table 3 and figure 3, it is found that communicative indicators, accuracy of glosssary, integrity and unity of ideas show same percentage of step 1 to 2, and have good interpretation. Other indicators, level of student progress, the accuracy of using letters, punctuations, glossary, sentences, bibliography, and source of images show increase percentage from step 1 to 2, and have good interpretation. Some improvement which isrecommended by language experts, there are some written spelling and punctuation which are not based on EYD (Ejaan Yang Disempurnakan), the usage of active and passive sentences which is not suitable should be repaired.

Value of inter-rater reliability is 0.65, it means good rater consistency. Based on the results of feasibility test and inter-rater reliability show good language. Then it can be tested to students and teachers.

d. The results of trial test to students and teachers

Trial test to students is conducted by researchers who are presented an online learning system and explained using website. Then the students scheduled to access website directly, and to do online learning activities such as reading material in PDF format, seeing the video, trying to express opinions through forum or chat, doing quiz and so on. After that, students filled out a questionnaire which was given by researchers to assess and give comments or suggestions regarding the online learning system.

Based on table 4, the indicators of clarity information on teaching materials, navigation buttons, and website as a supporter online learning have good interpretation. Meanwhile the indicator suitability material with standard of competence, basic competences, the indicators, questions on quiz/game, usage of language, interface design, and the usage of letters, pictures, animations, and videos show excellent interpretation. There are some corrections recommended by teachers, shadows on some images should be removed in order to they can be seen clearly and the rules of some standardization written questions on multiple choice quiz should be suitable.

Then the results of trial test to students (table 5) show good interpretation on all indicators. However, there are some improvementwhich is suggested by students, such as font size on *home*zoomed, the background color should be colored brighter, and speed of loading time is accelerated, in order students do not have to wait to open the website.

Based on the test results to students and teachers that have been outlined above so it can be concluded based online learning system Moodle 2.5.3 version that is good and fit to used for supplement the learning materials Colloids.

6. *Future Development*

After developing online learning based on Moodle version 2.5.3, it can test the effectiveness of online learning based on Moodle version 2.5.3 and development of online learning based on Moodle version 2.5.3 for other chemical materials.

D. Conclusion

Using internet and computers that is more increasing among teachers and students has not been supported by the presence of chemistry online learning in school. Therefore, researchers develop an online learning based on the needs analysis and characteristics of colloid which is using Moodle version 2.5.3.

Based on feasibilty test by media, materials, and language experts has shown good and excellent intepretation. The results of inter-rater reliability and the trial test for students and teachers haveshown good and excellent interpretation. It can be concluded online learning system based on Moodle version 2.5.3 is good and feasible to complete learning Colloid.

E. References

MarkPlus Insight. 2013. *Pengguna Internet Indonesia 74 Juta di Tahun 2013.*http://www.themarketeers.com/archives/Indonesia%20Internet%20Users.html#.UtKUUNKSxic. (Accessed on 12th January 2013).

Menteri Pendidikan Nasional. *Peraturan Menteri Pendidikan Nasional Republik Indonesia No 22 Tahun 2006*. Jakarta : Departemen Pendidikan Nasional.

__________. *Peraturan Menteri Pendidikan Nasional Republik Indonesia No 41 Tahun 2007*. Jakarta : Departemen Pendidikan Nasional.

Yehudit & Miri. 2003. A Web-Based Chemistry Course as a Means To Foster Freshmen Learning. *Journal of Chemical Education*. 80(9). 1084-1092

Tambovceva, T dan Artis, I. 2013. Development of Moodle : Case of Riga Technical University. *Proceedings of 2013 International Conference on Education and Educatinal Technologies.*

Margareta dan Rahela. 2007. E-Learning in Chemistry : To Use It or Not to Use it.*10th Intermational Multi-Conference*. Slovenija : University of Ljubljana.

Richey, R. dan James, K. 2007. *Design and Development Research*. New Jersey: Lawrence Erlbaum Associates Publishers.

Djaali dan Pudji. 2008. Pengukuran Dalam Bidang Pendidikan. Jakarta : Grasindo

Varjola, Irma. 2000. Use of The Internet In The Teaching of Chemistry in Finnish Schools: A Case Study. *Chemistry Education : Research and Practice in Europe*. 1(1). 121-128.

Shiang Kwei dan Thomas. 2007. The Effects of a Web-Based Learning Environment on Student Motivation in a High School Earth Science Course. *Education Tech Research Dev* (55). 169-192

CE-6

IMPROVING THE LEARNING PROCESS OF POLIFUNCTIONAL COMPOUND TOPIC BY USING JIGSAW COOPERATIVE LEARNING WITH MULTI LEARNING RESOURCES

Mitarlis, Sri Hidayati Syarief, Nurul Hidayati, Suyatno and Tukiran

Department of Chemistry, Faculty of Mathematic and Natural Sciences, Surabaya State University
Email: mitarlis@ymail.com

ABSTRACT

Organic Chemistry lecture had been held in the classroom meeting and laboratory experiment activity. Many topic were conducted experiment in laboratory activity representatively, such as the alcohol phenols, carboxylic acids, aldehydes ketones as well as other topics. Especially on poly functional compounds are not specific experiment title. Therefore the learning process of polyfunctional compound needs to be improved. The aims of this study to describe the implementation of Jigsaw Cooperative Learning Model in order to enhancing the learning process of Organic Chemistry 2 lecture on polyfunctional compounds topic. It used multi learning resources during the lecture. The study design used one shot case study post-test only design. Methods of data collection used observation, interview, and test methods. Data analysis used descriptive quantitative and qualitative. The study result shown that: The implementation of Jigsaw cooperative learning Model of all components of the average of the 3 observers get a score of 3.49 in good categories; almost all stages of Jigsaw Cooperative learning get score between 3.33 to 4.00 on good and very good category, except the award given syntax obtain an average score of 2.33 in quite well category; learning resources and media usage get score between 3.66 to 4.00 on very good category. The student learning outcome in polyfunctional compound topic with the details value of A^- to A 12.9 % ; B^- to B^+ 77.9%; and C^+ 7.4% on mastery learning category; as well as D 1,8% and E 0% on not mastery learning category. The students response about this learning, they interest on the matter 9.1%; learning process 66.7%; and learning resources 21.2%.

Keywords : polyfunctional compound , Jigsaw cooperative learning, multi learning resources.

INTRODUCTION

Organic chemistry is one of the compulsory subjects that must be taken for chemistry students and chemistry education students of chemistry department in Faculty of Mathematic and Natural Sciences Surabaya State University. The course consists of Organic chemistry I (2 credits), Organic chemistry II (3 credits), Organic Chemistry III (3 credits) and Organic Chemistry IV (2 credits) [**1**]. *Organic Chemistry* is one of the most important courses for second year undergraduate students majoring in chemistry especially Organic Chemistry 2 was hold on odd semester for student chemistry program study and even semester for student of education chemistry program study. In our teaching and learning process, the most commonly used teaching methods include lectures in classroom and doing experiment in laboratory. There are many topics in Organic chemistry 2 course. Course description are discussing about structure physical and chemical characteristics, as well as it synthesize, consists of alcohol compound; phenol; eter; aldehyde; keton; carboxylic acid and it derivates; amine; and polyfunctional compound. These topics were studied by learning activity in the class and doing experiment in the laboratory. The titles of the experiments represent the topics in course except polyfunctional topic there is no experiment title representatively. So the learning process of this topic needs more interesting and makes students more active. Student activity in ordinary learning process is not high. It's indicated when the lecturer gives question to the students; almost all of them were not give response until the third question. There are less than 10% only of students give answer, or 3 students from 54 students. This condition is very passive class.

There are many learning models had been develop to improve learning process, like cooperative learning, direct instruction, problem based learning, and others. The models can applied on many subject, or topic according to characteristic of them. In chemistry subject, we can apply one of the models, especially for polyfunctional compound topics. The polyfunctional compound topic in this course divided into 7 sub topics mainly dicarboxylic acid; double functional ester; hydroxyl acid; phenolic acid; keto acid; dicarbonyl compound; and diene compound**[2]**. The discuss about structure, characteristics, and specific basic reaction for each compound. Based on the characteristics there are many sub topics, the learning process can conduct by using cooperative learning model especially Jigsaw type.

The Jigsaw method is a cooperative learning technique in which students work in small groups. Jigsaw can be used in a variety of ways for a variety of goals, but it is primarily used for the acquisition and presentation of new material, review, or informed debate. In this method, each group member is assigned to become an "expert" on some aspect of a unit of study. After reading about their area of expertise, the experts from different groups meet to discuss their topic, and then return to their groups and take turns teaching their topics to their group mates. One of the benefits of the jigsaw process encourages listening, engagement, and empathy by giving each member of the group an essential part to play in the academic activity. Group members must work together as a team to accomplish a common goal; each person depends on all the others. No student can succeed completely unless everyone works well together as a team. This "*cooperation by design*" facilitates interaction among all students in the class, leading them to value each other as contributors to their common task **[3]**. (http://www.jigsaw.org/overview.htm).

Many students believe that the study of organic chemistry requires rote memorization. This is supported through the encyclopedic nature of introductory organic chemistry textbooks and their associated solutions manuals. However, help is available through an understanding of reactions and their underlying mechanisms. A reaction mechanism can be described using arrow pushing - the use of curved or curly arrows to show the movement of electrons through the various functional groups. Through this technique, applicable to any book or course, memorization can be minimized and the subject demystified. Of the many available online resources, this site focuses on making organic chemistry more approachable. There are many learning resources provide for students in this era. However when the globalization, market power, multinational, international as well as information technology advancement and the other trend in the world give influence significantly to the learning material, really we did not show yet a great changes in the teaching and learning process in college[4]. Learning resources were not teacher or lecturer only. In this research students can use textbooks, website by using their gadget, student book and worksheet as a guide of this activity. The problems will be answered in this study are: 1) How the implementation of Jigsaw Cooperative learning model in chemistry organic 2 on poly functional compound topic. 2) What the students learning outcomes on polyfunctional topic by learning process with cooperative learning model. 3) How the students response about learning process of organic chemistry II on polyfunctional topic.

This study aims to improve the learning process in the course of organic Chemistry 2, especially on the topic of polyfunctional compounds with Jigsaw cooperative learning by using a multi learning resources. In addition, to know the learning process of organic chemistry 2 subject on polyfunctional compound topic with Jigsaw cooperative learning by using multi learning resources, to know student learning outcomes and to student response. The benefit of this study is to provide an alternative learning model that can be used in the learning process of Organic Chemistry 2 especially on polyfunctional compound topic. For students with more learning can enable students in the learning process. For science, especially in learning process it can expand the using of learning models and multi learning resources in the lecture.

Benefits of learning resources are: can give direct learning experiences and real to students; present something that impossible to provide, visit or observe directly and really; can give wider presentation in the classroom; provide accurate and newest information; supporting to solve education/ instructional problem in micro or macro area; give positive information, if its benefits was regulated and planed precisely; can stimulate to think, posed, and develop furthermore**[5]**.

Which includes of learning resources are variety information, data science, human ideas , either in the form of printed materials (books , brochures , pamphlets , magazines , etc.) as well as in non-print form (movies , filmstrip , cassette , videocassette , etc.). Association for Education and Communication Technology (AECT) outlines that learning resources include: message, people, materials, tools, techniques and environments. All sources in the form of data, and the particular form that can be used by students in learning, either separately or combined to facilitate students in achieving the learning objectives or achieve a particular competence. Learning resource materials that are used and required in the learning process, which may include text books, print media, electronic media, speakers, surrounding environment; and so on that can increase the levels of students activity in the learning process. Learning resource is anything that is available around the learning environment that serves to help optimize learning outcomes. Optimizing the results of this study can be seen not only on the learning outcomes, but also the views of the learning process in the form of student interaction with a variety of learning resources that can provide a stimulus for learning and accelerate the understanding and mastery of science are studied **[6].**

Components of learning resources which used in the teaching and learning activities can be distinguished by the way that can be seen from the existence of learning resources as planned and utilized. Learning resources are deliberately planned (by design) are all sources of learning that has been specifically developed as a component of the instructional system to provide facilities-directed learning and formal. Learning resources as utilized (by utilization) that learning resources that are not specifically designed for learning purposes, but can be found, applied, and used for learning purposes. Based on the above concepts, learning resources is essentially an instructional system components include a message, people, materials, equipment, techniques and background (environmental) **[7].** http://www.m-edukasi.web.id/2013/07/pengertian-sumber-belajar.html. In this study learning resources was used in the classroom of Organic Chemistry 2 course on polyfunctional compounds are among others; student textbook of polyfunctional compounds, student worksheets, and other resources that are accessed from the internet through gadgets that owned by the students.

RESEARCH METHOD

The research design used one shot case study posttest only design. Methods of data collection in this research used observation, interview, and test methods. Data analysis used descriptive quantitative and qualitative. The instruments are used in this research; observation sheet of implementation of cooperative Jigsaw learning process using an instrument that adapted from the implementation of learning assessment instruments in the implementation Signs Professional Teacher Education and Training (PLPG) 2013 **[8]**. In this study, the instrument is modified as needed. Assessment scores using a range of 1 to 4, with criteria 1 =

less, 2 = adequate, 3 = good, and 4 = very good. Analysis of observational data using a learning instrument that has been compiled and analyzed quantitatively by the formula:

$$\text{Value} \quad = \frac{\text{total score components}}{\textit{number of components}}$$

Then the conversion described with ratings in the following categories:

Very well = $3.33 < \text{score} \le 4$
Good = $2.33 < \text{score} \le 3.33$
Adequate = $1.33 < \text{score} \le 2.33$
Less = $\text{score} < 1.33$ **[7]**

Student responses to the questionnaire calculated the percentage of students answer according to the number of items in the instrument. The test results were analyzed according to assessment system based on the Criterion prevailing in the Department of Chemistry, State University of Surabaya presented on Table 1[1].

Table 1. Converse of value in letter and number

Value	Score	Interval
A	4.0	$85 \le A \le 100$
A^-	3.75	$80 \le A^- \le 85$
B^+	3.5	$75 \le B^+ \le 80$
B	3.0	$70 \le B \le 75$
B^-	2.75	$65 \le B^- \le 70$
C^+	2.5	$60 \le C^+ \le 65$
C	2.0	$55 \le C \le 60$
D	1.0	$40 \le D \le 55$
E	0	$0 \le E \le 40$

Beside the kind of quantitative data there are qualitative data that can support the results of this research will be analyze by using descriptive qualitative.

RESULT OF THE RESEARCH AND DISCUSSION

Feasibility of learning process in Organic Chemistry 2 course by using Cooperative learning Jigsaw type with the multi learning resource can be seen on Table 2.

Table 2. Observational data of Cooperative learning models Jigsaw type in Organic Chemistry 2 course on polyfunctional matter

Observation aspects	**O1**	**O2**	**O3**	**Average**
Introduction				
A. Apperception and motivation				
1. Creating learning situations to focus attention	3	4	4	3,66
2. Asking students to express their opinions	3	3	4	3.33
3. Conveying learning objectives	4	4	4	4
4. Linking the present with previous lesson	4	3	4	3.66
B. Conveying of Competence and activity plan				
1. Communicating student competencies to be achieved	3	4	4	3.66
2. Informing activity Jigsaw cooperative learning model to polyfunctional compounds topic	4	3	4	3.66
Main Activity				
A. Mastery Learning Materials				
1. 1. Adjusting the material with the aim of learning	4	4	4	3.66

Observation aspects	**O1**	**O2**	**O3**	**Average**
2. 2. Linking material with other knowledge	3	3	4	3.66
3. 3. Presenting material in a proper discussion	4	4	4	4
4. 4. Presenting the material in a systematic	3	4	4	3.66
B. Implementation of educated learning strategies				
1. Conduct the learning process according to competences will be achieved	4	4	4	4
2. Facilitate student activity with multi learning resources	3	3	4	3.33
3. Conduct the learning process harmony	3	4	4	3.66
4. Comprehending class management	4	4	4	4
5. Conduct the learning process to grow positive habituation possibly	4	3	4	3.66
C. Implementation of Jigsaw Cooperative Learning				
1. Presenting information of polyfunctional matter	3	3	4	3.33
2. Organizing students in group of Jigsaw cooperative Learning	3	4	4	3.66
3. Guiding students in work and learning	4	4	4	4
4. Doing evaluation and presenting result of working in group	3	4	3	3.33
5. Giving reward	3	3	1	2.33
D. Use of source Learning / Media in Learning process				
1. Demonstrate skill in the use of learning resources	3	4	4	3.66
2. Demonstrate skill in the use of instructional media	3	4	4	3.66
3. Produce compelling message	4	4	3	3.66
4. Involving students in the use of learning resources	4	4	4	4
5. Involving students in the use of instructional media	4	4	3	3.66
Closing				
1. Making reflection by involving students	4	4	1	3.0
2. Provide follow-up to the next activity	3	4	3	3.33
Number of average score				**94.22**
Total average score	**3.49**			

Based on Table 2 shows that the learning process in the Organic Chemistry 2 course, especially on polyfunctional material using Jigsaw cooperative learning model can be implemented well, with score in range of 3.33 to 4 in the good and very good category. Overall aspect assessed get score 3.49 in the excellent category. The using of instructional media sources and get a score between 3.66 to 4 of all aspects on good and very good category. With the implementation of learning outcomes are included in the criteria of good and very good it will be seen also student learning outcomes by providing a test on the material polyfunctional compounds. Observation of student activity in the classroom of Organic Chemistry 2 course with Jigsaw cooperative learning model supported as shown at Figure 1.

Figure 1 Students are in groups: (A) original group (Group 7),
(B) The expert group material D: phenolic acids

In addition, we can observe the activities of students in a group discussion and use of learning resources supported by Figure 2.

Figure 2 (A) Students interaction in Jigsaw cooperative learning groups
(B) Interaction of students with learning resources (student book, gadgets for browsing teaching material)

Figure 3 Presentation of group work by students and clarification from lecturer

Figure 3 presents the student activities in the final stage of cooperative learning by presenting the results of the group discussions that have been finalized in the original group. In this activity lecturer as a facilitator will be clarify anything if needed to help straighten out the answer when the students presenting their work. In this study the end of the lesson according to the syntax of cooperative learning should no awards. However, the syntax does not look real by giving something to a student or group of students, but only as a compliment of verbal praise of lecturers as reinforcement on the performance of students either individually or in groups. This led to a score of adherence to cooperative learning as in Table 1 section C.5 get a score just 2.33, in quite well category.

After completion of learning in the classroom for Organic Chemistry 2 course on material of polyfunctional compounds that requires two meetings, than conducted the test. The results of the test on polyfunctional compounds material in Organic Chemistry 2 can be seen in Table 3.

Table 3 Results of test material with a polyfunctional compound Jigsaw cooperative learning using a variety of learning resources.

Letter	Score	Interval	Students	percent
A	4.0	$85 \leq A \leq 100$	2	3.7%
A^-	3.75	$80 \leq A^- \leq 85$	5	9.2%
B^+	3.5	$75 \leq B^+ \leq 80$	20	37.1%
B	3.0	$70 \leq B \leq 75$	13	24.1%
B^-	2.75	$65 \leq B^- \leq 70$	9	16.7%
C^+	2.5	$60 \leq C^+ \leq 65$	4	7.4%
C	2.0	$55 \leq C \leq 60$	0	0
D	1.0	$40 \leq D \leq 55$	1	1.8%
E	0	$0 \leq E \leq 40$	0	0

Based on Table 2 . Learning outcomes of students in the category of A^- and A grades at 12.9 % ; B^- to B^+ 77.9 % ; grade of C^+ 7.4 % in completed category and D 1.8 % ; as well as E 0 % in the incompleted category . Based on these data of classical mastery learning had been achieved greater than 80 % it is 90.8 %.

Student response to this study shows student data was pleased with the material 9.1 %; like in the learning process 66.7 %; and 21.2 % interest to the learning media/ learning resources. Based on these data the dominant student was pleased with varying learning process, through group discussions on the Jigsaw cooperative learning model type. Based on the result of students interviews they feel happy because they feel more energized and motivated to learn the material organic chemistry 2 by utilizing information technology as a learning resource. It is like Margareta (2014) statement that we do not make the technology as so rival, so as friends to make we no stop learning" [9]. In addition, learning becomes monotonous tasks can quickly respond and get clarification. Thus from this study an increase in the learning process with more activating and expanding student learning resources from a variety of sources was occurred.

CONCLUSSION AND SUGGESTION

The result of this study can be concluded that: 1)The implementation of Jigsaw cooperative learning Model of all components of the average of the 3 observers get a score of 3.49 in good categories; 2) Almost all stages of Jigsaw Cooperative learning get score between 3.33 to 4.00 on good and very good category, except the award given syntax obtain an average score of 2.33 in quite well category; 3) Learning resources and media usage get score between 3.66 to 4.00 on very good category. 4) The student learning outcome in polyfunctional compound topic with the details value of A^- to A 12.9 % ; B^- to B^+ 77.9%; and C^+ 7.4% on mastery learning category; as well as D 1,8% and E 0% on not mastery learning category. 5)The students response about this learning, they interest on the matter 9.1%; learning process 66.7%; and learning resources 21.2%.

REFERENCES

[1] Anonim, 2013. Buku Pedoman. Universitas Negeri Surabaya. Fakultas Metematika dan Ilmu Pengetahuan Alam.

[2] Solomons Graham, TW. and Fryhle, Craig B., 2011. Organic Chemistry. International Student Version. John Willey and Sons.

[3] Aronson Elliot. 2014. Jigsaw Classrom, Overview of the Technique. http://www.jigsaw.org/overview.htm diakses tanggal 20 Maret 2014.

[4] Utari Rahma, 2007. **Disarikan dari Forest, J. J.F & Altbach, P.G (ed). 2007.**

International Handbook of Higher Education. Dordrecht: Springer. Belajar di Perguruan Tinggi http://staff.uny.ac.id/sites/default/files/BELAJAR%20MENGAJAR%20DI%20PERGURUAN%20TINGGI.pdf

[5] http://zanikhan.multiply.com/journal/item/5399 Diakses hari jumat 14 desember 2012 pkl., 10.00 WIB.

[6] Anonym, 2011. Pengertian Media dan Sumber Belajar. http://info-makalah.blogspot.com/2011/07/media-dan-sumber-belajar.html diakses tanggal 17 Desember 2013

[7] Anonim, 2013. Pengertian Sumber Belajar. http://www.m-edukasi.web.id/2013/07/pengertian-sumber-belajar.html.

[8] Kemendikbud, 2013. Model Penilaian Kompetensi Peserta Didik.

[9] Margareta Maria. 2014. Guru dulu dan Sekarang. http://m.kompasiana.com/post/read/639874/3/guru-dulu-dan-sekarang.html

CE-7

IMPLEMENTATION OF JIGSAW MODEL WITH LESSON-STUDY-BASED ON STRATEGY AND METHOD OF CHEMISTRY INSTRUCTION SUBJECT (SMPK).

Muhammad Danial

(Lecturer from Chemistry Education FMIPA UNM)

Abstract

This research is aimed to increase the interactive activity and concept mastery of the students of chemistry education study program on Strategy and Method of Chemistry Instruction Subject through jigsaw model with lesson-study-based. Lesson study activity with steps plan, do and see was conducted at Chemistry Department of Mathematics and Science Faculty, State University of Makassar with 7 involved observers. Methods used in this research is learning cycle (plan, do, and see) and collaborated with several SMPK lecturer through collegiality principle and mutual learning. Present research was done on odd semester academic year 2012/2013 with four cycles. The research subject is students of chemistry education study program, received year 2010/2011.

Keywords: jigsaw, lesson study, instructional strategy and method

Introduction

Creation of effective and pleasant learning condition is very crucial on order to emerge and develop variety of competences of students. The development of student's competence include cognitive, affective, and pshycomotoric competence is the main goal of a learning process. Based on that, the application and elaboration of learning steps, strategy or learning type should be afforded, either in direct instruction, investigation-based learning, or cooperative-collaborative learning.

One learning types that has been known, applied, and developed is cooperative learning type jigsaw. This learning type required a fixed unique planning type. As example, times that used to organize specific skills required, the teacher can mapping how to make transition from class instruction to small groups instruction can run well (Arends, 2007)

This learning type counted as learning type that implement team appreciation, individual responsibility, and equal chance to success. According to Nur (2005) jigsaw could be used if the subject course in written narrative form and the learning goal emphasize to concept in descriptive material than skills. Continuously, Nur (2005) also explained that in jigsaw, especially jigsaw II, the student work in heterogeneous team. The students asked to learn a chapter or another subject to read and was given "expert sheet" that contain different topics to each team members, so when reading section began, students from different team with same topic met in an expert team to discuss their topic for 30 minutes. The experts then back to their initial groups and then teach their topic to the teammate alternately. In the end, the students give some quiz about all the topics and the score of that quiz become team score. The winner team could be appreciated with a prize or certificate. Using this way, the students are expected to be motivate to learn. Giving present technique can improving the student behavior to respond the tasks given and maximally the mean of present that they get.

This learning type can develop several abilities and competences, for example high thinking order competence and pedagogical competence that can affect to professional competence or mastery of subject concepts. The last competence is very important to the prospective teacher as well as the professional teacher. The concept mastery is the ability to understand and apply the lesson contentin every occasion or condition where that concept required. This ability can be trained through a knowledge activities and learning skills, for example lesson study activity. Lesson study is learningactivities studies collaboratively andsustainable based on collegiality principle and mutual learning to build learning community (Susilo, 2009). On lesson study implementation the teacher collaboratively: 1) studying the curriculum, formulating the learning objectives and student development objectives (life-skills development); 2) planning the learning to gaining the objectives; 3) performing and observing the research lesson; and 5) taking reflection to discuss the lesson investigated and improved on the next lesson planning (Susilo, 2009).

Relative with the aim to attaining the learning condition that effective to develop the professional competence or the concept mastery of educational students, in this occasion, we are the lesson study team carry out a learning research by implementing the steps of lesson study (plan, do, and see) to the lecture subject Strategy and Method of Chemistry Instruction (SMPK). This purposed to increase the knowledge and pedagogic competence to the chemistry teacher candidate. Besides that, this research also aimed to introduce the steps of cooperative learning type jigsaw. This lesson type include in simple cooperative learning, because the student only discuss and solve the problem given by teacher in the classroom.

All this time, lecture of SMPK subject was held by two lecturers, where the lecturer divide the lecture course for 16 times lecture process. So, each lecture get 8 times lecturing process include the evaluation. This method absolutely has a limitation, such as variation of information being lack because the lecturing course or discussion topics only from one lecturer. This method also not a solid team because the team working partially, so the learning until reflection to improve learning quality process not maximally transpired.

Based on that reason, SMPK lecturing through lesson study with open class implementation (watched and observed by observer from another study program) very important for continuous teaching profession development effort, so the professionalism and concept mastery of students continuously increased.

