[Chem. Pharm. Bull.
13(3) 345~351 (1965)]

UDC 547.457.1-386:546.56

47. **Zenzo Tamura and Motoichi Miyazaki**: Metal Complexes of D-Glucosamine and its Derivatives. V.*1 Spectrophotometric Investigations on Copper (II), Nickel (II), and Cobalt (II) Complexes of D-Glucosamine and the Preparation of Glucosamine-copper Complex.*2

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Very few informations have been found on the spectrophotometric investigations of metal complexes of sugars and none of the metal complexes of D-glucosamine had been reported before Ishidate, *et al.*1) described the nature of the complex formation between cupric ion and D-glucosamine (I) qualitatively in their work. They pointed out that the copper chelate had a molar ratio of 1:1 in the neutral solution from their spectral data.

However, further quantitative informations have not been obtained on the metal complexes of I.

In this report, the complex formations between I and metal ions such as cupric, nickelous, and cobaltous ion were studied respectively by spectrophotometry in visible region, and stability constants of the complexes were determined from the experimental data according to the method of McConnel-Davidson.2)

Furthermore, the authors tried to isolate the metal complexes of I in solid state.

There have been scarcely made observations as to the preparation of metal complexes of sugars and only very few informations are still available among them. Recently, Juvet3) prepared a lead complex of N-methyl glucamine and Tabe, *et al.*4) succeeded in preparing a ferrous complex of gluconic acid. However, none of the definite methods has been hitherto established for the preparation of metal complex of I.

In this report, the authors tried at first to prepare copper complex of I and it was found to be necessary to handle copper reagent and I in alkaline solution at relatively low temperature, with a rapid manipulation of the sample from the results of the preliminary experiments.

Otherwise, a reduction reaction had taken place, and I was decomposed by an existence of cupric ion in alkaline medium. However, the action of water soluble cupric salts upon I was found to be less value, because the solid prepared had no definite-value of elementary analysis. The most probable method preparing complex was to use the cupric hydroxide5) freshly prepared for the copper reagent. By this method, a 1:1 complex having a definite analytical value was obtained.

Experimental

Materials and Solutions——D-Glucosamine (I) was the same preparation used in a previous reports.*1 About $10^{-1}M$ stock solution was made by dissolving a certain amount of I in water. Three metal ion

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*1 Part Ⅳ: This Bulletin, 13, 333 (1965).

*2 Presented before the Monthly Meeting for the Pharmaceutical Society of Japan, Tokyo, September, 1963.

*3 Hongo, Tokyo (田村善蔵, 宮崎元一).

1) M. Ishidate, T. Sakaguchi, K. Taguchi, S. Kanao: Anal. Chim. Acta, **22**, 452 (1960).

2) H. McConnel, N. Davidson: J. Am. Chem. Soc., **72**, 3164 (1950).

3) R. S. Juvet: *Ibid.*, **81**, 1796 (1959).

4) H. Tabe, K. Okada: Ann. Report of Research Laboratory, Takeda Pharmaceutical Industries, Ltd., **21**, 1 (1962).

5) "Jikken Kagaku Koza" edited by the Chemical Society of Japan, Vol. 9, p. 387 (1958).

stock solutions of $10^{-1}M$ were made by dissolving $Cu(NO_3)_2$, $Ni(NO_3)_2$, and $Co(NO_3)_2$ of analytical grade in water respectively, without further purification.

Each stock solution of metal ions was standardized by EDTA titration using metal indicators. A $10^{-1}M$ aqueous solution of either HCl or NaOH was used to adjust the pH of the sample solution. To adjust the ionic strength of the sample solution $NaClO_4$ of analytical grade was used.

Apparatus and Procedure——All of the spectral measurements were made with a Cary automatic recording spectrophotometer Model 11 using a 10 mm. cell.

In the case of I-nickel system, a 20 mm. cell was used.

The sample solutions for spectral measurements were made by mixing the calculated quantities of the stock solution of I and of the metal ion stock solutions, respectively and it was diluted to make about $10^{-2}M$ solution with water. Prior to the spectral measurements, the pH of the sample solutions was adjusted to the desired value by a $10^{-1}M$ HCl or NaOH and measured with a Toa Dempa pH meter Model HM-5A equipped with a glass electrode and a calomel electrode as a reference. The ionic strength of sample solutions was maintained to $1M$ by adding $NaClO_4$.

