UDC 615.779.931-011:615.778.47

37. **Kiyomi Taguchi**: The Mixed Complex of Tetracycline–Metal Chelates. III.[*2] Chlorotetracycline–Metal Chelates with Isoniazid.

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The insoluble metal chelates of isoniazid (INAH) were isolated by Foye, *et al.*[1] They found that the molar ratio of the chelates varied with the pH at the time of preparation. That is 1:1 ratio of INAH to metal at acid side and 2:1 ratio at alkaline side of neutrality. The presence of 1:1 chelate makes the formation of a mixed complex similar to the tetracycline–metal chelates possible. The enhancement of effectiveness of INAH by copper(II) ion was reported by Erlenmeyer, *et al.*[2] on the basis of *in vitro* tests. Foye, *et al.*[1] observed, however, that the chelates of INAH showed *in vivo* antitubercular activities comparable to that of INAH itself. Albert[3] proposed the hypothesis that the 1:1 chelate (cationic complex) of oxine to copper is effective against bacteria greater than oxine. A function of 1:1 chelate will provide the connection with other ligands, including bacteria forming a mixed complex.

2:1 Complex (pH 4.5) 1:1 Chelate (about pH 6.0)

INAH–Co(II) Compound

The following experiments were carried out in order to study the formation of mixed complexes of chlorotetracycline (CT)–metal chelates (CT–Cu(II), CT–Co(II), CT–Fe(III), and CT–Th(IV)) with INAH on the acid side. The properties and composition of INAH–metal compounds were studied in order to avoid contamination as much as possible.

INAH-Metal Compounds—INAH–Cu(II) chelate, which was soluble in water, was isolated by the addition of ethanol to the aqueous solution of copper(II) nitrate and INAH at about pH 4. The chelate changed into insoluble form as prepared by Foye.[1] The cobalt(II) complex was obtained from hydrous methanol medium at about pH 4.5, and the cobalt(II) chelate was prepared at about pH 6.0. Iron(III) ion instantly decomposed INAH into isonicotinic acid and nitrogen. Thorium(IV) and zirconium (IV) ions did not form complexes with INAH.

Analytical results for the isolated complexes showed that INAH–Cu(II) chelate, INAH–Co(II) complex, and INAH–Co(II) chelate had 1:1, 2:2, and 1:1 ratio of INAH to metal, respectively. Molar ratio of soluble INAH–Cu(II) chelate could not be confirmed because of the unstableness of the chelate, but it is probably a 1:1 chelate. Molar ratios in aqueous solution were detected by the continuous variation method.[4] INAH–Cu(II) chelate had a maximum absorption at 730 mμ at pH 4.0 and INAH–Co(II) chelate had a maximum absorption at 500 mμ at pH 6.5, showing a great difference in absorbancy (Fig. 1). The experimental data of INAH–Cu(II) chelate mainly gave a composition of 1:1 at

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610 mμ and pH 3.0 or 4.3 (Fig. 2). The measurement at higher pH values could not be performed because of precipitation of the chelate. Results for INAH-Co(II) chelate gave a 1:1 ratio of INAH to cobalt at 500 mμ and pH 6.3 (Fig. 3). The measurement at a lower pH values, such as 2:1 complex, could not be performed because the difference of absorbancy between the complex and cobalt(II) nitrate was small. INAH-Cu(II) chelate was insoluble in water, alcohols, ether, and benzene. The copper(II) chelate which was soluble in water was obtained as mentioned already. The chelate, however, was unstable and changed into the insoluble form on contact with air, changing color from green to deep blue. The cobalt(II) complex was soluble in water, and slightly soluble in cold water and methanol. The cobalt(II) chelate prepared from cobalt(II) nitrate was soluble in water but the one prepared from cobalt(II) chloride was less soluble in water. These substances showed no melting point and were explosive at about 160°.

