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Studies on the Sulfur-containing Chelating Agents. XXIX.[1] Reaction of Cysteamine and Its Related Compounds with Copper

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The formations of two kinds of red–violet complex were observed in the reaction of cysteamine (2-mercaptoethylamine) with copper. They were considered to be mixed valence complex and that containing molecular oxygen respectively. Their structures and processes of the formations were presumed from the results of pH, potentiometric and photometric titrations, visible and infrared spectra, and the estimation of copper(I) in the complexes. Analogous results were obtained in some of the related compounds of cysteamine.

As to the mechanism of the radiation protection by some of the radiation protectors, such as cysteamine (2-mercaptoethylamine, abbreviated as MEA hereafter) and S-2-aminoethylisothiuronium salt, the complex formation of these protectors with metals of the metalloenzymes have been considered as one of the important factors.[3–6] Moreover, the participation of the radiation protectors to the redox system through the complex formation with some metal ions may have connections to the mechanism of the radiation protection to some extent. Further, the effectiveness of MEA may rest on its ability to break the chain reaction of hydroxyl radical which is apparently propagated by trace of iron or copper.[7] In consideration of above-mentioned background the basic study on the complex formation of MEA and its related compounds with copper which is one of the most important metals as to the metalloenzymes and the redox systems has been attempted. The complex formation of some thiol compounds with copper is complicated because it involves redox reaction between the ligand and copper, and hence its detailed study has been very little. Recently, in thiomalic acid,[8] mercaptoacetic acid[9] and penicillamine,[10] red–violet complexes were found in the re-

1) Part XXVIII: Y. Sugiura, Y. Hojo, and H. Tanaka, *Radioisotopes* (Tokyo), **19**, 184 (1970).
2) Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) W. Lohmann, *Progr. Biochem. Pharmacol.*, **1**, 118 (1965).
4) W. Lohmann, A.J. Moss, Jr., J.L. Sanders, B.J. Porter, and D.M. Woodall, *Radiation Res.*, **29**, 155 (1966).
5) W.O. Foye and J. Mickles, *Progr. Biochem. Pharmacol.*, **1**, 152 (1965).
6) E.D. Fultz, *Radiation Res.*, **34**, 544 (1968).
7) A. Albert, *Federation Proc.*, **20**, Suppl., No. 10, 137 (1961).
8) I.M. Klotz, G.H. Czerlinski, and H.A. Fiess, *J. Am. Chem. Soc.*, **80**, 2920 (1958).
9) U. Takeuchi, *Nippon Kagaku Zasshi*, **83**, 292 (1962).
10) Y. Sugiura and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 368 (1970).

action with copper (II) ion, and they were confirmed to be mixed valence complexes in which copper (II) and copper (I) are present. The instability constants of the copper complexes of MEA and some related compounds were determined by polarography and it was pointed out that there is a close correlation between those values and the radiation protective effects.[11,12] However, the structure and the formation mechanism of the MEA-copper complex has not been studied in detail. Hemmerich inquired the reaction of cystamine (di-2-aminoethyl disulfide, abbreviated AEDS hereafter) with copper(I) ion and observed the formation of the mixed valence complex.[13] In connection with the radiation protection, Schubert has assumed that the effective protectors can complex with copper(I) and their effects are explained in protecting copper(I) which is present in the metalloenzymes through the complex formation against the oxidation caused by the radiation.[14] This paper deals with the detailed investigations of the complex formations of MEA and its derivatives with copper. In addition, the reactions of cysteine or selenocysteamine (2-aminoethaneselenol) with copper were also studied as the comparative studies. The pH titration method and spectrophotometric method were mainly used.

Experimental

Materials——MEA·HCl was purchased from Nakarai Chemicals, or synthesized according to Mills and Bogert's method.[15] β,β-Dimethyl-MEA·HCl was synthesized according to Mills and Bogert's method[15] and recrystallized from dehydrated EtOH. N,N-Dimethyl-MEA·HCl was purchased from Tokyo Kasei Kogyo. Selenocysteamine·HCl was prepared according to the method described in the previous paper.[16] Cysteine and homocysteine were purchased from Nakarai Chemicals. The purities of these thiol and selenol compounds were confirmed to be more than 97% before use by the iodometric titration. AEDS·2HCl was synthesized according to Foye's method.[17] As the stable univalent copper compounds, $Cu(CH_3CN)ClO_4$ was synthesized according to Hemmerich's method.[18] Other reagents used were reagent grade materials.

pH Titration——Titrations were carried out under the atmosphere of nitrogen as previously reported.[16] The titrations on Cu(I) ion were carried out according to the method of Hemmerich.[13]

Measurement of Absorption Spectra——The absorption spectra in visible region were measured in solution by a Hitachi EPS-2 recording spectrophotometer and in solid state by a Shimadzu MPS-50L recording spectrophotometer. The absorption spectra in infrared region were measured by a Nippon Bunko Koken DS-301 spectrometer.

Isolation of Copper Complexes——(A) MEA·HCl or selenocysteamine·HCl (0.01 mole) was dissolved in 10 ml of H_2O and pH of the each solution was adjusted to 8.0 with dilute NH_4OH, or MEA·HCl or selenocysteamine·HCl was dissolved in 10 ml of the buffer solution of pH 8.0. Ten milliliters of solution containing 0.005 mole or 0.01 mole of $CuCl_2$ was added dropwise with stirring. In accordance with the addition of the solution of $CuCl_2$, pH of the solution dropped and at pH 3—4 precipitate appeared, and pH of the solution became 1.0 when the addition of $CuCl_2$ was complete. Stirring was continued for an hour after the addition of $CuCl_2$, and the product was collected and washed with H_2O followed by EtOH. The product was dried under vacuum on P_2O_5.

(B) MEA·HCl or selenocysteamine·HCl and $CuCl_2$ were dissolved in 20 ml of H_2O with a molar ratio of 1:1 (0.01 mole:0.01 mole). To this mixture dilute NH_4OH was added dropwise with stirring. At pH 7—8, violet (in the case of MEA) or dark gray (in the case of selenocysteamine) precipitate appeared, and the stirring was continued for 30 minutes. The product was collected and washed with H_2O followed by acetone. The product was dried under vacuum on P_2O_5.

The color and the results of the elemental analyses of these complexes are shown in Table I.