Spectral measurements were performed at a room temperature.

Preparation of Glucosamine-copper Complex——Two grams of I was dissolved in 10 ml. of water and to this solution, one gram of freshly prepared $Cu(OH)_2$ was added at a room temperature. The reactant was thoroughly mixed under mechanical stirring, which was kept for about five minutes until the solution appeared to show a deep blue coloration.

Then, $Cu(OH)_2$ was partially dissolved and the deep blue solution was filtered to remove the unreacted $Cu(OH)_2$ on a glass filter. To the filtrate, 50 ml. of abs. EtOH was added to make the solution turbid. The alcoholic solution was placed in an ice box over night, and was precipitated blue amorphous solid, which was filtered on a glass filter, and washed with a successive portion of about 50 ml. of 95% EtOH, and finally with abs. ether.

The precipitate was dried over $CaCl_2$ *in vacuo*. Blue powder, very hygroscopic. Yield, 1.09 g. *Anal.* Calcd.: (the assumed formula G Cu(OH)Cl, assumed mol. wt. 295.17) C, 24.41; H, 4.78; N, 4.75; Cu, 21.53; Cl, 12.01. Found: C, 24.59; H, 4.94; N, 4.95; Cu, 21.75; Cl, 10.60.

Copper content of the complex was determined directly by EDTA titration using murexide as an indicator. Chloride was precipitated as AgCl by adding 10% $AgNO_3$ solution, and the amount of AgCl was determined by EDTA titration using $K_4Ni(CN)_6$ as an indicator. The amount of I in the complex was determined by the improved Fehling method previously reported⁶⁾ and was found to be 99.2% of the theoretical amount which was calculated according to the assumed formula described above.

From the analytical data, the assumed formula seemed to be the most probable for the experimental formula of the complex. Nickel and cobalt complexes of I could not be obtained by this method.

Treatments of Spectral Data——The treatment of spectral data of the metal complex system is mainly based on the method of McConnel-Davidson.

This method is applied to the spectra of 1:1 complex system. If the complex formation is carried on in the following equation (1),

$$M+GH \overset{k}{\rightleftharpoons} GM+H^+ \qquad (1)$$

where,
M : metal ion
GH : protonated form of I
k : equilibrium constant defined as $\frac{[GM][H^+]}{[M][GH]}$
GM : 1:1 complex

[ ] denotes the concentration of each ionic species.
then, k is given by the equation (2)

$$k=\frac{x[H^+]}{(a-x)(b-x)} \qquad (2)$$

where,
x : the concentration of complex formed
a : the initial concentration of the metal ion
b : the initial concentration of the ligand

if the experimental condition is chosen as a=b, equation (3) is finally obtained according to McConnel-Davidson's method

$$\frac{a}{A}=\frac{1}{\varepsilon_c}+\left(\frac{1}{\varepsilon_c k}\right)^{1/2}\left(\frac{[H^+]}{A}\right)^{1/2} \qquad (3)$$

where,
A : the optical density of the sample solution measured at the definite wave length
ε_c : molar extinction coefficient of the complex formed

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6) Z. Tamura, M. Miyazaki: Japan Analyst, **12**, 470, 561 (1963).

Therefore, many A values of sample solutions having various pH values are measured and the a/A are plotted against the $([H^+]/A)^{1/2}$ graphically.

If there is a linear relation between a/A and $([H^+]/A)^{1/2}$, a 1:1 complex as shown in the equation (1) takes place in the complex formation reaction.

When the pKa of I is known, the stability constant of the 1:1 complex, log K_1 can be calculated as follows:

$$GH \overset{Ka}{\rightleftarrows} G+H^+ \qquad Ka=\frac{[G][H^+]}{[GH]} \qquad (4)$$

$$M+G \overset{K_1}{\rightleftarrows} GM \qquad K_1=\frac{[GM]}{[M][G]} \qquad (5)$$

therefore, $\log K_1=\log k+pKa$ (6)

where, Ka is the acid dissociation constant of I, and G stands for I. K_1 is the formation constant of 1:1 complex of I-metal, and the stability constant of the complex is given by log K_1.

