follow the reaction of GSSG and cystine with silver nitrate. Since GSSG and GSH were both subject to the same error the interpretation of this reaction is unaffected. The cystine concentrations on the other hand were correct, whereas the cysteine figures were 17% high. This reaction is therefore slower than it appeared and the difference in reactivity between the two substances greater. Details will not be given here, as work on these and other compounds is continuing.

SUMMARY

1. A method for the estimation of four thiols by potentiometric titration with silver nitrate is described. The titrations are carried out in buffered solutions, the pH used depending on the particular compound. In all cases one of two pH ranges, 1.8–2.2 or 6.5–7.0, can be used.

2. At pH < 2.5 the silver ion concentrations are sufficiently high for the titrations to be followed with a silver electrode.

3. At pH > 2.5 the silver ion concentrations are too low to be followed with a silver electrode and the appropriate silver thiol electrode must be used. The preparation of these electrodes is described and a simple thermodynamic treatment is given.

4. The method is applied to the estimation of six disulphides. These are first allowed to react with sodium sulphite to give one equivalent of the corresponding thiol. The factors governing the optimum pH for the reaction are discussed and the conditions for each compound are given.

5. The conditions under which the different silver mercaptides form stable complexes with additional silver are discussed.

6. This work disclosed errors in previous work on the estimation of thiols and disulphides. These are discussed and the corrections given.

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Complex-Formation between Polypeptides and Metals

1. APPLICATION OF VARIOUS EXPERIMENTAL METHODS TO THE GLYCINE-COPPER SYSTEM

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In recent years complex-formation between amino acids and various metals has been extensively studied with the aid of potentiometric titration (Albert, 1950, 1952; Monk, 1951; Perkins, 1952). The theory and practical application of the method has been fully described by Albert (1950), and the results obtained are summarized in the form of stability constants which for a simple ligand L^-

(such as an amino acid anion) and a divalent metal M are the equilibrium constants for the reactions

so that

$$K_1 = [ML^+]/[M^{2+}][L^-]$$

and

$$K_2 = [ML_2]/[ML^+][L^-].$$

The constants K_1 and K_2 may be combined in the expression $K_1 K_2 = K_s$, where K_s is the equilibrium constant for the overall reaction between the metallic ions and the amino acid, i.e.

When the method of potentiometric titration was applied to the study of complex-formation between cupric ions and the simplest polypeptide, glycylglycine, it became evident that the situation was essentially more complicated than with an amino acid such as glycine. With glycine in the zwitterion form one proton is liberated for each molecule of amino acid taken up by the metal. A preliminary consideration of the titration curve of glycylglycine in the presence of cupric chloride suggests that in this case at least two protons are liberated for every peptide zwitterion which reacts with the metal.

It seemed desirable, therefore, in order to obtain a confirmed interpretation of metal-complex formation in such a system to supplement the potentiometric titration data with information provided by other techniques. Those tested were copper-electrode potential measurements, electrophoresis, and spectrophotometric observations in the visible range of the spectrum. It seemed necessary in the first instance to test the value of these methods by applying them to the interpretation of the relatively well-understood copper-glycine system. This paper describes how these methods were applied and demonstrates that the results obtained from their use are consistent with the view that glycine reacts with copper according to eqns. 1 and 2 above.

EXPERIMENTAL

Materials

The copper salt used was A.R. $CuCl_2 \cdot 2H_2O$. The glycine was supplied by British Drug Houses Ltd. and found to be chromatographically pure. Before use it was dried *in vacuo* at 78°.

Methods

Potentiometric titrations. Three solutions were examined containing $CuCl_2 \cdot 2H_2O$ (0.04262 g.) and glycine in the molar ratio of 1:1, 1:2 and 1:6, respectively. The reagents in each case were dissolved initially in such a volume of boiled-out distilled water that the total concentration of copper at the mid-point of each titration was almost exactly 0.005 M.

The solution to be titrated was contained in a 150 ml. beaker fitted with a rubber bung which held a glass electrode, a saturated calomel electrode, a thermometer and a capillary glass tube through which N_2 was passed continuously for stirring and maintaining an inert atmosphere. Two additional small holes were made in the bung; one for insertion of the burette tip and the other (lined with a piece of glass tubing) for insertion of a copper electrode or for a fine pipette through which samples of the solution might be withdrawn for spectrophotometric analysis.

