[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY, ST. LOUIS UNIVERSITY AND DUQUESNE UNIVERSITY]

Copper(II) and Zinc Complexes of Some Amino Acids and Glycylglycine[1a,b]

BY NORMAN C. LI AND EDWARD DOODY

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The logarithms of the over-all complex formation constants of the anions of proline, β-alanine, norleucine, methionine and glycylglycine with copper(II) were determined polarographically at 25.0°. With the exception of β-alanine, these values, as well as the pK_2 of the amino acids and peptide, decrease in the order given. β-Alanine has the second largest pK_2 and yet its copper complex is less stable than the methioninate, probably because a six-membered chelate ring is less stable than a five-membered one. In addition to the formation constants, the number and formulas of these and other amino acid complexes with copper(II), and in some cases with zinc, have been determined by polarographic, potentiometric, conductometric and spectrophotometric methods. The four methods are in agreement with respect to the existence of the 1:2 complex. The conductometric and spectrophotometric methods reveal an additional 1:1 complex for all the α-amino acids investigated, but fail to show this for glycylglycine.

As part of a general program on studies of complex formation between different metallic and amino acid ions,[2a,b] this paper presents the results on the copper(II) and zinc complexes of some amino acids and glycylglycine, using the methods of polarography, potentiometry, conductometry and spectrophotometry. These studies are of importance in the elucidation of the more complicated systems in which metals interact with proteins and enzymes.

Experimental

Only reagent quality chemicals were used. The preparation and analyses of solutions have already been described.[2a] The amino acids were generously supplied by Merck & Co., Inc. The glycylglycine was the gift of Sigma Chemical Co.

The apparatus used for polarographic, potentiometric and conductometric measurements are similar to those used by Li and Doody.[2] The polarographic half-wave potentials, after correction for the *IR* drop, are reproducible to ±1 mv. For studies with the zinc complexes, a Beckman Model G *p*H meter was also used for the potentiometric *p*H titrations. Spectrophotometric measurements were made with a Beckman quartz spectrophotometer, Model DU.

Results

Polarographic.—Figure 1 shows plots of $(pK_2 - p\mathrm{H})$ *vs.* $-E_{1/2}$ at 25.0° for solutions containing 5×10^{-4} *M* $Cu(NO_3)_2$, indifferent electrolyte 0.06 *M* KH_2PO_4 plus KOH, and 0.04 *M* solutions of potassium prolinate, potassium β-alaninate, potassium glycylglycinate and potassium glycinate. The data are used to calculate the number of groups, *p*, coördinated to each copper(II) ion and the over-all formation constant, k_f, of the complex by the method of Li and Doody.[2b] The results so computed are shown in Table I.

It will be noticed in Fig. 1 that in the region where $(pK_2 - p\mathrm{H}) < 1$, the experimental points deviate from the lines. The reason is that as *p*H of the solution approaches the pK_2 of the amino acid, the concentration of A^-, the anion of the amino acid or peptide, becomes appreciable and equation 1 of Li and Doody[2b] now becomes

$$(E_{1/2})_c = p0.0296(pK_2 - p(K_2 + (H^+)) - 0.0296 \log K_f - p0.0296 \log T + (E_{1/2})_s \quad (1)$$

where T is concentration of undissociated amino acid plus A^-. In order to obtain linear plots of $(pK_2 - p\mathrm{H})$ *vs.* $E_{1/2}$, therefore, it is necessary that the difference between $p(K_2 + (H^+))$ and *p*H be negligible. This is seen to be the case only in the region $(pK_2 - p\mathrm{H}) > 1$, as shown in Fig. 1.