The SMPK subject that that become research object through lesson study learning is method and type of cooperative learning. So, this activity teach cooperative learning through cooperative learning method that sets in learning cycle of lesson study (plan, do and see)

Present research aimed to improve the concept mastery of educational student through the application of cooperative learning type jigsaw on the lecture course Strategy and Method of Chemistry Instruction (SMPK). This research was done by several SMPK lecture collaboratively (with collegiality and mutual learning principle).

This activity expected to be useful for: 1) the lecturer, to give information about implementation of lesson study, the importance of team collaboration to furnish and correct the lack of each other, so the learning quality will improve; and 2) to the head of faculty, this activity can be a media to evaluate the success of lecturing process, especially in the achievement of standard competence and basic competence of SMPK subject.

Research Methods

Present research is a lesson study research which apply cooperative learning type jigsaw on the SMPK lecture. The research subject is 43 students ofchemistry study program, Mathematics and Science Faculty, State University of Makassar received year 2010/2013. This research done in 4 cycles (plan, do and see activity).

The learning devices used this research were RPP, LKPD, Strategy of Chemistry Instruction textbook, and student's book for grade X, XI, and XII. The instruments used is observation sheets and SMPK concept mastery tests.

The steps of lesson study elaborated below:
1^{st} cycle:

Plan step:

Lesson study team meet and planned the implementation of lesson study on SMPK lecture at Chemistry Major. This meeting was headed by the team leader and discuss: 1) implementation time “plan” and “do” activity for the first meeting; 2) selection of lecturer as model for first, second, third, and fifth “do” activity; 3) selection of observer for first, second, third, and fifth meeting.

Do step:

The first “do” activity wasperformed by lecturer model who implement jigsaw learning model. Basic competence is the students should be able to understand and communicate various types of cooperative learning (KD-1). Methods which execute to gaining that competence is applying cooperative learning type jigsaw and preparing the learning devices, Models and Strategy of Science Instruction books, and student’s chemistry book for grade X, XI, XII that borrowed from Chemistry Library FMIPA UNM. The lecturing process watched by observer to observe the student activity and involvement in lecturing process. The observer expected to give any advice in the “see” activity for improvement of next “do” activity. The steps of jigsaw learning model showed in Table 1.

Table 1. The steps of jigsaw learning model.

Learning Steps	Learning Activity	Substances Required
Dividing the students into several teams	Divided the students into heterogeneous teams with has 5-6 members	
Dividing the students into expert team	Divided the students into expert team randomly from a team	
Reading	The students get the expert topics and asked to read the information related with the topics	Expert sheets (in this case, LKPD), literature (example: StrategiBelajarMengajar Kimia by Arifin, M et.al. 2005), and student’s chemistry book for grade X, XI, and XII (example: Chemistry Book for SMA/MA by Rachmawaty and Johari, 2007).
Team reports	The expert back to their initial group and then teach the other group member about their mastery topic	
Presentation and discussion	Each expert team presented their discussion results	The discussion summary of the expert group
Test	Individual test about all topics give to each students.	Quiz sheet for each students
Team recognition	The team score calculated and prepared certificate or another present to the winner team	Criteria appreciation sheets

Sources: Slavin (2005).

See steps and plan for 2ndcycle

The implementation of first "see" and plan for second cycle execute after the implementation of first "do" to evaluate and reflect the result of "do" activity. First chance given to the students to extend their opinion about lesson study implementation, and the next the observer to present their observation result and give suggestion for second "do" improvement.

2nd cycle

Do step

The "do" step activity was performed by lecturer model. The basic competence is the students should be able to identify the suitability of cooperative learning characteristic with chemistry concept in Senior High School.

See step and plan for 3rd cycle

The implementation of the second "see" activity and third "plan" executed after the implementation of second "do" to evaluate and reflect the second "do" activity. The first chance given to observer from inter-chemistry-major to elaborated their observation and give any advice to improvement of the third "do". Continuously, the second chance given to lesson study team from chemistry study program.

3rd Cycle

Do step

The third "do" activity was performed by model lecturer. The basic competence is the students should be able to communicate various methods of chemistry instructional methods (KD-1 for main course of Methods of Chemistry Instruction)

Third see step and fourth plan

The implementation of third "see" and fourth "plan" executed after the implementation of third "do" activity. The first chance given to observer from inter-chemistry-major to elaborated their observation and give any advice to improvement of the fourth "do". Continuously, the second chance given to lesson study team from chemistry study program.

4th Cycle

Do step

The fourth "do" step was performed by lecturer model. The basic competence is the student should be able to identify the suitability of the learning model characteristic with certain chemistry concept (KD-2 for main course of Methods of Chemistry Instruction)

See step

On the fourth "see", four lesson study team member decided to finish the lesson study observation activity on SMPK course, odd semester, academic year 2012/2013 at Chemistry Major FMIPA UNM.

Results and Discussion

1. Students activity during learning process

The activity of students during learning process was observed by observers. The observation results each lesson study cycles were explained below:

1st cycle

Plan step:

The meeting results is one lecturer agreed as lecturer model and 7 as observer. From this meeting also agreed that the first "plan" was executed by lecturer team of SMPK course.

The implementation of first "plan" concerted the lecturing activity of types of cooperative learning concepts. Substance discussed was lecturing strategic used, media used, time allocation, and other learning devices that support the course, the course content, and conclusion expected after the lecturing process. Agreement reached was the implementation of cooperative type jigsaw as lecturing strategy with LCD projector and white board as media. To support the lecturing process, lesson study team compromised to prepare learning source likehandbook and LKPD sheet about type and methods of learning based on strategy that will implemented to the students.

Do Step:

The first "do" step by lecturer model applied jigsaw method. The steps of jigsaw method have been showed in Table 1.

See step and second plan for 2nd cycle:

Generally, the observer commented that the learning process have been actively engage the students to find information and increasing the student's competence on concept mastery which expected. Even there are student from a study group irresponsive to the lesson. This is out of lecturer model attention during the class. Students have a notion that the implementation of lesson study coerce the students to fostering better learning based on the students and lecture content characteristic and make the students understand the subject matter deeply. For second "do" improvement, the suggestions of observers are to pay attention on whole student involvement and admonish the unwanted activity of students, such as if there are students that seems like look for information in their PC/ Laptop whereas they look for another things.

As lecturer model, observer's advice will follow-up, implement, and perfect for second "do" with basic competence (KD-2) to identify the suitability of types of cooperative learning with certain concept or content in chemistry at same class.

2nd cycle

Do step:

Steps that carried out to gaining the competence was the application of cooperative learning type jigsaw by lecturer model and then prepared the learning devices, Models and Strategy of Science Instruction books, and student's chemistry book for grade X,XI. And XII which borrowed from Chemistry Library, FMIPA UNM. The learning process was observed by observer team to view the active involvement of students during the learning process. After second "do" executed, lesson study team made second "see" activity to obtain information and suggestion of second "do" results.

See step and third plan for 3rd cycle

Generally, the observer commented that the learning process have been run well. The class activity have been engaged the student to constructed their knowledge and professional skills through literature reviewing, discussion, presentation, and sharing between presenter team and another teams. Nevertheless, there are member of presenter team that not get opportunity to give comment and answer to another team's question, because times up, and lecturer model instructed to finish the learning. For the improvement of third "do" observes advised to adjust time allocated with discussion topics range or the next course content. The lesson study team, especially lecturer model followed up the observer's suggestion to arrange the third plan with pay attention to suitability of time allocated with next discussion topics (third "do")

3rd cycle

Do step:

Steps that carried out to gaining the competence was the application of cooperative learning type jigsaw by lecturer model and then prepared the learning devices, Models and Strategy of Science Instruction books, and student's chemistry book for grade X,XI. And XII which borrowed from Chemistry Library, FMIPA UNM. The learning process was observed by observer team to view the active involvement of students during the learning process. After third "do" executed, lesson study team made third "see" activity to obtain information and suggestion of third "do" results.

Third see and fourth plan

In general, either the observer from chemistry study program or from outside stated that the learning process run interactively and high level RPP (Lesson plan) achievement. The process engage the students actively to construct their knowledge and professional skills through literature study, discussion, presentation and sharing between presenter team and the other teams. Nevertheless, there are students that cheat each other during the evaluation (quiz), because they are still sitting position (in circle) not arranged to normal position yet, so they have chance to share the answer during the test. For forth do improvement, the observer suggest to pay attention with student's sit arrangement during evaluation or another test like quiz and the other. The lesson study team, especially lecturer model followed up the observer's suggestion to arrange the fourth "plan" and perform fourth "do".

4th cycle

Do step:

Steps that carried out to gaining the competence was the application of cooperative learning type jigsaw by lecturer model and then prepared the learning devices, Models and Strategy of Science Instruction books, student's chemistry book for grade X,XI. And XII which borrowed from Chemistry Library, FMIPA UNM also followed the observer suggestion on third "see". The learning process was observed by observer team to view the active involvement of students during the learning process. After third "do" executed, lesson study team made fourth "see" activity to obtain information and suggestion of third "do" results.

See step:

On the "see" step. All four lesson study teams and observer stated that the learning process run very interactive and evaluation process run orderly. On the fourth "see", lesson study teams agree to finish to lesson study observation on SMPK course, odd semester, and academic year 2012/2013 at Chemistry Major FMIPA UNM.

2. Concept mastery

Concept mastery data as the student's learning achievement on SMPK course shown in Table 2.

Table 2. Concept mastery data from 1st cycle to 4th cycle (plan, do, see)

Score / cycle	**1st cycle**	**2nd cycle**	**3rd cycle**	**4th cycle**
Average score	72	78	82	87
The lowest score	65	71	72	75
The highest score	77	80	85	90

Based on that concept mastery data as the student achievement in table 2 shown that there are average score from 1st to 4th cycle increased. This indicated that the implementation of cooperative learning type jigsaw with lesson-study-based can increased student's concept mastery on SMPK course.

Conclusion

The implementation of lesson study gave positive contribution for students and lecture involved, such as:

1. Improve the interactive activity of educational students on SMPK course.
2. Increase the concept mastery especially for types of cooperative learning and instructional methods subject.
3. Produce the lesson plan that able to teach students effectively and awaking student's participation on learning process
4. Inculcate the understanding which the observation focus not on how the lecturer teach, but to the student's learning activity.

BIBLIOGRAPHY

Arends, R.I. 2007. *Learning to Teach*. New York: McGraw Hill Companies, Inc

Arifin, M. *et.al*. 2005.*Strategi Belajar Mengajar Kimia*. Malang: UM Press

Beaulieu, D. 2004. *Impact Techniques in The Classroom*. USA. Crown House Publishing Company LLC

Nur, Muhammad. 2008. *Pembelajaran Kooperatif.* Surabaya: Pusat Sains dan Matematika Sekolah Unesa

Rachmawati, M and JMC Johari. 2007. *Kimia 1 SMA dan MA kelas X*. Jakarta: Esis

Slavin, RE. 2005. *Cooperative Learning: Theory, Research, and Practice.* London: Allyman Bacon.

Susilo, H. dkk . 2009. *Lesson Study BerbasisSekolah.* Malang: Bayumedia Publishing

CE-8

THE INFLUENCE OF CONSTRUCTIVISM APPROACH IN DIRECT LEARNING TOWARDS STUDENTS' METACOGNITIVE AWARENESS AND LEARNING OUTCOMES IN THE TOPIC OF SOLUBILITY AND SOLUBILITY PRODUCT

Muharram [a*] , St. Hayatun Nur Abu [b], and Jusniar [c]

[a,b,c]*Makassar State University , Makasar, 90224, Indonesia*

ABSTRACT

The study was a quasi-experiment research which aimed to know: (i) the influence of constructivism approach in direct learning towards metacognitive awareness (ii) the influence of constructivism approach in direct learning towards student's learning outcomes on solubility and solubility product subject. The results of the study show that (i) there is no influence of constructivism approach in direct learning towards students' metacognitive awareness ($p = 0.240$, $n = 39$); (ii) there is influence of constructivism approach in direct learning towards students' learning outcomes ($p = 0.000$, $n = 39$). After the learning process, the average of learning outcomes value in control class is 72.25 which is smaller than experiment class , of which the average is 79.47. In the experiment class, 84.21% of the students achieved the standard of mastery and the percengatage of those who achive the standard in the control class is 70% s. The study also shows that there is correlation between metacognitive awareness and learning outcomes. In the experimental class, metacognitive awareness contributes 27.6% ($R = 0.526$, R square = 0.276) to the learning outcomes. While in the control class, metacognitive awareness is contributes 5.6% ($R = 0.237$, R square = 0.056) to the learning outcomes.

Key words: Constructivism Approach, Direct Learning, Metacognitive Awarenes, Learning Outcomes

1. INTRODUCTION

Learning process is an activity involving teacher and student. Teacher, as an educator, teaches knowledge, skill, and morale to students that are useful to their life. A teacher does not act as the only source of learning, however, he can be a facilitator for students to learn. Teacher plays an important role and task in learning process. An achievement obtained by students is not influenced by teacher's knowledge level of learned subject, but also it is determined by the approach and the model of learning that is used.

The problem of learning process is mainly caused by the incorrectness of the design and the plan of learning process causing the learning achievement becomes low and the objective of the learning is not achieved. The problem also occured in the learning outcome of students grade XI IPA MAN 1 in Watansoppeng, academic year 2011/2012 in the topic of Solubility and Solubility Product. The students was difficult to apply the concept and to solve problems because they just tend to memorize. The teacher was also difficult to apply appproach and model of learning beside conventional (behavioristic) since the students were accustomed to be directly informed without struggling to seek for their knowledge by themselves.

Direct learning is one of learning models that is frequently used by teachers. The learning model helps students to acquire intellectual knowledge and procedural steps. That kind of learning requires much effort of teacher to teach. In addition the direct learning is teacher-centered so it needs an approach ensuring the involvement o students in learning process. One of the approaches that can be used is constructivism approach. It refers to a learning approach that puts emphasis on the self-experience and involvement of students in a learning process. During learning process, students actively construct their knowledge. Moreover, it requires the role of teacher to help students establish the relationship between what they have known and what they are learning.

Solubility and Solubility product is one of the topics which requires students not to simply memorize formulas but also to fully understand concepts. Furthermore, the topic covers counting related to mathematical skill which is likely to be achieved in several steps of direct learning model, i.e. guided practice, feedback, and individual practice.

Constructivism approach involves the activities of students in asking question seeking for information, and doing an inquiry. It demands students to apply their favorable learning strategies which entails students' metacognitive awaraness. Basically, metacognition refers to learning ability about how to apply learning in which it well applies several activities. (Maulana, 2008). Students, with their metacognition, are aware of their strenghts and limitations in learning so they can make an improvement. (Susantini, E. dkk in Susana, 2011). Consequently, it is highly necessary to evoke students' metacognitive awaraness to manage their own learning to be more self-sufficient. Additionally, constructivism is a process of forming or constructing new knowledge in students' cognitive structure based on their experience (Sanjaya, 2008). Constructivism theory states that students should find and transform complex information by themselves, check new information by comparing it with previously known theories, and even revise the theories when they are not correct (Trianto, 2008).

Constructivism learning emphasizes on active, creative, and productive self-construction of understanding. Knowledge is a sequence of facts, concepts, and rules which are ready to apply. Humans should firstly construct their knowledge and give meaning through empirical experience (Muslich, 2009). During learning process, students construct their own knowledge through active involvement in which students, not teacher, are the centre of activities (Mulyana, 2011).

Learning activities in constructivism learning are directed to the empowerment of students' potency to have some required skills comprehensively (Srini & Lina, 2011). In that, teachers have important task in facilitating tha kind of process by (a) making students learn meaningfully and acquire relevant information; (b) leading students to acquire and apply their own ideas; and (c) making students aware of their own strategy application in learning.

There are several characteristics of constructivism learning, namely (1) providing learning experience in taking the advantage of students' own knowledge to create new knowledge construction process, (2) providing several learning experience alternatives, for instance, giving open problem to students to solve with various answers, (3) integrate learning process with realistic and relevant situations involving concrete experience, for example to understand a concept through the reality in everyday life, (4) integrating learning process that allows social transmission of interaction and collaboration between students and teacher, and among students, (5) utilizing some media including oral and written communication so that learning process bocomes more effective.

Metacognitive is an adjective of metacognition. Metacognition is often defined as "thinking about thinking". Metacognition can't be defined simply. Metacognition word contains the prefix "meta" and "cognition". Meta comes from Greek, which means after or exceed while cognition means skills related to thinking (Livingstone, 2003). Metacognitive consist of two main components, namely metacognitive knowledge and metacognitive regulation (Flavel in Danial (2010)). Metacognitive knowledge refers to knowledge about cognition as skills knowledge (skills) and work strategies for students and how and when using skills and strategies. Furthermore, metacognitive regulation refers to the activities that control a person's thinking and learning such as planning, monitoring comprehension, and evaluation (Schraw and Dennison in Danial (2010)). This metacognitive activities comes through four situations, namely (Maulana, 2008): (a) students are required to justify a conclusion or **keep** the objection, (b) cognitive situation in face the opening problem of the opportunities to make questions, (c) students are required to make a conclusion, judgment, and a right decision which requires carefulness in monitoring and regulating the cognitive process, (d) the students' situation in cognitive activities experience difficulty, for example in problem solving.

Metacognitive becomes very important because it is a knowledge derived from cognitive process and their products. Students' metacognitive awareness which develops shows that students are more accurate in controling and monitoring students learning (Miranda, 2010).

Learning outcome is achieved by students' score after learning. Learning outcomes can be measured by learning outcomes test. Generally, a teacher uses a test as a measuring tool. The results can give an illustration about students' mastery level to the learned lessn (Haling, 2007).

2. METHODS

This study was a quasi experiment using pretest-posttest control group design. It is consist of two variables, namely independent variable and dependent variable. The approach in direct learning as independent variable consists of constructivism approach and behaviorism approach. Meanwhile the dependent variables are metacognitive awareness and learning outcomes as dependent variable.

This research uses descriptive statistical analysis in students' learning outcomes description on solubility and solubility product subject for each experimental class, which includes mean, standard deviation, and the highest and the lowest scores. Students' learning outcomes can be categorized into complete and not complete based on minimal completeness criteria at MAN 1 Watansoppeng on solubility and solubility product subject. A student's learning outcome is categorized complete if the student's level of mastery is more than or equal to seventy, while students who have the degree of mastery less than seventy is categorized not complete. The score of students' metacognitive awareness was obtained by MAI questionnaire that convert it to students' score.

Students' score = $\frac{students'score}{total\ score}$ x 100

Total score is obtained from the number of item on questionnaire (52 items) multiplied by the highest score in each item (4). Total score is 52 x 4 = 208. Furthermore, score is categorized based on the categorization of metacognitive awareness (Green in Susana(2011)) which is shown at Table 1.

Table 1 Categorization of Metacognitive Awareness

Score Interval	Category	**Ket**
0-20	**Very Risk**	**Msb**
21-40	**Not developing**	**Bbb**
41-60	**Starting to develope**	**Mb**
61-80	**Well developing**	**Bb**
81-100	**Very well developing**	**Bsb**

Inferential statistic analysis was used in hypothesis test. Normality test and homogeneity test were done before testing the hypothesis, using SPSS 16 for windows. The Normality test of students' learning outcomes and metacognitive awareness was applied using SPSS 16 of *One-Sample-Kolmogorov-Smirnov Test* analysis. The criteria of the test is that if (p) $< \alpha = 0{,}05$ then the data is not normally distributed, conversely if (p) $> \alpha = 0{,}05$ then the data is normally distributed.

Homogeneity test was conducted to determine whether the data are homogeneous. Homogeneity test of students' learning outcomes variance data and MAI questionnaire use SPPS 16 of *Levene Statistic* analysis. The criteria of test is if (p) < α = 0,05 then variance of data group is not same, conversely if (p) > α = 0,05 then variance of data group is same.

Hypothesis test toward students' learning outcome and students' metacognitive awareness was done using SPSS 16 of *Covariance Analysis*. The formulation of hypothesis Ho is that there is no difference in students' metacognitive awareness between constructivism approach and behaviorism approach in direct learning. Meanwhile, that of H_1 is that there is difference in students' metacognitive awareness between constructivism approach and behaviorism approach in direct learning. The criteria of test if (p) < α= 0,05 then Ho is rejected; which means that there is difference in students' metacognitive awareness between constructivism approach and behaviorism approach in direct learning.

This study also used additional analysis to determine the relation between metacognitive awareness and learning outcomes. That relation was analyzed by simple linear regression analysis using SPSS 16. Metacognitive awareness correlated with learning outcomes is based on the significant value (p) < α= 0,05. R square value indicates the percentage contribution of metacognitive awareness toward learning outcomes.

3. RESULT AND DISCUSSION

a. The influence of constructivism approach in direct learning toward metacognitive awareness

Based on the result of the students' metacognitive awareness research, the students' metacognitive awareness both in experimental class and control class, before the learning is implemented, is in the category of well developing. After learning using constructivism approach and behaviorism approach is respectively applied in the experimental class and the control class, it shows that there is no significant difference between the two classes in the term of metacognitive awareness improvement. The increase average score of students' metacognitive awareness at experimental class is higher than control class. The average score at experimental class is 3,2326 higher than control class. There is no significant difference in students' metacognitive awareness between experimental and control classes caused by metacognitive awareness will not be presented without stages systematically and to improve metacognitive awareness takes time for students to control their thinking process.

The number of students with increasing metacognitive awareness from the pretest to posttest at experimental class is 23,95 % higher than control class. The improvement of metacognitive awareness is caused by students' knowledge about skill and work strategy, as well as students' ability to control their thinking and learning outcomes, for example planning, monitoring of comprehension, and evaluation. There are 5,26 % of students at the experimental class that have the same score at pretest and posttest. This result accordance to Susanti E., et.al (2004) in Susana (2011) that most of students improve their metacognitive awareness step by step, and the others are not improve. The students metacognitive awareness score decrease of pretest to posttest at control class is 29,21 % higher than experimental class. The decrease of metacognitive awareness is caused by some of students had difficulty in understanding MAI questionnaire, so that the answer of students is less precise with themselves. The students felt bored to read and understand every item because the number of item on MAI is pretty much. Most of students looked apathetic and rush to finish MAI even provided the maximum time in process.

The analysis of Covariance shows that p = 0,240 > α which implies that there is no difference in students' metacognitive awareness between constructivism approach and behaviorism approach in direct learning. Based on the analysis of difference in metacognitive awareness of students between those who are given treatment of constuctivism approach and those who are given behaviorism approach in direct learning and the hypothesis test, it can be concluded that there is no influence of constructivism approach in direct learning toward students' metacognitive awareness.

Students' metacognitive awareness at each class increases, but it's not significant, which means that the approach is different. In addition the result doesn't change significantly and the statistical test shows there is no influence. Based on the factors that can increase metacognitive awareness, not all students can increase their metacognitive awareness. Contructivism approach is not able to make students think systematically in solving the problem and making conclusion. Moreover, students' initial knowledge is low causing students feel difficult to relate the previous subject with topic of solubility and solubility to find new concept. Besides that, not all students make a question or answer the question from teacher. Another factor is that some of the students judge well themselves without keeping attention and understanding the process during learning which consequently causes their answers are less precise compared to the expected answers. These results are supported by Arifin (2012) which suggested that there is no significant difference in students' metacognitive awareness between PBI group and TPS group.

This research is also accordance to Danial (2010) research's result that there is no influence on learning strategy towards students' metacognitive awareness. The same opinion by Susana (2011) on her research's result, shows that metacognitive learning model is not influence to metacognitive awareness. However, the different opinion by Warouw (2009) in Thahir (2012), said that using learning strategy will influence students' metacognitive awareness.

The result on Miranda research also support the theory of Blake, Spence, dan Sheila (1990) in Miranda (2010), said that metacognitive ability can be improved if the students is allowed manage themselves in learning strategy. Constructivism approach in direct learning is considered can be built students' metacognitive awareness compare with behaviorism approach in direct learning. This approach give an opportunity of students to found and apply their own idea, so needed metacognitive awareness. Constructivism approach provide learning experience, it is make relation between the previous subject with the subject being studied, so that learning is a process of knowledge formation. Each students have a different task, so that they have to apply their own strategy. Besides that, students participate emotionally then interesting in study. Students suggested to make the right conclusion, consideration, and decision. Therefore, it needed learning strategy and the accuracy of students in monitoring and managing cognitive process (Maulana, 2008). Constructivism approach in direct learning shows learning process that related with metacognitive situation compare with behaviorism approach in direct learning. Behaviorism approach doesn't give opportunity to students on improve strategy, idea or thinking process. It is accordance to Anonym (2012) said that behaviorism approach means students expected have the same comprehension. It means what teacher know should be known by students

Based on that statement, it shows that measuring students' metacognitive awareness using MAI questionnaire is not able to record students' metacognitive awareness well. The students tend to hide their weaknesses but also they don't show their abilities, so their answers are less accurate, then there is no significant difference in students' metacognitive awareness between constructivism approach and behaviorism approach in direct learning.

b. The influence of constructivism approach in direct learning toward students' learning outcomes

Based on the result of the descriptive statistical analysis, there is no significant difference on THB pretest score before learning process between experimental class and control class. The difference result shows after learning process. Constructivism approach in direct learning on experimental class is better than behaviorism approach in direct learning on control class. The increase of the average students' learning outcomes on experimental class is higher than control class. Standard deviation of posttest on experimental class is lower than control class, the ability of students at experimental class are not really different. It's caused learning at experimental class using constructivism approach that require students to found or build their own knowledge by themselves, so the students can remember longer than the students who get knowledge from the teacher.

The result of inferential analysis using *SPSS for Windows* of *Covariance Analysis*, significance value $p = 0,000 < \alpha = 0,050$ shows that Ho is rejected, it means there is difference in students' learning outcomes between constructivism approach and behaviorism approach in direct learning on solubility and solubility product subject in Grade XI Science MAN 1 Watansoppeng. This result also supported by Elvinawati (2011) who concluded that the application of constuctivism and maps model concept can improve students' activity and learning outcomes on chemistry separation subject.

The difference in students' learning outcomes between constructivism approach and behaviorism approach in direct learning is caused emphasize on build comprehensive by themselves in constructivism approach in direct learning. Mulyana (2011) also said that during learning process, students build their knowledge by themselves and a center of activity is not a teacher but it is a student. Teachers will not be able give all their knowledge to students. Students should construct their knowledge. Therefore, students have to apply their own learning strategies so that they become independent students. When students have a different task, they can solve the problem by their own ways. The involvement of students in learning process to found knowledge by themselves and become an independent student can increase students' leaning outcomes. The difference of learning outcomes shows by students with behaviorism approach, the percentage of completeness and the average of learning outcomes still lower than learning outcomes with constructivism approach. Behaviorism approach doesn't give an opportunity to students on found knowledge by themselves because the subject is inform by teacher directly step by step.

