Preparation and Infrared Spectroscopic Studies of Chromium (III) – Glutamic Acid Complexes, Antidiabetic Supplement Candidates

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Abstract

A new chromium(III) supplement was needed as antidiabetic supplement for patient with type 2 diabetes mellitus. A series of chromium(III) complexes with L-glutamic acid has been synthesized as the candidates. Samples were analyzed by FTIR spectroscopy and their spectra were then compared to those of L-glutamic acid and chromium(III). All spectra of the samples possessed absorption bands at 542-525 cm $^{-1}$ which corresponded to Cr-O stretching vibration and 390-410 cm^{-1} bands which corresponded to Cr-N stretching vibration. In addition, several absorption bands of L-glutamic acid disappeared after its complexation to chromium(III). These facts indicate that the complex formation between chromium(III) and L-glutamic acid is proved.

Keywords : *Cr(III), L-glutamic acid, diabetes mellitus, supplement, FTIR spec*tra

1. Introduction

Diabetes is a degenerative disease that is causing morbidity in Indonesia. There are two types of Diabetes, namely type 1 DM (which appear from birth) and type 2 diabetes that appears due to poor lifestyle and diet. World Health Organization (WHO) predicted that the amount of diabetic patients in Indonesia will increase from 8.4 million at 2000 to 21.3 million in 2030. *International Diabetes Federation* (IDF) also predicted the similar condition. Prevalence of diabetes mellitus in Indonesia reaches 5,7% [(1,2)].

The management of Diabetes includes diet, exercise, supplement or nutraceutical and insulin. Nutraceuticals (often referred to functional foods) are natural bioactive or chemical compounds that have health promoting, disease preventing or medicinal properties**[(3)].** Well known nutraceutical usually come from organic compound. In the other hand, inorganic nutraceutical and the research about this topic is less popular. The role of inorganic compounds in the management of this disease has not been widely discussed by researchers. Inorganic or metal-containing medicinal compounds may contain either (a) chemical elements essential to life forms—iron for anemia or chromium for diabetes —or (b) non essential/toxic elements that carry out specific medicinal purposes—platinum as antitumor agents [(4)].

Chromium (III) plays a significant role in glucose metabolism. Supplement contains trivalent Chromium is needed for a person with type2 diabetes mellitus. Several inorganic Cr(III) complexes (200µgCr/kg body mass) restored glucose tolerance from a ≤2.8% per minute rate of removal of intravenously injected glucose. Biological function of chromium is not fully known yet. The diabetes relevant interaction of Cr (III) is with the hormone insulin and its receptors. This suggests that Cr (III) acts with insulin on the first step in the metabolism of sugar entry into the cell, and facilitates the interaction of insulin with its receptor n the cell surface[(5,6)]. Chromium increases insulin binding to cells, insulin receptor number and activates insulin receptor kinase leading to increased insulin sensitivity. Additional studies are urgently needed to elucidate the mechanism of action of chromium and its role in the prevention and control of diabetes[(7)].

The most popular chromium supplement is Chromium picolinate, $Cr(pic)_3$, a relatively well absorbed form of chromium (III). The disadvantage of $Cr(pic)_3$ is the effect of this compound in DNA damage[(8)]. Comparative studies of chromium(III) picolinate and niacin-bound chromium(III), two popular dietary supplements, reveal that chromium(III) picolinate produces significantly more oxidative stress and DNA damage. Administration of the supplement to rats has demonstrated for the first time that it can give rise to oxidative DNA damage in whole animals[(9)]. The search for compounds with novel properties to deal with the disease condition is still in progress.

Another form of Cr(III) supplement is Chromium ascorbate complex[(10).] But there is a direct relationship between the charge of the Cr(III) species and their reactivity with DNA. The positively-charged complexes displayed ultimate DNA-breaking properties, while the neutral and

negatively-charged complexes were almost inert. Yang[11] proposed D-phenylalanine, an amino acid, as a novel ligand for Chromium (III) complex. The product was $Cr(pa)_3$. Unlike chromium picolinate, $Cr(pa)_3$ does not cleave DNA under physiological conditions.