Decrease in pH[5] was expected in the chelate formation between INAH and metal ion. Marked drop in pH was observed in the reaction of INAH with copper(II) ion even at pH 2.8. In the interaction of INAH with cobalt(II) ion, decrease was not observed at a pH lower than 5, but was observed at pH 6.9 (Table I). These results showed that INAH

TABLE I. Decrease in pH in the Reaction of INAH with Metal

Metal	pH (Metal)[a)]	pH (INAH)[b)]	pH after mixing[c)]	Difference
Cu(II)	2.79	2.79	2.68	−0.11
	4.30	4.34	3.88	−0.42
Co(II)	5.20	4.82	5.03	....
	5.82	5.87	5.74	−0.08
	6.96	6.87	6.18	−0.69
CT-Cu(II) chelate	2.97	2.79	2.92	....
	5.11	5.03	4.44	−0.59

a) The concentration of metal nitrate solution was 1/200*M*. The pH of the solution was measured 2∼3 days later after adjusting the pH with 0.1*N* HNO_3 or 0.1*N* KOH for stabilization.

b) The concentration of INAH solution was 1/200*M*. The pH of the solution was adjusted with 0.1*N* HNO_3 or 0.1*N* KOH.

c) pH of the mixture was measured 2∼4 hr. later for stabilization after mixing equal volumes of each solution.

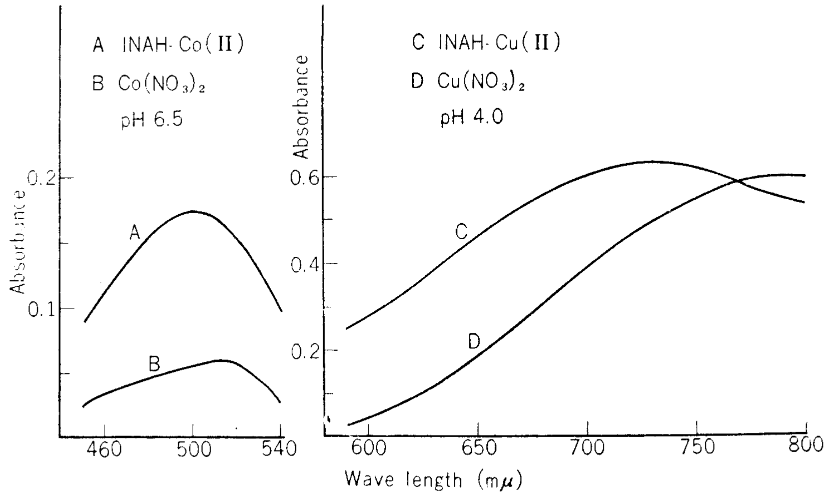

Fig. 1. Absorption Spectra of INAH-Metal Chelates

Concentration : A and B, 1/40*M*, C and D, 1/20*M*
pH of each solution adjusted with KOH or HNO_3

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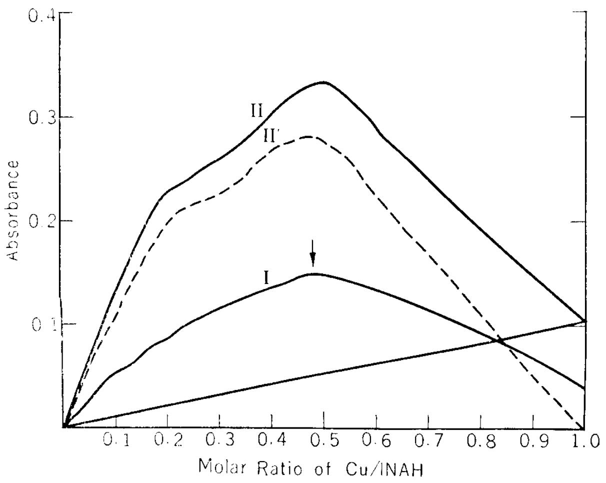

Fig. 2. Continuous Variation Study for Determination of INAH-Cu Ratio in Chelates (measured 3 hr. after adjusting pH of the solutions with KOH or HNO_3)