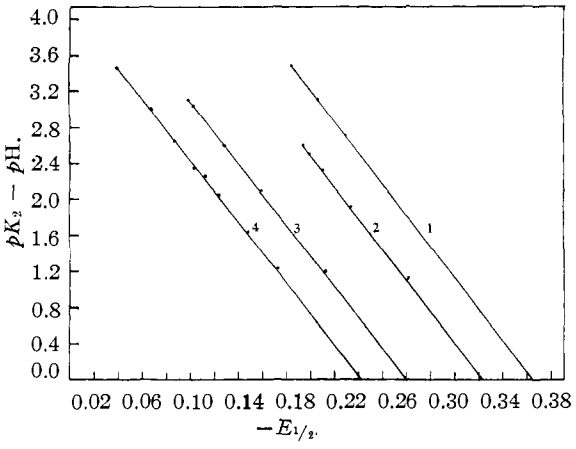


Fig. 1.—Change of half-wave potential with $(pK_2 - p\mathrm{H})$: 1, prolinate; 2, glycinate; 3, β-alaninate; 4, glycylglycinate.

Norleucinate and methioninate form a precipitate when used at 0.04 *M* concentration with 5×10^{-4} *M* copper(II) nitrate in 0.06 *M* potassium dihydrogen phosphate, the concentrations used for the other amino acids. To avoid precipitation we changed the compositions of the solutions to: 0.005 *M* potassium salt of norleucine or methionine, 5×10^{-5} *M* $Cu(NO_3)_2$, 0.015 *M* KH_2PO_4, plus KOH. In order to be sure that the over-all formation constants of the copper(II) norleucinate and methioninate complexes may be compared with the complexes studied in Fig. 1, we redetermined the formation constant of the glycinate complex in exactly the same way as norleucinate and methioninate. The value of log k_f for the copper glycinate complex now is 15.10, which is identical with the value found when the glycinate complex is studied in Fig. 1. For this reason the values of *p* and log k_f for the methioninate and norleucinate complexes

(1) (a) Data on copper complexes taken largely from the Ph.D. thesis of Brother Edward Doody, S.L.U., 1953. Zinc complex studies at D.U. with support of Grant No. 1496 from the Penrose Fund of the American Philosophical Society. (b) Presented before the Division of Physical and Inorganic Chemistry at the 124th ACS Meeting, Chicago, Ill., Sept., 1953.

(2) (a) N. C. Li and E. Doody, THIS JOURNAL, **72**, 1891 (1950); (b) *ibid.*, **74**, 4184 (1952).

are included in Table I and we are confident that comparisons of the over-all formation constants of these with the other complexes are completely valid.

The reversibility of the electrode reaction was proven for each analysis by determining the values of $E_{3/4} - E_{1/4}$ as was done by Li and Doody.[2b] The value of $(E_{3/4} - E_{1/4})$ for glycylglycinate, however, seems too high and a plot of log $i/(i_d - i)$ *vs.* $E_{d.e.}$ was made, from which the value of "n" is found to be 1.6. In our calculations for glycylglycinate, the value of "n" is taken to be 2, the same as for all the other complexes.

TABLE I

CALCULATED CONSTANTS FOR AMINO ACIDS AND THEIR COPPER(II) COMPLEXES

	pK_2	p	log k_f
Proline	10.60[3]	1.97	16.63
Norleucine	9.76[3]	1.98	15.20
Methionine	9.17[3]	1.98	14.75
β-Alanine	10.19[4]	2.02	12.89
Glycylglycine	8.22[4]	2.02	11.65
Glycine	9.69	2.00	15.10

It is seen in Table I that log k_f of the copper complexes of the α-amino acids decreases in the same order as the pK_2 values of the acids. This is as expected, since the stability of the complex is related to the basicity of the amino group of the acid.