Ochiai[12] explained the correlation of Cr(III) with glutamic acid, glycine, and cystein as GTF (*Glucose Tolerance Factor*). Based on this fact, there is a potential opportunity to develop a new Cr(III) complexes with glutamic acid as the ligand. In this work, preparation of Cr-Glutamate (Cr-Glu) was carried out in aqueous solution, by varied of three variables : reflux duration, pH and temperature. Ratio of Cr(III) : L-glutamic acid is 1:3.

2. Methods And Experimental Details

Chemicals used:

Chromium(III) chloride hexahydrates ($CrCl_3.6H_2O$) salts and L-Glutamic acid were commercially available of high purity (E-Merck). Both of them were diluted in distillate water. Sodium hydroxide was used to adjust the pH of the mixture. Synthesis of the complexes was carried out by refluxing the mixture in various time, pH and temperature. Infrared spectra were recorded with Shimadzu FT-IR 8300 spectrophotometer from 4000-400 cm-1 using KBr pellet technique.

Synthesis of Cr(III) – L-Glutamic Acid complexes

The Cr(III) complexes of L- Glutamic Acid ligand were prepared in 1:3 [metal:ligand] ratio. To a 50 ml water solution of Chromium (III) chloride (0.26 g, 1 mmol), ligand solution was added (0.4414g, 3 mmol) to obtain 1:3 ratio. The resulting mixture was stirred under reflux for 1h (then varied from 2,3,4 and 5 h) and 80^{o}C to obtain the precipitated complex. It was collected by Buchner filtration, washed with water, and dried in air. This method was based on the experiment of Yang[11]. The purple solid was weighing until a constant weight. In the study of the effect of pH, NaOH was added into the Cr(III) solution until reaches certain pH value (2.5;3;3.5;4.5; and 5).

3.Result and Discussion

The formation of the Cr(III) complexes was achieved by reaction of the ligand with Cr (III) salts in 1:3 [M: L] ratios. Color changes from green to purple indicate the occurrence of reaction. Product yield and physical data are presented in Table 1.

Table 1. Product yield and physical data of the complexes of Cr-glu

Predicted Compound	**M:L ratio**	**Product and physical data**								
$Cr(glu)_3$	1:3	Reflux duration (h), pH=4	yield (%)	color	pH (T = 80^{o}C)	yield	color	Temperature (^{o}C), pH =4	yield (%)	color
		1	88.40	purple	2.5	-	-	25	52.41	Reddish purple
		2	61.11	purple	3	-	-	40		purple
		3	62.55	purple	3.5	50.92	reddish purple	60	79.29	purple
		4	57.03	purple	4	63.14	purple	80	87.50	purple
		5	80.39	purple	4.5	81.48	bluish purple	100	88.45	purple
					5	83.01	blue	-	-	-

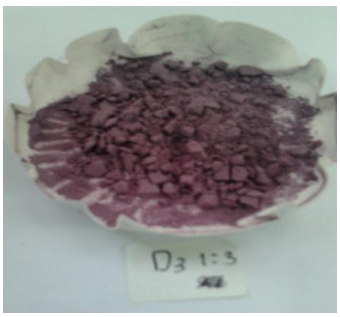

Fig.1. purple solid of $Cr(glu)_3$ complex

IR spectra of Cr (III) complexes.

Preparation of Cr - L glutamic acid complexes was carried out at various reflux duration (t) from 1 to 5 hours. FTIR spectra of these product were shown in Fig.2 and listed in Table 2.