Based on the difference of students' learning outcomes in constructivism approach with behaviorism approach in direct learning and hypothesis test, can be concluded that there is influence constructivism approach in direct learning toward learning outcomes. The number of students in each class at XI Science MAN 1 Watansoppeng only consist of 20 students, it makes teacher easier to explore their knowledge and manage students to found their own knowledge. The knowledge on students with constructivism approach is retained longer than students with behaviorism approach.

Solubility and solubility product subject is a subject that requires comprehension concept not only memorize the formula or material. The comprehension can be achieved if students thinking creative with their strategy to found knowledge or solve the problem. Comprehension concept will make students easy on solving the exercise then increase learning outcomes. It is accordance to Larasati (2007) for two cycles of learning, the result of her research said that learning pythagoras theorem is effective by constructivism approach.

This research also analyzes the relation between metacognitive awareness and learning outcomes. The relation between metacognitive awareness and learning outcomes can be determined by regression test. The result of data analysis shows that there is correlation between metacognitive awareness and learning outcomes at experimental class ($p = 0,001 < \alpha = 0,05$). The correlation coefficient between metacognitive and learning outcomes is 0,526. The percentage contribution of metacognitive awareness toward learning outcomes is 27,6 % or R square 0,276 while the remaining 72,4 % is influenced by other variable which not include on this model. The difference result is showed by control class, i.e. there is no relation between metacognitive awareness and learning outcomes ($p = 0,141 < \alpha = 0,05$). Low correlation coefficient between metacognitive awareness and learning outcomes is 0,237. The percentage contribution of metacognitive awareness toward learning outcomes is 5,6 % or R square 0,056.

Conclusion and Suggestion

Based on the research results and the discussion, it can be concluded that: (1) there is no influence of the approach in direct learning toward students' metacognitive awareness, (2) there is influence of the approach in direct learning toward students' learning outcome

Based on the research results, it is suggested that: (1) teacher can consider the use of constructivism approach as one of the alternatives in learning since the approach can increase students' activeness and make students independent implying that their learning outcomes improve (2) further research of the approach is necessary to conduct in aiming of obtaining valuable information of implementing constructivism approach effectively and efficiently. (3) the measurement of metacognitive awareness using MAI shall be supposed to be revised without changing its meaning which consequently be able to memorize the personal behavior of students.

REFERENCES

Arifin. 2012. *Perbandingan Kesadaran Metakognitif Dan Hasil Belajar Kognitif Biologi Siswa SMA Yang Diajarkan Menggunakan Model Problem Basedintruduction (PBI) Dengan Kooperatif Tipe Think Pair Share (TPS) (Studi Pada Siswa Kelas X SMA Negeri 1 Sungguminasa)*. Tesis. UNM

Danial, M. 2010. *Menumbuhkembangkan Kesadaran dan Keterampilan Metakognisi Mahasiswa Jurusan Biologi Melalui Penerapan Strategi PBL dan Kooperatif GI*. Disertasi tidak diterbitkan. Universitas Negeri Malang. Malang.

Elvinawati, 2011. Optimalisasi Pembelajaran Kimia Pemisahan Melalui Penerapan Pendekatan Konstruktivisme dan Model Peta Konsep. *Jurnal Exacta*, (online).Vol.IX.No.1.(http://repository.unib.ac.id/503/1/04.%20Elvinawati%20Hal.%2023-28.pdf, diakses 23 Mei 2013).

Haling. 2007. *Belajar dan Pembelajaran*. Badan Penerbit UNM: Makassar.

Lasati. 2007 Penerapan Pendekatan Konstruktivisme pada Pembelajaran Teorema Pythagoras di Kelas 8 SMP. Jurnal Pendidikan Inovatif, (online), Vol.23, Nomor 1. (http://jurnaljpi.files.wordpress.com/2009/09/vol-3-no-1-dwi-lasati.pdf, diakses 23 Mei 2013).

Livingstone,J. 2003. *Metakognitive An Overview*. (http://gse.buffal.cdu/gwell/files/coursesfolder/documents/livingstonemetakognition.pdf. diakses 23 Mei 2013)

Maulana. 2008. Pendekatan Metakognitif Sebagai Alternatif Pembelajaran Matematika Untuk Meningkatkan Kemampuan Berpikir Kritis Mahasiswa PGSD. *Jurnal Pendidikan Dasar,* (online). Nomor 10 (http://file.upi.edu/Direktori/JURNAL/PENDIDIKAN_DASAR/Nomor_10Oktober_2008/Pendekatan_Metakognitif_Sebagai_Alternatif_Pembelajaran_Matematika_Untuk_Meningkatkan_Kemampuan_Berpikir_Kritis_Mahasiswa_PGSD.pdf, diakses 24 Desember 2012)

Miranda. 2010. Dampak Pembelajaran Metakognitif dengan Strategi Kooperatif terhadap Kemampuan Metakognitif Siswa dalam Pelajaran Biologi di SMA Negeri Palangkaraya. *Jurnal Penelitian Kependidikan*. (online) TH.20, NO. 2, Oktober 2010 (http://www.scribd.com/doc/95515105/96, diakses 24 Desember 2012).

Mulyana. 2011. Laporan PTK: Kontribusi Penerapan Model Pembelajaran "Card Sort" Berbasis Pendekatan CTL Terhadap Peningkatan Hasil Belajar Siswa VII. SMPN 1 Cadasari, (online), (http://4shared.com/document/BnvA57H1/Laporan_PTK_Kelas-7.html, diakses 24 Desember 2012)

Sanjaya, W. 2008. *Strategi Pembelajaran Berorientasi Standar Proses Pendidikan*. Jakarta : Prenada Media Group.

Siroj, R.A. 2004. *Pemerolehan Pengetahuan Menurut Pandangan Konstruktivistik*. (http://www.depdiknas.go.id/Jurnal/43/rusdy-a-siroj.htm, diakses pada tanggal 2 Februari 2013).

Susana. 2011. *Pengaruh Model Pembelajaran Metakognitif terhadap Kesadaran Metakognitif, Keterampilan Metakognitif, dan Hasil Belajar Kogntif Siswa SMN 1 Palopo*. Tesis. Program Pascasarjana UNM.

Srini & Lina. 2011. *Peningkatan Kualitas Pendekatan Pembelajaran Kimia (Kma 523) Menggunakan Tugas Strategi Konstruktivis Dan Blended Learning* . Makalah disajikan dalam Seminar Nasional Penelitian, Pendidikan dan Penerapan MIPA, Universitas Negeri Yogyakarta, 2 Juni 2012.

Thahir. 2012. *Pengaruh Metode Survey, Question, Read, Recite, Review Terhadap Kemampuan Metakognitif Dan Hasil Belajar Materi Ekosistem Siswa SMA*. Tesis. UNM.

Trianto. 2008. *Mendesain Pembelajaran Kontekstual (Contxtual Teaching and Learning)* di Kelas. Jakarta: Cerdas Pustaka Publisher

Trianto, 2009. *Model-Model Pembelajaran Pembelajaran Inovatif Berorientasi Konstruktivistik*. Jakarta: Prestasi Pustaka

Anonim,. 2012. Teori Belajar Behavioristik. (http://id.wikipedia.org/wiki/Teori_Belajar_Behavioristik, diakses 14 Juli 2013).

CE-9

THE USE OF WEB-BASED ASSISTANCE IN MULTIMODAL CHEMISTRY LEARNING AT SENIOR HIGH SCHOOL TO IMPROVE STUDENTS' MOTIVATION

Nuke Ajeng Prabawati[1] and Jaslin Ikhsan[2]

The Department of Chemistry Education, Faculty of Mathematics and Science
Yogyakarta State University, Karangmalang, Yogyakarta 55281 INDONESIA
[1]nuke.ajeng.p@gmail.com, [2]jaslinikhsan@gmail.com

Abstract

The development of information and communication technology (ICT) affects significantly education sector. Nowadays, students are very familiar with ICT, such as computer and Internet which has the advantage to use as media in chemistry learning. The use of student-friendly application of a web based learning management system (LMS) that completed by chemistry multimedia can be benefit for chemistry learning because the multimedia in the web can be accessed anywhere at any time as potential assistance for students. Learning was managed through various learning strategies in the combination of web-based assistance and face-to-face learning in cooperative model of student team achievement division (STAD) which called multimodal learning. This research investigated the effects of multimodal learning on chemistry towards students' motivation.

This research was an experimental research to measure the improvement of students motivation due to multimodal learning. The samples of this research were 2 groups of students, which are experimental group consisting of 30 students and the control group having 31 students. Samples were from grade X of SMA N 7 Purworejo. The difference of chemistry learning between students from the experimental group and control group was learning with and without multimodal learning, respectively. The students' motivation was collected using a questionnaire, observation, and analyzed statistically. Students' motivation from one group was compared to the another group using independent sample t-test, and the improvement of students' motivation was analyzed using paired-sample t-test.

The result of this research showed that students' motivation of group with multimodal learning was higher significantly than that without multimodal learning.

Key words: Multimodal Learning; Cooperative Learning; Web-Based Learning; Learning Management System.

INTRODUCTION

The phenomenon of globalization presents education systems around the world with both challenges and opportunities (Kagan and Stewart, 2005). Students nowdays are native ICT persons. They are familiar with ICT such as gadget, computer, and their application. This condition should be considered as opportunities to improve methods of learning that can strengthen the motivation of students. This is the challenge for teachers to provide more application or media of learning and help student to solve the difficulties encountered by students, including chemistry.

Chemistry is the academic discipline concerning on the study of the composition, structure, properties, and speed of change of matter (George Philander, 2008). Chemistry is intangible and considered as a difficult matter by learners. This is a challenge for teachers to create exciting learning model that can help students to comprehend the chemistry matter. One of alternatives to solve such problems is made by using a multimodal learning in the chemistry learning process. Multimodal learning is a classroom strategy that supports students' acquisition of new knowledge by pairing digital tools with requisite other strategies (Casey Heather, 2012).

Integration various teaching was a teaching–learning is known as multimodal learning (Dominic, 2012). In the implementation of multimodal learning, teacher can use some related media that suitable with chemistry matter or even use one of learning management system (LMS) like edmodo. Teacher can also combine the learning system with cooperative learning model.

Cooperative learning is a strategy in which small groups of students with different levels of ability engage in a variety of activities to improve their understanding of the topic (Eugene, 2009). There are certain types of cooperative learning. One of them is student team achievement division. It is a collaborative learning strategy in which small groups of learners with different levels of ability work together to achieve a shared learning goal (Tiantong, 2013).

This research investigates the use of various strategies of learning accomplished by the media run in the methods of cooperative STAD and its effect on the students' motivation. There are 4 purposes in this research. The first is to find out whether there are differences in the initial motivation and motivation of students who apply late STAD cooperative learning multimodal web-based on material reduction and oxidation. The second purpose is there a difference in motivation of learners who implement cooperative learning multimodal web-based group investigation compared to implement STAD cooperative learning on the web without material reduction and oxidation. For the third purpose is there any difference in increased motivation of learners who implement cooperative learning multimodal web-based group investigation compared to implement STAD cooperative learning on the web without material reduction and oxidation. And the last whether there is a significant increase on the motivation of learners who implement cooperative learning multimodal web-based group investigation.

RESEARCH METHOD

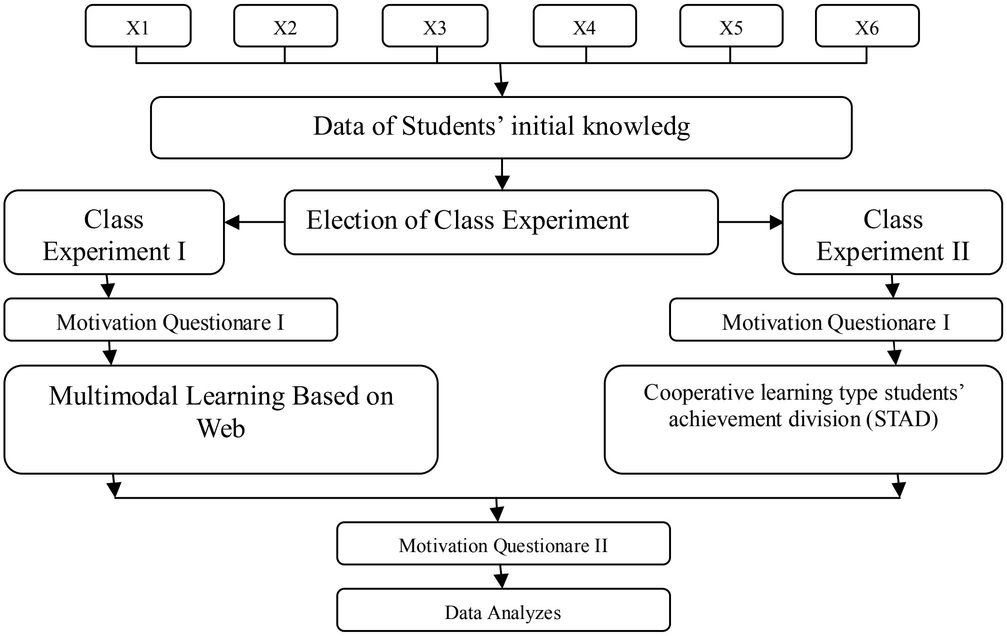
This research is an experimental research which has one factor, two samples, and one covariable. The factor is the implementation of cooperative multimodal learning of group student team achievement division (STAD), based on web. Two samples are students from class experiment and control. The class experiment I was class that used cooperative multimodal learning of student team achievement division (STAD) based on web and class control is class that implement cooperative of student team achievement division (STAD) without web. The covariable is students' chemistry prior knowledge taken from the school documentation.

There are three variables in this research. They are independents variable, controlled variable, and dependent variable. The independent variable is the implementation of cooperative multimodal learning of student team achievement division (STAD) based on web. The controlled variable is the students' chemistry prior knowledge, and the dependent variable is the students' motivation on chemistry learning. Chemistry learning motivation was measured by using a questionnaire, and the collection was done before and after the experiment.

Population of this experiment was grade tenth students of science class at SMAN 7 Purworejo in 2013/2014 academic year consisting of 156 students. The sample was chosen by purposive sampling method which was then divided into class experiment and control.

The instruments of this research consist of lesson plan, questionnaire and observation check list. The lesson plan was prepared for five meetings for each class. The lesson plan for class experiment is the lesson plan that implement cooperative multimodal learning of student team achievement division (STAD) based on web and the lesson plan for class control is the lesson plan that implement cooperative of student team achievement division (STAD) type without web. The instrument should be valid and reliable. The questionnaire for motivation was validated by construct validation method. The design of this experiment can be observed in the diagram 1.

Diagram.1 : Flow Chart of Research



The media that used in this experiment class are power point, prezi, and e-book. This media has been validated by the validate (chemistry teachers and peers). There are some relevant notes from validatory about the look and lay out of writing such as writing a reaction, phase, or other type errors. While the content of the media was good. Notes from validatory were then used to revise and corrected the errors.

Data analysis included normality and homogeneity tests, before the paired sample t-test and the independent sample t-test. Normality was to find out that the data are in normal distribution. Homogeneity test was the test to check the homogeneity of the population. Paired sample t-test is to analyze the improvement of students' motivation before and after treatment, and independent sample t-test was to determine the difference of students' motivation from the experimental group compared to that from the controled group. When the improvement of students' motivation was significant, therefore, average normalized gain test was conducted.(Meltzer in Wiyono, 2013).

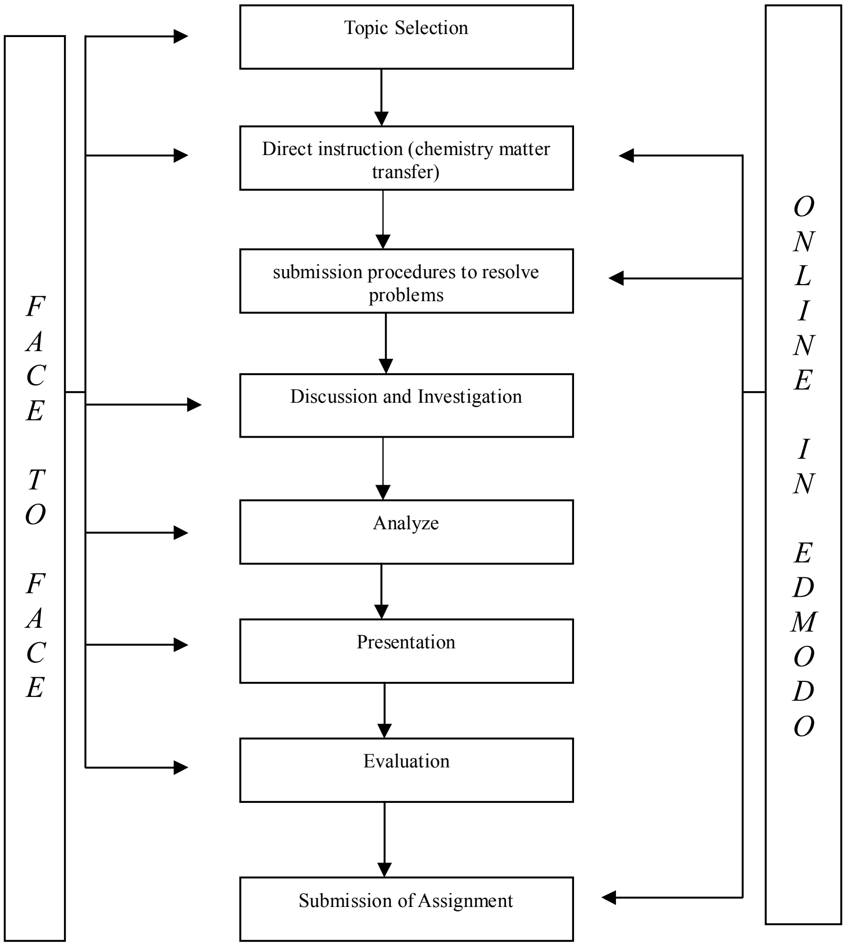
RESULT AND DISCUSSION

1. Design of the cooperative multimodal learning of student team achievement division (STAD) type based on web

Cooperative multimodal learning of student team achievement division (STAD) based on the web was conducted for five meetings. i.e. Four times of meetings was for teaching learning and once for the final test. Teaching and learning were always delivered with a group setting. Teacher divided students into six equal heterogen groups with different levels of students competencies. According to Slavin (2010), Student Team Achievement division (STAD), learners are grouped into several groups with diverse members of ability, gender, race and ethnicity. Students were not permitted to pick their own teams. Educators delivered learning materials in a face to face meeting and then the students were given a task that must be completed in a group to ensure that all members of the group have completed the lesson well. In this group, the smart student must help the other students who got difficulties in understanding learning matters. At the end of meeting, all learners acquired individual quiz on teaching materials and at the time the individual should not help each other because it was about team achievement division to which a reward was given to a good team.

Each group received instruction consisting of the list of work procedures, learning materials in the format of multimedia (e-book, Prezi and Power Point) in Edmodo, so the group can start to investigate, analyze, and synthesise the topic. Learning of Chemistry in this research was designed by using the mixture of face to face mode and other modes with the use of some multimedia. This study of learning was called multimodal learning. In this experiment, there were four of learning strategies has been implemented. They were demonstration, experiment, discussion, and games. In the first meeting, learning was on the topic of reduction and oxidation reactions in daily life by demonstration approach. With this approach, students' interest toward chemistry could be built and their motivation increased. At the second meeting, experimental strategy of learning was applied. In this meeting, students understood the learning matter well. At the third meeting, discussion strategy was utilized. At the last meeting, learning in games was chosen to study the compound nomenclature. In each meeting, students from all groups can ask the teacher about the tasks. The results of their task/assignment were presented in front of the class and uploaded to edmodo, so the other group was able to download and do cross check about their answers. Each group finally presented group discussion result, and the other groups raised questions. When the presentation and question time was over, each group answered questions listed on the work sheet. The best performance group will be given more stars or points that were accumulated in the final meeting. Teacher gave a reward to the best team. Teacher also had to confirm and emphasized the answers in the discussion group. So, this went to minimize the misconception that may occur. Teacher and students took the conclusion from learning process. The design of multimodal learning in the class of experiment was presented in the diagram 2.

Diagram.2 : Description of the Class Experimental



Based on the results, teaching learning in the class of experiment was more active than that in the class of control. Students were enthusiastic and they understood the topic well. So, the implementation of cooperative multimodal learning of student team achievement division (STAD) based on web was able to provide good learning environment generating students' joy of learning so that students learned independently, active, and high motivation.

2. Normality and Homogenity of Data

The data of students' motivation were analyzed for normality using SPSS 16.0. For the class experiment, the significance of the students' final motivation data was (p) 0.200 > (α) 0.005. The significance for class control was (p) 0.101 > (α) 0.005. All significances of the data were higher than 0.05, all sets of data were in normal distribution. The homogeneity test results showed that the sample was homogen, as showed by the significant value was more than 0.05, (p) 0.479 > (α) 0.005.

3. The Effect of Multimodal Learning toStudents' Motivation

Analysis of data using paired sample t-test showed that the significance (p) value was 0.000 < (α) 0.005. It meant that the motivation of students improved significantly in class experiment due to the multimodal learning, but in the class control, the motivation did not improve, as the p value was (p) 0.138> (α) 0.005.

In the independent sample t-test, the result analysis showed that the significant (p) value is 0.000 < (α) 0.05. It meant that there was a significant difference between students' motivation in class experiment and class control due to multimodal learning based on web. There was also significant magnitude improvement of the class experiment compared to class control, as can be seen from the significant value of (p)0.000 < (α) 0.005. The gain test result showed that the significant improvement of students' motivation in class experiment was fair not quite high, with the average normalized gain test results of 0.26 (26%) of motivation improvement. The factors that may influence the improvement were:

a. Cooperative multimodal learning of student team achievement division (STAD) based on web was able to increase students' interest and the curiosity. As stated by Latifah (2008) that cooperative learning can strengthen students' motivation in different ways, like encouraging group development, promoting social and academic interaction among students, and encouraging successful group participation. This research showed that students' curiosity increased significantly after learning reduction and oxidation matter using the student team achievement division (STAD) in Edmodo. Besides that, teacher also gave some quiz in each face-to-face learning process and if students could solve the problems fast and right, they got more stars/points.
b. Cooperative multimodal learning of student team achievement division (STAD) based on web provided good learning environments. Fathul Imam (2013), said that Edmodo is a social networking platform that was designed as a medium of interaction between teachers and students in the online learning environment that was safe to share data, information and educational contents in the form of writings, documents, video, audio, photos, calendar, links that can be distributed either by teachers and students as well as special content in the form of grades, quizzes, event activities, assignments and polls by teachers that can be done anytime and anywhere, as well as parents can control the course of the study. So, student interaction with the teacher was more intensive and it can help students to better understand chemistry matter easily.
c. Cooperative multimodal learning of student team achievement division (STAD) based on web made learning more attractive and fun. Scott Amstrong and Jesse Palmer (1998) stated that use of STAD in the social studies classroom made learning fun, and students could understand the contents of learning easier.
d. Multimodal learning that completed by some media can give opportunities for students to choose media that were appropriate to their interest. Casey Heather (2012) said that the use of technology and digital modes of texts offered further opportunities for engagement, in part, because students found these tools inviting, but also because they had more opportunities to work with materials that were specific to their individual needs.

Based on the result, the conclusion is the cooperative multimodal learning of student team achievement division (STAD) based on web can improve student's motivation.

CONCLUSION AND SUGGESTION

The conclusion of this research are.

1. There was significant difference between initial motivation and final motivation of students in class that implemented multimodal learning based-web on material reduction and oxidation.

2. There was significant difference of motivation of students from the class that used multimodal learning based on web with the class which used cooperative learning type STAD without web.
3. There was signifficant difference of magnitude improvement of students' motivation from the class that used multimodal learning based on web with class which used cooperative learning type STAD without web.
4. There was little signifficant improvement of the students' motivation in the class that use multimodal learning based on web.

Authors would like to suggest:

1. If further research or similar research is carried out, the media should be validated empirically beforehand to see how effective the media when used by learners.
2. If a similar study is conducted, instructional media that are developed should be more innovative, variatif, and as attractive as possible so that students will be more interested in using it.

REFERENCES

1. Sharon Lynn KaganandVivienStewart,. (2005). *Education in a Global Era*. Phi Delta Kappan. Vol. 87. p185-187.
2. Ed. S. George Philander. (2008).*Encyclopedia of Global Warming and Climate Change.* Vol. 1. Thousand Oaks, CA: SAGE Publications. p189-193.
3. Heather Casey,.(2012).*Multimodal Learning Clubs.* Middle School Journal. Vol. 44. p39-48.
4. Dominic W. Massaro. (2012). *Multimodal Learning*: *Encyclopedia of the Sciences of Learning*. Universitas California, p. 1.
5. Eugene F. Provenzo, Jr. (2009).*Encyclopedia of the Social and Cultural Foundations of Education.* Ed. Vol. 1. Thousand Oaks, CA: SAGE Publications. p182-183.
6. MonchaiTiantong andSanitTeemuangsai,. (2013).*Student Team Achievement Divisions (STAD) Technique through the Moodle to Enhance Learning Achievement*. Vol. 6. p85-92.
7. Latifah A. Alharbi. (2008). *The Effectiveness of Using Cooperative Learning Method on ESL Reading Comprehension Performance, Student's attitudes toward CL, and Student's Motivation toward Reading of Secondary Stage in Saudi Public Girls' Schools*. Dissertation. West Virginia University.
8. Fathul Imam. (2013). *Efektifitas E-learning Melalui Media Edmodo Terhadap Hasil Bealajar Peserta Didik Pada Materi Statistika SMA.* Thesis of IKIP PGRI Semarang : Semarang.
9. Scott Armstrong and Jesse Palmer. (1998). Student Teams Achievement Divisions (STAD) in a twelfth grade classroom: Effect on student achievement and attitude. ***Journal of Social Studies Research***. Vol. 22.1 . p3.
10. Robert E Slavin. (2010). *Cooperative learning teori riset dan praktek.* Bandung: Nusa Media.
11. Wiyono. (2013). Pembelajaran Matematika Model Concept Attainment Meningkatkan Kemampuan Pemecahan Masalah Materi Segitiga. Journal of Education Research and Evaluation. Vol. 1. P51-54.

CE-10

THE DEVELOPMENT OF INORGANIC CHEMISTRY LEARNING MODEL BASED ON PORTFOLIO ASSESSMENT

Ramlawati[1] and Melati Masri[2]

[1,2]Chemistry Department, Makassar State University
Email: ramlawaty65@yahoo.com

Abstract

This study is a research and development that aims to produce the Inorganic Chemistry in learning model based on portfolio assessment. The research and development model was used is the Gall et al. (2003) model which consists of four stages: a preliminary study , the model design phase, design development and testing models phase. The subjects were 46 students of the third semester of Chemical Education who enroll a course of Inorganic Chemistry II in 2013/2014. Pilot phase model using a pre-experimental design. Implementation of the portfolio in the lecture through five stages, namely : planning assessment portfolio, portfolio collect, select and reflect on selected products, improve and evaluate products, utilizing the results of the assessment portfolio. The results showed that the Inorganic Chemistry Learning model based on portfolio assessment of generated include in the valid and practical criteria. The effectiveness of the model is 73.6 %.