Results and Discussion

Copper (II) Complex

The spectra of sample solutions at various pH values were measured at a 1:1 ratio of I to cupric ion whose concentration was $2.303\times10^{-2}M$, and an ionic strength was 1. These spectra are shown in Fig. 1.

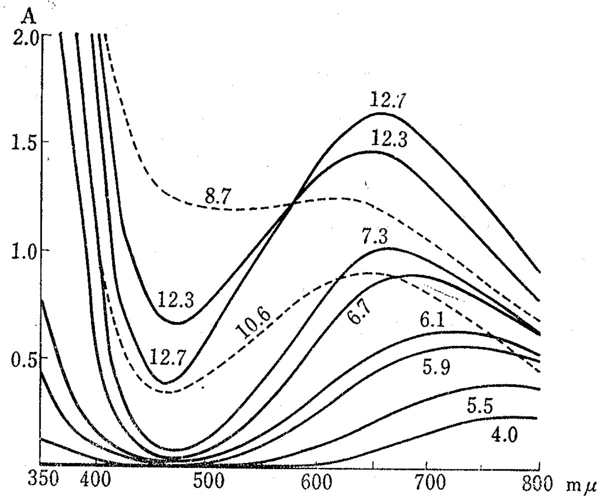

Fig. 1. Absorption Spectra of 1:1 Glucosamine-copper (II) Complex System at Various pH Values

concentration: $2.303\times10^{-2}M$ $\mu=1(NaClO_4)$

In the solutions of pH 8.7 and 10.6, precipitates which resembled to copper hydroxide or other hydrolysis products deposited and the absorption measurements were carried out with the supernatant after being centrifuged. The precipitates were redissolved at pH 12.3 and 12.7, in clear solutions. The wave length at a maximum absorption had a tendency to shift from about 800 mμ to 600 mμ as an increase of pH values.

Furthermore, a marked increase of optical density was found in the range between 500 mμ and 350 mμ. Similar phenomenon has been observed by Kubota[7] on cupra ammonium complex.

He described the relation between the absorption bands and the configuration of ligand groups of cupra ammonium complex, and found that an increase of absorbance near ultraviolet region was one of the features of hydroxo complex which was formed by the coordination of hydroxyl group to normal complex. It seems to be reasonable to consider the probability of coordination of hydroxyl group in the case of copper complex of I from the experimental data.

Moreover, this probability was supported by the fact that an increase of optical density was also found in the aqueous solution of the prepared copper complex having a composition of I to copper in 1:1 ratio, which will be described later in this report, at near ultraviolet region.

Another set of spectra of sample solutions at various pH values is shown in Fig. 2.

Measurement was made with the solution in the 2:1 ratio of I to cupric ion whose concentration was $8.630\times10^{-3}M$ and an ionic strength was 1. There was a similar tendency

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[7] M. Kubota: Nippon Kagaku Zasshi, **62**, 509 (1941).

which was seen in the 1:1 spectra to the case of 2:1 spectra. Namely, the spectral variance with an increase of pH values was also observed. However, there were such differences between 1:1 and 2:1 spectra that no precipitation was perceived in 2:1 spectra throughout the whole range of pH values measured and that relatively, lower absorption was measured in the range from about 500 mμ to 350 mμ in 2:1 spectra.

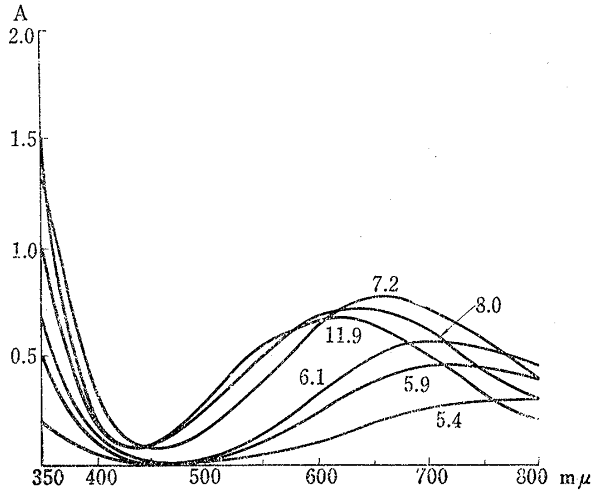


Fig. 2. Absorption Spectra of 2:1 Glucosamine-copper (II) Complex System at Various pH Values

concentration: I $1.726\times10^{-2}M$
cupric ion $8.630\times10^{-3}M$, $\mu=1(NaClO_4)$

Considering from these experimental results, it seems to be reasonable to assume that the complex formation between I and cupric ion would be complicated, and the species of complexes were not always the same in 1:1 and 2:1 sample solutions.