The alkali used for titration was carbonate-free 0.1 N-KOH standardized against weighed portions of potassium hydrogen phthalate and stored in a waxed bottle from which it was delivered by a 2 ml. burette with drawn-out tip. The alkali was added in a series of small portions each of which generally contained 0.1 equiv. KOH/molecule of chelating agent present in the solution. After each addition of alkali, the pH of the solution was determined with a glass electrode and a Beckman Model G pH meter. All the experiments were done at 20°.

If we define $\bar{n}$ as the average number of ligand molecules bound by each metal ion at any stage in the process of complex-formation (Bjerrum, 1941) and $[L^-]$ as the corresponding concentration of free chelating species, then, provided that certain conditions are satisfied, K_1 and K_2 can be calculated from eqns. 3 and 4 without actual measurement of the free metal-ion concentration being made (Albert, 1950):

$$K_1 = \frac{\bar{n}}{(1 - \bar{n}) [L^-]}, \quad (3)$$

$$K_2 = \frac{(\bar{n} - 1)}{(2 - \bar{n}) [L^-]}, \quad (4)$$

$[L^-]$ may be evaluated from the equation

$$\beta [L^-] = [L_0] - [B^+] - [H^+], \quad (5)$$

where

$$\beta = \frac{2[H^+]^2}{K_a K_{a'}} + \frac{[H^+]}{K_a},$$

K_a (the ionization constant of the NH_3^+ group) $= [L^-] [H^+] / [{}^+HL^-]$; $K_{a'}$ (the ionization constant of the $COOH$ group) $= [{}^+HL^-] [H^+] / [{}^+HLH]$, $[L_0]$ = total concentration of amino acid and $[B^+] =$ the concentration of base added to the solution at any point. The corresponding value of $\bar{n}$ may be found from

$$\bar{n} [M] = [L_0] - \alpha [L^-], \quad (6)$$

where $\alpha = [H^+]^2 / K_a K_{a'} + [H^+] / K_a + 1$ and $[M_0]$ = total concentration of metal.

In applying these equations we have used concentrations instead of activities since all solutions examined were sufficiently dilute to ensure that the activity coefficient of every ionic species was nearly unity. The problem of obtaining the best values of the constants K_1 and K_2 has been considered by Irving & Rossotti (1953). This method, though valuable in the simpler cases, would not seem to be readily applicable to the more complex situations considered in the following papers of this series and so is not employed here. It is doubtful whether the improvements in the values which would be obtained by such arithmetical refinements would be of practical significance in work of the present type.

When values for K_1 and K_2 have thus been estimated at restricted points on the titration curve of glycine in the presence of cupric ions, their validity throughout the entire process of complex-formation may be tested by calculating the theoretical titration curve which they imply for the copper-glycine system and comparing this with the experimental curve.

If $[L_0]/[M_0]$, the ratio of the molar concentrations of ligand and metal in a solution, is denoted by X , then at any pH the concentration $[L^-]$ of free chelating anions in this solution is given by

$$K_1 K_2 \alpha [L^-]^3 - K_1 K_2 (X - 2) [M_0] [L^-]^2 + K_1 \alpha [L^-]^2 - K_1 (X - 1) [M_0] [L^-] + \alpha [L^-] = X [M_0]. \quad (7)$$

The value of L^- found from this equation may then be inserted in eqn. 5 to obtain the amount of base required to bring the solution to the chosen pH.

Copper-electrode potential measurements. The potential of a copper electrode, partially immersed in a solution containing cupric ions, may be read against a calomel electrode using a suitable potentiometer in which the null point is detected by a high-resistance valve voltmeter. The current taken by our instrument was of the order 10^{-11} A, so that while a reading was being taken, polarization of the electrode was reduced to a minimum.

The electrodes used were lengths of copper wire of about 1 mm. in diameter. Before use, an electrode was washed in $n\text{-NH}_3$ followed by distilled water. It was then electroplated in a solution containing 15% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O} + 5\%$ (w/v) H_2SO_4 . When covered with an even film of copper, the wire was rinsed in distilled water and placed in the titration vessel. After each pH reading had been taken during a titration, a measurement of copper-electrode potential was made. When K_1 and K_2 have been calculated from the potentiometric titration data, $[M^{2+}]$ may be found at any pH from the equation

$$[M^{2+}] = [M_0] (1 + K_1[L^-] + K_1 K_2[L^-]^2).$$

The theoretical electrode potential corresponding to any value of $[M^{2+}]$ may then be calculated using the equation

$$E = E^0 - \frac{RT}{zF} \ln c,$$

where E^0 = standard potential of copper electrode and c = concentration of cupric ions, and the observation that in an 0.001M solution of pure $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ the potential of a copper electrode read against our saturated calomel electrode was 0 mv at 20° . The use of the above equation was subject to the provision that the activity coefficient of the copper in our solution should be practically unity. The copper-electrode potential values obtained experimentally were compared with those calculated from these equations.