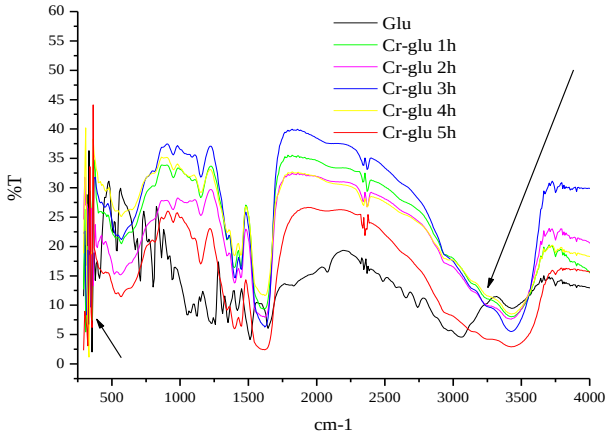

Fig 2. FTIR spectra of L-Glutamic acid (black) and Cr-Glu complexes (color) in variation of reflux duration (t)

Table 2. Infrared vibration of L- Glutamic acid (Glu), Cr (as $Cr(OH)_3$,and the complexes, cm^{-1}

Vibration	Glu [13]	Glu (exp)	Cr- Glu, (by reflux duration, t)	Cr- Glu (by pH)	Cr- Glu, (by T)	$Cr(OH)_3$ (exp)	Ref 2 [14]
υC=O	1684	1643.35	1604.77 - 1620.21→	1604.77	1404.18	-	-
υ_{as} COO^-	1556-1560 (450-470)	1512.19	-	-	-	-	-
υ_s COO^-	1404 (316)	1419,61	1396.46-1404.18	1404.18	1396.46-1404.18	-	-
δ COH	1264-1450	1257,59	-	-			
υ C-O	1120-1253	1126.73; 1257.59; 1150	1149.57-1157.29	1149.57	1149.57-1157.29	-	-
δ CH_2	1440 s	-	1442.75 -1450.47	1442.75-1450.47	1442.75-1450.47	-	-
δ C-H	1323, m 1130 w	1311.59 1125 ←	1342.46→ 1149.57 -1157.29 →	1342.46 1149.57	1342.46 1149.57		-
υ N-C	1260 mw	1257.59 ←	-	-	-	-	-
Υ_t CH2, δ CH	1225 sh	1226.73	-	-	-	-	-
Υ_t CH2, δ C-H	1187 mw 1130 m	1150 mw 1130 m	1149.57-*1157.29*	1149.57-	1149.57-	-	
υ C-C	1074vw	1075	1087.85 -1095.57	1087.85	1087.85		
υ C-C	1040 vw	1056.99	1049.28-1056.99	1049.28	1049.28	-	
υ C-C	1018w	-	-	-	-		
Cr-O strech	542 – 525	- -	570.93- 560 540.07-*516.92*	540.07	570.93 540.07	- -	
Cr-O strech			354.9-	347.19	339.47		348
Cr-O			401-416	424.34	401.19		400
Cr-N		-	447-455	478.35	447.49	- ; 385	442
Cr-Cl		-	330-324	347.9	331.76		
O-H		3000-3200	3200-3500	3200-3500	3200-3500	3200-3600	

υ = stretching; υ_s = symmetric stretching ; υ_{as} = asymmetric stretching; w = weak intensity ; Υ_t = twisting ; Υ_w = wagging ; Υ_r = rocking. Other References : 13)-17).

This spectra showed a clear pattern which shows the difference between the ligand (glutamic acid- black line) with the complex produced (colored lines). The characteristic

absorption in the IR spectra of complexes is listed in Table 2. Comparison of the infrared spectral data of complexes and ligand confirmed that complexation has occurred as significant shifts in the bands of the OH groups were observed in the region 3000-3500 cm^{-1}. The IR spectra of Cr(III) complexes showed the expected characteristic υ_{as} COO^- band in the region 1556-1582 cm^{-1} (Barth, 2000)[13] and 1512.19 (this work) are disappeared due to metal coordination.