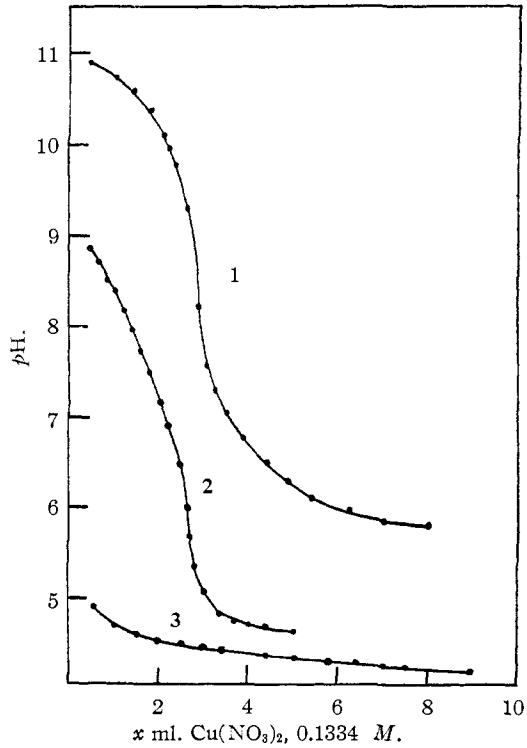
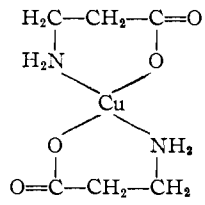


Fig. 2.—Potentiometric titration: curve 1, 13.93 ml. 0.054 M β-alaninate, diluted to 50 ml., plus x ml. $Cu(NO_3)_2$, p = 2.05; curve 2, 15 ml. of 0.050 M glycylglycinate, diluted to 50 ml., plus x ml. $Cu(NO_3)_2$, p = 2.25; curve 3, 7.5 ml. of 0.1 M β-alanine, diluted to 50 ml., plus x ml. $Cu(NO_3)_2$.

(3) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 84.

(4) C. B. Monk, *Trans. Faraday Soc.*, **47**, 288 (1951).

β-Alanine has a very large pK_2 approaching that of proline and yet the β-alaninate complex is very much less stable. An explanation is that the chelate would have the structure



Since the amino group is in the β-position, a six-membered ring is necessitated. This possibly is less stable than a five-membered ring for a copper chelate.

From Table I it is seen that the glycylglycinate complex is the least stable. Klotz, *et al.*,[5] postulated an increase in complex formation in going from copper glycinate to copper glycylglycinate. Our data therefore contradict the postulate of Klotz, *et al.*[5] It may be mentioned that our log k_f for the copper(II) glycylglycinate complex, 11.65, is in excellent agreement with Monk's value[4] of 11.66, obtained from pH measurements.

Potentiometric.—The potentiometric titration of β-alaninate and glycylglycinate with copper(II) ion are shown in Fig. 2, curves 1 and 2, respectively. The ratios of the ligand to copper, p, are 2.05 and 2.25 when the ligands are β-alaninate and glycylglycinate, respectively. In a reverse titration of copper with the ligands, the values of p are 1.99 and 2.12 for β-alaninate and glycylglycinate, respectively. The shape of these curves indicates very strongly that these complexes are far less stable than the complexes in which the α-amino acid ion is the ligand.[2b] This result and the values of p obtained confirm the polarographic results on these two complexes. No break is observed for β-alanine in curve 3, and the explanation is that the repulsion of the positively charged NH_3^+ to the copper cation weakens the affinity of the carboxylate anion for the same cation. A summary of potentiometric results is given in Table II.

TABLE II

POTENTIOMETRIC STUDIES OF RATIOS OF AMINO ACID IONS TO METALLIC CATIONS

	P
$Zn(NO_3)_2$ added to β-alaninate	2.07
β-Alaninate added to $Zn(NO_3)_2$	2.12
$Cu(NO_3)_2$ added to prolinate	2.10
$Cu(NO_3)_2$ added to norleucinate	1.98
$Cu(NO_3)_2$ added to glycylglycinate	2.25
$Cu(NO_3)_2$ added to β-alaninate	2.05[a]
Methioninate added to $Cu(NO_3)_2$	2.13[a]
Glycylglycinate added to $Cu(NO_3)_2$	2.12
β-Alaninate added to $Cu(NO_3)_2$	1.99[a]

[a] Precipitate formed during titration.

Conductometric.—Groups of curves which are typical of the conductometric titrations of all the α-amino acid ions investigated with zinc and copper

(5) I. M. Klotz, I. L. Faller and J. M. Urquhart, *J. Phys. Colloid Chem.*, **54**, 18 (1950).

are shown in Fig. 3, curves 1 and 2. The ordinates are the reciprocal of resistance corrected for dilution. There are two breaks in each curve and the results obtained show that the complex is of the MA type when the metal cation is in excess and of the MA_2 type when the amino acid ion is in excess.