In the case of cysteine, the similar phenomena were observed, but complexes were not obtained in pure state, because of the instability of complexes or the contamination by cystine which is insoluble inH_2O.

11) E.C. Knoblock and W.C. Purdy, *Radiation Res.*, **15**, 94 (1961).
12) E.C. Knoblock and W.C. Purdy, *J. Electroanal. Chem.*, **2**, 493 (1961).
13) P. Hemmerich, "The Biochemistry of Copper," ed. by J. Peisach, P. Aisen, and W.E. Blumbert, Academic Press, New York and London, 1966, pp. 15—34.
14) J. Schubert, *Sci. Am.*, **5**, 40 (1966).
15) E.J. Mills and M.T. Bogert, *J. Am. Chem. Soc.*, **62**, 1173 (1940).
16) H. Tanaka, H. Sakurai, and A. Yokoyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 1015 (1970).
17) W.O. Foye, A.M. Hebb, and J. Mickles, *J. Pharm. Sci.*, **56**, 292 (1967).
18) P. Hemmerich, *Experientia*, **19**, 488 (1963).

Table I. Analysis and Color of MEA or Selenocysteamine Copper Complexes

Exp.	Mixing ratio (ligand: copper)	Ligand: MEA Formula		Analysis (%) C	H	N	Ligand: Selenocysteamine Formula		Analysis (%) C	H	N
(A)	2:1	$C_4H_{14}O_2S_2N_2Cu_2Cl_4$ (red–violet[1])	Calcd.	10.55	3.11	6.16	$C_2H_6SeNCuCl$ (brown [3])	Calcd.	10.82	2.73	6.31
			Found[a]	10.77	3.26	6.48		Found	10.66	3.23	5.94
(A)	1:1	(red–violet [1])					$C_4H_{12}Se_2N_2Cu_3Cl_4$ (dark gray [4])	Calcd.	8.30	2.10	4.84
								Found	8.70	2.76	4.90
(B)	1:1	$C_4H_{12}S_2N_2Cu_3Cl_4$ (red–violet [2])	Calcd.	9.91	2.50	5.78	$C_4H_{12}Se_2N_2Cu_3Cl_4$ (dark gray [4])	Calcd.	8.30	2.10	4.84
			Found	10.15	3.29	5.84		Found	8.32	2.60	4.63

a) Elemental analysis was made on the red–violet complex which was formed from the yellowish white complex by the exoposure to air.

Possible Structures of Complexes

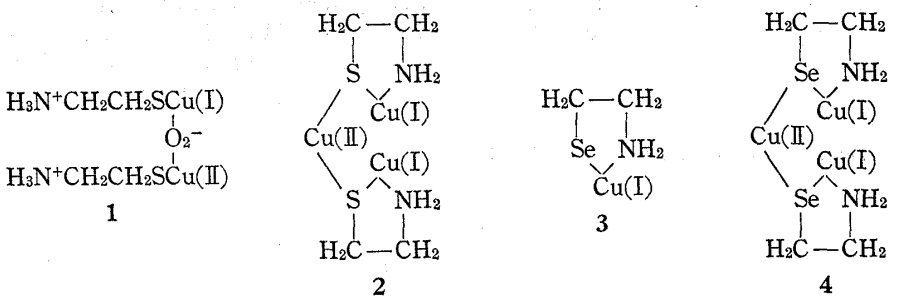

Chart 1

Potentiometric Titration——Potentiometric titrations were carried out by the use of platinum–calomel electrode connected to a Hitachi–Horiba F-5 pH meter. Fifty ml of 0.008M $CuCl_2$ solution was titrated with the solution of MEA·HCl (0.1M) with the addition of equal volumes of equimolar solution of NaOH (0.1M) at the same rate, and potential was measured after each addition of certain volume of MEA·HCl and NaOH, and the color change of the solution was also observed.

Photometric Titration——Photometric titrations were carried out under essentially the same condition as in the potentiometric titration, with a Shimadzu QV-50 spectrophotometer and its photometric titration apparatus. In the case of cysteine and β,β-dimethyl-MEA, the concentration of the solution was 0.01M.

Estimation of Copper(I) in MEA-copper Complex——The amount of univalent copper in the mixed valence copper complex of MEA was estimated by the use of cuproin according to Poillon and Dawson's method.[19]

Result

pH Titrations

In order to discuss the structure and the formation mechanism of MEA-copper complex, pH titrations on the systems of MEA-copper(II), MEA-copper(I) and AEDS-copper(I) in various ratios were carried out. In addition, pH titrations on the systems of cysteine–copper(II) and selenocysteamine–copper(II) were also carried out. The observations during each titration are summarized in Table II.

The white or yellowish white precipitates or solutions observed in the titrations No.1, 2, 4 and 9 were turned to red–violet when they were exposed to air, and the color faded gradually. Red–violet colors observed during the titrations were stable. In comparison of MEA

19) W.N. Poillon and C.R. Dawson, *Biochem. Biophys. Acta*, 77, 27 (1963).

Table II. Observation in pH Titration

System	No.	Mixing ratio (ligand: copper)	Observations in the course of titration
MEA·HCl–Cu(II)	1	1:0.3—0.4	yellow clear solution throughout the titration
	2	1:0.5	a=1.0, yellowish white precipitate
	3	1:0.6—1.8	a=0.7 (pH 3.5), red–violet clear solution a=1.0—1.1 (pH 4.7), fading of red–violet color or red–violet precipitate
Cysteine-Cu(II)	4	1:0.3—0.5	yellowish white turbidity throughout the titration
	5	1:0.6—0.7	a=0.96 (pH 3.6—3.9) white turbidity a=1.6—1.7 (pH 8.7—9.0) red–violet turbidity a=1.82 (pH 9.5—9.8) clear red–violet solution
	6	1:0.9—1.1	white or slightly red–violet turbidity throught titration
	7	1:1.3—2.0	white turbidity throughout titration
Selenocysteamine·HCl–Cu(II)	8	all ratio	yellow or pale–green turbidity throughout titration
MEA·HCl–Cu(I)	9	1:0.4—2.0	light yellow solution throughout titration
AEDS·2HCl–Cu(I)	10	1:0.5—2.0	a=0—1.0 (pH 6—7) red–violet solution a=1—1.8 fading of red–violet color a=1.8—2.0 (pH 9—10) red–violet solution