Key words: Learning Model, Inorganic Chemistry, Portfolio Assessment.

INTRODUCTION

The implications of changes in the curriculum is alteration paradigm in the education assessment system, i.e. assessment system that emphasizes on learning outcomes (assessment of learning) becomes holistic assessment that includes planning, processes, and outcomes of learning (assessment for learning). The assessments typically use traditional assessment (e.g., true- false, multiple choices, fill in, short answer, and essay) turns into the implementation of various types of authentic assessment. That is, the assessment conducted by educators for learning should be corresponding to the real world situation. This assessment is intended to educators and learners be able to monitor the process, progress, and improvement of student learning outcomes. it is consist of the principles implementation of the assessment proposed by the National Education Standards Agency (BSNP) that educational assessment is the series process of activities to analyze and interpret the data about process and learning outcomes of students who carried out a systematically and continuously, so that the results of these assessments can be a significance information in decision-making (BSNP, 2009).

One of the assessment systems appropriate in this objective is portfolio assessment. Portfolio assessment is an assessment system that uses a collection of student work that demonstrates achievement or improvement obtained by the students from learning process (Stiggins, 1994). Therefore, the assessment should give an idea of the learning process, the assessment of learning is not only carried out at the end of learning but also executed during the learning process. In other words, assessment is an integral part of the learning process.

Portfolio assessment included in the authentic assessment. This assessment can be used to determine the level of achievement and competence development of learners based on collection of work from period of time (Arifin, 2011). Therefore, the works or tasks that are documented by the students are all aimed to the achievement of competencies or standards that have been targeted in the study.

In order to the implementation of an integrated assessment of learning, then this assessment system can be a basis in the development of learning model. Inorganic Chemistry learning with assessment-based on learning model that allows students to collect a portfolio of tasks associated with materials Inorganic Chemicals more a description of the concept of reading and monotonous. It supports students to improve their competence through the completion of the portfolio tasks.

A Key element of the development model is to improve the competence of student learning by working on the tasks and feedback. Feedback in portfolio assessment requires the students to reflect on and self-assessment. These elements are the basis for developing learning models of Inorganic Chemistry. Assessment reform Group (ARG) revealed that the success of role assessment in improving learning is determined by five key factors, namely: 1) the availability of effective feedback to students; 2) the active involvement of students in learning; 3) teaching adjustment to adjust the assessment results; 4) recognition of the influence of assessment on motivation and self-esteem of students; and 5) the students need to be able to judge themselves and know how to fix it (ARG, 1999).

Accompanying the principle that portfolio assessment is an integral part of learning process, the characteristics of learning model was developed based on the characteristics of learning model proposed by Arends (1997), which has: 1) learning objectives to be achieved; 2) The theoretical rationale 3) teaching behaviors necessary for these models can be implemented successfully; and 4) the learning environment necessary for learning objectives can be achieved.

The objective of development Inorganic Chemistry-based on learning model portfolio assessment is to improve students' competencies as defined in the course syllabus of Inorganic Chemistry. Rational development of theoretical models based on the principle of portfolios assessment for learning (Rate, 2008; Barret, 2005). Assessment for learning is designed to improve student learning (Black, 2004: Black et al., 2004). In applying the learning portfolio, the student task was selected to illustrate the lesson. The portfolio serves as a formative assessment are used to improve student learning (Barrett, 2006). Assessment for learning is implemented to provide feedback as a means of improving student learning. Critical comments provided by lecturer as feedback to the student portfolio assignments that encourage them to reflect on themselves to improve their learning.

This learning model is also developed from the constructivist learning theory that come from the idea of Piaget and Vigotsky. Piaget's view of constructivism and Vigostky can contiguous in Piaget's constructivism learning process that emphasizes the internal activities of the individual objects encountered and the experience of the person. While the grouping of students in the learning process refers to Vigotsky constructivism process that emphasizes social interaction in the process of knowledge construction for the social environment (Rusman, 2010).

Teaching behavior that is expected to occur in Inorganic Chemistry-based learning model portfolio assessment, namely the syntax: portfolio assessment plan, gather a portfolio, selecting a portfolio of evidence, feedback and reflection portfolio, and utilization of portfolio assessment. A support system feasibility study model is the presence of the RPP, textbooks, and sheet portfolio assessment.

METHODOLOGY

This study is research and development that uses models Gall et al. (2003) to Develop and validate products. Stages of the study consists of four main stages, namely: 1) a preliminary study that includes activities and studies Empirical studies of literature, requirements analysis, concept analysis; 2) planning and designing the product; 3) the development and initial product testing; and 4) implementation and validation of the product. Flowchart of the implementation of the study is shown in Figure 1.

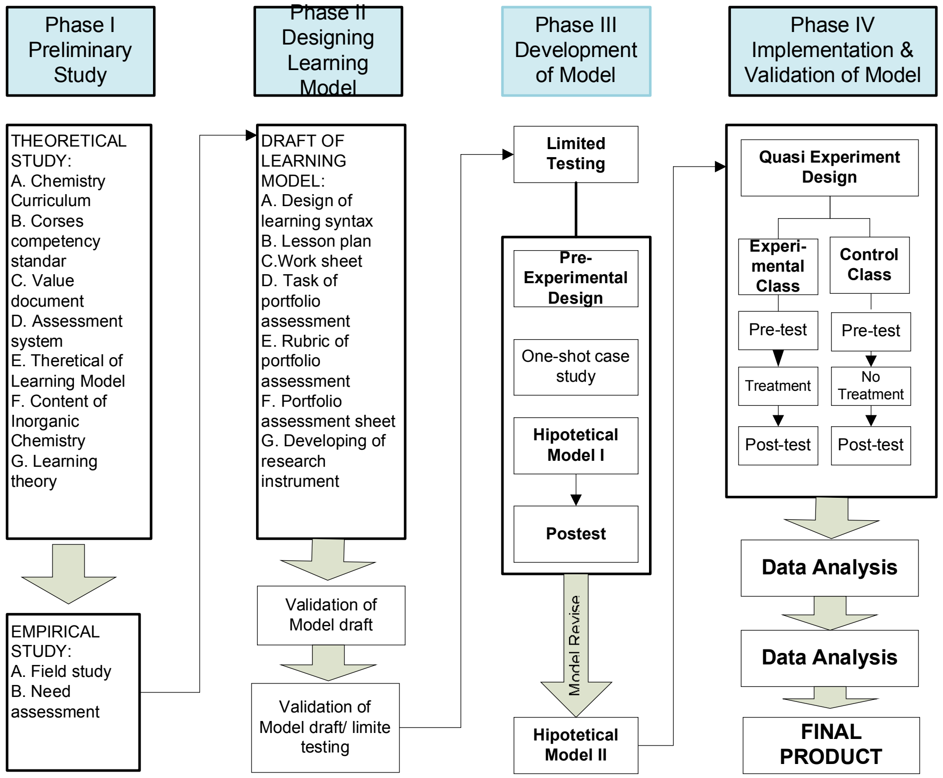


Figure 1 Flowchart of the Implementation Research

The devices and instruments model that have been developed validated by three experts. The device model has been validated was tested further limited by using a type pre-experimental design with a one-shot case study. The modelt was implemented in one class of Chemical Education, State University of Makassar that consists of 45 students in the first semester of 2013/2014. The instrument used to collect the data is in the form of achievement test multiple choices. The object of research is the subject of Inorganic Chemistry II and the subject matter metallic bond, ionic bond, Metallurgy, and Metals Alkali. The portfolio implementation process in the lecture through five stages, namely: planning a portfolio assessment, collecting the products that have been made, select and reflect on selected products, improve and evaluate products, utilizing the results of the assessment portfolio. The components of portfolio assessment in the course include the individual tasks and task groups on any subject matter. The tasks in the portfolio students are admitted feedback in the form of comments and returned on a weekly basis. Components portfolios in learning were assessed using a portfolio assessment rubric.

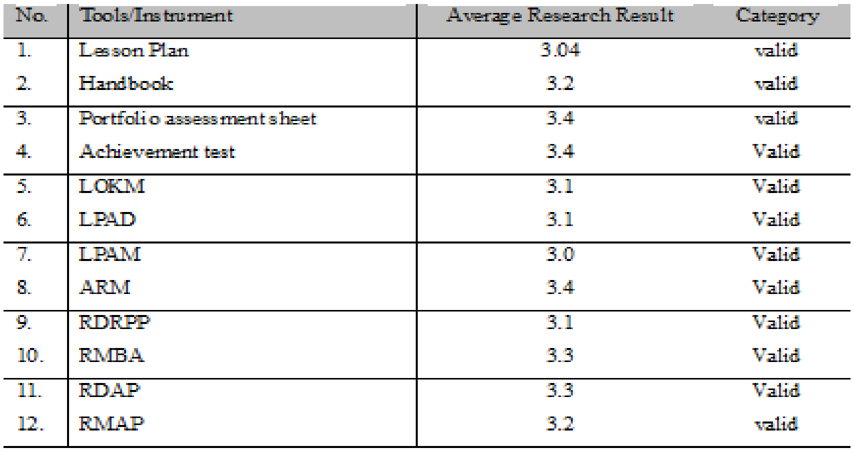
The outcomes learning data were processed using descriptive statistics. The criteria used to determine the value of the student achievement category are five-point scale based on categorization techniques standards set by the Ministry of National Education.

RESULT AND DISCUSSION

In general, the implementation of this study consists of four phases. The first year of research only carried out up to the stage three. In the first stage, a review of theoretical and empirical, such as: (1) analysis of curriculum, standards of competence and type of assessment is applied to the subject of Inorganic Chemistry; (2) analysis of the value of the course of Inorganic Chemistry; (3) Inorganic Chemical analysis of the material, as well as the theoretical studies (4) portfolio assessment; and (5) learning theory and learning models. The results of the analysis, the source of information used to design the instructional design models.

The second stage is the design stage and designs the learning model. At this stage, the design of learning tools that support the learning model of Inorganic Chemistry II based portfolio assessment and instruments associated with developing model. Learning device was developed through textbooks, portfolio assessment sheet and rubrics assessment, lesson plans, and research instruments such as achievement test and questionnaire responses of students to the learning model used. This initial design was then evaluated by a preliminary field validation sheet and testers using open-ended questions to provide feedback. Revisions were prepared based on their responses. Final revisions were made based on this feedback. The results of validation tools and instruments of learning models show that the devices and instruments are in the category of valid research. Table 1 presents the summary results of the assessment and research instruments.

Table 1 The Results of Validation Device Assessment and the Research Instrument



No.	Tools/Instrument	Average Research Result	Category
1.	Lesson Plan	3.04	valid
2.	Handbook	3.2	valid
3.	Portfolio assessment sheet	3.4	valid
4.	Achievement test	3.4	Valid
5.	LOKM	3.1	Valid
6.	LPAD	3.1	Valid
7.	LPAM	3.0	Valid
8.	ARM	3.4	Valid
9.	RDRPP	3.1	Valid
10.	RMBA	3.3	Valid
11.	RDAP	3.3	Valid
12.	RMAP	3.2	valid

Information:
RPP = lesson plan
LOKM : model implementing observation sheet
LPAD: lecturer activities observation sheet
LPAM: student activities observation sheet
ARM: student respond questionnaire
RDRPP: lecturer respond to the portfolio assessment rubric
RMBA: student respond to the handbook
RDAP: lecturer respond to the portfolio assessment
RMAP: student respond to the portfolio assessment

The third stage is the development stage. At this stage, the trial is limited to models that have been validated by using pre-experimental design with the type of one-shot case study. The subjects were students in one class that consist of 45 students who took a course of Inorganic Chemistry II. Subject matter that is the object of testing: Metal Bonding, Ionic Bonding, Metallurgy and Alkali Metals.

The implementation of trial in four sessions. Based on the analysis of data obtained by an average amounting 73.6 student results are included in the high category. Students gain a minimum value of 40 and a maximum value of 92 is the ideal score of 100. The Distribution category of learning achievement by students at the time limited trials are presented in Figure 2. On the picture appears that around 64.44% of the students have learning achievement at the high category . A small part of achievement by student learning outcomes is at medium and low categories.

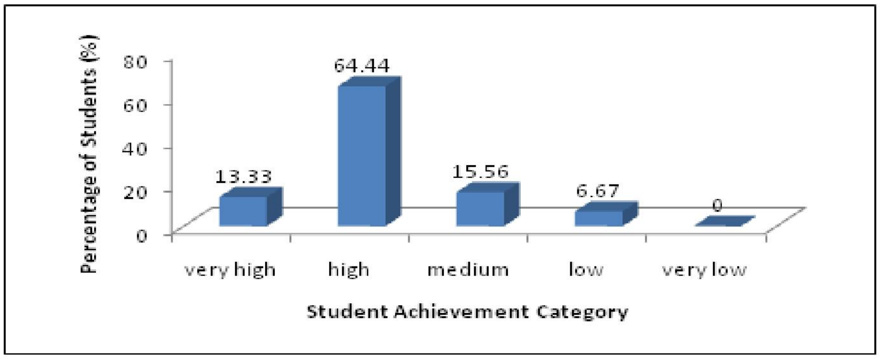


Figure 2 Distribution of Student Category by Learning Outcomes in the Course of Inorganic Chemistry II

Figure 3 presents the distribution of student achievement of learning outcomes in the material Metal Bonding, Ionic Bonding and Metallurgy and Alkali Metals. In the picture appears that the percentage achievement of student learning outcomes in all three materials is generally same, although the cognitive aspects of student achievement on the third matter have not been fully achieved. The success of the role assessment in improving learning is determined by five key factors. These five factors are: 1) the availability of effective feedback to students; 2) the active involvement of students in learning; 3) adjustment of teaching with assessment results; 4) recognition of the influence of assessment on motivation and self-esteem of students; and 5) the students needed to be able to judge themselves and know how to fix it (ARG, 1999).

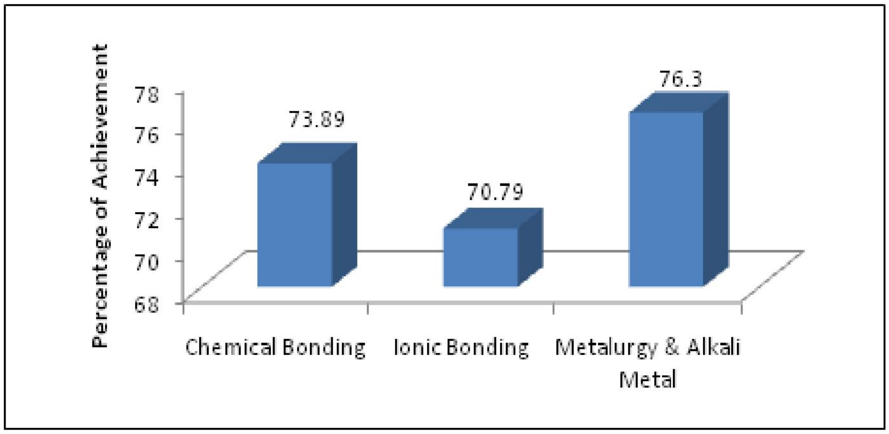


Figure 3 Distribution Percentage of Student Achievement on Different Topic

The concepts in materials Chemical Bonding and Metallic Bonding generally consist of abstract concepts and the concepts stated principles, it is relatively difficult to be understood by the students in lectures. The use of portfolio assessment in the lecture has effectiveness respectively 73.89 and 70.79. In the material metallic bonding, which is somewhat elusive concept among others on view in the molecular orbital theory explains bonding of metals and calculation of atomic number and mass of the unit cell.

Slightly different with subject matter Metallurgy and Metal group that consist of more alkali in abstract concepts with concrete examples and concepts expressed symbol. Through the implementation of portfolio assessment in the course enables students more easily grasp the concepts of the material. The feedback through comments for improvement admitted to the student portfolios enables them to evaluate themselves against the learning outcomes, thus spurring them to be able to repair their learning. Ramlawati (2012) states that through the feedback admitted by the lecturers allow the students to know their strengths and weaknesses, so that they can improve their learning process.

CONCLUSION

Based on the result of the research that has been obtained, it can be concluded that the model-based on portfolio assessment of learning Inorganic Chemicals has met the criteria for a valid and practical. Effectiveness of the learning model that measured the percentage of achievement of learning outcomes obtained for 73.8%, and at the high category.

BIBLIOGRAPHY

Arends, R. (1997). *Classroom Instruction and Management.* New York: McGraw-Hill Book Co.

Assessment Reform Group (ARG). (1999). *Assessment for Learning: Beyond the Black Box*. Cambridge: University of Cambridge School of Education. Available: http://assessmentreformgroup.files.wordpress.com/2012/01/ beyond_blackbox.pdf [03 February 2012].

Barret, H. (2006). “Using Electronic Portfolios for Classroom Assessment”. Dalam *Connected Newsletter,* **13***,* (2)*,* 4-6.

Black, P. (2004). *The nature and Value of Formative Assessment for Learning*. King’s College London.

Black, P., Harrison, C., Lee, C., Marshall, B., and Wiliam, D. (2004). “Working Inside the Black Box: Assessment for Learning in the Classroom. Dalam ” *Phi Delta Kappan*, **86**, (1), 9-21.

Ramlawati. (2012). Pengembangan Model Asesmen Portofolio Elektronik untuk Meningkatkan Keterampilan Generik Sains mahasiswa pada Praktikum Kimia Anorganik. *Disertasi.* UPI Bandung: Not Published.

Rusman, 2010. *Model-Model Pembelajaran*. Bandung: Raja Grafindo Persada.

Stiggins, R. J. (1994). *StudentCentred Classroom Assessment*. New York: Merrill.

CE-11

THE DEVELOPMENT OF ANDROID MOBILE GAME AS SENIOR HIGH SCHOOL LEARNING MEDIA ON RATE REACTION AND CHEMICAL EQUILIBRIUM

Resti Yektyastuti[1*], Jaslin Ikhsan[2], Rr. Lis Permana Sari[2]

[1]*Postgraduate Study Program, Yogyakarta State University,*
[2]*Department of Chemistry Education, Faculty of Mathematics and Science,*
Yogyakarta State University
**restiyektyastuti@yahoo.com*

Abstract

The use of Android mobile phone is growing fast, also among senior high school students. Student with Android mobile phone can do many things. They are chatting, browsing, reading books, and also they love playing games with this smart device. It is good to develop an educational game operated in Android mobile phone so the students can learn everywhere and everytime by playing games in their device.

This research was a development research on Android mobile game as senior high school learning media, especially on rate reaction and chemical equilibrium. The Android mobile game was developed by adapted ADDIE (i.e analysis, design, development, implementation, evaluation) method. It was reviewed by expert of chemistry, expert of multimedia, and peer-reviewers. The quality of this Android mobile game was determined based on the aspect of subject matter, language, operating proccess, audio visual, and software design. The assessment data are collected from 5 chemistry teachers and 25 senior high school students by using Likert quistionnaire.

The results shows that the Android mobile game has very good quality. Students said this mobile game is interesting, joyfull, and make chemistry learning more attractive.

Key words: android, rate reaction, chemical equilibrium, ADDIE

INTRODUCTION

A learning process is a process of communication that will not take place optimally in the absence of learning media. Learning media is a tool that can be used to facilitate students' understanding of the competencies which need to be mastered from a subject. One alternative media that can be used is game.

From the literature review conducted by Funk (2002), game applications can improve students' interest in learning, speed of information processing and problem solving, while increasing social sensitivity and academic ability. With game applications, students are encouraged to solve problems in a particular subject by completing training questions contained in the application. This kind of game application commonly referred as the educational application games.

Educational application games can be presented in a variety of devices such as computer, video, and mobile phone. Games that work on the mobile phone have several advantages when compared to the games that run on other devices. Distribution of the mobile phone technology in the society and also the ease of access device, anywhere and anytime, making game applications installed on the device is very popular.

The use of mobile phone in society will always follow trend towards the development of technology used in the mobile phone itself. Mobile phone with Android operating system is now becoming a new trend in the selection of mobile phone devices. According to Dwi Andi Susanto (2012, in www.merdeka.com), Android mobile phone users in the mid-2012 increased four-fold compared to 2011. In 2011, the Android mobile phone users is only about 100 million people while in mid-2012, users had increased to about 400 million people. Mobile phone Android offers an open operating system that allows users to add any application to the mobile phone beyond the basic applications are provided. It also makes Android mobile phones became very popular.

Building educational application games based Android mobile phone can be an alternative learning media. This application game need to be designed to fit the Content Standards of subject. Specifications of subject matter in the media are considered important to enhance the depth and discussion in the media. Therefore, this study focuses on the extent to which Android-based educational application game as a learning media can be developed and utilized in accordance with the existing learning design, to create a fun, effective, and new learning environment, for the achievement of learning goals. To achieve these objectives, this research is devoted to develop Android-based educational application game for high school student. Chemistry learning on reaction rate and chemical equilibrium requires a lot of practice in resolving problems associated with it. Learning in this material will be colored by the new experience of learning by using educational application game on the Android mobile phone.

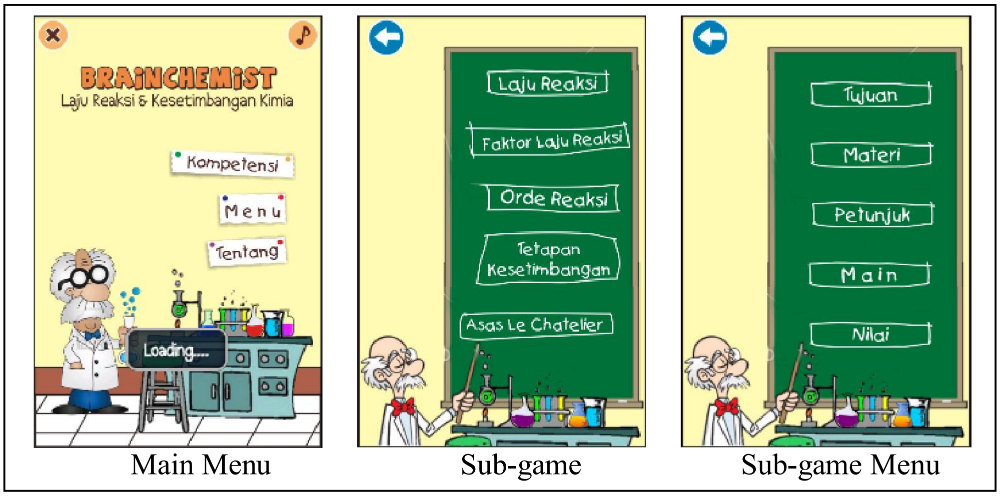
Android-based mobile game "Brainchemist" offers a new exciting and fun learning because by playing the game, students can improve the understanding of the material presented in the game. The aims of this study is to design and create a an Android-based mobile game on reaction rate and chemical equilibrium. It is expected to be an alternative media learning which is interesting, fun, and can be used by anyone, anytime, and anywhere. Quality assessment of mobile game "Brainchemist" will be given by chemistry teachers and students of high school class XI science to see their assessment and how they response.

DISCUSSION

Mobile game “Brainchemist” was developed by using ADDIE (Analisys-Design-Development-Implementation-Evaluation) method. This development model generally includes 5 steps: analysis, design, development, implementation and evaluation, but this study was limited to the implementation step only. The initial product mobile game "Brainchemist" reviewed by 1 subject matter expert, 1 information and technology expert, and 3 peer-reviewers. Mobile game "Brainchemist" assessed by 5 chemistry teachers and 25 high school students using questionnaire with Likert scale. The assessment was based on 5 aspects, they were subject matter, language, operating process, audio visual, and software design. The data were analyzed to determine the quality of mobile games in accordance with the table of ideal criteria assessment. In addition, the results of the assessment were also obtained data on how student responses to the mobile game.

The product of this study is mobile game "Brainchemist" on reaction rate and chemical equilibrium in Android Application Package file format (.apk). This game presents in Indonesia language and can only be operated on the mobile phone with Android operating system. This game was developed using Eclipse Indigo program with Java programming language.

Mobile game "Brainchemist" is a two dimensional game in the form of quiz, containing questions for practicing and for helping the understanding of reaction rate and chemical equilibrium. The material presented in this game was adapted from Chemistry Content Standards by *Badan Standar Nasional Pendidikan* (Indonesian: *National Education* Standards Board). It is all about reaction kinetics, chemical equilibrium, and factors that influence it. Figure 2 showed some game display and available menu button.



Main Menu Sub-game Sub-game Menu

Figure 2. Mobile game “Brainchemist” display

Mobile game "Brainchemist" present in an attractive and colorful layout. The images, sound effects, music, and game rewards also enhance its attractiveness. Mobile game "Brainchemist" consist of 5 sub-games, they are sub-games of reaction rate, factors affecting the reaction rate, reaction order, Le Chatelier’s principle, and equilibrium constant. Question type in the sub-game reaction rate is right-wrong questions, while the sub-game reaction order is short field questions. Other 3 sub-games are presented in multiple choices type of question.

Each sub-game provides an option game with a fight against the clock or without against the clock. The time allocated for playing the game was different for each sub-game, depending on the level of difficulty of the questions presented. Each game set consists of 5 questions that come out randomly according to available question bank. Feedback right or wrong answers immediately appear when one question has been answered. After answering five questions, resume game results will appear to show rewards, scores obtained, and a discussion.

The results of the quality assessment of mobile game "Brainchemist" by chemistry teacher shows an average score of 110.6. Based on the assessment ideal criteria table, this score qualifies Very Good because it lies within the range of scores > 105. Maximum score mobile game quality assessment "Brainchemist" by teachers is 125, so the average score of 110.6 indicates the ideal percentage of 88.48%. Details of quality assessment results by chemistry teachers can be seen in Table 1.

Table 1. Quality Assessment of Mobile Game "Brainchemist" by Chemistry Teacher

No	Assessment Aspect	Average Score (	Range of Score	Quality Categories	Maximum Score	Ideal Percentage
1.	Subject matter	27.2	> 25.2	Very Good	30	92.33 %
2.	Language	8.6	> 8.4	Very Good	10	86.00 %
3.	Operating Process	17.8	> 16.8	Very Good	20	89.00 %
4.	Audio and Visual	34.2	> 33.6	Very Good	40	85.50 %
5.	Software Design	22.8	> 21	Very Good	25	91.20 %
Overall Score		**110.6**	**> 105**	**Very Good**	**125**	**88.48 %**

Quality mobile game "Brainchemist" is also rated by the students, but without including aspect of subject matter. The result of the quality assessment by the students shows an average score of 80.76. Based on the ideal assessment criteria table, this score qualifies Very Good because it lies within the range of score > 79.8. Maximum score mobile game quality assessment "Brainchemist" by students is 95, so that the average score of 80.76 indicates the ideal percentage of 85%. Details of student assessment results can be seen in Table 2.