Wave length : 610 mμ

Concentration of INAH + Cu(ii) : $0.04M$

I Absorbance of INAH-Cu(ii) chelate + Cu(ii) at pH 3.0

II Absorbance of metal + the chelate at pH 4.3

II' Absorbance related to metal-INAH chelate at pH 4.3

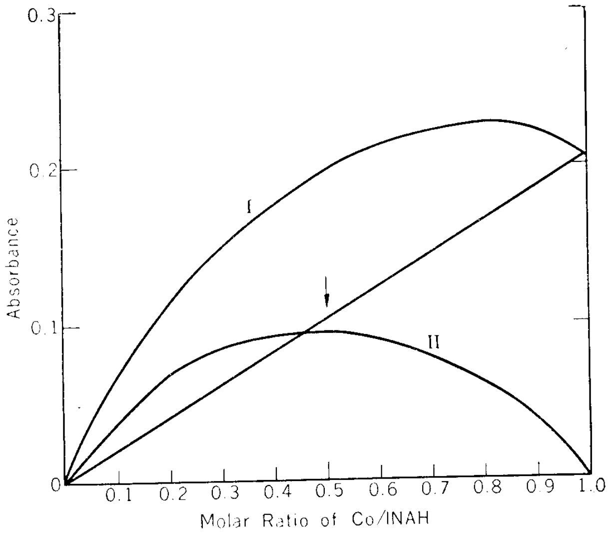

Fig. 3. Continuous Variation Study for Determination of INAH-Cu Ratio in Chelates (measured 3 hr. after adjusting pH of the solutions with KOH or HNO_3)

Wave length, 500 mμ, pH 6.3

Concentration of INAH + Co(ii), $0.1M$

I Absorbance of Cobalt(ii)+ INAH-Co(ii) complex

II Absorbance related to INAH-Co(ii) complex

formed a chelate with copper(ii) ion, and a complex with cobalt(ii) ion at pH lower than 5 and a chelate at pH higher than 5.

Mixed Complexes—Mixed complexes were mainly isolated as crystalline powder by a little addition of ether to the solution of INAH and CT-metal chelate in hydrous methanol on the acid side of neutral. Purification of the mixed complexes was carried out by repeated washing, reprecipitation, and recrystallization. The mixed complexes of CT-Cu(ii), CT-Co(ii), and CT-Fe(iii) chelates with INAH were isolated. Iron(iii) ion decomposed INAH, but CT-Fe(iii) chelate formed a mixed complex without decomposition. CT-Th(iv) chelate did not form a mixed complex with INAH. The interaction between INAH-metal compound and CT·HCl was also studied. Insoluble INAH-Cu(ii) chelate hardly reacted with CT·HCl in a hydrous methanol medium at room temperature, but the soluble chelate of INAH-Cu(ii) reacted readily. The mixed complex of INAH-Co(ii) with CT·HCl was isolated.

Analytical results showed that CT-Cu(ii)-INAH formed a 1:1:2 ratio (CT:Cu:INAH) at about pH 5, and a 1:1:1 ratio at about pH 4. CT-Fe(iii)-INAH and CT-Co(ii)-INAH mixed complexes were obtained in 1:1:1 ratio at about pH 4 or 5. The mixed complex prepared from soluble INAH-Cu(ii) and CT·HCl did not show a definite composition. The mixed complex of INAH-Co(ii) complex with CT·HCl was separated, having the same composition as the one prepared from CT-Co(ii) chelate. These mixed complexes were soluble in hot water and hot methanol, and slightly soluble in cold water and cold methanol, and insoluble in ether, acetone, benzene. It was observed that the color of the solution of CT-Cu(ii) and CT-Co(ii) chelates was somewhat intensified by the addition of INAH.

The absorption curves of the mixed complexes showed a similar shape as those of CT-metal chelates in the near-ultraviolet region (Fig. 4). The mixed complexes were

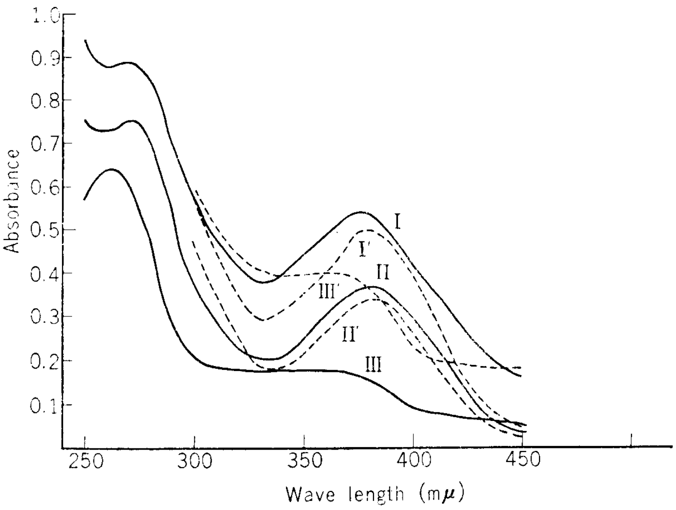

Fig. 4 Absorption Spectra of Mixed Complexes

I CT-Cu(II)-INAH I' CT-Cu(II)
II CT-Co(II)-INAH II' CT-Co(II)
III CT-Fe(III)-INAH III' CT-Fe(III)
Solvent : MeOH

explosive at about 170°.