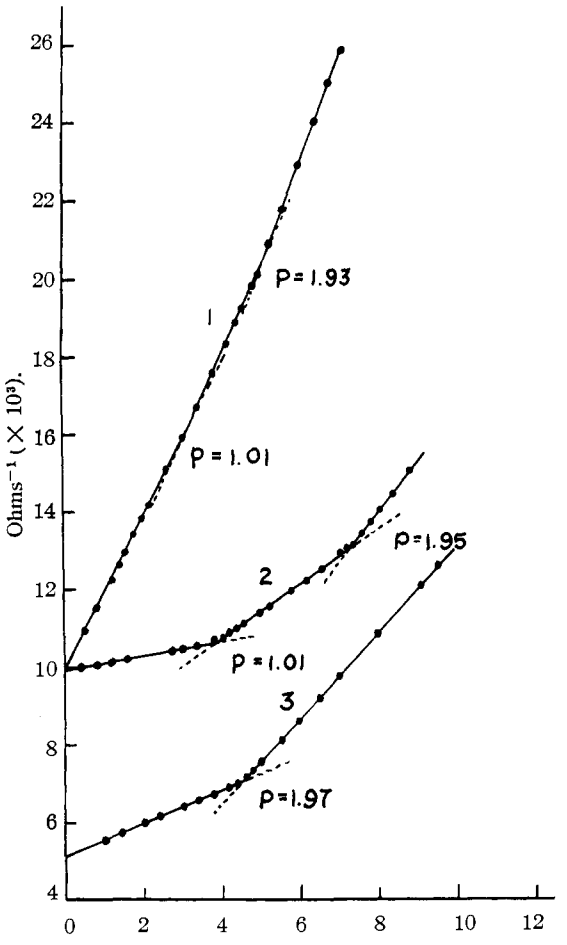


Fig. 3 —Conductometric titration: curve 1, 1.00 ml. of 0.1247 *M* $Cu(NO_3)_2$, plus 29 ml. water, plus *x* ml. 0.050 *M* arginine; curve 2, 1.00 ml. 0.1247 *M* $Cu(NO_3)_2$, plus 29 ml. water, plus *x* ml. 0.0333 *M* potassium serinate; curve 3, 1.00 ml. of 0.1143 *M* $Cu(NO_3)_2$, diluted to 15 ml., plus *x* ml. of 0.050 *M* potassium glycylglycinate.

In the case of glycylglycinate, as shown in Fig. 3, curve 3, there is only one break corresponding to the 1:2 (MA_2) complex. This behavior is different from the complexes in which the ligand is an α-amino acid ion. Conductometric titration curves are too numerous to include in this paper and the results are summarized in Table III. Where there are two numbers, the first and second numbers correspond to the first and second breaks, respectively, in the conductometric curves.

Spectrophotometric.—The spectrum of copper (II) ion in an aqueous serine solution is highly sensitive to *p*H. Table IV lists the wave length, λ_{max}, at which maximum absorption occurs, the molar extinction coefficient, ϵ_{max}, at that wave

TABLE III

CONDUCTOMETRIC STUDIES OF RATIOS OF AMINO ACID IONS TO METALLIC CATIONS

	p
$Zn(NO_3)_2$ added to glycinate	2.26; 1.05
$Zn(NO_3)_2$ added to glycylglycinate	1.78; ..
$Zn(NO_3)_2$ added to β-alaninate	2.06; ..
$Cu(NO_3)_2$ added to glycylglycinate	2.00; ..
Glycylglycinate added to $Cu(NO_3)_2$	.. ; 1.97
Phenylalaninate added to $Cu(NO_3)_2$	1.15; 2.24
Serinate added to $Cu(NO_3)_2$	1.01; 1.95
$Cu(NO_3)_2$ added to serinate	1.97; 0.98
Arginine added to $Cu(NO_3)_2$	1.01; 1.93
$Cu(NO_3)_2$ added to arginine	1.90; 0.98
Argininate added to $Cu(NO_3)_2$	0.99; 1.96[a]
Lysine added to $Cu(NO_3)_2$	.96; 1.96[a]
Lysinate added to $Cu(NO_3)_2$	.92; 2.03[a]
Methioninate added to $Cu(NO_3)_2$	1.01; 2.04[a]
Glutamate added to $Cu(NO_3)_2$	0.99; 2.02

[a] Precipitate formed during titration.