Table 2. Quality Assessment of Mobile Game "Brainchemist" by High School Student

No	Assessment Aspect	Average Score (	Range of Score	Quality Categories	Maximum Score	Ideal Percentage
1.	Language	8.16	6.8< ≤8.4	Good	10	81.60 %
2.	Operating Process	17.16	> 16.8	Very Good	20	85.80 %
3.	Audio and Visual	34.04	> 33.6	Very Good	40	85.10 %
4.	Software Design	21.40	> 21	Very Good	25	85.60 %
Overall Score		**80.76**	**> 79.8**	**Very Good**	**95**	**85 %**

The comparison of the ideal percentage of each aspects in quality assessment of mobile game "Brainchemist", both based on the chemistry teachers and high school students is presented in Figure 3.

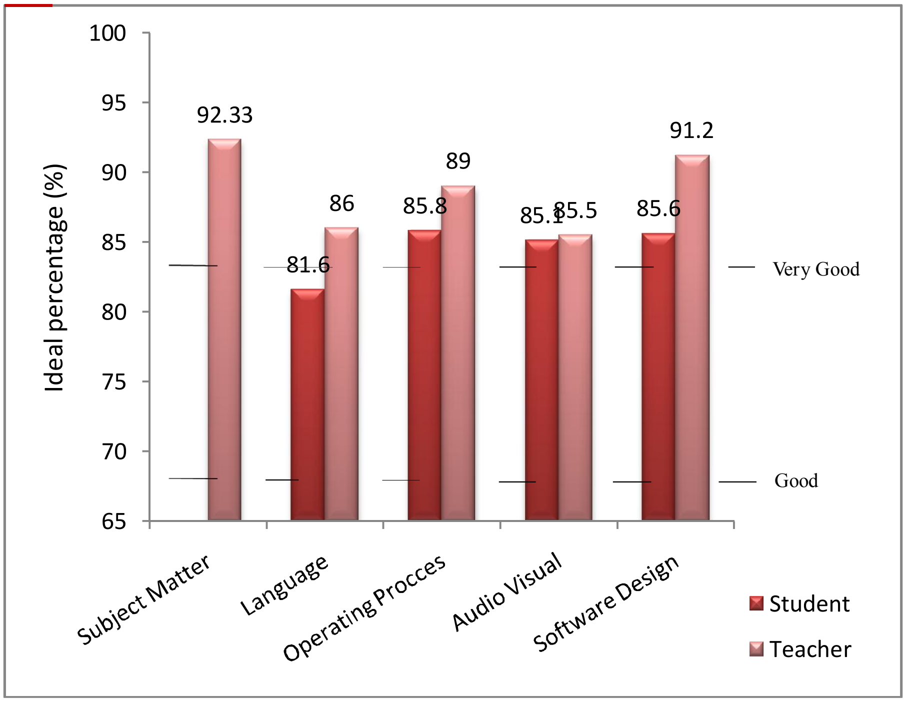


Figure 3. Ideal Percentage of *Mobile Game* “Brainchemist”

Data of student’s responses indicate that mobile game "Brainchemist" is interesting, joyfull, and make chemistry learning more attractive. Although the subject matter presented in the mobile game is limited and can not be used to measure mastery learning, generally this game can be used as a learning media that support the learning process for reaction rate and chemical equilibrium. This game has met the criteria of good learning media according to Mulyanta, they are relevance, convenience, attractiveness, and benefit.

CONCLUSION

Mobile game “Brainchemist” as chemistry learning media for senior high school on reaction rate and chemical equilibrium has successfully developed through analysis, design, development, and implementation steps. Quality of mobile game "Brainchemist" as chemistry learning media based on chemistry teacher assessment shows an average score of 110.6 Based on the ideal assessment criteria table, this score qualifies Very Good because it lies within the range of scores $\overline{X} > 105$. Maximum score mobile game quality assessment "Brainchemist" by teachers is 125, so the average score of 110.6 indicates the ideal percentage of 88.48%. Quality of mobile game "Brainchemist" as chemistry learning media based on high school student assessment shows an average score of 80.76. Based on the ideal assessment criteria table, this score qualifies Very Good because it lies within the range of score $\overline{X} > 79.8$. Maximum score mobile game quality assessment "Brainchemist" by students is 95, so that the average score of 80.76 indicates the ideal percentage of 85%. Based on this assessment, the mobile game "Brainchemist" fit for using as chemistry learning media for senior high school on reaction rate and chemical equilibrium. Students assume the media is interesting, joyfull, and make chemistry learning more attractive.

REFERENCES

Azhar Arsyad. (1996). *Media Pembelajaran*. Jakarta: RajaGrafindo Persada.

Bermani Munthe. (2009). *Desain Pembelajaran.* Yogyakarta: Pustaka Insan Madani.

Cahya Dwi Wahyudi. (2010). Pengembangan Permainan Who Wants to be a Great Chemist? sebagai Media Pembelajaran Kimia untuk Siswa Kelas XI. *Skripsi Program Studi Pendidikan Kimia FMIPA UNY.*

Daryanto. (2010). *Media Pembelajaran*. Yogyakarta: Penerbit Gava Media.

Dewi Padmo. (2004). *Teknologi Pembelajaran*. Jakarta: Pusat Teknologi Komunikasi dan Informasi Pendidikan.

Dwi Andi Susanto. (2012). *Pengguna Android di Tahun 2012 Meningkat 4x Lipat.* Downloaded from http://www.m.merdeka.com/teknonogi/pengguna-android-di-tahun-2012-meningkat-4x-lipat.html.

Eko Putro Widoyoko. (2011). *Evaluasi Program Pembelajaran.* Yogyakarta: Pustaka Pelajar.

George Boeree. (2009). *Metode Pembelajaran dan Pengajaran.* Penerjemah Abdul Qodir Shaleh. Yogyakarta: Ar-ruzz Media.

Malachowski. (2002). *ADDIE Based Five-Step,Method Towards Instructional Design.* Diambil pada 23 Februari 2012 dari http://fog.ccsf.cc.ca.us/~mmalacho/OnLine/ADDIE.html

Punaji Setyosari. (2012). *Metode Penelitian Pendidikan dan Pengembangan.* Jakarta: Kencana Prenada Media Group.

Stephanus Hermawan S. (2011). *Mudah Membuat Aplikasi Android*. Yogyakarta: Penerbit ANDI.

Sugiyono. (2007). *Metode Penelitian Kuantitatif Kualitatif dan R&D.* Bandung: Penerbit Alfabeta.

Tim Pengembang JENI. (2008). Permainan Edukatif Berbasis Mobile Sebagai Media Pembelajaran Matematika dan Bahasa Inggris. *Laporan Penelitian Java Education Network Indonesia.*

http://www.eclipse.org

CE-12

IMPLEMENTATION OF GUIDED-INQUIRY TO PROMOTE STUDENTS' METACOGNITIVE SELF REGULATION IN XI GRADE

Rusly Hidayah, Fitria Dwi Lestari

Chemistry Education, Faculty of Mathematics and Sciences
Surabaya State University, Jl. Ketintang, Surabaya

ABSTRACT

This study aims to describe the ability of metacognitive self-regulation of XI grade students of SMAN 2 Lamongan after implementation guided inquiry learning model on reaction rates material. One group pretest-postest design was used in research. Students in XI grade of SMAN 2 Lamongan as result subject. Metacognitive Activities Inventory (MCA-I) used as pretest and postest instruments while achievement, metacognitive test and interviews test were conducted in the end of learning. Non-test instruments were class management and students activities observation sheets. The results showed that the students self-regulation has increased especially in monitoring, followed by evaluating and planning skill. As many as 66.67% students in low level jump to moderate and 13.04% students in moderate jump to high level. This result are supported with students learning outcomes that can reach classically mastery of learning is 86.11%, metacognitive test and interview that show same level as in MCA-I data, students activities during learning shows that the activities support self-regulation in monitoring skill dominantly, implementation of guided inquiry is effective with good and very good criteria, so it can promote metacognitive self-regulation.

Keywords: *guided-inquiry, metacognitive self –regulation*

INTRODUCTION

Quality of education in Indonesia so far remained much improved in comparison with the quality of education in other countries. One of the data about quality of education related to the Human Development Index (HDI). HDI data released by the United Nations Development Program/ UNDP on November 2, 2011, it appears that Indonesia is in the bottom rank among the ASEAN countries. Indonesia is categorized as a country with the Medium Human Development and only ranks 124 of 187 countries in the world.

Indonesia's lack of achievement has been the concern of many educators. The government has organized the improvements improve the quality of education. But it seems not give satisfactory results. These results should be recognized for education in Indonesia is only visible from the ability to memorize facts, concepts, theories or laws. Although many children are able to present a good level of memorization of the material it receives but in fact they often do not understand the substance of the material (*Pusat Kurikulum Badan Penelitian Dan Pengembangan Departemen Pendidikan Nasional, 2007*). Students' lack of understanding is also because students do not have the awareness about how to learn (*Pusat Kurikulum Badan Penelitian Dan Pengembangan Departemen Pendidikan Nasional, 2007*). If students are able to understand how to learn or understand how to apply metacognitive skill when studying a variety of subjects including chemistry then received information can transfer into long-term memory and will be more meaningful.

Based on the results of student questionnaire on metacognitive abilities, as much as

14.28% of students who use the planning skills, 17.85% of students who use the monitoring skill, and 25% of students who use evaluating skill. Beside that, results of interviews with 28 students of SMA Negeri 2 Lamongan in Class XI IPA 5 as much as 53.57% of students stated that the reaction rate of the material is a difficult material.

Generally, based on the results of student questionnaire above , shows that the self -regulatory metacognitive skill of students of class XI IPA 5 SMAN 2 Lamongan is still low with a percentage of less than 50%. This is because there are now learning chemistry is still not able to develop metacognitive skill of the students' self-regulation. Based on the above explanation, it seems clear that the related process of learning, metacognitive skill which includes planning, monitoring, and evaluating skills should always be empowered. One of the ways is through the implementation of Guided Inquiry Learning Model.

Guided inquiry learning model is chosen because it needs active role of students in learning so that students are expected to use metacognitive abilities as much as possible and also in implementation of guided inquiry, the teachers still provide direction and guidance to the students. And it absolutely makes the students become more focused on the process of investigation conducted.

Based on the background above, the problem can be formulated: 1). How about activities of students in XI grade SMA Negeri2 Lamongan after implementation of guided inquiry learning model in reaction rates material? 2) How about feasibility of guided inquiry learning model in reaction rates material in XI grade SMA Negeri 2 Lamongan? 3). How about learning outcomes of students XI grade SMA Negeri 2 Lamongan after implementation of guided inquiry learning model in reaction rates material?

The purpose of this research: 1). To describe activities of students in XI grade SMA Negeri 2 Lamongan after implementation of guided inquiry learning model in reaction rates material 2) To describe feasibility of guided inquiry learning model in reaction rates material in XI grade SMA Negeri 2 Lamongan 3)To know learning outcomes of students XI grade SMA Negeri 2 Lamongan after implementation of guided inquiry learning model in reaction rates material.

Research Method

This research is a pre-experimental design with the objects are students of XI Grade of SMAN 2 Lamongan. The design study is one group pretest-posttest. The design of this study can be described as follows: (Sugiyono, 2010: 75)

$$\boxed{\mathbf{O_1} \quad \mathbf{X} \quad \mathbf{O_2}}$$

Explanation:
O_1 : Pretest score of metacognitive self- regulation of students
X : Treatment
O_2: Postest score of metacognitive self- regulation of students

Learning device used in this study were developed by the researchers themselves which will be validated by the chemistry lecturer. The device consists of: a) Syllabus b) Lesson Plan c) Students Worksheet

While the research instrument used in this study include: a) Metacognitive Activities Inventory (MCA-I) b) Metacognitive skill test c) Interview Sheet d) Observation sheet e) Students Learning Outcomes Test.

Data analysis that is used in this research is explained below:

a. Analysis of MCA-I

There are two techniques of analysis used in this study, which is the first quantitative data analysis techniques to determine whether there is any effect of implementation of guided inquiry learning model of the metacognitive self-regulation skill of students. Previously calculated values of metacognitive self-regulation skill of students with the following procedures:

1) The score of students' metacognitive self - regulation is obtained by calculating the total score of the students' answers on the Likert scale (always to never). For items positive always answer get the score 5, Often get the score 4, sometimes get score 3, seldom get score 2, and never get score 1. As for the negative items have a value that is the opposite of the positive items.

2) Calculate the average score of metacognitive self-regulation skill of students from the pretest and its standard deviation. The measurements using the formula (Sudjana, 2005):

$$\bar{x} = \frac{x_i}{n} \text{..} (1)$$

with:

$\bar{x}$ = average score of metacognitive self-regulation

x_i = total of students metacognitive self-regulation score

n = number of students

While the standard deviation (SD) was measured by using the formula (Sudjana, 2005):

$$s = \sqrt{\frac{(x_i - \bar{x})^2}{n-1}} \text{..} (2)$$

with:

x_i = the data score of student *i*

$\bar{x}$ = the average score of the overall metacognitive skill

3) Grouping students into three level, namely (Pulmones, 2007):

a. Low-level with a range of values (minimum average score until ≤ ((-SD)+ $\bar{x}$)).

b. Moderate-level with a range of values > ((-SD)+$\bar{x}$ until ≤ ($\bar{x}$ +(SD)).

c. High-level with a range of values > (($\bar{x}$ + (SD) until > (($\bar{x}$ + (SD) s/d nilai maximum average score).

4) Calculate the average of the students' metacognitive ability posttest results using the formula (1).

5) Comparing the average value of the initial capability with the average value of the final capability. This is done by conducting a descriptive analysis of their skill in each component of the planning, monitoring, and evaluating. This description is supported by the results of interviews of representatives of each group of students and the level of metacognitive skill test results.

6) Determine the average self-regulatory metacognitive capabilities include: planning, monitoring, and evaluating of all students by using the formula (1).

7) Determine the components of which are crucial to the self-regulatory metacognitive skill of students, whether planning component, monitoring, or evaluating by looking at the average results.

b. Analysis of Metacognitive Skill Test

It is analyzed by the way of students to do all of the question in this test. Maximum score given to such questions is 4 and the minimum score given is 1. Then calculated the average score for that question then the results are grouped based on the criteria of the scale range of 1.3 to each group as follows:

Scores for high levels of self-regulation: 2.8 - 4.0

Scores for intermediate level of self-regulation: 1.4 - 2.7

Scores for the low level of self-regulation: 0 - 1.3

c. Analysis of Interview Test

It is analyzed descriptively.

d. Analysis of Students activities

Observations were made every five minutes during the learning takes place that dominant in a group. Students activities percentage is calculated by using formula below:

$$\text{\% category of students activities} = \frac{\text{amount of each activity}}{\text{Amount of all activities in 90 minutes}} \times 100\%$$

e. Analysis of Guided Inquiry Learning Model Feasibility

It is analyzed descriptively then determined the score of teacher's activities by using scale 1-5. The data is gain is analyzed to get the average of *KMP (Kriteria Mengelola Pembelajaran)* by using formula below:

KMP = ————————

Then the score is converted to the criterion shown below:

No.	Score	Criterion
1	0-1,1	Very bad
2	1,1-2,2	Bad
3	2,2-3,3	Enough
4	3,3-4,4	Good
5	4,4-5	Very Good

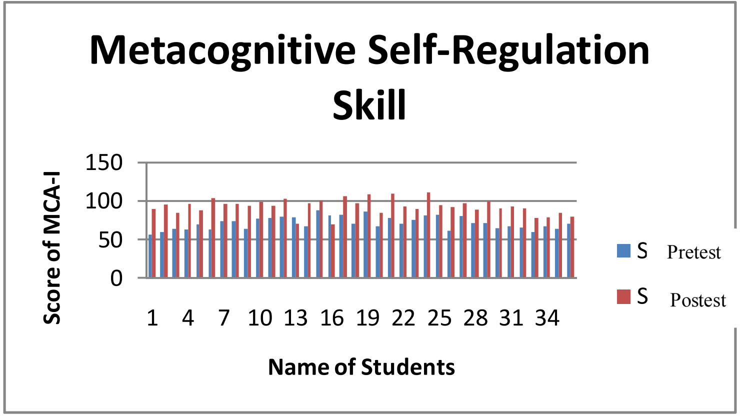
f. Analysis of Students Learning Outcomes.

It is analyzed to know mastery of students. Classically, a class is said to pass the study if there is a minimum of 75% of students who had received grades ≥ 80 according to the Complete Standard Minimum value set at SMAN 2 Lamongan for chemistry subjects was calculated as follows:

Student learning outcomes = (Score obtained) / (maximum score) x 100

DISCUSSION

a) Metacognitive Self-Regulation Skill of Students



Generally, there is an increase from pretest score to posttest score of students' metacognitive self-regulation skill. The students self-regulation has increased especially in monitoring, followed by evaluating and planning skill. As many as 66.67% students in low level jump to moderate and 13.04% students in moderate jump to high level b) Students' activities recorded some decline and rise of the meeting 1, 2, and 3. Most of the activities of the student demonstrate their metacognitive self-regulation skill. c) Feasibility of guided inquiry learning model is effective, based on the average score at meetings either category 1 (3.75), meeting 2 and 3 including the excellent category (4.52 and 4.53) d) Learning Outcomes of Students

No.	Characteristics	Test Result
1.	Number of Students	36
2.	Number of incompleteness students	31
3.	Number of completeness students	5
4.	Classically mastery learning	86,11%

CONCLUSION

Metacognitive self-regulatory ability of students has increased, especially in the aspect of monitoring, evaluation and follow aspects of the lowest on the planning aspects. A total of 66.67% of students low group category, go to the middle group and 13.04% of students get into the middle class category groups the high group category. Metacognitive ability test results and interview students showed levels of self-regulatory metacognitive abilities of students in accordance with the results of the MCA-I. Percentage of student activity dominant in activities that support the monitoring aspect. Feasibility study in the form of a teacher's ability to manage the learning gets good and excellent categories that support the improvement of self-regulation metacognitive abilities of students. Students learning outcomes achieved by classical completeness percentage of 86.11% .

REFERENCES

Arends, Richard. I. 2012. *Learning to Teach Ninth Edition.* New York: TheMcGraw-Hill Companies, Inc.

Branch, Jennifer dan Oberg, Dianne. 2004. *Focus on Inquiry*, (online), (http://education.alberta.ca/media/313361/focusoninquiry.pdf accessed on July, 5 2013).

Hamruni. 2012. *Strategi Pembelajaran*. Yogyakarta: Insan Madani.

Kementerian Pendidikan Nasional. 2007. *Naskah Akademik: Kajian Kebijakan Kurikulum Mata Pelajaran IPA*. Jakarta: Pusat Kurikulum Badan Penelitian Dan Pengembangan Departemen Pendidikan Nasional.

Keenan, dkk. 1984. *Kimia Untuk Universitas Jilid 1 Edisi Keenam.* Jakarta: Erlangga.

Kuhlthau, Carol C, dkk. 2007. *Guided Inquiry: Learning in the 21st Century*. London: Libraries Unlimited, Inc.

Martin, Lisa dan Hansen. 2002. *Defining Inquiry*, (online), (http://people.uncw.edu/kubaskod/NC_Teach/Class_2_Teach_Strat/Teaching_Strategies/DefiningInquiry.pdf accessed on July, 5 2013).

Pulmones, Richard. 2007. *Learning Chemistry in Metacognitive Environment,* (online), (http://www.dlsu.edu.ph/research/journals/taper/pdf/200712/pulmones.pdf accessed on Pebruary, 12 2013).

Riduwan. 2007. *Skala Pengukuran Variabel-Variabel* Penelitian. Bandung: Alfabeta.

Sandi-Urena, Guillermo Santiago. 2008. *Design and Validation of A Multimethod Assessment of Metacognition and Study of the Effectiveness of Metacognitive Interventions*. Dissertation Clemson University, (online), (http://etd.lib.clemson.edu/documents/1219850998/umi-clemson-1711.pdf accessed on July,8 2013).

C-13

IMPROVING STUDENTS' CRITICAL THINKING AND CHARACTER SKILL THROUGH CHEMSISTRY LECTURE

Sri Poedjiastoeti

Jurusan Kimia FMIPA
Universitas Negeri Surabaya, Indonesia

ABSTRACT

The aims of this research are to develop learning kit to improve characters and critical thinking skills of students. This research is designed based on research and development model (R & D) and focused on development, and limited trial. Instruments used are validation sheet for lesson plan/Satuan Acara Perkuliahan (SAP), assessment sheet of accomplished SAP, observation sheet of student activity, observation sheet of character skill, abservation sheet of self assessment character skill, and quetionarre sheet of students' respon. Validation sheet is analyzed using Likert scale, and the others are analyzed using descriptive method. The results show that learning kit for Stoichiometry in General Chemistry lecture feasible to use and can improve students' criticalthinkingskill, butnot so significant. Thatisrelevanttostudents' activity record that shows lack of askingactivity. Therefore, goodcharáctershowedbyallstudentsduringthelecture.

Keywords: critical thinking, character, chemistry

INTRODUCTION

One of Indonesia'snational development mission is to create a nation with great competitiveness. Priority of this mission is increasing human resources quality (RJPN 2005 – 2025). This statement is clarified with others policy below it, such as vision of strategic plan Kementrian Pendidikan Nasional on 2010-2014 are improving professionalism human resources on education and providing learning facility. The strategic goal of Direktorat Jendral Pendidikan Tinggi is provide great quality of high education as required by national development demand. More specific, the goals of strategic plan of Universitas Negeri Surabaya are to improve (1) continuing science and education to create professional educational human resources, (2) elementary and midlle school, and (3) high quality learning kit for elementary and middle school (Unesa, 2011). Thus policy headed to great plan to improve human resources to face global competition on 21st century. That could be done by giving high quality learning to Indonesian students to improve their thinking skill.

On the other hand, found the fact how Indonesian students are and what skill that require nowadays. PISA and TIMSS studies shows that education should improve not only routine manual and routine cognitive, but also complex communication and expert thinking skill. The result of this study was known that Indonesian students were lack of expert thinking skill. Five skill measured by TIMSS test are (1) *Understanding Simple Information*, (2) *Understanding Complex Information*, (3) *Theorizing, Analyzing, and Solving Problems*, (4) *Using Tools, Routine Procedures, and Science Processes*, (5) *Investigating the Natural World*. Skills (2), (3), (4), dan (5) are indicator for *expert thinking*and we got low score for thus.

High order thinking skill concept on this paper would is refer to *21st century skills*concept that have been studied in USA *The Partnership for 21st Century Skills* (P21) established on 2002 as a coalition that unite society of businessman, educator, and legitimator to study how important *21st century skills* for all student is. That partnership presented a viewpoint for *21st century teaching and learning*and well known as *21st Century Students Outcomes* dan *Support Systems*. Nowdays, it has been realized that innovation and learning skills give the differences between the *ready to work* student and the other one. Focused on creativity, critical thinking, problem solving, learning strategies, communication, and collaboration are essential subject to prepare strudents' future.

The low level of thinking skill of Indonesian people did not mean that they have no potential thinking skill. Research result of PSMS Unesa showed that low skill was just happened because they had no occasion to learn it. Habitually, teachers make learning activities based on national exam latticework. As we knew, this latticework was not represented all skills the students should have to graduate (Nur, 2008).

In line with that, a research to train process skill for deaf students have been done (Poedjiastoeti, et.al.,2007; Poedjiastoeti, S., 2008). The result of this research showed that, deaf student could master process skill in chemistry learning by using learning kit and interactive multimedia. Learning kit was designed with writing to learn strategies to accommodate their inability to hear. This learning kit consisted of worksheet to do some experiments. Writing to learn strategies gave opportunity to them for expressing their thought. Interactive multimedia was designed with more visual stimulus and attractive motion. In addition, it contained chemistry daily activities and phenomenons to give experience learn from daily life for students. Both of them, learning kit and interactive multimedia, used hand signal language to accomodate students need.Process skll trained was basic process skill, such as observing, measuring, recording data, and applying procedure. at the end of research we knew that they could master process skill and had ability to be traind higher level thinking skill.

By training higher level of thinking skll for students' university, hoped that would result professional educational human resources. Furthermore, they would teach younger generation with good thinking skill, as required. Based on this reason, this research, focused on critical thinking and character skill. The aims of this research is to create learning kit model is that can teach critical thinking an character skill in Stoichiometry topic of General Chemistry Lecture.

METHOD

This research planned based on research and development (R & D) method. The phase of research and development design are preliminary study, development, and limited trial phase (Sukmadinata, 2010). Instruments used are validation sheet for lesson plan/Satuan Acara Perkuliahan (SAP), assessment sheet of accomplished SAP, observation sheet of student activity, observation sheet of character skill, absorvation sheet of self assessment character skill, and quetionarre sheet of students' respon. Instruments analyzed using description method. Likert's scale was used for validation sheet, and percentage was used for the others. Table 1 shows the Likert's scale.

Table 1

Score	Criteria
0,0 – 1,0	Poor
1,1 – 2,0	Enough
2,1 – 3,0	Good
3,1 – 4,0	Very good

(Riduwan, 2005)

RESULT AND ANALYSIS

Learning Kit for General Chemistry

Validated syllabus and SAP of Stoichiometry in General Chemistry lecture implemented on real lecture for bachelor degree class of Chemistry Education 2013. Developed learning kit designed for topic Stoichiometry and refered to main competency: (1) Using ICT in learning process; (2) Mastering base concept in General Cemistry; (3) Desaining and doing an experiment; and (4) Responsibility in mastering concept and experiment. Stoichiometry topic elaborated to 4 subtopics, (1) definition of stoichiometry, (2) calculation of stoichiometry, (3) limitting reactant, and (4) percent yield (Glencoe Science Chemistry Matter and Change, 2002; Brady, 2004; and Chang, 2005). First of all, the students learned sciencetific method and properties of matters.

SAP was planned for three lecture, on the 7th, 8th, and 9th lecture of General Chemistry. On 7thlecture,students studied the definition and calculation of stoichiometry. At the beginning of lecture, they've done the structural task about reaction between vinegar and baking soda based on LKM – 1. Using dialy material aimed to related theory on lecture with their life. Students found that there is quantitave relationship between number of reactants and the number of products they got. Therefore, students could identify problem formula, explain the aims, give hypothesis, and determine variables (manipulation, respons, and control variable). Given hypothesis tested through experiment. Because of that, student should determine what material needed on their experiment based on the procedural instruction. While experimenting, hoped that students could organize the data they got and followed by good analyzing, so they could give the right conclusion. Through this activity, students' critical thinking and character skill would be trained.

As well on 8th lecture, it began with practical task given on 7th lecture. The aim of the task was to find relationship between calculation of stoichiometry with limiting reactant based on LKM – 3. This activity would stimulate student to improve their process skill. In addition, stoichiometry concepts would be trained using LKM – 2 about definition of stoichiometry, calculation of stoichiometry, and LKM – 4 for limitting reactant, and percent yield.The last lecture, 8th, held test to measure students' comprehension for stoichiometry concepts using critical thinking problems, and psychomotoric skill of using buret.