To determine the composition of the complex formed in the solution, McConnel-Davidson's method was applied to the 1:1 spectra. After the treatment of spectral data, a linear relation was found between a/A and $([H^+]/A)^{1/2}$ in the copper complex as shown in Fig. 3.

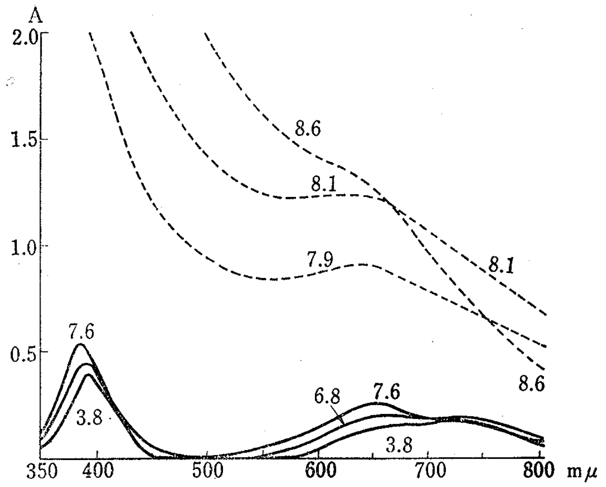


Fig. 4. Absorption Spectra of 1:1 Glucosamine-nickel (II) Complex System at Various pH Values

concentration: $4.045\times10^{-2}M$
$\mu=1(NaClO_4)$, cell length: 20 mm.

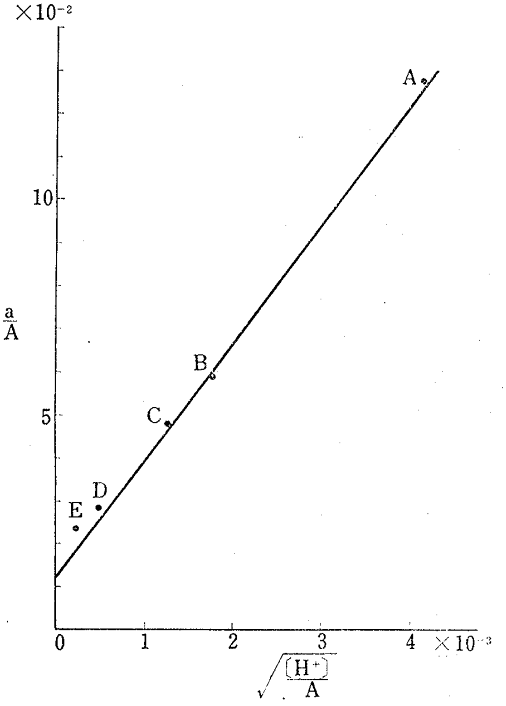


Fig. 3. Relation between $\frac{a}{A}$ and $\sqrt{\frac{[H^+]}{A}}$ at 650 mμ

I : Cu=1:1 concentration: $2.303\times10^{-2}M$
pH: A, 5.5; B, 5.9; C, 6.1; D, 6.7; E, 7.3

In Fig. 3, it is demonstrated that a 1:1 complex would form in the 1:1 sample solution under experimental conditions. However, two experimental points of pH 6.7 and 7.3 deviated slightly from the line and this fact suggests that McConnel-Davidson's method is inapplicable around these pH regions.

The concentration of unbound form of I may be increased by accelerating the degrees of dissociation of protonated form of I in these pH regions where the amount of unbound form of I can not be negligible as compared with that of the protonated form.

Concerning to the limit of the application of their method, it may be also considered that the formation of hydroxo complex will be formed arround these pH regions. Accordingly, it may be assumed that other species of complexes than the normal 1:1 complex can exist in the solution corresponding to the pH variance.

Further attempts were made to examine the composition of copper complex by using the molar ratio method and continuous variation method, but it was found impossible to obtain enough data for the establishment of composition. The impossibility may be due to the nature of the complex, especially the relatively low stability and high susceptibility of hydrolysis of the complex.