a=moles of KOH added per mole of ligand

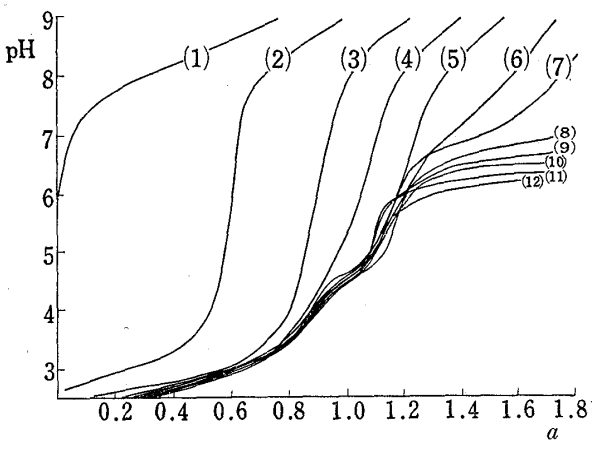

Fig. 1. pH Titrations of MEA·HCl with Cu(II)

MEA: Cu(II) (1) 1:0.0 (2) 1:0.317 (3) 1:0.395 (4) 1:0.493 (5) 1:0.583 (6) 1:0.691 (7) 1:0.815 (8) 1:0.992 (9) 1:1.092 (10) 1:1.270 (11) 1:1.398 (12) 1:1.805
concentration of MEA·HCl: 5.0×10^{-3}M
a=moles of KOH added per mole of ligand

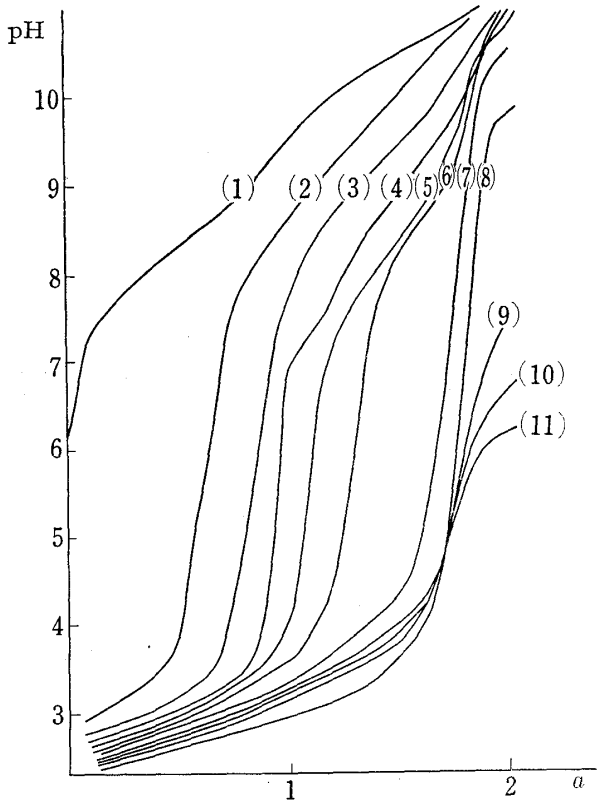

Fig. 2. pH Titrations of Cysteines with Cu (II)

cysteine: Cu(II) (1) 1:0 (2) 1:0.3 (3) 1:0.4 (4) 1:0.5 (5) 1:0.6 (6) 1:0.7 (7) 1:0.9 (8) 1:1.0 (9) 1:1.1 (10) 1:1.3 (11) 1:2.0
concentration of cysteine: 5.0×10^{-3}M
a=moles of KOH added per mole of ligand

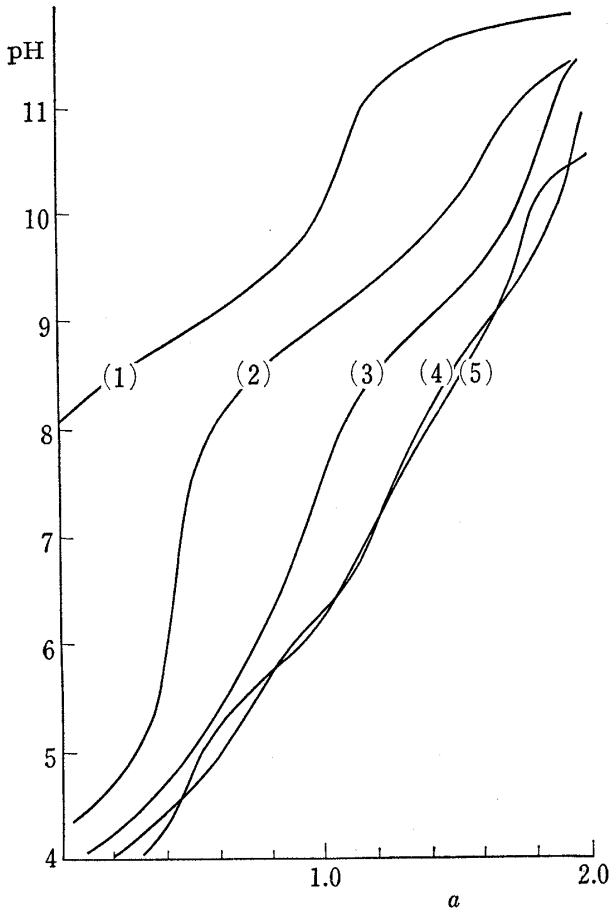

Fig. 3. pH Titrations of MEA·HCl with Cu(I)
MEA: Cu(I) (1) 1:0.0 (2) 1:0.4 (3) 1:0.8 (4) 1:1.0 (5) 1:2.0
concentration of MEA·HCl: 2.0×10^{-3}M
solvent: H_2O–acetnitrile (1:1)
$Cu(CH_3CN)ClO_4$ was used as Cu(I) ion.
a=moles of KOH added per mole of ligand