Electrophoresis. A drop of a solution containing CuCl_2 and glycine at any pH, when placed on a filter-paper strip moistened with glycine solution of equal concentration and pH to whose ends a potential is applied, will move according to the effective mean charge on the metal at that pH. However, in order to determine the displacement of the spot due to electrophoresis alone it is necessary to take into account the behaviour of the spot due to electroendosmosis and adsorption of the filter paper. The extent of movement due to electroendosmosis may be measured by placing initially alongside the test spot a drop of a solution containing molecules which are uncharged and have no tendency to be adsorbed on the paper. A suitable marker is glucose. The displacement of this marker will then be due to electroendosmosis alone.

Any tendency for the test spot to be adsorbed on the paper may be determined by comparing its movement with that of the marker substance when no current is passed and the liquid phase is allowed to move slowly along the paper due to a siphoning effect alone.

Strips of Whatman no. 54 chromatographic filter paper 5 cm. in width were used. At two points on a line perpendicular to the long axis of the filter paper were placed respectively a 5 μl . drop of the copper-glycine solution being examined and, 2.5 cm. away, a 5 μl . drop of a 2% (w/v) glucose solution as marker of the electroendosmotic

flow. It was thought desirable in any experiment to apply the drops on a line whose distance from the mid-point of the strip would be approximately equal to that of the mean position of the spots detected at the end. When the spots applied to a strip were dry, the strip was wetted in a solution of glycine equal in concentration to that of the test spot and at the same pH, care being taken not to move the spots. The wet strip was then placed between two pairs of glass plates in such a way that the central part of the paper, free of the plates, was tautly suspended while the last few cm. at each end dipped down into glass troughs containing some of the glycine solution used to wet the paper. The glass plates were held between two large glass plates resting on a block. The sides of the small chamber in which the central part of the paper was suspended were closed by insertion of suitable pieces of thick glass plate. A piece of platinum foil was partially immersed in the solution in each trough and the potential between these electrodes had a gradient of about 5 v/cm. along the paper strip.

When the current had been passed for 5 hr. the papers were removed and quickly dried in a horizontal position by a current of hot air. Each strip was then divided lengthwise. The half containing the copper-glycine spot was sprayed with an aqueous 0.1% solution of sodium diethyl-dithiocarbamate. Reaction between the copper and this substance led to the immediate appearance of a light-brown spot. The other half was sprayed with a 3% solution of *p*-anisidine hydrochloride in *n*-butanol and dried for 10–15 min. at about 110° when the glucose, which gave a brown spot, was seen to have moved towards the cathode.

In order to find whether the copper-glycine solution was adsorbed on the filter paper, similar experiments were done in which no current was passed and glycine solution was put in only one trough of each pair. The liquid then flowed slowly over the paper towards the empty trough. After the same period of 5 hr. the papers were dried and sprayed as before.

When the resultant electrophoretic movement of the copper in a particular copper-glycine solution at any pH has been measured experimentally, it can be compared with that predicted from consideration of the expected distribution of the metal as free ions and glycine complexes. These forms exist in the ratio

$$[M^{2+}]:[ML^+]:[ML_2] = 1:K_1[L^-]:K_1 K_2[L^-]^2 \quad (8)$$

at any pH, the copper gradually losing its positive charge as complex-formation proceeds.

Spectrophotometric observations. Visible changes in the colour of a copper-glycine solution during titration with alkali may be attributed to the formation of successive complexes having different absorption spectra. If these spectra were known and also the relative amounts in which the complexes are present at any pH value it would be possible to calculate the composite absorption curve for the solution at that pH assuming that the spectral contributions of the various complexes are additive. Absorption curves thus calculated could then be compared with those observed spectrophotometrically.

The experimental observations were made with a Unicam SP. 500 spectrophotometer, a sample of each solution being withdrawn at intervals during the course of titration, then replaced in the titration vessel after its optical density had been measured at 10 $m\mu$. intervals throughout the visible range of the spectrum.

By using the equilibrium constants obtained from the potentiometric titration experiments, the distribution of the metal in a solution at any pH is found from expression (8) above.