Nickel (II) Complex

The spectra of sample solutions at various pH values were measured with a 1:1 ratio of I to nickelous ion whose concentration was $4.045\times10^{-2}M$ and an ionic strength was 1.

These spectra are shown in Fig. 4.

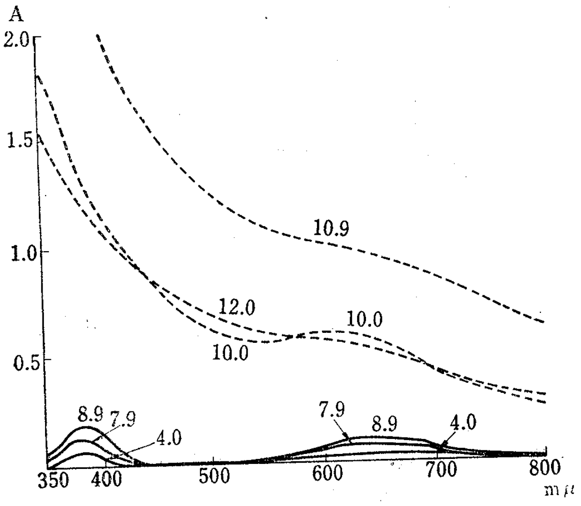


Fig. 5. Absorption Spectra of 2:1 Glucosamine-nickel (II) Complex System at Various pH Values

concentration : I $6.316\times10^{-2}M$
nickelous ion $3.158\times10^{-2}M$ $\mu=1(\text{NaClO}_4)$

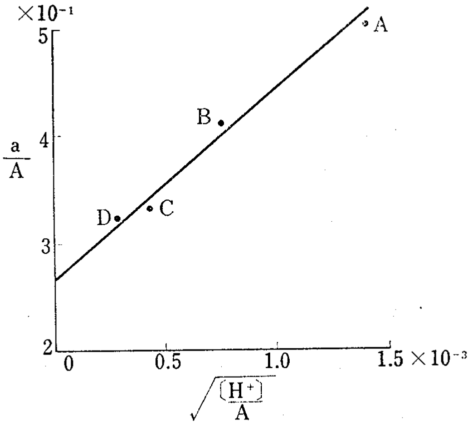


Fig. 6. Relation between $\frac{a}{A}$ and $\sqrt{\frac{[H^+]}{A}}$ at 650 mμ

I : Ni=1:1, concentration : $4.045\times10^{-2}M$
pH : A, 6.8; B, 7.2; C, 7.6; D, 7.9

As to the sample solutions of pH values of 7.9, 8.1, and 8.6 respectively, faintly greenish blue precipitates formed on standing after the measurements of absorption. In the pH range from 3.8 to 7.6, the absorptions were relatively low, and the pH range from 7.9 to 8.6, the absorptions increased. The spectra of sample solutions at various pH values were measured with a 2:1 ratio of I to nickelous ion whose concentration was $3.158\times10^{-2}M$ and an ionic strength was 1. These spectra are shown in Fig. 5.

The analysis of nickel complex by McConnel-Davidson's method is shown in Fig. 6.

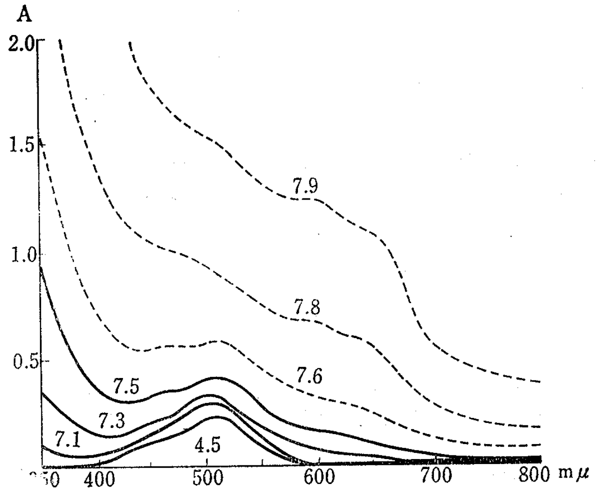


Fig. 7. Absorption Spectra of 1:1 Glucosamine-cobalt (II) Complex System at Various pH Values

concentration : $6.002\times10^{-2}M$ $\mu=1(\text{NaClO}_4)$

A linear relation is seen in the case of nickel complex as well as the copper complex, and a 1:1 complex may be expectedly present in the sample solutions.