Result and analyze learning kit consist of: (1) accomplishment of SAP, (2) students' activity on lecture, (3) result study, (4) observed and self-assesment character skill,and (5) students response.

1. Accomplishment of SAP

The SAP developed was designed to improve students' critical thinking and chacarter skill through General Chemistry Lecture on Stoichiometry topic. The validation of learning kit developed showed that it 's feasible to use and could be used for testing. While testing, the accomplished of SAP was observed of two observer. Observation result showed SAP have been done well as written on Table 2. All learning step was done well.

Table 2. The Accomplishment of SAP

Learning step	Lecture7the			Lecture8the		
	Acc	N-Acc	Score	Acc	N-Acc	Score
Preliminary phase						
1. Opening lecture and doing *class setting*	√		3	√		3
2. Doing apperception	√		4	√		3,5
3. Motivating student	√		5	√		4
Main phase						
4. Guiding the observation of the result of structural task (experiment)	√		3,5	√		4
5. Guiding discussion of the result of structural task (experiment)	√		1	√		4

6. Giving accentuation for the conclusion of the result of structural task (experiment)	√		3	√		2
7. Giving information	√		4	√		3
8. Applying given information	√		3,5	√		2
9. Guiding students work on team	√		3	√		3,5
10. Guiding students present their discussion result	√		4	√		4

Learning step	Lecture 7th			Lecture 8th		
	Acc	N-Acc	Score	Acc	N-Acc	Score
Finishing phase						
11. Guiding students summarize their discussion result	√		2	√		3
12. Giving reinforcement for the best team	√		3	√		3
13. Giving structural task for next lecturer	√		4	√		3

Note: Acc :accomplished N-Acc : not accomplished

Critical thinking skill was trained through *LKM – 2: Stoichiometry Calculation* and *LKM – 4: Determine Limitting Reactant and Percent Yield* that have been done on team, there're 7 teams. Not all of critical thinking component was on thus LKM, it just used the one which are relevant to Stiochiometry topic. They are: 1) interpretation (significant coding), 2) analyzing (analyzing argument), 3) evaluation (evaluating argument), 4) interfering (interfering alternatives and making conclusion), and 4) explanation (explain result). The result of LKM – 4 showed on Table 3.

Table 3. LKM – 4 Determine Limitted Reactant and PerccntYield

No.	Critical Thinking Component	Description
A.1	Explanation/ Explain result	Well done by all team, average score 4.4/5
A.3	Interpretation/ Significant coding	Well done by all team, average score 5/5
A.5	Interpretation/ Significant coding	Almost all teams made mistake on it
B.1	Interfering/ Interfering alternative	Some teams gave the wrong answers, just two teams done it well
B.3	Evaluation/ Evaluate argument	Some teams gave right answers along with good argument
C.4	Explanation/ Explain result	Well done by almost all team
C.5	Interpretation/ Making conclusion	Some teams haven't done it yet, so the result could not be analyzed

LKM – 1 :Reaction between Acetic Acid Solution and Baking Soda and LKM – 3:Reaction between Sodium Carbonat Solution and Plumbum (II) Nitrat Solution designed to improve students' process skill. Considering that chemistry is science, which is developed from experiments, so chemical experiment was used on this lecture. Because of that, learning and assessment for chemistry should concern scince characteristic as a product and process. (Ibrahim, et al, 2010).In addition, students' ability of process skill could be observed through assessment of experiment report. The process skill are formulating problem, making hypothesis, determining variable, organizing data, and making conclusion. The report sent on *softfile* format to familiarize using ICT for lecture. Generally, their process skill increased in LKM – 3, because they made a reflection in LKM – 1, so they could revise the wrong one. Increasing process skill showed on Table 4.

Table 4. Increasing Students' Process Skill

Process Skill Component	LKM – 1 : ***Reaction between Acetic acid solution and Baking soda(s)***							LKM – 3: ***Reaction between Sodiumcarbonat solution and Plumbum (II) Nitrat* solution**						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Formulating problem	-	√	√	√	-	-	-	√	√	V	√	√	-	-
Making hypothesis	√	√	√	√	√	-	-	-	-	√	√	√	√	-
Determining variable	√	√	√	-	√	-	√	√	√	√	√	√	√	√
Organizing data	√	-	-	-	-	-	-	√	√	√	√	√	√	√
Making conclusion	-	√	-	√	-	-	-	-	√	√	√	-	-	-

Students' character skill improved by doing activities through cooperative learning that focused of being polite, honest, and accurate. Students learned to be polite when discussion was held. They learned to be honest when reporting the result of team discussion and the result of experiment. On other side, they learned to be accurate when solving the problem in LKM.

2. Students' Activity

Students' activity was observed by two observer. It was not a general observation, every observer just observed a

team and their member. Figure 1 shows the activity score for 10 students during the lecture.

Based on that, can be stated that students' activity on 7th lecture better that on 8th lecture. On 7th lecture, all students was so active and marked good activeness score (>0.5 of 1.0), but just one students was marked active with activeness score >0.5 of 1.0. Based on observers' note, on lecture 7th all students did a experiment. For addition, on 8th lecture, the team gave presentation. The complete result showed on Figure 1 and Table 5.

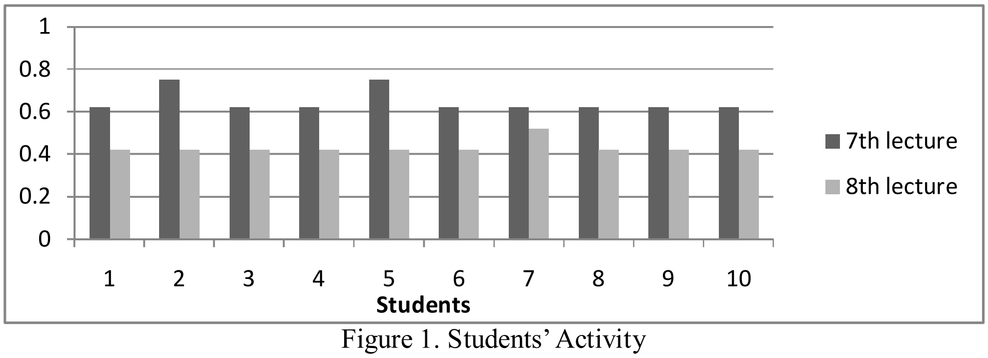


Figure 1. Students' Activity

Based on Table 5 known that the worst is asking activity, both of mutual questioning on preliminary learning and discussion. The students needed more guidance to improve how to communicate the ir idea which is one of process skill components.

Table 5. Students' Activity

No	Activities	%	
		7th lecture	8th lecture
1.	Doing mutual questioning about preliminary concept	0	0
2	Observing the result of structural task	100	100
3	Doing mutual questioning about structural task	10	10
4.	Apllying concept on given problems	100	100
5.	Discussing on team	100	100
6.	Presenting the result of team discussion	100	0
7.	Doing mutual questioning about the result of team discussion presented	20	0
8	Doing experiment	100	-

3. Observation and Self-Assesment Character Skill

Character skill of 10 students observed by two observers. Their result would be compared with students' self assessment. Go on Table 6 for complete result.

Generally, while lecturer students showed good character skill, especially for being accurate. All of observed students did the task as it instructed, solving calculation problem based on the steps, and reporting perfectly. But, this good result should be compared with students' result study, both of in example problem and comprehension test.

Students' needed more improving for their honest to communicate their own argument and the habit for raising hand before giving argument. The score for thus two activity was not so good, but it didn't mean that they lack of character. In some cases, found that students' too shy or too afraid to communicate their idea, question, or argument, so they asked the other to do it for them. This habit could be classified as not being honest. This type of students did not have good process skil, considering that communicating skill is one of process skill component. In addition, by doing that, they would classified as unactive students (Lickona and Davidson, 2005 & Facione, 1990).

Table 6 Students' Character Skill

Character Skill Components	(%)	
	7th lecture	8th lecture
Honest		
1. Reporting the result study include the references	100	100
2. Reporting the observation result as it found in fact	100	100
3. Communicating their own opinion	60	60
Polite		
1. Being good listeneron group disscussion	100	100
2. Not cutting off the others word roughly	100	100
3. Raising hand before asking or giving argument	40	20
Accurate		
1. Doing the tasks as it instructed	100	100
2. Solving calculation problem follow the steps	100	100
3. Reporting result of the task correctly as instructed	100	100

4. Students' Result Study

Students' result study consist of: (1) mark of pretest and posttest for stoichiometri comprehension and critical thinking skill, and (2) psychomotoric skill. Based on data, known that students' stoichiometry comprehension was improved. Average rate 18.6 for pretest increase to 56.29 in posttest.Although it significantly increased, but it's not good enough. Because number of students that not passed the standart (grade C, >56 rate) is 19 of 38 students. In depth, 18 students got >55 rate, the highest rate is 89and the lowest is 39 of 100 rate. It showed how heterogenous the class is. Pretest andposttest rate analyzed to know students' critical thinking skill on Stoichiometry topic. The result of this analysis showed on Table 6.

Almost all of thus critical thinking component showed increasing. It meant this learning kit can improved students' critical thinking skill. For addition, most of students are usual using shortcut steps to solve the stoichiometry calculation problems in pretest, so they got some difficulties to solve it with the right steps. So, it's a homework rof next research to make morehighlite for preliminary concepts to avoid misconception. Not all of critical thinking skill was trained on this lecture, but only some relevant critical thinking skill. Actually, there are six critical thinking skills and the subtopics included. They are (1) interpretation (classifying, signcificancy coding, clarifying); (2) analyzing (studying ideas, identifying argument), (3) evaluation (evaluating claim, evaluating argument); (4) interferring (questioning proof, looking for alternative, making decision), (5) explanation (giving result, approving procedure, giving argument); and (6) self regulation (studying their self, evaluating her self) (Lai, 2011)

Table 6. Students' Critical Thinking Skill Based on Stoichiometry Comprehension Test

No.		Critical Thinking Component	Score	
Pretest	Posttest		Pretest	Posttest
2 a	1 a	Interpretation/ significancy coding	0,97	0,93
2 b	1 b	Interfering/looking for alternatives	0,15	0,75
5	2	Interferring/making conclusion	0,17	0,50
9	5	Interferring/making conclusion	0,05	0,63
11	6	Explanation/explain argument	0,15	0,51
12	8	Interferring/making conclusion	0,04	0,27

5. Students' Response

Through students' response would be known how the student responed during lecturer. It said that most of the command for thinking critically and behaving in good character was received well by the students. Students' crtical thinking skill needed more improvement through every lecturer they'll do. Their character skill for being honest, polite, and accurate were good enough and needed more improvement for the others component of character skill. So, it needs to keep integrating character skill in every lecturer.

CONCLUSION

The testing result of developed learning kit showed that it could improve students' critical thinking but not so significant. In line with the the lack of students' asking activity in other hand, students' gave good mark for polite, honest, and accurate character skill.

PREFERENCES

Brady, James.E.2004. *General Chemistry.Principle and Structure. 4th*. ed. New York. John Willey and Sons, Inc.

Chang, Raymond. 2005. *General Chemistry The Essential Concepts Third Edition.* USA: McGraw Hill.

Facione, Peter A.. 1990. *The Delphi Report.* California:Califonia Academic Press.

Glencoe Science. Chemistry: Matter and Change

Ibrahim, Muslimin. 2009. *Hasikat IPA*¸.Surabaya:Unesa University Pres.

Kementerian Koordinator Bidang Perekonomian. 2011. *Masterplan Percepatan dan Perluasan Pembangunan Ekonomi Indonesia 2011 – 2015 Republik Indonesia*. Jakarta: Kementerian Koordinator Bidang Perekonomian.

Lai, Emily R. June. 2011. *Critical Thinking: A Literature Review. Research Report*. Pearson.

Lickona, T., and Davidson, M. (2005). *Smart & Good High School: Integrating excellence and ethics for success in school, work, and beyond.* Cortland, N.Y.: Centre for the 4th and 5th Rs (Respect & Responsibility)/Washington, D.C.: Character Education Partnership.

Nur, Mohamad. 2008. *Pengukuran Kemampuan Logika Peserta didik dan Guru YLPI Al Hikmah Surabaya.*

Partnership for 21st Century Skills (USA).

Poedjiastoeti, Sri. 2008. Development of Student Activity Sheet (LKS) in Subject Science Orented Direct Instructional for Senior Highschool with Special Need Education (SMA-LB). *Proceeding of The 2nd International Seminar of Science Education.*18-10-08. ISBN.978-979-98546-4-2.

Poedjiastoeti, Novita, D., Miseri. 2007. Recognation of Chemistry Laboratory Equipment to Increase Study Science in Senior Highschool for Special Education (Deaf). *Proceeding of The 1st International Seminar of Science Education.*27-10-07. ISBN 979-25-0599-7.

Presiden Republik Indonesia. *Lampiran Peraturan Presiden Republik Indonesia Nomor 5 Tahun 2010 Tentang Rencana Pembangunan Jangka Menengah Nasional (RPJMN) Tahun 2010 – 2014. Buku 1 Prioritas Nasional. 2010*. Diperbanyak oleh: Kementerian Perencanaan Pembangunan Nasional/Badan Perencanaan Pembangunan Nasional (BAPPENAS).

Rencana Strategis Direktorat Jenderal Pendidikan Tinggi 2010 – 2014.

Rencana Strategis Kementerian Pendidikan Nasional 2010 – 2014.

Rencana Strategis Unesa Tahun 2011-2015.

Riduwan. 2005. *Variabel dan Skala Penelitian*. Bandung:Alfa Beta

Sukmadinata, Nana Saodih. 2010. *Penelitian dan Pengembangan (R & D).* Bandung:Rosda Karya

Thiagarajan, S, Semmel, D.S., & Semmel, M. I. 1974. *Instructional Development for Training Tachers of Exceptional Children: A Sourcebook*. Minneapolis, Minnesota: Indiana University.

Undang-Undang Republik Indonesia Nomor 17 Tahun 2007 Tentang Rencana Pembangunan Jangka Panjang Nasional Tahun 2005 – 2009.

CE-14

MISCONCEPTION PREVENTION OF SENIOR HIGH SCHOOL STUDENTS ON CHEMISTRY CONCEPTS USING SEVERAL INQUIRY-BASED LEARNING MODELS

Suyono

The Faculty of Mathematics and Sciences, The State University of Surabaya
Email: ionunesa@yahoo.co.id

Abstract

The aim of this study was to prevent misconceptions of senior high school students on chemistry concepts using several inquiry-based learning models. The meaning of prevention in this research was the proportion of students misconceptions smaller than the proportion of students knowing concept. Chemical concepts that learned and/or tested were the properties of colligative solution, chemical equilibrium, redox reactions, and reaction rate. The implemented inquiry-based learning models were guided inquiry, open inquiry, modified inquiry, and LC 7E. The combination of the implemented model and learned concepts produced 6 (six) learning packages. The targets were students in 6 (six) of representative Senior High Scholl in 6 cities as Unesa target areas (Surabaya, Sidoarjo, Sumenep, Gresik, Bojonegoro, and Jombang). During the implementation used one group pretest posttest design. The results revealed that : (1) the student conception in six cities before the teaching learning was dominated by student without knowing concept and by student with misconception potential; (2) from 6 (six) implemented learning packages showed the variety of student misconception, namely 11.00%; 14,80%; 11,5%; 18,5%; 22,0%; and 11,1% respectively. All these percentage were smaller than the percentage of student knowing concepts.

Key words: misconception, guided inquiry, open inquiry, modified inquiry, LC 7E

INTRODUCTION

Misconception is the most interesting phenomenon in learning to be investigated. Misconceptions on various chemistry concepts have occurred on high school students. This fact was found in Turkey (Demircioglu et al., 2005) and also in other countries where it is reported as Cheung (2008), Camacho & Good (1989), Banerjee (1995). In Indonesia, especially in the six districts/cities included intarget areas of Unesa (Sumenep, Surabaya, Sidoarjo, Gresik, Bojonegoro, and Jombang) the same facts were also found (Rohmawati and Suyono, 2012; Delhita and Suyono, 2012; Arif and Suyono, 2012). These findings strengthen the statement ofBarke et al. (2009) that a number of chemical concepts are often understood by the students' misconceptions. The misconception has become globalized problematic.

The burden misconception born by the student has to be handeled together, college intervention (LPTK) is needed to assist teachers in senior high school. Repairing misconceptions is not easy, as said Barke et al. (2009), but the stakeholders who committed to the quality of education in Indonesia should not disregard on this phenomenon. Efforts to reduce chemistry misconceptions in high school students have also been conducted by various parties or researchers, but have not reached a wider target and involve chemistry teachers who are in the target schools. Through funding sources ofDitlitabmas decentralization (TA 2013), researchers have conducted dissemination for prevention and remediation models of chemistry misconceptions on high school students in six districts/cities as mentioned above. Prevention of chemistry misconceptions used several inquiry-learning based model, while remediation which is consecutive stages of prevention stage used learning strategies based on conceptual change strategy. This article will only reports a summary of the results of the prevention phase. The selection of learning models to prevent student's misconceptionsis very important. This is in accordance with Pekmez's instructions (2010) *"the selection of teaching methods has an important factor in preventing students' misconceptions*."

Researchers tried to prevent chemistry misconceptions that often presented in high school students using the several inquiry based-learning model as researcher as researchers have argued as follows. By inquiry approach, students are given greater opportunities to learn chemistry concepts by netting (nested) common exercise of thinking skills that are commonly done by scientists, students are expected to comprehendthe way of scientists' working and thinking so gaining insight according to scientists understood, students have no chemistry misconceptions. Barthlow (2011) have shown positive results of this idea that through scientific inquiry learning, students' chemistry misconceptions can be replaced to the true concepts.

The learning model known to accommodate the syntax or a series of scientific inquiry phases and selected to prevent chemistry misconceptions of high school students were: guided inquiry, open inquiry, modified inquiry and LC7E. Chemistry concepts that learned and/or tested includedcolligative properties of solutions, chemical equilibrium, redox reactions and reaction rate. The implemented learning model were guided inquiry, open inquiry, modified inquiry and LC 7E. The combination of the implemented learning models and learned concepts generated 6 (six) learning packages as shown in Table 1.

RESEARCH METHOD

The research design used in this study was one group pretest-posttest design as presented in Figure 1.

O_1 X_1 O_2

Figure 1 Research Design

Description:

O_1 = pretest, mapping student preconceptions before prevention phase
X_1 = treatment, learning topreventmisconception using inquiry-based learning
O_2 = posttest, mapping the students' conceptions after learning topreventmisconception

The research subject was senior high school students in chemistry classes determined as the implementation and dissemination classes. From every District/Municipal, one high school whichits teachers have positive responds and participate actively in conducting research aimed at the improvement of student's misconceptions.The High School name from each district/city is shown in Table 1.The level of implementation accommodatedlearning practices conducted by chemistry teachers (the university students who are researcher) who developed and tested learning packages and instrument package. The level of dissemination accommodatedlearning practices conducted by chemistry teachers in target schools. Chemistry teacher as dissemination actors (teacher disseminator) taught chemical concepts to students using scenarios and learning packages that have been developed by teacher implementer, absolutely by adjustments. Efforts to understand the learning packages, learning scenarios and learning materials through two activities: workshops andscanning.

In order the research findings representing the feasibility of learning packages, the quality oflearning process implemented by teachers and the instructional effect of learning packages on prevention, then it wasmade treatment replication as many as two replications. Every packet was applied in two implementation classes (the first and second research classes) and intwo dissemination classes (first and second research classes). First and second research classes were guided by the same chemistry teacher. To maintaining theresearch feasibility, implementation and dissemination activities of learning packages wereimplemented in high schools targets are listed in Table 1 by crossed, except to Surabaya city. For Surabaya city, SMAN 2 Surabaya as the research target for implementation while SMAN 22 Surabaya for dissemination.

RESULT AND DISCUSSION

Result

The percentage data of students who potentially misconceptions (misconceptions profile at preconception status) and the percentage data of student misconceptions after learning process in every target schools are shown in Table 1.

Table 1 Summary of Research Findings

Learning Packages	Target Schools/Teacher Name	% MC Students		Dissemination Schools/Teacher Name	% MC Students	
		Before	After		Before	After
Guided Inquiry-Colligative Properties of Solutions	SMAN 2 Surabaya/Rosalina Eka Permatasari	16.2	11.0	SMAN 22 Surabaya/Ida Kurniawati	35.5	36.0
Open Inquiry-Chemical Equilibrium	SMAN 1 Kebomas Gresik/Marjuki	34.7	14.8	SMAN 1 Sumenep/Maswiyanto	25.6	31.9
Modified Inquiry-Chemical Equilibrium	SMA Neg 1 Kabuh Jombang/Arif Imam Subagyo	37.5	11.5	SMA Neg Model Terpadu Bojonegoro/Purwanto	49.5	38.5
Modified Inquiry-Redox Reactions	SMAN 1 Sidoarjo/Wahyu Juli Hastuti	10.5	18.5	SMAN Kabuh Jombang/Putut & Eko	9.5	19.0
LC7E-Redox Reactions	SMAN Model Terpadu Bojonegoro/Agus Sri Hono	25.0	22.0	SMAN 1 Kebomas Gresik/Khotmatuz Zuhrufah	26.5	23.0
Guided Inquiry-Rate of Reaction	SMAN 1 Sumenep/Darminto	25.2	11.1	SMAN 1 Sidoarjo/Masrutji Handayani	20.3	13.4

Note: MCbefore learning process = potentially MC

Additional data to complete the data in Table 1 is described as : (1) The structure of students'preconceptions in all target schoolshad similarities. The structure of student preconceptionsin a class consisted of student unknowing conceptsin the largest proportion, followed by the proportion of student misconceptionand the last wasknowing concepts. If there was a difference, only in the comparison of the proportion difference, but in the same order, (2) Prevention learning in the sixth learning packages has been implemented by the teachers, generally having very good quality and (3) The structure of student conception after the prevention stagewas different.Learning the same chemistry concepts, using the same learning model, commonly being conducted by the same teacher, using the same learning packages, and getting the same assessment of learning quality which was equally good, still generated the different structure of conception and even still leaved student's misconception.

Based on the data in Table 1, it can be concluded: (1) there is no classamong the target schools that is not populated by student misconception, (2) the percentage of student misconception are very greatly among the schools (one to another) and (3) after preventionusing inquiry-based learning in every target school, there isstill found a large percentage of student misconception, (4) inquiry-based learning implemented in some schools producesthe percentage of studentmisconception which is smaller than the percentage of students who previously could potentially misconception, but at some other schools just the opposite (5) inquiry-based learning actually has not yet prevented student misconception successfully to the lowest point.

Discussion

1. The Potential of Student's Misconceptions

Before participating formally in chemistry learningat school, a number of studentshave actually brought several certain concepts.There are several concepts which are accordance with scientific concept but some of those are also not.By relying to the experts' statement that the initial conception is less complete or less perfect so it is needed to design a formal learning in schools aimed to fix that rudimentary of misconception. When students are not involved yet in formal learning,sothe student preconception which are not in accordance with the scientific concept should not be convicted asmisconception, the more appropriate term isthat students is stated have alternative conception (Barke et al. 2009). This study uses the term of potential misconception that is also known knowing and unknowing concepts.From the research data,before prevention, the structure of misconception (preconception) was dominated by the percentage ofunknowing concepts, the following percentage of student misconception and then knowing concepts. This fact almost happened at all learning packages that have been practiced included the learning packages1, 2, 4 and 6, except for the learning packages 3 and 5 which was dominated by the percentage of student misconception, the following percentage of unknowing concepts and then knowing concepts.When found the largest percentage of unknowing conceptsin the earlylearning process, it wasactually equity. The differences status of student misconception indicates that before learning process in the class, students have brought certain concepts into a scheme in their cognitive structure. Scheme in student's cognitive structure would later take part in the process of assimilation and accommodation of new information. This is one of the important things that must be considered by the teacher. The teacher must know the potential of student conceptions and then attempt to awaken ourselves that all possibilities can occur as the result of assimilation and accommodation processes that apply to students of different schemes in their cognitive structure. By knowing the status of preconception students, teachers can use it in considering the learning plan, including the use it in the formation of cooperative groups.

2. The Success of Prevention Using Inquiry-Based Learning Model

Inquiry-based learning is proven to prevent student's misconception while not maximum. The prevention efforts of student misconception conductedwell by teachers proven can actualize students who are potentially unknowing conceptsand misconceptionto turn into knowing conceptat the end of learning process. When it is stillfound student misconception (potentially student misconceptionchange into student misconception) so that the advantages of inquiry learning have not been able to reconstructmisconception resistance that occurs in most students' cognitive structures. As known, misconception have resistant properties or difficult to change and tend to persist eventhough the students have been taught the concept in the right way, commonly way used by scientiststhat is scientific inquiry (Barke et al. 2009).

3. The Unsuccessful Prevention of Chemistry Misconceptions on Student

The positive facts as stated previously did not appear to the six practiced learning packages. The unsuccessful (students do not actualize themselves) was found to occur in some learning packages that is characterized by the fact that the implementation of inquiry learning still leaved percentage of student misconception even there has exceeded the proportion of student's knowing concept. The number of student'sknowing concepthas been smaller than student misconception after prevention stage occurs at: (1) learning package 3, dissemination class was the first and second research classes and (2) learning package 4,dissemination class was the first and second research classes. Discussion of the phenomenon is that in term constructing the concepts, students assimilate and accommodate new experiences with the understanding is already exist in the cognitive structure.In the process of assimilation and accommodation, an anomaly can be created (Suparno 1997). The anomaly can be prevented by extending the exercise, the ways to construct a concept. According to Fogarty (1991), exercise the ways to construct a concept would be meaningful if it would be nested process skills (implementation of scientific approach) and thinking skills. The nested process skills and/or skills have been conducted as the integral part of is inquiry-based learning model used in to prevent student misconception. When there were still found a large number of student misconceptionbecause of having process skills, practical exercise of thinking in two or three meetings is really not enough to avoid the occurrence of anomalies in the student's cognitive structure.