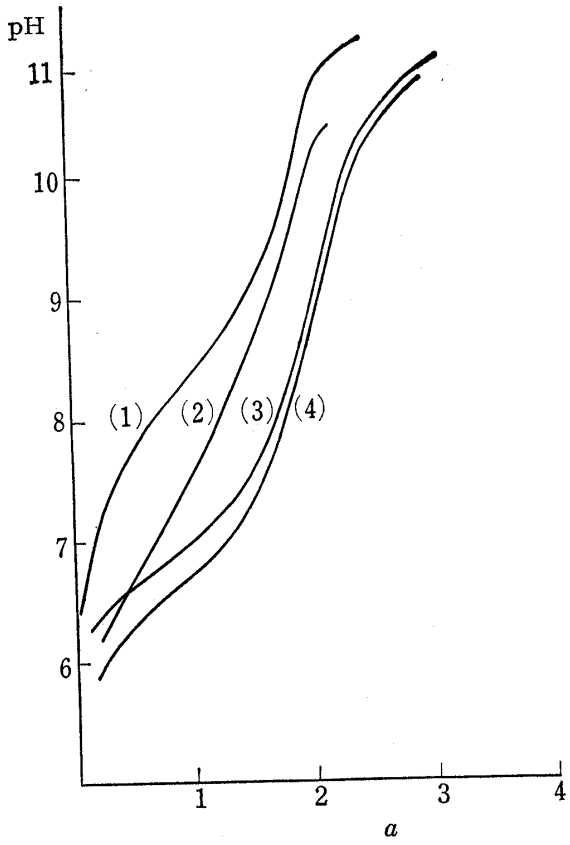

Fig. 4. pH Titrations of AEDS·2HCl with Cu(I)
AEDS: Cu(I) (1) 1:0.0, (2) 1:0.5, (3) 1:1.0, (4) 1:2.0
concentration of AEDS·2HCl: 1.0×10^{-3}M
solvent: H_2O–EtOH (1:1)
$Cu(CH_3CN)ClO_4$ was used as Cu(I) ion.
a=moles of KOH added per mole of ligand

with cysteine, considerable difference in the pH range in which the red–violet complex is present were observed. In selenocysteamine, violet color was not observed in all cases and yellow or slightly green turbidity which may be due to the hydrolysis of copper (II) ion was seen throughout the titrations. The titration curves are shown in Fig. 1, 2, 3, and 4.

Potentiometric and Photometric Titration

In order to discuss the structure of the red–violet complex which was observed in the pH titrations, potentiometric titration and photometric titration on the MEA and copper (II) system were carried out. In addition, the same experiments were carried out on β,β-dimethyl-MEA, N,N-dimethyl-MEA, cysteine or homocysteine for the comparison with MEA.

In MEA, β,β-dimethyl-MEA and cysteine, in the potentiometric titrations the solutions became red–violet by the additions of the ligands and the intensity of the color increased in accordance with the additions of the ligands, and the color of the solution turned to yellow sharply when the ratio of the ligands to copper became 2 or above as shown in Fig. 5, 6, and 7. The photometric titrations of these systems showed the comparable results to that of the potentiometric titrations as shown in Fig. 5. In the case of MEA, the absorbance became maximum at the points where the ratios of the ligands to copper were 5—6 to 4, and in the case of β,β-dimethyl-MEA and cysteine it became maximum at the point of ligand-to-metal ratio 2:1. On the contrary, in N,N-dimethyl-MEA, the red–violet color was not observed and the solution showed slightly greenish blue color and no remarkable change of potential at the point

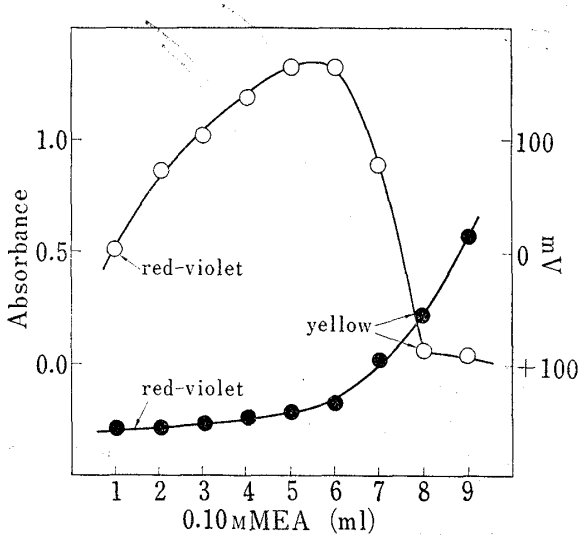

Fig. 5. Potentiometric and Photometric Titrations of MEA with Cu(II)

concentration of Cu(II): 0.10M (4 ml)
wavelength: 480 mμ
●—●: potentiometric titration
○—○: photometric titration

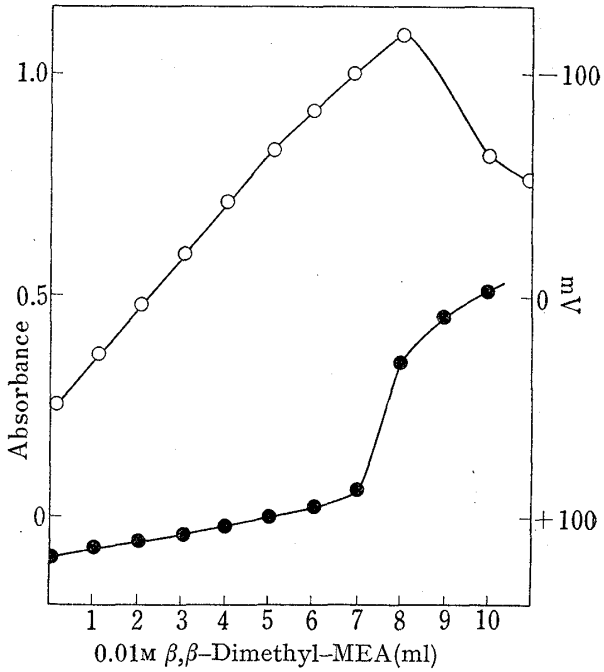

Fig. 6. Potentiometric and Photometric Titrations of β,β-Dimethyl-MEA with Cu(II)

concentration of Cu(II): 0.01M (4 ml)
wavelength: 550 mμ
●—●: potentiometric titration
○—○: photometric titration

where the ratio of the ligand to copper was 2 to 1, was observed. In homocysteine, the red–violet color was not observed, even in the alkaline medium (pH 10—12) where the red–violet color was observed in the case of cysteine, and the change of potential was small.