TABLE IV

DEPENDENCE OF λ_{max} AND ϵ_{max} ON *p*H, FOR COPPER–SERINE COMPLEX $Cu(NO_3)_2$, 0.0066 *M*

*p*H	λ_{max}, mμ	ϵ_{max}
2.97	687	17
5.15	635	38
6.46	623	49
10.50	617	50

length, as a function of *p*H for a copper–serine solution in which the ratio of copper to serine is 1:2. It is seen that as the *p*H is increased, the values of

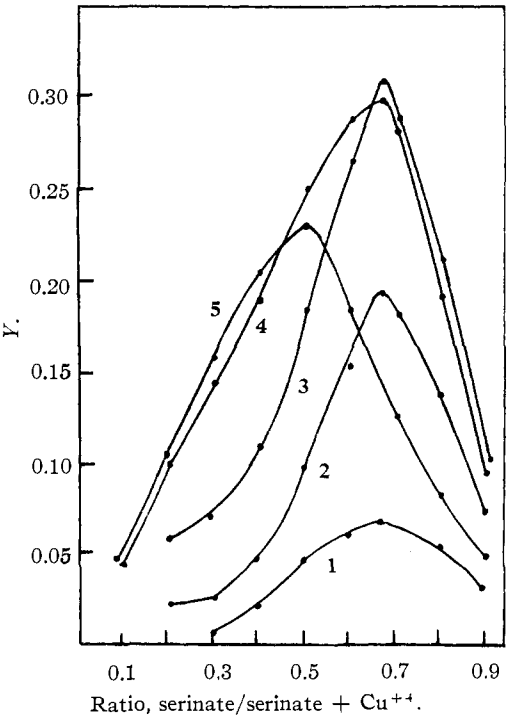


Fig. 4.—Continuous variations applied to potassium serinate with $Cu(NO_3)_2$: 1, 500 mμ, *p* = 2.0; 2, 550 mμ, *p* = 2.0; 3, 600 mμ, *p* = 2.0; 4, 650 mμ, *p* = 2.0; 5, 725 mμ. *p* = 1.0.

λ_{max} become smaller and ϵ_{max} become larger. This behavior is similar to that described for copper–glycine solutions reported by Klotz, *et al.*,[5] the shift of the absorption peak to shorter wave lengths being parallel to the ease of removal of a proton from $-NH_3^+$ as the *p*H is raised.

Since it seems evident that the chelating agent to Cu^{++} is the anion of the amino acid, this is the species used in all subsequent spectrophotometric determinations.

The method of continuous variations[6] was applied to the copper nitrate–potassium serinate system at several different wave lengths in order to find the ratio of the copper and serinate ions in the complex. Solutions of copper nitrate and potassium serinate, each 0.02 *M*, were prepared and a series of mixtures containing x ml. of the copper solution and $(10 - x)$ ml. of the serinate solution were made up in 10-ml. volumetric flasks. The absorbancy of each solution was measured with respect to water. Figure 4 shows the result obtained when the increase in absorbancy over that predicted for no reaction, Y, is plotted against the mole ratio of the serinate for several wave lengths. At 725 mμ the absorption maximum occurs at a mole ratio of 50%, indicating that the ratio of serinate to copper in the complex is unity, *i.e.*, the complex is CuA. At lower wave lengths, however, the maximum occurs at mole ratio of 0.67, indicating a 1:2 (CuA_2) complex.