The concept is the result of human thought derived from the facts and phenomenastated in the definition (Dahar 2011). The concept are generalization of facts which is have the same characteristics (Ibrahim 2012). Students' failure to build concepts (unknowing concepts) may be due to students fail to make generalizations based on the data to establish the definition by themselves (reading a textbook or through practical activities) or data provided by the teacher through paperworks given to students. Students' failure in making generalizations may be due to the lack frequency ofthinking exercise at this level. Student's failure to build a correct concept, in the sense that accordance with an agreed conception of scientists (students misconception) seems to be more influenced by factors derived from students. This statement is based on the opinions of experts in the fields of learning, the results of previous studies and facts currently that apply to the entire class of research on the entire learning packages which are implemented and disseminated. The essence of the experts' statements in the field of learning associated with the source of misconceptions originating on students is that: (1) before being involvedin formal learning process at school, a student has brought a certain concept (preconception), which became a schematic in the cognitive structure showing the functions as the repellent filter of new knowledge deliberately intervened by teachers through learning so that students failed to improve their wrong conception, (2) student's alternative conceptions were able to explain the problems that occurred (Suparno , 2006) so that students do not want to change it and (3) that student's initial knowledge played an important role in learning (Suyono and Hariyanto, 2011). Muallifah (2013) and Ahmad (2013) found that students' preconceptions to be one of the most dominant factor which is recognized by students as a cause of failure to understand the concept correctly. The fact currently that apply to all research target classes on the entire learning packages which is implemented and disseminated are that students' prior conceptions structure is still dominated by potential unknowing concepts and followed by potential student misconception. This means that the potential student can not be entirely prevented despite the prevention have been carried out. That potency is real still stuck on students' cognitive structures. Scheme in the cognitive structure of students has not been functioning as the facilitator for the new knowledge gained through learning processthat found student misconception at the end of learning process.

CONCLUSION AND SUGGESTION

Conclusion

1. The structure of students’ preconceptions in all research target schools had similarities. The structure of students’ preconceptions in a class consisted of potentially unknowing concept in the largest proportion, followed by the proportion of student misconception then knowing concept. If there was a difference, only in the comparison of the proportion difference, but in the same order.
2. The structure of students' conceptions after the prevention phase is different. Learning the same chemistry concepts, using the same learning model, commonly being conducted by the same teacher, using the same learning packages, and getting the assessment of learning quality which was equally good, still generated the different structure of conception and even still leaved student misconception.

Suggestion

1. In learning practice, teacher should not assume that the identified preconceptionsas ‘unknowing concept’and ‘misconception’ would just disappear as advised by Barke et al. (2009). In fact, after prevention, the students who endure the burden of chemistry misconception still exist. Chemistry teachers at school are always encouraged and facilitated to eliminate ‘unknowing concept’and ‘misconception’on students by finding an appropriate learning strategy for their students. The implementation of inquiry model to prevent studentmisconception still requiresdeep thought in order to gaining a repair mechanism of student conceptionwhich hopefully gives a most good result.
2. Improvement of student misconception through remediation program are an inevitability because there is evidence that prevention efforts using various learning models recommended by various experts still leaves students misconceptions. According to Trumper (1997), a learning cored conceptual change can be considered as a strategy to reduce student misconception.

REFERENCES

Ahmad. 2013. *Reduksi Miskonsepsi Asam Basa melalui Inkuiri Terbuka dan Strategi Conceptual Change.* Tesis. Pascasarjana Unesa Surabaya.

Arif, M. Agus Al dan Suyono dan. 2012. Penerapan Model Pembelajaran Konflik Kognitif untuk Mereduksi Miskonsepsi Siswa pada Materi Pokok Larutan Elektrolit dan Non Elektrolit. *Prosiding Seminar Nasional Kimia Unesa*, Februari 2012.

Banerjee, C. A. 1991. Misconception of Students and Teachers in Chemical Equilibrium. International Journal of Science, 13(3): 355-362.

Barke, Hans-Dieter; Al Hazari, Al; and Yitbarek, Sileshi. 2009. *Misconceptions in Chemistry, Addressing Perceptions in Chemical Education*. Berlin Heidelberg: Springer-Verlag.

Barthlow, Michelle J. 2011. *The Effectiveness of Process Oriented Guided Inquiry Learning to Reduce Alternative Conceptions in Secondary Chemistry*. Disertation. Liberty University.

Camacho, M., Good, R. 1989. Problem solving and chemical equilibrium: Successful versus unsuccesful performance. *Journal of Research in Science Teaching* 26 (1989), 251.

Cheung, Derek. 2008. *Using Think-Aloud Protocols to Investigate Secondary School Chemistry Teacher's Misconceptions About Chemical Equilibrium.*

Dahar, R.W. 1988. *Teori-teori Belajar*. Jakarta: Ditjen Dikti-P2LPTK.

Delhita, A. dan Suyono. 2012. Penggunaan *Think-Aloud Protocols* untuk Mengatasi Miskonsepsi Siswa pada Materi Pokok Stoikiometri di SMA Khadijah Surabaya. *Prosiding Seminar Nasional Kimia Unesa,* Pebruari 2012.

Demircioglu, G.; Ayas, A.; dan Demircioglu, H. 2005. Conceptual Change Achieved Through a New Teaching Program on Acids and Bases. *Journal: The Royal Society of Chemistry,* Vol. 6, No. 1, Januari 2005.

Fogarty, R. 1991. *The Mindful School: How to Integrate the Curricula.* Palatine: Iri/Skylight Publishing, Inc.

Hasan, Saleem, Diola Bagayoko, dan Ella L. Kelly. 1999. Misconceptions and The Certainty of Response Index (CRI). *Journal: Physics Education,* Vol. 34, No. 5, hal. 294-299, http://iopscience.iop.org/0031-9120/34/5/304, diakses tanggal 13 Januari 2011.

Ibrahim, M. 2012. *Konsep Miskonsepsi dan Cara Mengatasinya*. Surabaya: Unesa University Press.

Muallifah, Lilik. 2013. *Prevensi dan Reduksi miskonsepsi Kesetimbangan Kimia Siswa SMA Negeri 1 Kandangan Kediri*. Tesis. Pascasarjana Unesa Surabaya.

Pekmez, E.S. 2010. "Analyzing of Students' Misconceptions About Chemical Equilibrium". *International Conference on New Trends in Education and Their Implications*. Antalya-Turkey.

Pekmez, E.S. 2010. "Using Analogies to Prevent Misconceptions About Chemical Equilibrium". *Asia-Pasific forum on Science Learning and Teaching, Vol. 11.*

Rohmawati, Laily dan Suyono. 2012. Penerapan Model Pembelajaran *Conceptual Change* Untuk Mereduksi Miskonsepsi Siswa Pada Materi Pokok Asam dan Basa di Kelas XI IA SMAN 2 Bojonegoro. *Prosiding Seminar Nasional Kimia Unesa*, Februari 2012.

Suparno, P. 2005. *Miskonsepsi dan Perubahan Konsep dalam Pendidikan Fisika*. Jakarta: Grasindo.

Suparno, P. 1997. *Filsafat Konstruktivisme dalam Pendidikan*. Yogyakarta: Kanisius

Suyonodan Hariyanto. 2011. *Belajar dan Pembelajaran*. Bandung: PT. Remaja Rosdakarya.

Trumper, Ricardo. 1997. "Applying Conceptual Conflict Strategies in the Learning of the Energy Concept." *Research in Science & Technological Education,* Vol. 15, Issue 1.

CE-15

THE DEVELOPMENT OF ANDROID-BASED MOBILE LEARNING MEDIA AS CHEMISTRY LEARNING FOR SENIOR HIGH SCHOOL ON ACID BASE, BUFFER SOLUTION, AND SALT HYDROLYSIS

Yogo Dwi Prasetyo[1*], Jaslin Ikhsan[2], and Rr. Lis Permana Sari[2]

1 Science Education Department, Postgraduate Study Program, UNY*
Email : prastyogo@gmail.com
2 Chemistry Education Department, Mathematics and Sciences Faculty, UNY

ABSTRACT

This research is a development research in chemistry education. The aims of this research were (1) to develop android-based mobile learning media for chemistry learning in senior high school on acid base, buffer solution, and salt hydrolysis, (2) to determine the quality of mobile learning media based on evaluating by five chemistry teachers, and (3) to evaluate the quality and to measure the response of senior high school students to the media.

The model of development was adapted from ADDIE (Analyze, Design, Development, Implementation, dan Evaluation) model. The early product was reviewed and commented by research supervisors subject matter expert , technology and information expert and, peer reviewers, and then revised. The revised product was evaluated and reviewed by chemistry teachers and was field tested by Senior High School students. The results of the evaluation was analyzed to determine the quality of product.

The result of this development research were android-based mobile learning media for chemistry learning in Senior High School on acid base, buffer solution, and salt hydrolysis. This mobile learning media can be applied on Android mobile phone. Analysis data showed that the quality of the media was very good with ideal percentage of 89.40% based on chemistry teachers evaluations and was good with ideal percentage of 76.30% based on students review. Students response that the media was interesting, joyfull, and attractive. In conclusion, the media can be applied very well for chemistry mobile learning media on acid base, buffer solution, and salt hydrolysis.

Keywords: mobile learning media; android; and acid base

INTRODUCTION

Developments in science and technology drive the process of learning to be more applicable and attractive as an effort to improve the quality of education. In general, the learning process that takes place at this time is between the students face to face with students and teachers in the classroom (conventional learning). According to Burrowes (2003), the conventional learning emphasis on the completion of the subject matter without giving sufficient time for students to reflect on the material presented, linking with prior knowledge, or apply in real life. Further stated that the conventional learning have characteristics, namely: teacher-centered learning, there is passive learning, interaction among students is less, there is no cooperative groups, and assessment sporadic. Lessons are conducted in conventional lead to student learning is not effective and do not feel motivated students, causing students less or no understanding of material provided by the teacher (Daryanto, 2010: 2). Success of learning is not only determined by the teacher and the student, but also is influenced by learning media and teaching materials used. Liliek Setiono (2009), said that the use of learning media in teaching and learning can generate interest and a new desire, motivating and stimulating learning activities, and even carry psychological effects on students.

Information and communication technologies change the location of learning from the classroom to place anywhere and anytime students can learn. Thus, the evolution of communications technology push on the evolution of the location and time of study. Learning is no longer just a place in the school and in the classroom, learning can happen anywhere as long as there are teaching materials and students feel comfortable with the situation (Mandy Akhirul A, 2009).

According to I Made Agus Wirawan (2011: 316), the use of mobile phone technology has not only focused as a media of communication, or entertainment, but also has been used as a learning media. One media that based mobile phone that can be used by teachers in the learning and application has not been developed is a mobile game based learning (mGBL). mGBL is an application form of the game that contains subject matter and constructed in accordance with the level of education and also adapted to the prevailing curriculum and running on mobile phone devices.

According to Muhamed Ally Pieri Micelle (2009: 193), learning by using mobile learning make student learning becomes interesting and fun. The learning process will be effective when the students are in a state of happy and unhappy. The students will feel fear, worry, anxiety, feeling uncomfortable that can lead not optimal results when student learning is too forced (Eko Susanto, 2009: 19-20). Accordingly, the application of mobile gaming as a learning media can be developed and utilized in accordance with the existing learning design, to create a new learning environment, effective, and enjoyable to facilitate the achievement of learning goals.

Mobile Game "Brainchemist", the learning media of chemistry can be used as an alternative to chemical learning media fun and accessible. Mobile Game "Brainchemist" is adapted from the game Brainjiggle and the game BrainJuice that has been modified so that display just the relevant with chemical materials.

Chemistry learning can not be separated from definition of the learning and chemistry itself. Chemistry is the science of searching for answers to what, why, and how natural phenomena related to the composition, structure and properties, changes, dynamics, and energetics of substances. There are two point related, the chemical can not be separated, chemistry as the products (chemical knowledge in the form of facts, concepts, principles, laws, and theories) and chemistry as the process of scientific work (Mulyasa E., 2006: 132-133) .Success in achieving the chemistry learning objectives is influenced by several factors. For example, teaching and learning strategies, methods and approaches to teaching and learning resources or learning media.

The development of learning media, both for formal and non-formal education, a curriculum that applies is the primary reference that must be considered. In addition, ease of use, attractive and also usefulness must be considered. Criteria of good learning media ideally includes 4 main things (Mulyanta, 2009: 3-4), namely: the suitability or relevance, convenience, attractive, and usefulness.

Products of learning media are developed to run on Android-based mobile phone. Android is the operating system platform for mobile devices that is open source. Android has various advantages as software that uses computer code base that can be distributed openly (open source) so that the user can create a new application in it.

Develop of mobile game "Brainchemist" using Eclipse Indigo. Eclipse is an IDE (Integrated Development Environment) to develop software and can run on any platform. The advantages of Eclipse that makes it popular is its ability to be developed by the user with a component called a plug-in (www.eclipse.org).

According to Dwi Cahya Wahyudi (2010: 30), Amelia Handayani Burhan (2012: 61), Nur Rachma Kartika and Achmad Lutfi (2012: 178), chemistry learning media can be used as a interesting media for independent learning and motivate students in the learning activities.

Based on the above four studies, the researcher developed a mobile game "Brainchemist" as a chemistry learning media SMA/MA in acid-base material, buffers and salts hydrolysis.

RESEARCH METHOD

Development of media should be done using the right development model. One model that is often used in the research is the development model ADDIE. Several phase in the ADDIE Model is as follows (Mulyanta and M Marlong Leong, 2009: 5):

1. Analysis phase
2. Design phase
3. Development phase
4. Implementation phase
5. Evaluation phase

The development research follows of the ADDIE model. The phase in this research are phase analysis (analysis of curriculum, analysis of media creation benefit), the design phase (mobile game design and preparation of assessment instruments), stage of development (manufacture of mobile games and review by supervisors, material experts, IT experts, and peer reviewers), the implementation phase (mobile game used by teachers and students) and the evaluation phase (evaluate the quality of learning media). The form of research instrument is a questionnaire to assess the quality of media produced, adaptation of existing assessment instruments (Romi Satria Wahono, 2006 in aspect and learning media assessment criteria). Assessment criteria include five aspects of the assessment are to matter and task, linguistic, materialize, audio visual display, and software engineering (without matter and task aspects for students). The results are converted from qualitative data to quantitative data, tabulated and analyzed using the ideal criteria for determining the quality of the media (Eko Putro Widoyoko, 2011:238).

RESULT AND DISCUSSION

The result of this development is a product of the mobile game "Brainchemist" as a chemistry learning media for Senior High School in acid-base, a buffer solution, and salt hydrolysis matter. The products of mobile game "Brainchemist" as a learning media is apk application form that can be run with android based mobile phone. Mobile game "Brainchemist" contain materials or matters, and the discussion of acid-base, buffer solution, and salt hydrolysis.

1. Data Quality Product from Reviewers

Quality mobile games "Brainchemist" are assessed by the reviewers first (5 senior high school chemistry teachers in Yogyakarta). The results of the assessment show that the quality of mobile games "Brainchemist" are included in the category of Very Good (SB) with an overall average score of 111.8 and ideals percentage of 89.4%. Assessment of every aspect can be seen in table 1.

Table 1. The quality of every aspect assessment by reviewer

Criteria	Number Indicator	Average Score ( )	Maximal Score	Ideals percentage(%)	Category
Matter and task	6	26,6	30	88,67	SB (>25,2)
Linguistic	2	8,6	10	86	SB (>8,4)
Materialize	4	18,4	20	92	SB (>16,8)
Audio visual display	8	35,2	40	88	SB (>33,6)
Software Enginering	5	23	25	92	SB (>21,0)
Overall	**25**	**111,8**	**125**	**89,4**	**SB** **(>105,1)**

Based on the assessment of every aspect can be made by the graph of quality of every aspect versus the ideals percentage. The graph can be seen in Figure 1.

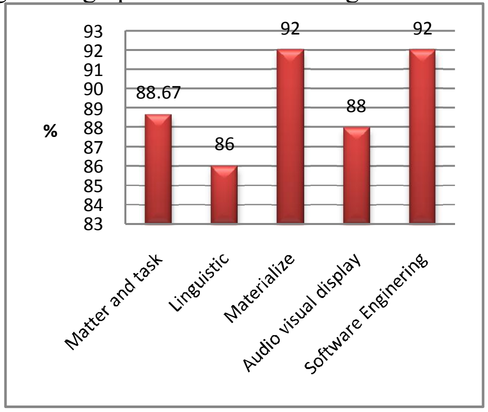


Figure 1. The graph of quality of every aspect versus the ideals percentage

The assessment by reviewer show that aspects of matter and task is included in the *very good* category (SB). Ideals percentage of matter and task aspect is 88.67%. When it is compared to the ideals percentage of the other aspects, ideals percentage of the matter and task aspect is the second largest. Percentage of 88.67% in the aspects of matter and task can not be separated from matter and task presented in the mobile game "Brainchemist" which has been adapted to the content standards of curiculum. The material presented is also adapted to the knowledge of high school students. Answer questions and discussion contained in the game are also one that are proper to the students' knowledge.

Linguistic aspects get the lowest ideals percentage than any other aspect, 86%. This is because it is less raw linguistic aspects by a reviewer. This is because the use of language in the game is still too much when compared to the games that circulate in the society. However, the language used is not rigid but the language used is the communicative language and does not cause double interpretation so that students can more easily understand. Overall aspects of language are included in the very good category (SB) and proper for use in a mobile game "Brainchemist" as a learning media for students of Senior High School.

Based on the assesment by reviewer, materialize aspects are included in the very good category (SB). Ideals percentage of materialize aspects get the highest aspect compared to other aspects, 92%. This is because of the reward each end games make students motivated to try again to obtain a desired reward by students. The interesting thing about the presentation of the material is to make the students do not feel bored easily in learning with mobile game "Brainchemist". In addition, the ability to use the media in frequent times and advantages of the media can attract the students to use this media as a supplement facility in the study.

Based on the assesment by reviewer, audio visual display aspects are included in the very good category (SB) by the ideals percentage which are also quite prominent, 88%. In all aspects of audio visual display mobile game "Brainchemist" as a chemistry learning media on Senior High School in acid-base material, a buffer solution, and salt hydrolysis can be concluded that the size of the text and pictures are very good so that there are illustrations to facilitate understanding of students in learning material presented text. The clarity of the colors used in the illustrations is very good so that students can focus on the presentation of the material which is completed by the pictures. In addition, the contrasting background color, font size is large enough, and the font type is also adapted to the user, that is the students.

Aspects assessment of software engineering also get very good (SB) of the reviewer with the highest ideals percentage than other aspect, 92%. This is because mobile games "Brainchemist" contain elements of creativity and innovation in the manufacture of new learning media. Formerly, there are still very few mobile developers who implement their ability to create learning media based mobile phone. Ease of use and ease of operation of the mobile game "Brainchemist" make the students have no difficulty in learning the material. Students who are are not familiar to the game can read the instructions for using the mobile game "Brainchemist" so that it is not difficult to use of mobile game "Brainchemist".

2. Data quality by Students

Quality mobile game "Brainchemist" also assessed from the perspective of the students. The data quality assessment is generated from the 24 students of class XI semester II SMA N 9 Yogyakarta. Assessment from students is slightly different to the reviewer, the students did not assess aspects of matter and task. Based on the assessment of the quality by students, quality mobile games "Brainchemist" included in the category of good (B) and has an average score of 76.3 with 80.3% percentage of ideals. Assessment of every aspect of the students can be seen in Table 2.

Table 2. The quality of every aspect " based on the assessment by students

Criteria	Number Indicator	Average Scor ($\bar{X}$)	Maxi-mal Score	Idelas Percentage (%)	Category
Linguistic	2	7,8	10	78	B ($6,8<\bar{X}\leq 8,4$)
Materialize	4	16,1	20	80,5	B ($13,6<\bar{X}\leq 16,8$)
Audio Visual display	8	31,3	40	78,25	B ($27,2<\bar{X}\leq 33,6$)
Software Enginering	5	21,1	25	84,4	SB ($\bar{X}>21,0$)
Overall	**19**	**76,3**	**95**	**80,3**	**B** **($64,6<\bar{X}\leq 79,8$)**

Ideals percentage quality assessment mobile game "Brainchemist" by every aspect of the assessment are presented in Figure 2.

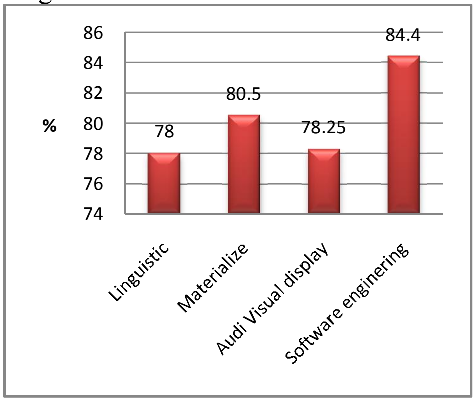


Figure 2. Quality Graphics of Every aspect of ideal percentage mobile gaming "Brain-chemist" on the assessment by students

Ideals percentage of linguistic aspects obtain the lowest, with ideals percentage 78%. Language used is a correction for researchers to pay attention to the preferred language by students. Because the age of the young studentss will prefer the communicative language for teenagers and not cause double interpretation. However, the assessment of language by studentss are included in good category (B), so that the aspects of linguistic fit for use in a mobile game "Brainchemist" to help students in learning / study.

Based on the assessment by students, materialize aspects included in good category (B) with ideals percentage of 80.5%. Ideals percentage of materialize aspect quite prominent than other aspects. This is because the implementation of mobile game "Brainchemist" is presented to the students in an interesting way. In addition, any student who uses a mobile game "Brainchemist" appropriately reward will be given the results of his work. Reward will stimulate students to try again to play, and the play went unnoticed by students and learning materials chemistry. Mobile game "Brainchemist" has the ability to be used continuously but not the same task/matter and out randomly. Mobile game "Brainchemist" has advantages over other learning media that can keep students interested in using it. The advantages of mobile game "Brainchemist" such as: simple, easy to use anywhere, anytime, and without requiring the use of the boot process.

Aspects of audio visual display included in good category (B) according assesment by students. Ideals percentage of audio visual display aspects is also quite prominent than other aspects. Aspects of audio visual appearance ideals percentage 78.25% gain. This percentage indicates that students like the look of the mobile game "Brainchemist" as a learning media that can help students to perform learning activities with an attractive illustration that accompanied the picture to make it easier to understand the material.

Based on the assessment by learners, software engineering aspects is include in the very good category (SB) with ideals percentage is the highest, 84.4%. This is because the mobile game "Brainchemist" is made with the latest learning innovations that can be used simply by using mobile phones making it easier for students to learn. Ease of touch and drag function in the mobile game "Brainchemist" is considered as to support ease of use. Mobile game "Brainchemist" also comes with use instructions that explain how to use the software game, so that students who do not know how to use can be guided by the instructions for use. In addition, for the development of learning media for science and technology development of mobile game "Brainchemist" This is very promising, because the use of mobile phone as a learning media is still little use.

Students were asked to rate other than product quality are also given open-ended questions that are packed in assessment instruments to determine student response. The response of students who are interested and excited to learn by using mobile gaming as many as 23 people or 95.8% and who are interested in learning more after playing the mobile game "Brainchemist" as many as 23 people or 95.8%.

The product of this research is mobile game "Brainchemist" which has a minimum specification devices to run on android based mobile phone, operating system Android Froyo 2.2, 256 Mb RAM, and 600MHz processor. File size of mobile game "Brainchemist" of 15 MB can be installed on an external mobile phone memory. Example of display in a mobile game "Brainchemist" can be seen in Figure 3.

ASAM DAN BASA

I. Teori asam basa Arrhenius

a. Asam

Menurut Arrhenius, asam adalah zat yang dalam air akan melepaskan ion hidrogen (H^+) atau ion Hidronium (H_3O^+).

Contoh :

$HCl(aq) + H_2O(l) \rightarrow H_3O^+(aq) + Cl(aq)$

b. Basa

Menurut Arrhenius, basa adalah

Sebanyak 40 mL larutan CH_3COOH tepat bereaksi dengan 20 mL larutan NaOH 0,15 M. Konsentrasi CH_3COOH tersebut adalah....

A. 0,075 M
B. 0,05 M
C. 0, 4 M
D. 0, 45 M
E. 0,75 M

Figure 3. Screnshoot matter of media

CONCLUSION AND SUGGESTIONS

Conclusion

Mobile game "Brainchemist" as a chemistry learning media for Senior High School in acid-base material, a buffer solution, and salt hydrolysis qualified in accordance with the categories specified quality media has been successfully developed. Quality of mobile game

"Brainchemist" as a chemistry learning media for Senior High School in acid-base material, a buffer solution, and salt hydrolysis based assessment by reviewer scored an average of $\overline{X}$ = 111,8 ($\overline{X}$ > 105,1), thus including the categories of very good (SB) with ideals percentage of 89.4%. Quality of mobile game "Brainchemist" based on assessment by students scored an average of $\overline{X}$ = 76,3 (64,6 < $\overline{X}$ ≤ 79,8), so it is included in the category Good (B) with the ideals percentage of 80.3%. Based on this assessment, the mobile game "Brainchemist" fit for use as a learning media or as reference students in learning.

A total of 95.8% of students believe learning by use the mobile game "Brainchemist" more interesting and fun, and as much as 95.8% of students are also interested in further study chemistry after playing Mobile Game "Brainchemist". These results indicate Mobile Game "Brainchemist" can make students become interested in studying chemistry with a nice atmosphere.

Suggestion

Suggestions can be submitted by researchers associated with the development of this research is a kind of learning media needs to be developed to be operated by using another OS, such as iOS, Blackberrry, or Windows phone. Learning media similar to other chemical materials need to be developed in order to be used as a complementary reference for students. Mobile Game "Brainchemist" needs to be tested in teaching of chemistry in senior high school to determine the extent of benefits and drawbacks.

BIBLIOGRAPHY

Amelia Handayani Burhan. (2012). Pengembangan Chemistry Game Seri Stoichiometry Academy sebagai Media Pembelajaran Kimia untuk Siswa SMA/MA Kelas X. *Skripsi Program Studi Pendidikan Kimia FMIPA UNY.*

Azhar Arsyad. (2011). *Media Pembelajaran*. Jakarta: PT Raja Grafindo Persada.

Cahya Dwi Wahyudi. (2010). Pengembangan Permainan Who Wants to be a Great Chemist? sebagai Media Pembelajaran Kimia untuk Siswa Kelas XI. *Skripsi Program Studi Pendidikan Kimia FMIPA UNY.*

Daryanto. (2010). *Media Pembelajaran*. Yogyakarta: Gava Media

E. Mulyasa. (2006). *Kurikulum Tingkat Satuan Pendidikan*. Bandung: Remaja Rosdakarya

Eko Putro Widoyoko. (2011). *Evaluasi Program Pembelajaran*. Yogyakarta: Pustaka Pelajar

Eko Susanto. (2009). *60 Games untuk Mengajar*. Yogyakarta: Lukita.

I Made Agus Wirawan. (2011). Pengembangan Desain Pembelajaran Mobile Learning Management System Pada Materi Pengenalan Komponen Jaringan. *Jurnal Penelitian dan Pengembangan Undiksa.*5(3).hlm. 312-324

John D Latuheru. (1998). *Media Pembelajaran dalam Proses Belajar Mengajar Masa Kini.* Jakarta: Depdikbud.

Ketut Jaliantara. (2009). *Pendekatan Pembelajaran Konvensional.* Diakses dari http://edukasi.kompasiana.com/2009/12/20/pendekatan-pembelajaran-konvensional/. Pada tanggal 16 Mei 2012, Jam 22.00 WIB

Liliek Setiono. (2009). *Membangun Media Pembelajaran.* Diakses dari http://ilmukomputer.org/2009/06/10/membangun-media-pembelajaran/. pada tanggal 24 Februari 2012, Jam 20.00 WIB

Mohamed Ally. (2009). *Mobile Learning: Transforming The Delivery Of Education And Training.* Canada: AU Press.