A sharp band at 1643 cm^{-1} in the ligand due to ν C=O was also shifted to lower frequency(1620,21-1604.77) in the complexes. Moreover, the appearance of additional weak bands in the region 401-447 and 540.07-532.35 cm^{-1} which were attributed to ν(Cr-O) and ν(Cr-N), respectively, confirmed complexation.

Infrared spectrum confirmed the formation of the complex by m-C-O (1563cm^{-1}) and m-N–H (3535 cm^{-1}) and the band shifting by about 40 and 30 cm^{-1} respectively. The shifting of the moderately sharp absorption band in the free ligand (3000–3500 cm^{-1}) to about 600 cm^{-1} may be attributed to the reorganization in intramolecular hydrogen bonding after chelation. New absorption bands in the far IR region around 390 cm^{-1}, 330 cm^{-1}) and 542-525 cm^{-1} can be assigned to the Cr–O and Cr–N bonds[14]. In this work, these bands appear at 385-410 cm^{-1}, 324-337 cm^{-1} and 447.49 cm^{-1} - 424,34 cm^{-1}. Based on the stoichiometry and spectral studies, the product obtained is a complex containing a 1:3 ratio of chromium to glutamate ($Cr(glu)_3$)

The complexation also observed in various pH according to the character of metal ion in water solution which influenced by pH, included Cr^{3+}. The highest concentration of Cr^{3+} is reached at pH 4,0- 4,5.[18]. Complexes from samples of pH 2,5 and 3 were not obtained. FTIR spectra of these products were shown in Fig.3.

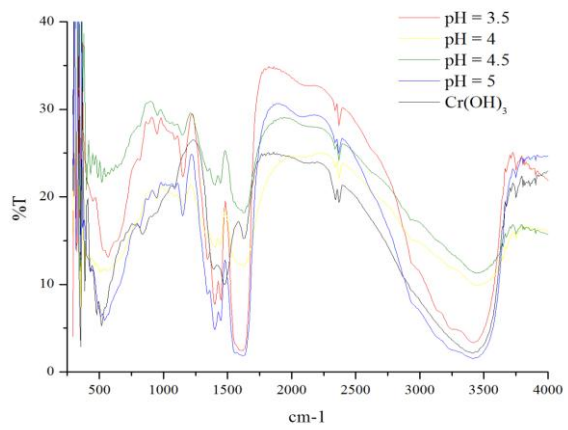

Fig. 3. FTIR spectra of Cr-Glu complexes in various pH

The effect of temperature was studied from 25°C (room temperature) – 100°C. There is no significant differences between their pattern in FTIR spectra (Fig.4). The optimum temperature is 80°C according to the product yield and the physical properties. Preparation at 100°C need an oil bath, which means increasing the cost of the process.

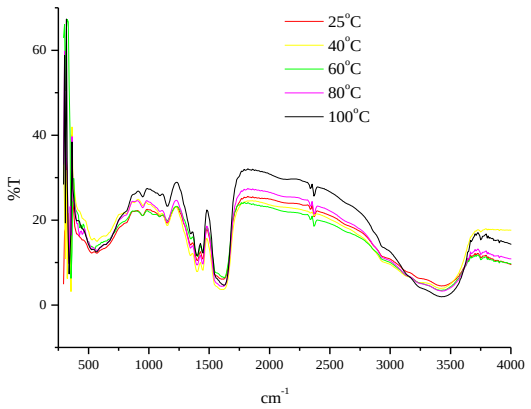

Fig.4. FTIR spectra of Cr-Glu complex in various reflux temperature

4.Conclusion

The formation of Cr-Glutamate complexes was not depend on the reflux duration but depends on the pH and temperature. The optimum pH is 4,0-4,5 and the optimum temperature is 80°C. Infrared spectra of these complexes show the disappearance of several bands of L-Glutamic acid after complexation. The new characteristic bands of Cr-O and Cr-N vibration, indicate that the formation of the complexes is proved.

5.References

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