Absorption Spectra

When MEA and copper (II) ion were mixed in the ratio of 1 to 0.5—1 in the buffer solution of pH 7.5, the red–violet color was observed but it faded very rapidly. In the ratio of 1 to 0.4, white precipitate was formed immediately after the mixing of MEA and copper (II) ion, and the precipitate disappeared and the solution turned to red–violet when the solution was exposed to air, but the color faded so rapidly. In these cases, the decolorization was too rapid that the absorption spectra of these red–violet solutions could not be measured. On the contrary, in the ratio of 1 to 0.2—0.3, when air bubbled into the solution, yellowish white precipitate which was formed immediately after the mixing of the ligand with copper (II) ion began to dissolve and the solution was turned to red–violet gradually. The color of this solution was considerably stable and the absorption spectrum of this solution showed a broad maximum at 490—500 mμ as shown in Fig. 8 (curve I). When MEA and copper (II) ion was mixed in the ratio of 1 to 0.5 in the buffer solution of pH 10.9, the solution became red–brown and it showed a shoulder at 420 mμ (Fig. 8, curve II). In the case of β,β-dimethyl-MEA, the color of the solution was stable enough to measure its absorption spectrum.

Copper Complex

The yellowish white precipitate isolated from the solution containing MEA and copper (II) in the ratio of 2 to 1, was stable in vacuum desicator and kept its color, but when the complex was exposed to air, it turned red–violet gradually. The absorption spectrum of the red–violet complex in the solid state showed broad absorption maximum at 480 mμ similarly to the case of the solution, as shown in Fig. 8 (curve V). In the infrared absorption spectra of both yellowish white and red–violet complexes, the absorption band of thiol group was

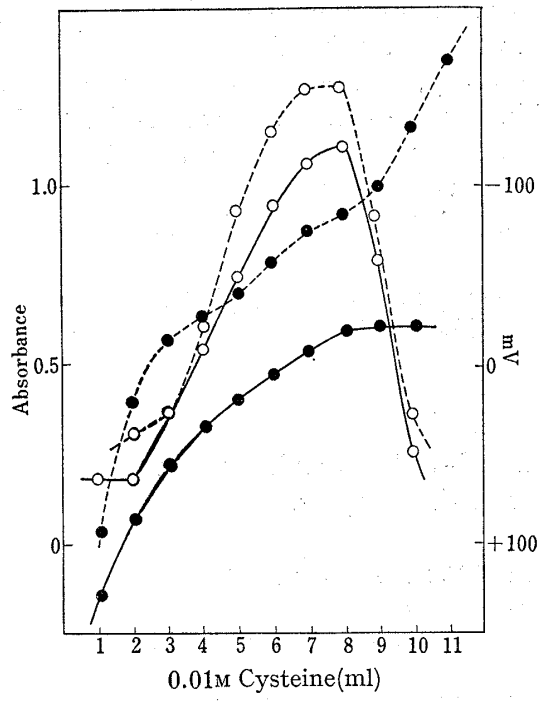

Fig. 7 Potentiometric and Photometric Titrations of Cysteine with Cu(II)

concentration of Cu(II): 0.01M (4 ml)
wavelength: 440 mμ
●-----●: pH 10 } potentiometric titration
●——●: pH 12 }
○-----○: pH 10 } photometric titration
○——○: pH 12 }

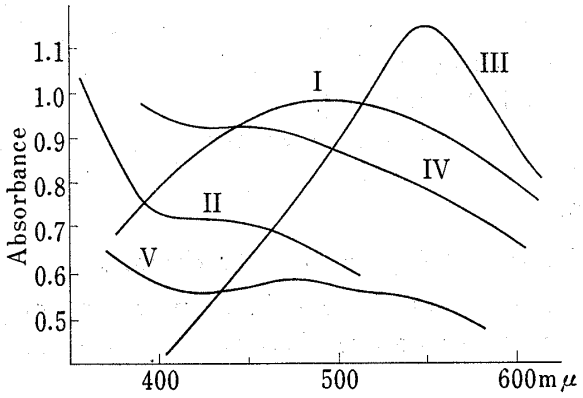

Fig. 8. Absorption Spectra of Copper Complexes

I MEA:Cu(II)=1:0.2 (pH 7.4)
II MEA:Cu(II)=1:0.5 (pH 10.9)
III β,β-dimethyl-MEA:Cu(II)=1:1 (pH 7.6)
IV cysteine:Cu(II)=1:0.5 (pH 11.0)
V MEA:Cu(II)=1:0.5 (solid)
concentration of MEA: 4×10^{-3}M
concentration of β,β-dimethyl-MEA: 2×10^{-3}M
concentration of cysteine: 4×10^{-3}M

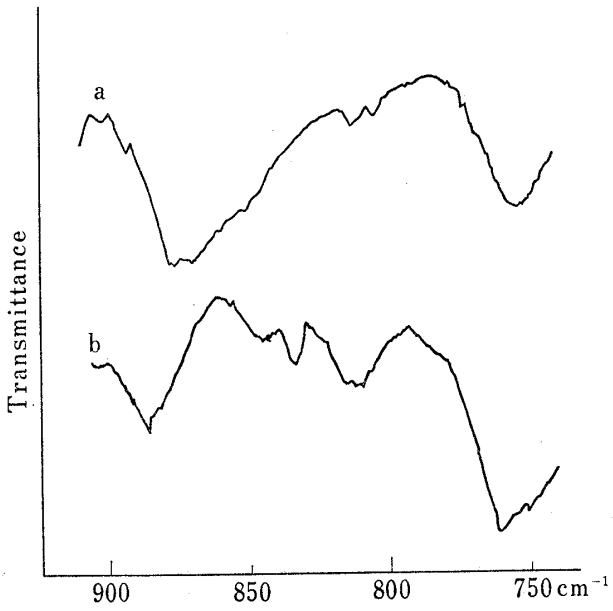

Fig. 9. Infrared Spectra of MEA Copper Complexes (Nujol)

a : yellowish white complex
b : red-violet complex (complex [I])