Cobalt (II) Complex

The spectra of the sample solutions having various pH values are shown in Fig. 7.

They were measured for a 1:1 ratio of I to cobaltous ion whose concentration was $6.002\times^{-2}M$ and an ionic strength was 1. The spectral variances were slightly below about pH 7.

The pink-red sample solution was changed the color to brownish red as an increase of pH values. At pH around 7.6, a greenish precipitate was formed, therefore, the spectra of the three sample solutions of pH 7.6, 7.8, and 7.9 respectively were measured after removing the precipitates by centrifugation.

The spectra of the sample solutions at various pH values were measured with a 2:1 ratio of I to cobaltous ion whose concentration was $3.001\times10^{-2}M$ and an ionic strength was 1. These spectra are shown in Fig. 8.

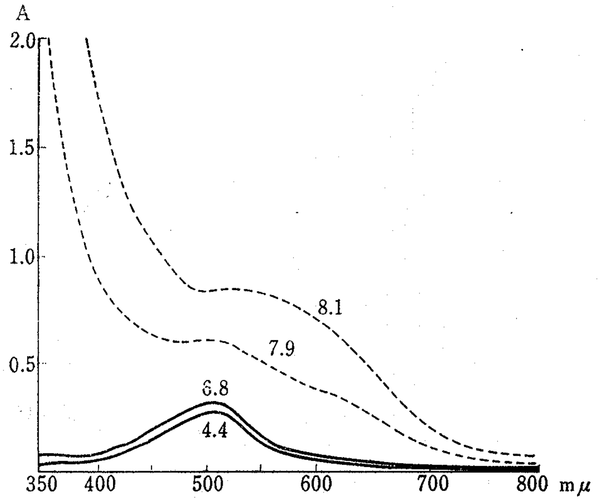


Fig. 8. Absorption Spectra of 2:1 Glucosamine-cobalt (II) Complex System at Various pH Values

concentration: I $6.002\times10^{-2}M$
cobaltous ion $3.001\times10^{-2}M$ $\mu=1(NaClO_4)$

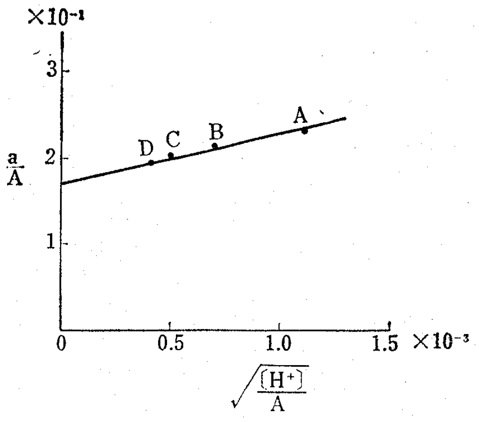


Fig. 9. Relation between $\frac{a}{A}$ and $\sqrt{\frac{[H^+]}{A}}$ at 510 mμ

I : Co=1:1, concentration: $6.002\times10^{-2}M$
pH: A, 6.5; B, 6.8; C, 7.1; D, 7.3

In the case of 2:1 spectra, the spectral variances were also scarce as in the case of 1:1 spectra, but no precipitation occurred in the whole range of pH measured.

To establish the composition of cobalt complex, McConnel-Davidson's method was also applied to the 1:1 spectra of cobalt complex. The result is given in Fig. 9.

A linear relation was found and hence a 1:1 complex would form in the solution.

The Stability Constants of Copper-, Nickel-, and Cobalt-complexes

The equilibrium and stability constants of these three metal complexes were calculated by the treatment of spectral data using the 1:1 spectra.

The results are given in Table I.

Table I. Equilibrium and Stability Constants of Three Metal Complexes of D-Glucosamine (I): I to metal ion 1:1

Complex	Molar extinction coefficient (ε_c)	Equilibrium constant (k_1)	Stability constant (log K_1)	Concentration (M)	Ionic strength (μ)
(I)-Cu	84 (650 mμ)	2×10^{-5}	3	2.303×10^{-2}	....
〃	84 (650 〃)	3×10^{-5}	3	3.044×10^{-2}	1M $NaClO_4$
(I)-Ni	4 (650 〃)	9×10^{-6}	2	4.045×10^{-2}	〃
(I)-Co	6 (510 〃)	4×10^{-5}	3	6.002×10^{-2}	〃

Copper complex has the same magnitude of stability constant as that of cobalt complex, but both complexes have a larger stability constant than nickel complex in experimental conditions.