Discrimination of Mixed Complexes—CT·HCl did not form a compound with INAH under the conditions under which the mixed complexes were prepared, and was separated from the mixture of CT·HCl and INAH by isolation and purification. The fact that CT–Th(IV) did not form a mixed complex indicated also that contamination of INAH was not caused by the method used in the preparation. Therefore, INAH in the mixed complexes isolated is not a contaminated substance.

If INAH in the preparation was brought about by a displacement reaction (CT–metal+INAH → CT+INAH–metal), the solubility and color of the products would be similar to the single complex (INAH–metal, CT–metal). However, the nature of the mixed complexes isolated was different from the single complex. The facts afore-mentioned showed that INAH in the mixed complex isolated, was not a contaminated substance, and combined with the metal.

Experimental

Reagents—Isoniazid (Daiichi Seiyaku Co., Ltd.), and other reagents were the same as those described in Part II.*2

Analyses of the Compounds—For the INAH-metal compounds, assays of metal, water of hydration, C, H, and N were conducted. Assays of metal, CT, water of hydration, and some elements were conducted for the mixed complexes. NO_3^-, Cl^-, and INAH were determined semiquantitatively. Formula of the mixed complexes was calculated from the results. Assays of CT, metal, and water of hydration were carried out by the BuOH-$Th(NO_3)_4$ extraction method, ashing to metal oxide, and drying in vacuum at 90～100°, as mentioned in Part II.*2 NO_3^- and Cl^- were detected by Griess' reaction or by diphenylamine and by $AgNO_3$, respectively. Detection of INAH was carried out by testing for the hydrazine group with ammoniacal $AgNO_3$ solution, followed by silver-mirror formation and evolution of N_2.

Preparation on INAH-Metal Compounds—INAH-Cu(II) chelate : A solution of 1 g. of INAH in 5 cc. of water was added to the solution of 2 g. of $Cu(NO_3)_2$ in 4 cc. of water. A green solution was instantly obtained. A green precipitate which was soluble in water, was obtained by the addition of 60 cc. of EtOH to the green solution after standing for a few min. The product was collected on a sintered glass filter. The product turned quickly to an insoluble substance, changing color to deep blue. The insoluble substance was washed with water and EtOH, and then dried over silica gel. The preparation should be carried out below 40° to avoid further decomposition. NO_3^- was positive. *Anal.* Calcd. for $C_6H_6ON_3$-$Cu(NO_3)_2$(INAH-$Cu(NO_3)_2$) : C, 27.30; H, 2.28; N, 21.33; Cu, 24.1. Found : C, 27.05; H, 2.44; N, 20.29; Cu, 20.70.

$(INAH)_2$-$Co(NO_3)_2$ complex : A solution of 1 g. of INAH in 4 cc. of water was added to a solution of 1.5 g. of $Co(NO_3)_2$ in 5 cc. of MeOH. The solution was kept at about 40° for 0.5 hr. after adjusting the pH to 4.5. If a residue remained, it was removed. The solution was then stored in a refrigerator for 1 hr. A pink crystal was obtained and collected on a sintered glass filter. The product was kept at about 40° as a suspension in 5 cc. of water for some min. and then filtered after cooling the mixture. The product was washed with cold water and MeOH, and dried over silica gel. NO_3^-, positive. *Anal.* Calcd. for $C_{12}H_{14}O_2N_6\cdot Co(NO_3)_2\cdot 2H_2O$ ($(INAH)_2$-$Co(NO_3)_2\cdot H_2O$) : C, 29.78; H, 3.38; Co, 12.34; H_2O, 3.78. Found : C, 29.11; H, 3.79; Co, 12.9; H_2O, 3.50.

By the use of $CoCl_2$ instead of its nitrate, the complex having a 2:1 ratio of INAH to Co was obtained. *Anal.* Calcd. for $C_{12}H_{14}O_2N_6$-$CoCl_2\cdot 3H_2O$ ($(INAH)_2$-$CoCl_2\cdot 3H_2O$) : C, 31.63; H, 4.42; Co, 12.90; H_2O, 11.8. Found : C, 31.73; H, 4.20; Co, 14.4; H_2O, 11.6.