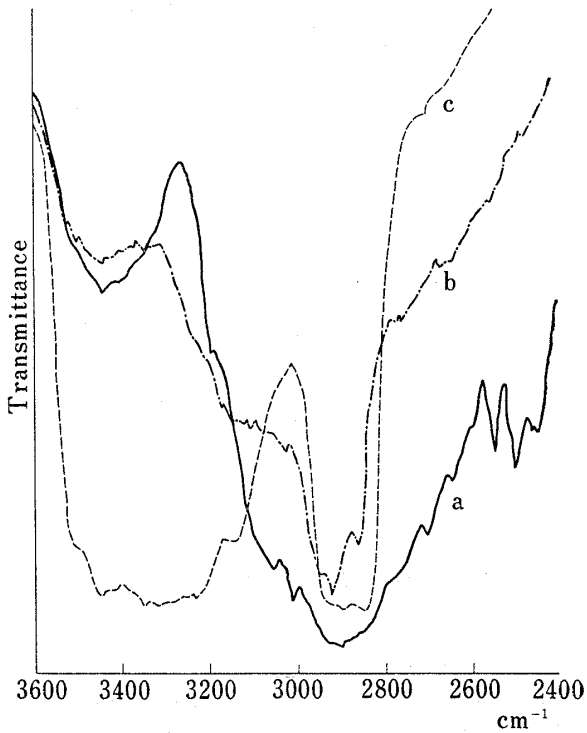

Fig. 10. Infrared Spectra (Nujol)

a : MEA·HCl
b : yellowish white complex and red-violet complex (complex [1])
c : red-violet complex (complex [2])

not observed. The spectra of these complexes resemble each other as a whole, but a week band observed at 875 cm^{-1} in the yellowish white complex, whereas two bands were observed in the red–violet complex at 885 cm^{-1} and 845 cm^{-1} respectively, in the corresponding region, as shown in Fig. 9. Both of these complexes dissolved in alkaline solution show red–violet color. In addition, the infrared spectra of the red–violet complexes represented as [**1**] and [**2**] listed in Table I are shown in Fig. 10. In the complex [**1**] a band of the ammonium ion was observed at 3050—3100 cm^{-1} whereas in the complex [**2**] a band of the coordinated amino group was observed at 3300 cm^{-1}.

In the case of selenocysteamine, two kinds of copper complexes [**3**] and [**4**], were isolated as shown in Table I, although red–violet color was not observed in the course of the pH titration.

Estimation of Copper (I) in the Complex

The results of the estimation of copper (I) in the red–violet complex, are shown in Table III as the percentage of copper (I) in the total copper of the complex.

Table Ⅲ. Estimation of Cu(I) in Mixed Valence Complex

Mixing ratio MEA:Cu(II)	pH	Ratio of Cu(I) to total Cu %
1:0.6[a]	5	68[b]
	8	46[b]
	5	65[c]
	8	59[c]
1:0.75[a]	5	60[b]
	8	45[b]
	5	60[c]
	8	51[c]
1:0.8[a]	5	47[b]
	8	38[b]
	5	56[c]
	8	46[c]
1:0.5[d]	10	49[b]
	10	50[b]
	10	52[c]
	10	51[c]

a) In solution
b) EDTA was used for the removal of Cu(II) after the decomposition of the complex.
c) *p*-Chloromercuribenzoic acid was used for the removal of mercapto group after the decompotition of the complex.
d) In solution which was obtained by dissolving the yellowish white complex isolated in alkaline solution in air.

Discussion

Reaction of MEA and Copper

The pH titration curves of the reaction of MEA and copper (II) ion are complicated as seen in Fig. 1. The titration curves obtained in various molar ratio of MEA and copper (II), are different from each other, and it is impossible to assume the independent formation of copper(II) complex or copper (I) complex. The red–violet complexes which were observed in the titrations should be considered as mixed valence complex, which has been found in the reactions of some thiols and copper (II) ion.[8–10] A yellow complex, which is considered to be copper (I) complex, was regarded to be only the product in the reaction of MEA and copper-(I) ion from the titration curves shown in Fig. 3. In the red–violet complex, it is considered

that copper (I) and copper (II) necessarily co-exist as seen in Table III. It is characteristic that the red-violet complex is formed in acid region (pH 3.5—4.7) in the presence of a little excess of copper (II). In the acid pH range where the red-violet complex is formed, ammonium group of the protonated MEA does not dissociate and cationoide type MEA-copper (II)–copper(I) mixed valence complex is formed. In higher pH region, red-violet precipitate is formed and the reaction process can not be followed by the observation in the titration, but the red-violet precipitate may be considered as chelate type MEA-copper (II)–copper (I) mixed valence complex in which ammonium group is dissociated. This complex corresponds to the complex [**2**] in Table I which was isolated from alkaline solution. The coordination of amino group in this complex can be assumed from the infrared spectrum (Fig. 10). In the titration of AEDS and copper (I) ion in 50% ethanol, the formation of the red-violet complex was observed in the range from pH 6 to 7 and pH 9 to 10 ($a=2$). The complex observed at $a=2$ may be regarded as the complex [**2**]. As we could not find the reliable and simple method to confirm the valence state of copper in the red-violet complex produced from MEA and copper (II) and determine the ratio of copper (I) to copper (II), the determination of copper (I) by cuproin[19] which was used for the determination of copper (I) in hemocyanine[20] was applied tentatively for the estimation of the ratio of copper (I) to total copper in the red-violet complexes. From the results shown in Table III, although they vary to some extent, it may be considered that 50—60% of total copper exist in the state of copper (I). In conclusion, when the ratio of copper (II) to MEA is more than 3:4, the reaction of MEA and copper (II) may be described as in Chart 2.

$$2H_3N^+CH_2CH_2SH + 2Cu(II) \rightleftarrows (H_3N^+CH_2CH_2S\text{-})_2 + 2Cu(I) + 2H^+ \quad (1)$$

$$2H_3N^+CH_2CH_2SH + 2Cu(I) \rightleftarrows 2H_3N^+CH_2CH_2S\text{-}Cu(I) + 2H^+ \quad (2)$$

$$2H_3N^+CH_2CH_2S\text{-}Cu(I) + Cu(II) \rightleftarrows \begin{matrix} & & Cu(I) & \\ & & | & \\ H_3N^+CH_2CH_2S & & & \\ & \diagdown & & \\ & & Cu(II) & \\ & \diagup & & \\ H_3N^+CH_2CH_2S & & & \\ & & | & \\ & & Cu(I) & \end{matrix} \quad (3)$$

$$4H_3N^+CH_2CH_2SH + 3Cu(II) \rightleftarrows$$

$$(H_3^+NCH_2CH_2S\text{-})_2 + \begin{matrix} & & Cu(I) & \\ & & | & \\ H_3N^+CH_2CH_2S & & & \\ & \diagdown & & \\ & & Cu(II) & \\ & \diagup & & \\ H_3N^+CH_2CH_2S & & & \\ & & | & \\ & & Cu(I) & \end{matrix} + 4H^+ \quad (4)$$

5

Chart 2

Complex [**5**] could not be isolated but its structure can be presumed as shown in Chart 2, from pH of the solution. Complex [**5**] corresponds to the protonated species of complex [**2**].