Mulyanta. (2009). *Tutorial Membangun Multimedia Interaktif Media Pembelajaran.* Yogyakarta: Universitas Atma Jaya Yogyakarta

Nana Syaodih Sukmadinata. (2008). *Metode Penelitian Pendidikan.* Bandung: PT. Remaja Rosdakarya.

Rachma N. Kartika dan Achmad Lutfi. (2012). Memotivasi Siswa Belajar Materi Asam Basa

Melalui Media *Permainan Ranking One Chemistry Quiz*. *Jurnal Pendidikan Kimia UnesaVol. 1 No. 1, Hlm. 174-179 Mei 2012*

Romi Satrio Wahono. (2011). Aspek dan Kriteria Penilaian media pembelajaran. Diakses dari http://romisatriowahono.net/2006/06/21/aspek-dan-kriteria-penilaian-media-pembelajaran. pada tanggal 20 Februari 2012, Jam 08.00 WIB.

Sri Rahayu. (2002). Kecenderungan Pembelajaran Kimia di Awal Abad 21. *Jurnal Matematika, Ilmu Pengetahuan Alam, dan Pengajarannya, Tahun 31 No.2 Universitas Negeri Malang.*

Stephanus Hermawan S. (2011). *Mudah Membuat Aplikasi Android*. Yogyakarta: Penerbit ANDI.

Tim Pengembang JENI. (2008). Permainan Edukatif Berbasis Mobile Sebagai Media Pembelajaran Matematika dan Bahasa Inggris. *Laporan Penelitian Java Education Network Indonesia.*

Wiwik A A. (2009). *Antara* Game*, Pendidikan dan HP (Game mobile Learning sebagai Wacana Pendidikan*. Diakses dari http://m-edukasi.net/artikel-mobile-learning-isi.php. pada tanggal 9 Juni 2012, Jam 13.00 WIB.

CE-16

ENGAGING STUDENTS IN SOCIAL EMOTIONAL LEARNING: THE ROLE OF DILEMMA STORIES IN CHEMISTRY LEARNING

Yuli Rahmawati, Nurbaity, and Marheni

Chemistry Department, Jakarta State University
e-mail: yuli.chem@gmail.com

ABSTRACT

The research aimed to engage students in social and emotional learning in chemistry learning. Dilemmas stories have been engaged students in values awareness through critical thinking and dilemma thinking. The research was conducted in Secondary schools in Jakarta. Interpretive study as a methodology provided deep engagement for both researcher and participants. The VLES (Values Learning Environment Survey), semi-structured interviews, and classroom observations were used as methods of collecting data. The VLES obtained perceptions of student engagement with the story, the teacher's supportive role, critical thinking skill development, and students' learning to listen actively and think about the chemistry learning. The results show that the students and the researcher engaged in critical reflection on their values, collaboration with others, on their roles in participating in environmental education, social issues, and in deep chemistry learning.

Keywords: social emotional learning, dilemma story, chemistry learning, action research

INTRODUCTION

The aim of education is not only to enhance students' understanding, but more important is to educate young people as agents of change who understand their character and culture. Character education and culture become an important issue in the curriculum at various levels. According to Chang and Munoz (2007), the most important factor in the formation of character is how young children cope with the problems in their society. One way to educate students to understand their character, culture, including their values is through the use of dilemmas stories. The dilemmas stories relate to everyday life and current issues which have dilemmas. According to Scardamalia , Bransford , Kozma , and Quellmalz (2012) there are important skills possessed by the students when they finish school and work, for example, the ability to:1) solve the problem , 2) make decisions , 3) work independently , 4) speak effectively and 5) work effectively with the use of technology. In this context, dilemmas stories help students to develop their critical thinking, problem solving skills, decisions making, and working with others which related to developing students' soft skills.

This innovative approach to socially responsible science education which earlier developed in Australia to address the National Framework for Values Education in Australian Schools and the National Goals for Schooling in the Twenty-First Century, as outlined in the Adelaide Declaration (1999), which argue that Australia's future depends on a solid foundation for young Australians' intellectual, physical, social, moral, spiritual and aesthetic development (Department of Education, Science & Training, 2005). Some examples of dilemmas stories that have been developed in the context of several countries (Taylor & Taylor, 2009) are: Mining dilemma, Rice fish dilemma, Nuclear power dilemma, Climate change dilemma, Dilemmas from Pakistan, etc.

The paper is the part of the results in study of dilemma story teaching in chemistry learning. At this learning process, students act as the main character in the story, so that students felt that the story happened to them. In everyday life, students are often faced problems which associated with science issues. Problems in daily life often create conflicts that require them to make decisions. Dilemmas stories educate students to integrate their knowledge with dilemmas in everyday life which allow them to engage in deep learning.

THEORETICAL PERSPECTIVES

Soft Skills

Character education is one important aspect of the educational process. According to Arthur (2008), character education focuses on student basic values and behaviour. Arthur and Wilson (2010) point out that education should 'produce' workers who are professional and qualified citizens, which not only the generation who have the knowledge, but also a willingness to keep learning and personal skills and values. According to Hutcheon (1999), school plays important role in establishing the character and culture of the students, especially in facing problems within an increasingly complex society and multicultural .

According Robbles (2012) soft skills are interpersonal qualities, also known as people skills, and personal attributes that one possesses. According to Rao (2010), soft skills are all skills, personality, and behavior that help beyond technical capabilities, such as team building, leadership, motivation, time management, and emotional, social, and personality Intelligence. Soft skills attributes include shared values , motivations, behaviours, habits, character and attitude. This attribute is owned by everyone with different levels which is influenced by the habits of thinking. Soft skills is a difficult competence to be defined as it is invisible and not immediate developed, while hard skills are skills that can produce something that is tangible and immediate (visible and immediate) which can be assessed from a technical or practical test.

Dilemma Story

According to Settlemaier, (2003), dilemmas stories are used for stimulating students' reflect critically on their values and beliefs through dilemma situation. The Dilemma stories can be in different forms such as summary of problematic situation, film, role-play, and stories (Settlemaier, 2003). In that study teachers had used specially prepared ethical dilemma stories to engage students in critical reflective discourse and collaborative decision-making on the ethical implications of science and its uses (Settelmaier, 2003, 2009). This approach also contributes to the recent call to 're-imagine' science education as an exciting, authentic, investigative and meaningful experience for all students (Tytler, 2007). In this respect, a good ethical dilemma teaching story – like all classic moral dilemmas - has no final solution, thus allowing for prolonged thinking, reflection and learning. Content knowledge – chemistry, biology, physics, mathematics concepts and skills - is essential in order to understand and to find solutions to the ethical dilemmas embedded in the stories. Thus dilemma story pedagogy does not aim at replacing content-based science education but rather aims to enhance its quality and relevance to modern day students by 'adding value' to their learning.

Values Learning Environment Survey (VLES)

The VLES was developed with three specific goals: (a) to promote values learning: the instrument provides teachers of science (and other school subjects) with a clearly articulated framework for establishing a values learning classroom environment; (b) to assess values learning: the instrument provides teachers with a relatively simple method for assessing the quality of students' engagement in values learning; and (c) to monitor values learning: the instrument allows teachers and researchers to trace students' long-term values learning development. the VLES was designed in order to obtain measures of students' and teachers' perceptions of 6 key factors of the classroom learning environment that our earlier research shows (Settelmaier, 2009) are conducive to values learning: critical self-reflection, empathic communication, critical social thinking, deep engagement, collaborative decision-making, teacher support. Each of these factors became initially a 7-item scale, later reduced to five. The questionnaire has two versions for measuring perceptions of the learning environment as experienced (a) by students and (b) by teachers. Previous research has established that student achievement is optimal when students perceive a close match between their preferred and actual learning environments (Fraser & Fisher, 1983). The instrument has a five-point Likert-type frequency response scale. For readability purposes on the questionnaire we re-named the scales: 'The Dilemma Story' (deep engagement); 'The Teacher' (teacher support); 'Learning to Work Together' (collaborative decision-making); 'Learning to Listen' (empathic communication); 'Learning to Think' (critical self-reflection); and 'Learningabout Science' (critical social thinking). Statistical analysis revealed that the Cronbach alpha coefficients lie in the range of 0.76-0.91, indicating satisfactory internal consistency of all scales.

RESEARCH DESIGN

This study implied interpretive study. Interpretive is used in real situations, rather than in contrived, experimental studies, since its primary focus is on understanding the research context. The participants in this study are students in chemistry classes from two schools in Jakarta. Multiple research methods (interviews, questionnaire (The VLES), and classroom observations) were used to provide the integrated pictures of the research. Dilemmas stories that are applied in this research was created and assessed by lecturers, teachers, and students' teachers. The criteria used to assess the stories are the dilemma in the story, a story with a chemical linkage, the truth of chemistry concepts in the story, and the story and the language used. The classroom observations were conducted during the research to provide the picture of classroom environment. Then, the VLES was used to assess students' perceptions on their classroom environment. The researchers conducted the interview with the students.

RESULT

Dilemmas stories teaching has been developed in Indonesia context. Development of dilemmas stories in relation chemistry topics and problems in Indonesia developes throughout the study. The study involved three steps: 1) dilemmas stories development, 2) VLES modified in Bahasa, 3) Implementing dillemmas stories teaching in chemistry learning. Thus the results of research is divided into three main parts, namely dillemmas stories development, realibitas VLES instruments in Bahasa, and implications of dilemmas stories.

Dillemmas Stories Development

This dilemma story development through several steps. Stories are created by the research team. Furthermore, the stories was assessed through rubric of dilemma story assessment. Description of the results of the assessment dilemma story are described in this section . There are 8 dilemmas stories has been developed in this study : 1) Hg (Merkuri) in skin whitening, 2) Food Preservative, 3) Sea Water, 4) Jelantah Oil, 5) Heavy Metals in Mineral Water, 6) ABS (Alkyl Benzene Sulfonate) in Detergents, 7) Carbonic Acid in soft drinks , and 8) Pesticide.

Results of the story through the assessment rubric given to 12 people consisting of experts and students. Rubric used to have indicators : dilemmas in the story, chemistry concept, benefits for students, language , and application chemistry in the story. Each question consists of five choice likert response scale assessment: Strongly Disagree, Disagree, Agree , Strongly Agree with a score of 1 , 2 , 3 , and 4. Assessment rubric of each story is presented below (Table 1):

Table 1. Assessment of Stories

No	**Criteria**	Mean of Dilemmas Stories							
		1	2	3	4	5	6	7	8
1	Dilemmas stories in relation to daily lives	3.5	3.5	3.2	3.5	3.3	3.7	3.3	3.4
2	Dilemmas stories in relation to chemistry concepts	3.3	3.3	3.0	3.1	3.0	3.0	3.1	2.8
3	Dilemmas stories motivate students and develop students' critical, creative thinking, and problem solving	3.3	3.4	3.3	3.3	3.2	3.3	3.3	3.2
4	Language and story setting are interesting	3.1	3.0	3.0	3.2	3.2	3.0	3.2	3.0
5	Dilemmas stories can be used in chemistry learning	3.5	3.3	3.0	3.1	3.1	3.3	3.3	3.3

There are some suggestions and feedback from the lecturers, teachers, and student teachers in relation for stories improvement:

Integrated learning outcomes in the introduction of the story
(Lecturer feedback, 23 September 2013)

The effect of waste and heavy metals could increasingly students awareness of the environment
(Student teacher feedback, 24 September 2013)

The story climax is not really interesting, should be made more dramatic, so that students are more engaged in dilemmas stories
(Teacher feedback, 24 September 2013)

Realibility of VLES Instrument

The questionnaire is about students' perceptions on the learning activities when we used dilemma story. There are 24 questions with 6 scales of deep engagement, teacher support, collaborative decision-making, empathic communication, critical self-reflection, and critical social thinking with five point likert scale with a response of strongly disagree, disagree, unsure, agree, strongly agree with scores of 1,2,3, 4, and 5. Results using the SPSS statistical analysis. The reliability of each scale can be seen in Table 2

Table 1. Internal Consistency (Cronbach Alpha Coefficient), Means and Standard Deviations

Scale	**Alpha Reliability**	**Mean**	**Standard Deviation**
Deep engagement	0.485	4.26	0.67
Teacher support	0.597	4.28	0.62
Collaborative decision-making	0.631	4.34	0.62
Empathic communication	0.716	4.26	0.58
Critical self-reflection	0.698	4.12	0.65
Critical social thinking	0.608	4.36	0.64

Number of students (N) = 128

Reliability can be determined by the internal consistency of each variable using Cronbach alpha reliability coefficient (Brown , 2007; Newby & Fisher , 1997). Overall VLES instrument that has been modified and adapted in the Indonesian context , reliability has been measured at 0.866 . While each scale in Table 2 above indicates that the alpha reliability coefficient obtained ranged from 0.48 to 0.71 . This indicates that the instrument reliable , because it has an alpha value above 0.50 . However, if viewed in each scale, then the value of reliability story remains to be improved to achieve an alpha value above 0.5 , this can be done with the repair of the story, or the questionnaire itself, especially on the language used . Mean value of each category is high at over 4.12 to 4.34. From these data, it can be seen that students respond positively to dilemmas stories learning. However, based on the characteristics of the research on interpretivism paradigm , the data used is more focused on students' responses to the learning itself which can be seen in the descriptions of implications of dilemmas stories .

Implications of Dilemmas Stories

The dilemmas story has been improved through the feedback from stories assessment. Then it followed by implementation of dilemmas stories teaching in 4 classes at two high schools in Jakarta. Most students are interested in dilemmas stories teaching. Students' deep engagement with dilemma stories and the dilemma learning experience is crucial for the success of this type of pedagogy (Settelmaier, 2009). The teacher's role as a facilitator, agent provocateur, or devil's advocate is vital within our values learning environment. The teacher is more of a guide than a 'dispenser of values'. Given that science teachers often find themselves in the role of the person who 'has all the knowledge', taking a step back and allowing students to engage actively in their values learning can be a challenge. Students feel the impact of learning dilemmas stories teaching are expressed through VLES questionnaire and interviews. Based on the interview, can be categorized into several implications learning stories dilemmas: 1) Deep chemistry learning , 2) Critical reflections on their values and beliefs, 3) Sharing and negotiating ideas, 4) Working with others, and 5) Learning from the stories. A description of each of the implications can be seen below :

Deep Chemistry Learning

That students' engagement with the dilemma stories seemed positive, and most interviewees described the experience as 'fun' and as 'different' from usual science classes. The teacher's role as a facilitator, agent provocateur, or devil's advocate is vital within our values learning environment. The teacher is more of a guide than a 'dispenser of values'. Given that science teachers often find themselves in the role of the person who 'has all the knowledge', taking a step back and allowing students to engage actively in their values learning can be a challenge. Interviews and observations indicated that teachers seemed to receive mostly positive feedback on their facilitator role, their engagement with the students, and for trying something new and different, as stated by the students bellow:

I like dilemmas stories in learning chemistry because more connected with chemistry in daily lives and more memorable
(Student Interview 3, Mercury Dilemma, SMU A)

I like it, because it becomes not a boring lesson
(Student Interview 4 , Sodium Benzoate Dilemma, SMU B)

I love it , because chemistry becomes very interesting
(Student Interview 5 , Sodium Benzoate Dilemma, SMU B)

I think its unique because it is a real story, it is easier to understand because the story is related to daily life
(Student Interview 6, Sodium Benzoate Dilemma, SMU B)

Confuse, first , because I do not like to wear cosmetics. Second , because I do not know the chemical content in cosmetics and their functions
(Student Interview 7, Mercury Dilemma, SMU A)

I agree completely of using dilemmas stories teaching , we become more understanding of chemistry. It is because we study chemistry usually only by learning theories and calculations
(Student Interview 1, Mercury Dilemma, SMU A)

I agree using dilemmas stories teaching, because we know how the application of chemistry in daily lives
(Student Interview 2, Mercury Dilemma, SMU A)

Critical reflections on their values and beliefs

When forced to make a decision students seem to engage in both critical self reflection and critical thinking (Settelmaier, 2009). Critical self reflection occurs when a student reflects on his/her own values in order to solve a problem. Critical thinking, on the other hand, is well documented in the literature as an analytic, systematic problem-solving approach that builds largely on existing knowledge, as stated by the students:

If such challenging story, I realise, I can do improvement for my environment, thinking about hazardous metals and how its impact
(Student Interview 1, Mercury Dilemma, SMU A)

Actually I'm confused, between our business and other lives
(Student Interview 8, Mercury Dilemma, SMU A)

Yes , I was confused at the time of solving the problem
(Student Interview 4 , Sodium Benzoate Dilemma, SMU B)

I am confused, because I had to put my position as myself. I'm confused what to do for my family and the other people
(Student Interview 13, Sodium Benzoate Dilemma, SMU B)

No, I can directly answer the first question when I faced with the problem that , because I positioned it as a junior high school kid so I choose my parents
(Student Interview 5, Sodium Benzoate Dilemma, SMU B)

I become aware of more careful in the use of drugs, especially cosmetics
(Student Interview 7, Mercury Dilemma, SMU A)

Sharing and negotiating ideas

Here, the focus is on developing emotional intelligence, empathy, the ability to accept and consider other people and their ideas. Most students reported that they felt their voices were listened to and their opinions accepted as legitimate. Students seemed to appreciate the opportunity to hear about other people's thoughts and opinions, and indicated the importance of knowing what others think and feel in order to care.

I love the discussion, because we can know other opinions as well as we can exchange ideas
(Interview 9 Students, Sodium Benzoate Dilemma, SMU B)

I realized, I need to think deeply about my ideas
(Student Interview 10, Mercury Dilemma, SMU A)

I love the discussion, because it combines our thinking with thoughts of friends, because sometimes we thought wrong
(Student Interview 11, Mercury Dilemma, SMU A)

Working with others

Collaborative decision making involves student groups negotiating a decision and/or a compromise in order to solve a problem as a group. Furthermore, students draw on both their own values and increasingly on evidence provided to them through their science learning to make informed decisions. One of the key issues raised during the ethnographic inquiry was the importance of recognising difference in opinions and values and working with it. Students reported that through the group-work and the discussions they seemed to become more aware of different opinions.

Thus, through these stories I can collaborate with friends
(Student Interview 11, Mercury Dilemma, SMU A)

It is better to ask a friend if there is not yet understood, because sometimes with the teachers, I am reluctant to ask
(Student Interview 2, Mercury Dilemma, SMU A)

Learning from the stories

Other implication of dilemmas stories teaching that students learn from the story presented. Story dilemma Mercury and Sodium Benzoate which applied to help the students learn to be aware in using cosmetic products, as well as preservatives in foods.

More careful in the use of drugs, especially cosmetics
(Student Interview 7, Mercury Dilemma, SMU A)

Food materials, plastic packaging, keep it mind every time using any chemical products. Whar are chemical ingredients in products, the dangerous. We have to understand its good or not for ourselves, others, or environment
(Student Interview 1, Mercury Dilemma, SMU A)

The problem of plastic, the plastic is almost everywhere much which contributes to pollution. We also have to be aware of any dangerous substances in food
(Student Interview 11, Mercury Dilemma, SMU A)

More careful in choosing products
(Student Interview 12, D Mercuryilemma, SMU A)

I suggest not to use sodium benzoate and sell with new innovations such as selling fruit ice using only sugar without preservatives and do not too much worry of preparation in order to keep the fruit fresh
(Student Interview 13, Sodium Benzoate Dilemma, SMU 30)

We may eat instant noodles, but not too often, instant noodles can be substituted with eggs or other foods. Although the food more expensive than instant noodles, our health is more valuable than money
(Student Interview 14, Sodium Benzoate Dilemma, SMU 30)

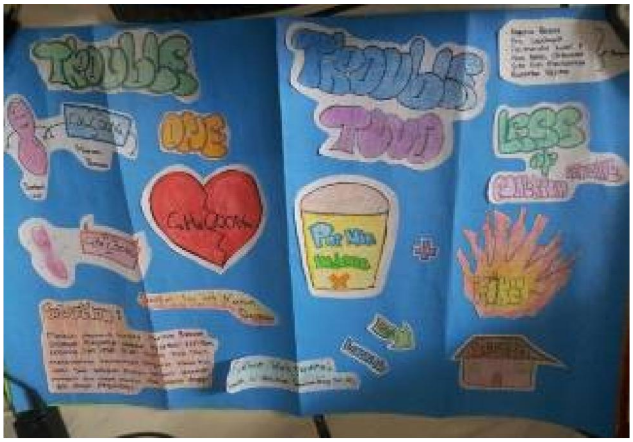
There some pictures of dilemmas stories teaching. Students created the poster as the results of their reflections on dilemmas stories teaching:

Picture 1. Teacher reads the story

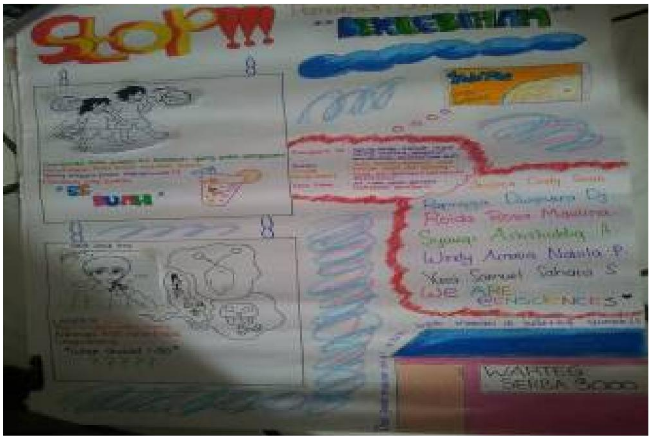
Picture 2. Students listen the story

Picture 3. Students discussion in pair

Picture 4. Students discussion in group for solving the problems



Picture 5. Students Poster on Sodium Benzoat Dilemma

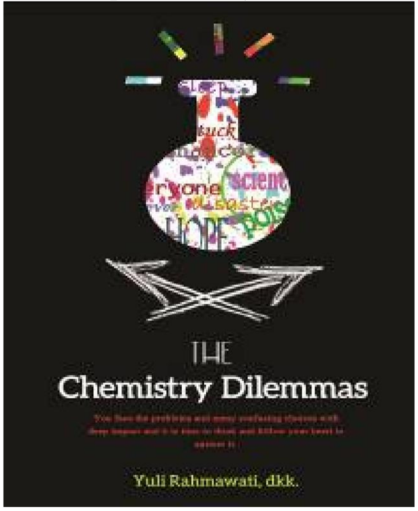


Picture 6. Students Poster on Sodium Benzoat Dilemma

Picture 7. Students Poster on Mercury Dilemma

Picture 8. Students completed VLES questionnaire

The interview and pictures above show how students learn from dilemmas stories. Even some of them try to find alternative solutions to the use chemicals. This is a positive impact on student awareness for a healthier life. The results show that the students are interested and motivated to learn chemistry as it is related to daily lives. Students also learn to understand and reflect on their values. Students learn to cooperate with others share and negotiate their ideas for solving problems on dilemmas presented. Dilemmas stories from this study are represented on the booklets and dilemmas stories weblog . The front page booklet dilemmas stories can be seen in Figure 9, whereas weblog address is http://chemistrydilemmas.wordpress.com/



Picture 9. Booklet of Dilemmas Stories

This study is still continue and the quality of the story be continuously improved by integrated topics related to chemistry learning on curriculum in 2013, particularly in the character education and social emotional learning.

CONCLUSION

The results of our study indicate that the students engaged positively in a social emotional learning. Research dilemmas stories on learning of chemistry has produced a collection of stories that can be used on engaging students in chemistry learning. Instruments Values Learning Environment Survey (VLES) has been modified in Bahasa and adapted to the context of learning chemistry in Indonesia which can be used in further research. Dilemmas stories teaching has implications for chemistry learning: 1) Deep chemistry learning, 2) Critical reflections on their values and beliefs, 3) Sharing and negotiating ideas, 4) Working with others, and 5) Learning from the stories. These results are consistent with earlier research and add further evidence to the claim that a socially responsible science education based on ethical dilemma story pedagogy offers a promising means to develop critical skills with which students can engage as socially responsible citizens in informed decision-making about the appropriate use of science for addressing global issues such as climate change and environmental sustainability (Settelmaier, 2009). Stories dilemma can be used as a chemical alternative learning approaches in learning the characters in Indonesia, especially its association with Curriculum 2013 in middle schools.

REFERENCES

Arthur, J. (2008). Traditional approaches to character education. In L.P. Nucci & D. Narvaez, *Handbook of Moral and Character Education*. UK: Routlegde.

Arthur, J. & Wilson, K. (2010). In new research directions in character and values education in the UK. T. Lovat et al. (eds.), *International Research Handbook on Values Education and Student Wellbeing.* Springer.

Brown, JD. (2007). Statistics corner. *JALT Testing & Evaluation SIG Newsletter*, 11(2),retrieved from http://jalt.org/test/bro_12.htm

Chang, F. & Marco A. Muñoz, M.A. (2007). School personnel educating the whole child: impact of character education on teachers' self-assessment and student development. *Journal of Personnel Education and Evaluation*, *19*, 35-49.

Department of Education, Science and Technology (2005). National framework for values education in Australian schools. Canberra, ACT: Commonwealth of Australia.

Fraser, B. J. & Fisher, D. (1983). Use of actual and preferred classroom environmentscales in person-environment fit research. Journal of educational Psychology, 75, 303-313.

Hutcheon, P.D. (1999). *Building character and culture*. Wesport, Connecticut: Praeger.

Newby, M., & fisher, D.L. (1997). An instrument for assessing the learning environment of a computer laboratory. *Journal of Educational Computing Research. 16,* 179-190.

Rao, M.S. (2010). *Soft skills enhancing employability: Conneting campus with corporate*. New delhi: LK. Internasional Publishing House.

Robbles, M.M. (2012). Executive perceptions of the top 10 soft skills needed in today's workplace. *Business Communication Quarterly*, 75(4) 453– 465

Scardamalia, M. , Bransford, J. , Kozma, B. , & Quellmalz, E. (2012). New assessments and environments for knowledge building. In P. Griffi n et al. (eds.), *Assessment and Teaching of 21st Century Skills.* Springer.

Settelmaier, E. (2003). Transforming the culture of teaching and learning in science: The promise of moral dilemma stories. Unpublished PhD, Curtin University of Technology, Perth, AUS.

Settelmaier, E. (2009). Adding zest to science education: Transforming the culture ofscience classrooms through ethical dilemma pedagogy. Saarbrucken: VDM

Taylor, E. & Taylor, P.C. (2009). Dilemmas stories on http://www.dilemmas.net.au/mod/resource/view.php?id=121.

Tytler, R. (2007). Re-imagining science education: Engaging students in science forAustralia's future. Camberwell, Vic: ACER Press.