INAH-Co(II) chelate : A solution of 1 g. of INAH and 1.8 g. of $Co(NO_3)_2$ in 10 cc. of water was kept for 30 min. at about 40° adjusting the pH to 6.0~6.4 with KOH solution. A pinkish crystalline precipitate occurred by the addition of 5cc. of EtOH to the solution while chilling in an ice bath. The product was collected, washed with EtOH and EtOH-water (1:1), and dried over silica gel. NO_3^-, positive. *Anal.* Calcd. for $C_6H_6ON_3$-$CoNO_3\cdot H_2O$(INAH-$CoNO_3\cdot H_2O$) : Co, 20.02; H_2O, 6.51. Found : Co, 20.93; H_2O, 7.60.

Use of $CoCl_2$ instead of its nitrate gave the chelate having 1:1 ratio of INAH to Co. The chelate was less soluble in water, so that it was washed with water. A pink crystalline powder was obtained. *Anal.* Calcd. for $C_6H_6ON_3$-$CoCl\cdot 2H_2O$ (INAH-$CoCl\cdot 2H_2O$) : Co, 22.2; H_2O, 13.56. Found: Co, 23.20; H_2O, 13.94.

Preparation of the Mixed Complex—$(INAH)_2$-Cu-CT mixed complex : A solution of 0.15 g. of INAH in 2 cc. of water was mixed with a solution of CT-Cu(II) chelate prepared from 0.2 g. CT·HCl and 0.2 g. $Cu(NO_3)_2$ in 20 cc. of MeOH. A small amount of precipitate occurred which dissolved when the pH was adjusted to 5.0. The solution was kept in a refrigerator for about 1 hr. after addition of Et_2O until precipitation began. A greenish yellow crystalline powder was obtained. It was washed with several portions of MeOH and Et_2O-MeOH (1:1), and dried over silica gel. *Anal.* Calcd. for $C_{34}H_{35}O_{11}N_8Cl$-$Cu\cdot HCl\cdot 2H_2O$ ($(INAH)_2$-Cu-CT·HCl·$2H_2O$) : N, 12.61; Cu, 7.16; CT·HCl, 57.9; H_2O, 4.06. Found : N, 12.27; Cu, 7.55; CT·HCl, 57.0; H_2O, 4.26.

INAH-Cu-CT mixed complex : A solution of 80 mg. of INAH in 2 cc. of water was added to a solution of CT-Cu(II) chelate prepared from 0.2 g. of CT·HCl and 0.2 g. of $Cu(NO_3)_2$ in 3 cc. of water. The solution was adjusted to pH 4 with *N* HCl. Then 2 cc. of $NaClO_4$ solution (10%), adjusted to pH 4 with $HClO_4$, was added to the solution. The greenish yellow precipitate was collected, washed with 5 cc. of hot water (40°), and then with several portions of cold water. The product was dried over silica gel. The occurrence of precipitates by the addition of perchlorate may be due to the formation of a perchlorate of CT.[6] *Anal.* Calcd. for $C_{28}H_{27}O_9N_5Cl$-$Cu\cdot HClO_4\cdot 3H_2O$ (INAH-Cu-CT·$HClO_4\cdot 3H_2O$) : Cu, 7.80; CT, 58.6; H_2O, 4.42. Found : Cu, 7.76; CT, 64.6; H_2O, 5.75.

INAH-Co(II)-CT mixed complex : A solution of 70 mg. of INAH in 1 cc. of water was added to a solution of CT-Co(II) chelate prepared from 0.2 g. of CT·HCl and 0.2 g. of $Co(NO_3)_2$ in 3 cc. of hot MeOH (50°). Recrystallization was carried out by chilling after addition of small amount of Et_2O. Fine crystals were obtained, washed with Et_2O-MeOH(1:1), and then dried over silica gel. *Anal.* Calcd. for $C_{28}H_{27}O_9N_5Cl$-$Co\cdot HCl\cdot 3H_2O$ (INAH-Co-CT·HCl·$3H_2O$) : N, 9.19; Co, 7.73; CT·HCl, 68.2; H_2O, 7.07. Found : N, 8.87; Co, 7.79; CT·HCl, 65.9; H_2O, 6.57.