The yellowish white complex was stable in vacuum, it turned to red-violet when exposed to air, both in the state of the solution and the solid. The result of the elemental analysis of the yellowish white complex was not reasonable for the simple copper (I) chelate. Further, a band of the ammonium ion is observed at 3050—3100 cm^{-1} in both yellowish white and red-violet complexes, as shown in Fig. 10. The yellowish white complex dissolved in alkaline solution to give clear red-violet solution in air, and the absorption spectrum of this solution shows a maximum at 490 mμ (Fig. 8). The absorption spectrum of the red-violet complex in solution (λ_{max} 490 mμ) and that measured in solid state, of the red-violet complex resulted from the yellowish white complex in air coincide each other. The ratio of

20) I.M. Klotz and T.A. Klotz, *Science*, **121**, 477 (1955).

copper (I) in this complex was determined to be 50% almost exactly in alkaline solution (Table III[d]).

From the observation in the absorption spectra, the complex which showed curve I in Fig. 8 is considered to be a complex containing molecular oxygen, while that showed curve II is considered to be a complex which does not contain oxygen (complex [**5**]). The difference in the absorption coefficient seen in Table IV may be explained by the contribution of the molecular oxygen. In infrared spectrum of this complex absorption bands which may be assigned as O–O stretching vibration were observed at 845 and 885 cm^{-1} as shown in Fig. 9. Recently in various complexes which contain molecular oxygen, characteristic O–O stretching vibration band is observed in the region from 830 cm^{-1} to 890 cm^{-1}.[21] From the infrared spectra and the result of the elemental analysis the red–violet complex observed in the presence of air, is different from the red–violet complex observed in the absence of air and may possibly be regarded as the complex involving molecular oxygen, and the structure may be considered as **1** in Chart 1, in solid state, the structure of hemocyanine[20] being referred, although at present any direct evidence of the existence of molecular oxygen in the complex has not been obtained. The change of yellow to red–violet by aeration into the solution after titration may also be regarded as the similar type of reaction. The reaction of MEA and copper (II) in the excess of MEA may be described in Chart 3, from the above–mentioned considerations.

$$2H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SH + 2Cu(II) \rightleftarrows (H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-})_2 + 2Cu(I) + 2H^+ \quad (5)$$

$$2H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SH + 2Cu(I) \rightleftarrows 2H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}Cu(I) + 2H^+ \quad (6)$$

$$4H_3N^+\text{-}CH_2\text{-}CH_2\text{-}SH + 2Cu(II) \rightleftarrows (H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-})_2 + 2H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}Cu(I) + 4H^+ \quad (7)$$

$$2H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}Cu(I) + O_2 \longrightarrow \begin{matrix} H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}Cu(I) \\ \vdots \\ O_2^- \\ \vdots \\ H_3N^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}Cu(II) \end{matrix} \quad \mathbf{1}$$

Chart 3

In the case of hemocyanine,[20] it was assumed that one electron is taken away from O_2 by copper (I) to give O_2^- as perhydroxyl ion and then one copper (I) is converted into copper (II) to result a complex in which molecular oxygen is absorved. The formation of complex [**1**] may be explained analogously to the case of hemocyanine. For the confirmation of the involving of the molecular oxygen, the detailed quantitative study on the absorption of oxygen to the complex will be necessary in future. It is very interesting that the mixed valence copper complex of MEA is relatively unstable in some cases, as shown in Table II, in comparison with those of other thiols, such as thiomalic acid,[8] mercaptoacetic acid[9] and penicillamine,[10] the fact that MEA is characteristically effective as a radiation protective agent among these thiols being considered.

In the case of selenocysteamine, it is characteristic that complex [**4**] in Table I was always obtained when the mixing ratio of selenocysteamine to copper (II) is 1:1. The infrared spectra of the selenocysteamine copper complexes were not clear enough in the region from 2800 to 3500 cm^{-1} to judge whether the amino group coordinates to copper or hydrogen ion. However, the structures of complexes [**3** and **4**] may be assumed as shown in Chart 1, analogously to the MEA-complexes, from the results of the elemental analyses.

Reaction of Cysteine with Copper (II)

The complex formation of cysteine with copper (II) ion has been studied in detail in parallel with the redox reaction. Robin and Day described that cysteine does not give red–violet

21) Y. Saito, *Yuki Gosei Kagaku Kyokai Shi*, **26**, 943 (1968).

color with copper (II).[22] Recently Lohmann studied the reaction of cysteine or reduced glutathione with copper (II) by the use of electron spin resonance spectroscopy and found that the signal of copper (II) disappears in accordance with the increase of the concentration of the thiol, namely the content of copper (II) decreased into 20—30% when the ratio of cysteine to copper (II) is 1 to 1, and copper (II) disappeared completely when the ratio is 2 to 1, and copper (II) was present in 10—15% when the ratio of 4 to 3, and the signal of copper (II) recovers again by the exposure to air,[23] but the color of the complex was not mentioned. From the titration curves (Fig. 2), the reaction of cysteine with copper (II) can be discussed analogously to the case of MEA. It is characteristic that in cysteine, the mixed valence complex is formed in alkaline solution unlike the case of MEA and the absorption maximum