The absorption coefficients at various wavelengths to be attributed to the free cupric ion were obtained directly by spectrophotometric observations on a solution containing 0.005M-CuCl₂. Those of the complex ML₂ were taken as a first approximation to be identical with the absorption spectrum of the solution containing cupric ions and glycine in the ratio of 1:6 at a pH value in the neighbourhood of 10 where practically all of the copper is present in this form.

If we now consider the experimentally observed absorption curve of the solution containing copper and glycine in the molar ratio of 1:1 or 1:2 at a pH where the species ML⁺ is predominant, we can derive the absorption curve of this complex using those of M²⁺ and ML₂ already obtained and the calculated ratio in which these three species exist.

Thus if M²⁺, ML⁺ and ML₂ are present at a particular pH in the amounts α_1 , α_2 and α_3 respectively, and their absorption coefficients at a given wavelength are E_1 , E_2 and E_3 , respectively, then $E_0 = \alpha_1 E_1 + \alpha_2 E_2 + \alpha_3 E_3$ is the observed absorption of the solution at that pH. E_3 may then be found by substitution of the values known for the other quantities in the equation. This process may be repeated until the absorption coefficient of ML⁺ has been calculated for every λ at which spectrophotometric examination of the solution has been made.

These absorption coefficients have been derived to fit the experimentally observed absorption curve of a particular solution at one pH. Consequently, slight adjustments may have to be made to them and to the absorption coefficients chosen for ML₂ in order that they combine with those of M²⁺ at all pH values to give the best possible agreement with the observed absorption curves of all three solutions examined.

Fig. 1. A, titration curve of solution containing 18.76 mg. glycine (0.005M) + 42.63 mg. CuCl₂·2H₂O (0.005M) in 48.75 ml. B, change with pH in potential of copper electrode immersed in above solution. —, observed; ○, calculated.

RESULTS

The values calculated here for the ionization constants of the amino and carboxyl groups of glycine at 20° are $K_a = 10^{-9.86}$ and $K_a = 10^{-2.22}$ at concentrations of 0.01 and 0.1M respectively. These values are the same as those given by Albert (1950).

From the potentiometric titration data of the 1:1 and 2:1 glycine-copper solutions, the values

Fig. 2. A, titration curve of solution containing 37.52 mg. glycine (0.01M) + 42.63 mg. CuCl₂·2H₂O (0.005M) in 47.5 ml. B, change with pH in potential of copper electrode immersed in above solution. —, observed; ○, calculated.

Fig. 3. Titration curve of solution containing 112.57 mg. glycine (0.03M) + 42.63 mg. CuCl₂·2H₂O (0.005M) in 42.5 ml. —, observed; ○, calculated.

calculated for the stability constants of the copper-glycine complexes are $K_1=10^{8.5}$ and $K_2=10^{7.0}$. These combine to give $K_1=10^{15.5}$ which is the value reported by Albert (1950). By the use of these values of K_1 and K_2 the expected titration curves of solutions containing CuCl_2 and glycine in the molar ratios of 1:1, 1:2 and 1:6 and also the copper-electrode potential expected at various points in these titrations were calculated. In Figs. 1-3 these theoretical curves are compared with those observed. The titration of the solution containing equimolar quantities of CuCl_2 and glycine was not

continued above pH 6, since a blue gelatinous precipitate, presumably $\text{Cu}(\text{OH})_2$, appears about this stage. This precipitation is due to the fact that reaction between glycine and cupric ions does not end with the formation of the complex ML^+ even when the reagents are present in equimolar concentration. Some of the dichelate ML_2 complex exists also in equilibrium with free cupric ions, some of which will be precipitated as $\text{Cu}(\text{OH})_2$ when the pH of the solution rises above 6.

The results of the electrophoresis experiments with solutions containing CuCl_2 and glycine in the

Table 1. *Electrophoretic behaviour of copper-glycine complexes*

Change with pH in the electrophoretic movement of the copper in a solution containing 0.005 M CuCl_2 + 0.01 M glycine. Figures given are distances traversed by the copper-glycine and glucose spots on a filter-paper strip to which a potential of 5 v/cm. was applied. Corrections are made for electroendosmosis and adsorption. All distances are measured as positive in the direction of the cathode. The theoretical change with pH in charge on metal is shown by distribution of metal in form of free ions and glycine complexes. G^- represents the glycine anion.