INAH-Fe(III)-CT mixed complex : A solution of 0.15 g. of INAH in 1 cc. of water was added to a solution of CT-Fe(III) chelate prepared from 0.2 g. CT·HCl and 0.2 g. $FeCl_3$ in 5 cc of MeOH. Brown crystals were obtained by the addition of Et_2O. They were collected after keeping in a refrigerator for a few min., washed with Et_2O-MeOH(1:1), and dried over H_2SO_4. *Anal.* Calcd. for $C_{28}H_{27}O_9N_5Cl$-$FeCl\cdot HCl\cdot 4H_2O$ (INAH-FeCl-CT·HCl·$4H_2O$) : C, 41.35; H, 3.35; Fe, 6.49; CT·HCl, 63.4; H_2O, 8.85. Found : C, 41.47; H, 3.68; CT·HCl, 65.7; H_2O, 9.40.

INAH-Co(II)-CT mixed complex from INAH-Co(II) complex : 0.2 g. of $(INAH)_2$-$Co(NO_3)_2\cdot H_2O$ and 0.3 g. of CT·HCl were dissolved into 5 cc. of MeOH by warming to 50°. A pinkish yellow precipitate was obtained by the addition of 5 cc. of Et_2O to the solution and the mixture was kept in a refrigerator for 0.5 hr. The precipitate was collected, washed with two portions of 5 cc. of water to remove free INAH and CT·HCl, and then dissolved in 10 cc. of MeOH. A precipitate was obtained by the addition of 20 cc. of Et_2O to the solution. It was collected, washed with water, and dried over silica gel. Cl^-, positive, and NO_3^-, slightly positive. *Anal.* Calcd. for INAH-Co-CT·HCl·$2H_2O$(mol. wt., 745.44) : Co, 7.91; CT·HCl, 69.2; H_2O, 4.84. Found : Co, 7.04; CT·HCl, 72.6; H_2O, 5.79.

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Summary

Isoniazid (INAH) was studied on the formation of mixed complexes with chlorotetracycline(CT)-metal chelates with Th^{4+}, Fe^{3+}, Cu^{2+}, and Co^{2+} in acid medium. INAH-metal compounds were also studied. INAH-Cu(II) chelate, and INAH-Co(II) complex and its chelate were isolated as crystalline powder showing 1:1, 2:1, and 1:1 ratio of INAH to metal, respectively, from hydrous methanol. Molar ratio of the compounds in aqueous solution was measured by the continuous variation method. Iron(III) ion decomposed INAH to isonicotinic acid, but the mixed complex of CT-Fe(III) chelate with INAH was formed without decomposition. The mixed complex of CT-Cu(II) with INAH gave different compositions in accordance with the acidity of the preparation. CT-Co(II) chelate formed a mixed complex, but not CT-Th(IV) chelate. The molar ratio of the mixed complexes was mostly 1:1:1 (CT:metal:INAH). These mixed complexes were isolated in crystalline powder form and were soluble in methanol and hot water. Remarkable changes in color due to the formation of a mixed complex were not observed. Reaction between INAH-metal and CT-HCl was also studied.

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38. **Kiyomi Taguchi**: The Mixed Complex of Tetracycline-Metal Chelates. IV.[*2] Chlorotetracycline-Copper(II) Chelate with 4,4'-Bis(dimethylamino)-thiobenzophenone (Thio-Michler's Ketone).

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In the previous papers, mixed complexes of chlorotetracycline (CT)-metal chelates with penicillin G or isoniazid were reported. In the present work, thio-Michler's ketone (TMK) having a thione group as a complex functional group different from the ligands of the former was studied concerning the formation of mixed complexes with CT-Cu(II) chelate.

TMK-Cu(II) complexes were also investigated. TMK is a specific reagent for detection of copper, mercury, and precious metals.[1]

TMK-Cu Complex—TMK produced a reddish purple color with copper(II) nitrate in acetone-methanol medium, showing a maximum absorption at 500 mμ (Fig. 2-III). TMK produced, however, an indigo blue color with copper(II) chloride, and the absorption maxima moved to 530 and 645 mμ (Fig. 2-IV). This color change was not observed with halogen ion or copper(I) chloride.

The continuous variation method[2] was applied for the determination of molar ratio of TMK to copper(II) nitrate in acetone. The result shown in Fig. 3 indicates a 2:1 ratio of TMK to copper(II).

TMK-Cu(II) (dusky brown) was prepared as a perchlorate by addition of sodium perchlorate solution to the solution of TMK and copper(II) nitrate in acetone-methanol.

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