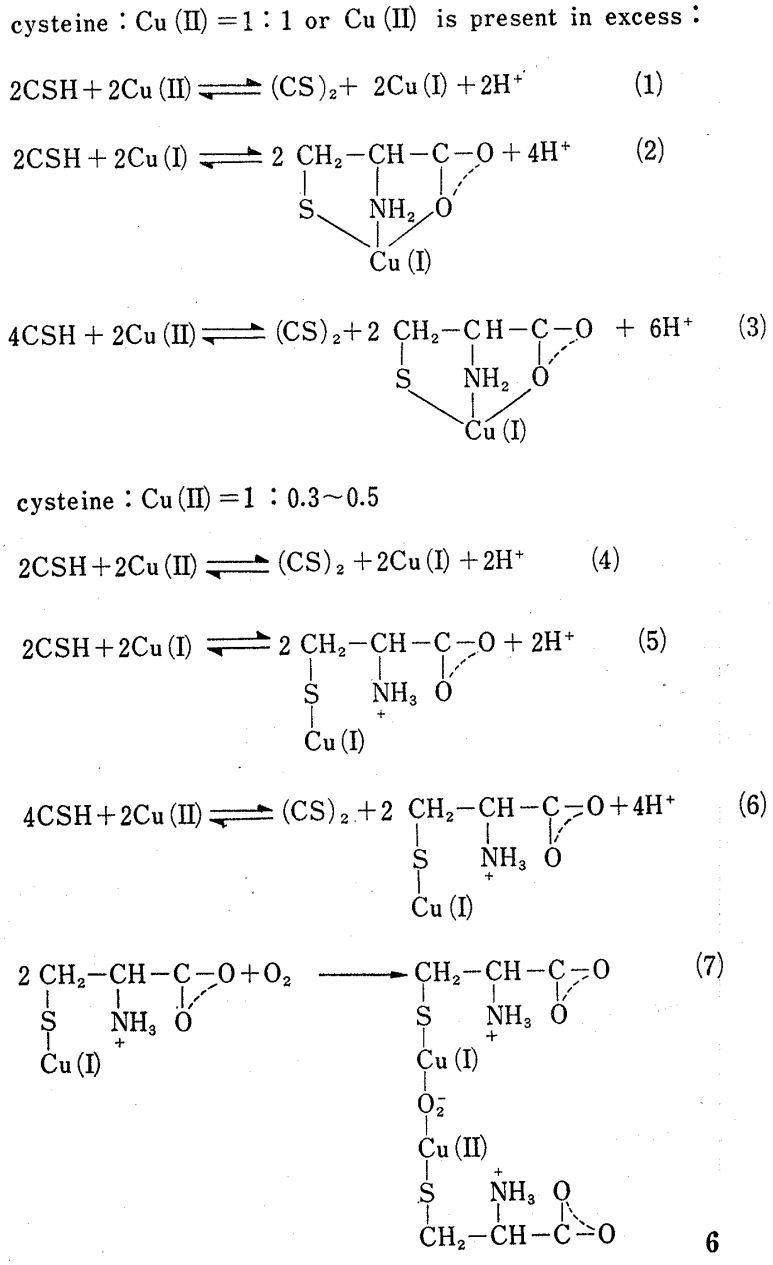

Chart 4-1

22) M.B. Robin and P. Day, *Advanc. Inorg. Chem. Radiochem.*, **10**, 247 (1967).
23) W. Lohmann, M. Momeni, and P. Nette, *Strahlen-Therapy*, **134**, 590 (1967).

cysteine : Cu(II) = 4 : 3

$$2CSH + 2Cu(II) \rightleftharpoons (CS)_2 + 2Cu(I) + 2H^+ \quad (8)$$

$$CSH + Cu(I) \rightleftharpoons CH_2-CH(S-Cu(I))-C(=O)(NH_3^+) + H^+ \quad (9)$$

$$CH_2-CH(S-Cu(I))-C(=O)(NH_3^+) + Cu(II) \rightleftharpoons \text{7} + 2H^+ \quad (10)$$

7

$$4CSH + 3Cu(II) \rightleftharpoons (CS)_2 + 7 + 6H^+ \quad (11)$$

CSH : $CH_2-CH(SH)-C(=O)(NH_3^+)$ $\quad$ $(CS)_2$: $\left(O=C(O)-CH(NH_3^+)-CH_2-S-\right)_2$

Chart 4-2

(shoulder) is observed at shorter wavelength than the case of MEA (Fig. 8, curve IV). The reaction of cysteine with copper (II) can be assumed in Chart 4 by the analogous considerations to the case of MEA. The structures of two kinds of the mixed valence complexes may be presumed as [**6** and **7**] respectively, and they correspond to complexes [**1** and **2**] in the case of MEA respectively.

Reaction of the Thiol Compounds Related to MEA and Cysteine with Copper (II)

The formation of the mixed valence copper complex in some thiol compounds related to MEA and cysteine, are summarized in Table IV. For the formation of considerably stable mixed valence copper complex, the mercapto and the amino groups may necessarily be free and apart from each other by a chain of two carbon atoms. Homocysteine and N,N-dimethyl-MEA do not form the red–violet mixed valence copper complex, not likely to cysteine and MEA. In N,N-dimethyl-MEA, mere redox reaction may be considered in the reaction with copper (II) ion. As seen in the comparison between cysteine and homocysteine, five membered chelate ring may be necessary for the formation of the mixed valence copper complex. The substitution at β-position of MEA as in β,β-dimethyl-MEA may have effect to stabilize the mixed valence copper complex. The similar relation has been reported in cysteine and penicillamine which is β,β-dimethylcysteine. In penicillamine the mixed valence copper complex is very stable and can be isolated from the solution.[10] In the mixed valence copper complex of β,β-dimethyl-MEA, absorption maximum was observed at longer wavelength, and the molar absorptivity was about two times greater than of MEA at 420 mμ. In cysteine and penicillamine, the similar relation is seen in Table IV. In order to discuss the relationship between the chemical structure of the chelating agents and the ability of the formation of the mixed valence copper complex, enough informations on the chelating and reducing ability of the many related chelating agents toward copper (II) ion must be necessary.

Table IV. Data obtained from Absorption Spectra of Mixed Valence Complex

Ligand	λ_{max}(mμ)	$\varepsilon^{a)}$
MEA	490	1200
MEA	420 (sh.)	370
β,β-Dimethyl-MEA	550	500
Cysteine	440 (sh.)	400
β,β-Dimethyl-cysteine (penicillamine)	520	800

a) These values were calculated with respect to the concentration of Cu(II) from Fig. 8.

In conclusion, for the explanation of the above-mentioned complicated reactions, two processes, namely, the combination of the complex formation and the redox reaction and the contribution of the oxidation by oxygen, may necessarily be considered for the formation of the mixed valence copper complex. The characteristic reactions of MEA and cysteine with copper (II) may be related to the mechanism of their radiation protective activity to some extent.