pH	...	...	...	...	...	3.0	5.0	8.0	10.0
Average observed movement of copper-glycine spot (cm.)						9.7	5.3	4.7	4.4
Average observed movement of glucose spot (cm.)						3.9	4.2	5.0	4.4
Calculated movement of copper-glycine spot due to electroendosmosis (glucose movement $\times R_F$ of copper-glycine spot)						3.3	3.4	4.7	4.4
Net movement of copper-glycine spot due to electrophoresis						6.4	1.9	0.0	0.0
Distribution of copper (%)									
						Cu^{2+}	79.0	4.6	—
						CuG^+	20.8	65.0	3.8
						CuG_2	0.2	30.4	96.2
								—	0.6
								99.2	99.4

Table 2. *Copper-glycine complexes*

A: distribution of copper in form of cupric ions and copper-glycine complexes at various pH values in solutions containing 0.005 M CuCl_2 , $2\text{H}_2\text{O}$ and different concentrations of glycine. B: comparison of observed and calculated optical densities of the solutions at various pH values. The figures are $\log I_0/I \times 10^3$ and have been adjusted to refer to a solution in which $[\text{Cu}] = 0.005 \text{ M}$ at each pH. G^- represents the glycine anion.

A. Distribution of metal as				B. Optical densities at ($\text{m}\mu$.)							
pH	Cu^{2+} (%)	CuG^+ (%)	CuG_2 (%)	600		650		700		750	
				Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
(a) Glycine concn., 0.005 M (composition of solution as in Fig. 1)											
3.49	69.8	29.8	0.4	—	—	49	49	73	72	86	84
4.22	40.4	57.0	2.6	—	—	81	83	108	108	115	114
5.33	18.5	72.2	9.3	64	68	109	114	136	136	135	133
6.30	14.0	73.3	12.7	72	75	120	122	140	141	136	136
(b) Glycine concn., 0.01 M (composition of solution as in Fig. 2)											
3.35	58.6	40.5	0.9	—	—	62	62	88	86	97	96
4.41	13.7	73.3	13.0	74	76	122	122	141	142	135	136
5.25	2.5	56.0	41.5	122	124	160	161	155	156	130	131
6.51	0.1	19.0	80.9	181	181	201	201	158	159	108	109
(c) Glycine concn., 0.03 M (composition of solution as in Fig. 3)											
4.14	4.7	65.4	29.9	110	106	152	155	156	151	135	135
8.84	—	—	100.0	208	209	217	219	159	159	97	97
9.60	—	—	100.0	208	209	220	219	161	159	99	97

molar ratio of 1:2 are given in Table 1. These show that the positive charge on the metal is gradually reduced in magnitude with increase in pH.

The distribution of the metal in the form of free ions and these complexes at various pH values in the three solutions examined are given in Table 2A. By means of these figures and the absorption curves of the species given in Fig. 4, the optical density expected for each solution at several pH values was calculated. In Table 2B a selection of these figures is compared with the experimental observations.

DISCUSSION

The purpose of this paper is chiefly to describe, test and justify, methods for use in studying the formation of complexes of Cu^{2+} with simple polypeptides. On potentiometric titration little comment is required except to say that the results agree closely with those in the literature. Observations have been made not only with the usual 2:1 glycine- CuCl_2 molar concentration ratio but also with 1:1 and 6:1 ratios. The agreement of the theory with the experimental data has been shown by what we consider the best method in such investigations, namely by calculating the titration curve of the solution from the values of K_1 and K_2 and com-

paring this with the experimentally observed curve. This is of importance in dealing with more complicated systems since the error of the experimental observations may be approximately estimated, while that of derived constants cannot be so readily found.

Our experience with the copper electrode suggests that this type of electrode is of only limited value due to the experimental difficulties involved in its use. In particular, it was difficult to ensure stability of the electrode especially when the cupric-ion concentration was low. Below a cupric-ion concentration of about 10^{-7}M the electrode readings became quite unreliable. It is to be remembered that measurements in solutions of low cupric-ion concentration will be complicated by the reaction $\text{Cu}^{2+} + \text{Cu} \rightleftharpoons 2\text{Cu}^+$ which takes place at the electrode. This reaction has an equilibrium constant

$$K = [\text{Cu}^+]^2/[\text{Cu}^{2+}] \approx 10^{-6}.$$

Because of this it has been found by Tourky & Wakkad (1948) that a copper electrode immersed in a solution of pure cupric sulphate will give reliable indication of the cupric-ion activity only when this actually exceeds 10^{-4} . However, in solutions containing copper-glycine complexes the concentration of Cu^{2+} at any pH will be kept relatively constant, since even if some Cu^{2+} is reduced to Cu^+ in the vicinity of the electrode, the Cu^{2+} concentration will be replenished by dissociation of the complexes. This buffering action may account for the comparatively satisfactory readings of the electrode in our solutions down to a cupric-ion concentration of about 10^{-7}M .