These values of stability constants for the three metal complexes are fairly consistent with those obtained by pH titration method previously, because, considering from the experimental facts that the values obtained spectrophotometrically here are influenced by the concentration of I, which is fairly higher than that of I in pH titration studies.

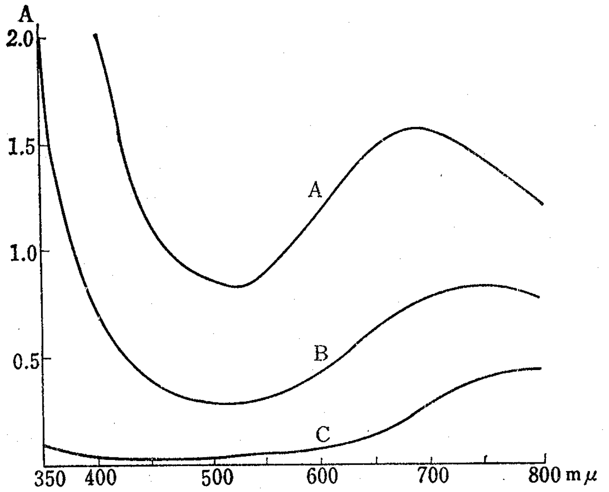


Fig. 10. Absorption Spectra of the Prepared Glucosamine-copper Complex in Aqueous Solution

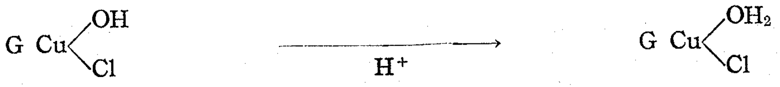
concentration : G Cu(OH)Cl $2.032\times10^{-2}M$
pH : A, 5.8; B, 5.3; C, 2.5

The Spectral Behaviors of the Glucosamine-copper Complex Prepared

The spectra of the aqueous solutions having the concentration of $2.302\times10^{-2}M$ at pH values of 5.8. 5.3, and 2.5, respectively, were measured.

The results are shown in Fig. 10.

The initial pH value of the complex solution when dissolved was about 6. The strong absorption was perceived in the range from about 350 mμ to 500 mμ at both pH 5.8 and 5.3. However, at pH 2.5, the absorption disappeared. Then, it may be considered that the disappearance may be due to the following mechanism by neutralization of the hydroxyl group of the prepared complex with proton in acidic pH region as indicated below :



$$\mathrm{G\ Cu}\begin{matrix}\mathrm{OH}\\ \mathrm{Cl}\end{matrix}\ \xrightarrow[\mathrm{H^+}]{}\ \mathrm{G\ Cu}\begin{matrix}\mathrm{OH_2}\\ \mathrm{Cl}\end{matrix}$$

The spectral behaviors of the complex prepared will offer one of the supports for the formation of hydroxo complex which was previously mentioned at the spectra of copper complex.

The authors wish to express their deep gratitude to Prof. Emeritus M. Ishidate of the University of Tokyo for his encouragements and helpful advices. They are also grateful to the members of micro analytical laboratory of this Faculty for elemental analysis and measurements of spectra.

Summary

Spectrophotometric behaviors of copper-, nickel-, and cobalt-complexes of D-glucosamine (I) were investigated in visible region. It was found that three metal ions formed a complex having a 1:1 ratio of I to a metal ion respectively.

Equilibrium and stability constants were also determined by the application of McConnel-Davidson's method. Both stability constants of copper- and cobalt-complexes were of the same magnitude, but that of nickel-complex was lower than the other.

A possibility of the presence of the hydroxo complex in the complex formation between I and metal ions was also ascertained by the analysis of the spectra at near ultraviolet region.

Furthermore, the preparation of copper complex in solid state was carried out, and a 1:1 complex was obtained. From the analysis of the prepared complex, GCu(OH)Cl seemed to be the most probable experimental formula for the complex.

(Received December 7, 1964)