The electrophoresis experiments indicate that the change with pH in the sign and magnitude of the resultant charge on the metal in a solution containing copper and glycine in the ratio of 1:2 corresponds well with the degree of metal chelation as calculated from the values found for K_1 and K_2 . It is also important to note that there is no apparent reversal of the sign of the charge on the metal, for in the next paper it will be shown that such a reversal is in fact observed with copper and glycylglycine in alkaline solution.

Very good agreement is obtained between the optical density measurements made on each of the three solutions over a range of pH and the figures calculated by combining the characteristic absorption curves of M^{2+} , ML^+ and ML_2 in proportions calculated from a knowledge of the values of K_1 and K_2 .

The degree of reproducibility of the observed sets of figures, and the sensitivity of the theoretical sets of figures to changes in the values taken for K_1 and K_2 , make the agreement between the two sets a reliable check on the validity of the interpretation of the process of complex formation in this system.

Fig. 4. Computed absorption curves of free cupric ions and copper-glycine complexes. Curve A, Cu^{2+} ; curve B, CuG^+ and curve C, CuG_2 . (G^- represents the glycine anion.)

The method of spectrophotometric analysis is therefore considered the best of the three methods used here for testing the results derived from potentiometric titration experiments. It proved to be of particular value in a later study of the combination between cupric ions and diglycylglycine, where the reaction constants of the system could not be evaluated from the titration data alone.

SUMMARY

1. The following methods have been studied with a view to their application to the reactions between metals and polypeptides in aqueous solution: (a) potentiometric titration, (b) copper-electrode potential measurements, (c) electrophoresis, and (d) spectrophotometric observations.

2. When these methods were applied to the study of complex formation between glycine and cupric ions they confirmed the view that two complexes ML^+ and ML_2 are formed.

3. Even in alkaline solution and with an excess of glycine there was no evidence from the electro-

phoretic observations of the formation of a complex with a negative charge such as would be found if one cupric ion combined with more than two glycine anions.

4. Absorption curves in the visible range of the spectrum have been calculated for the copper-glycine complexes.

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Complex-Formation between Polypeptides and Metals

2. THE REACTION BETWEEN CUPRIC IONS AND SOME DIPEPTIDES

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The reaction of cupric ions with peptides has been studied qualitatively by a number of workers, often with the object of shedding light on the biuret reaction. Kober & Sugiura (1912) described the colours produced on the addition of alkali to solutions containing cupric ions along with a di-, tri- or tetra-peptide in the metal:peptide molar ratio of 1:1, and tried to correlate these colours with the formation of bonds between the metal and nitrogen atoms. This work was extended by the spectrophotometric investigations of Kober & Haw (1916) with various peptides, some of considerable complexity. More recently, information on the absorption coefficients of the copper complexes of di- to octa-peptides has been supplied by Plekhan, Gavrilov & Poddubnaya (1942), Plekhan & Gavrilov (1948) and Plekhan (1951). These workers, and also Rising, Parker & Gaston (1934) and Wenaas (1937), who isolated crystalline copper complexes of tri- and tetra-glycylglycine, respectively, postulated the existence (in a copper-peptide complex) of links between the metal and the atoms of the peptide

bond. Confirmation of the existence of this linkage in metal-peptide complexes has been obtained by Chouteau & Lenormant (1951) and Chouteau (1951) by means of infrared spectrophotometric measurements.

Monk (1951) studied the effect of the presence of glycylglycine on the solubility of cupric iodate at various pH values. On the assumption that the observed increase in solubility is due to complex-formation between the cupric ions and the peptide and that two complexes are formed as with glycine, he calculated the following stability constants for these complexes:

$$K_1 = [CuG^+]/[Cu^{2+}][G^-] = 10^{6.0}$$

and $K_2 = [CuG_2]/[CuG^+][G^-] = 10^{5.6},$

where G^- represents the glycylglycine anion. These results were confirmed by potentiometric-titration experiments. The solutions studied in this work contained an excess of peptide and were apparently examined in the pH range 4-8.