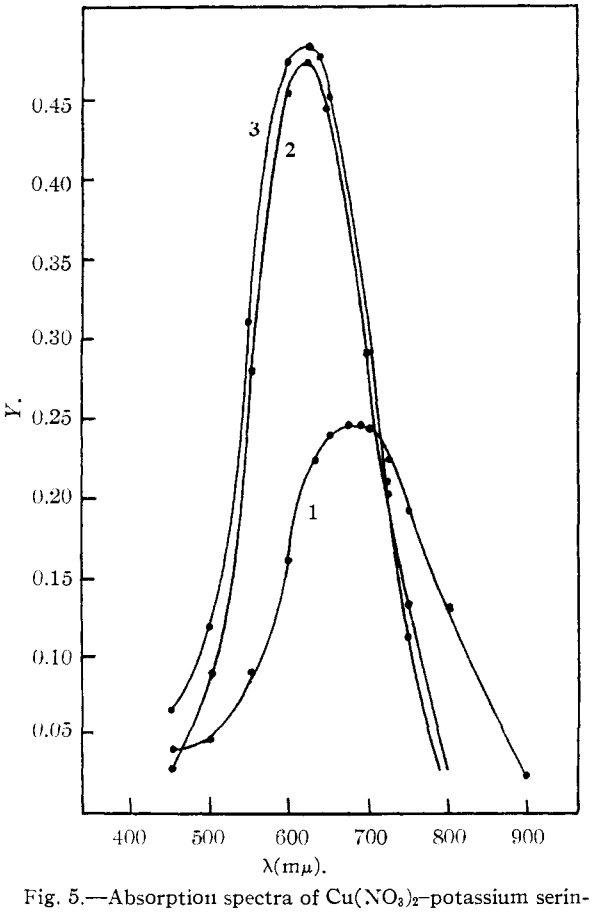


Fig. 5.—Absorption spectra of $Cu(NO_3)_2$–potassium serinate mixtures containing 0.01 *M* $Cu(NO_3)_2$ and: curve 1, 0.01 *M* serinate, *p*H 5.72; curve 2, 0.02 *M* serinate, *p*H 7.82; curve 3, 0.03 *M* serinate, *p*H 8.92.

(6) P. Job, *Ann. chim.*, **11**, 97 (1936); W. C. Vosburgh and G. R. Cooper, THIS JOURNAL, **63**, 436 (1941).

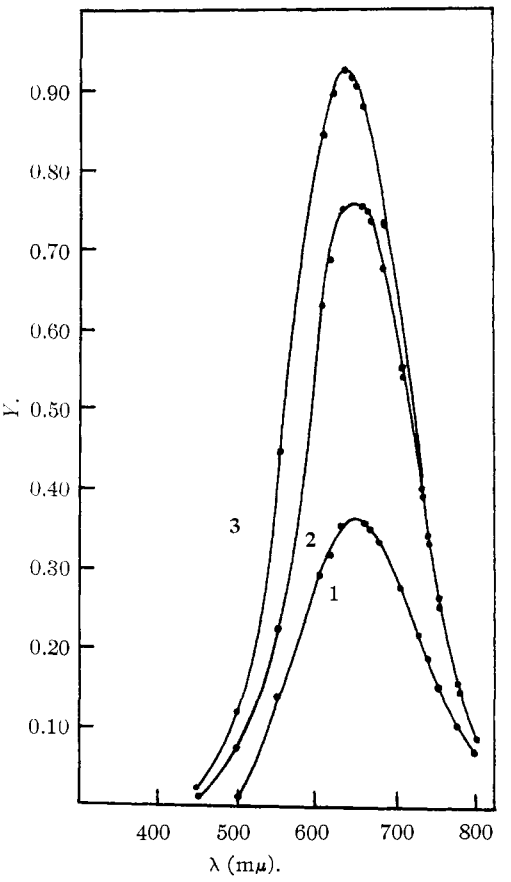
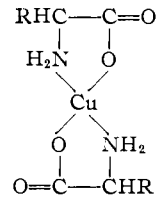


Fig. 6.—Absorption spectra of $Cu(NO_3)_2$–potassium glycylglycinate mixtures containing 0.01 *M* $Cu(NO_3)_2$ and: curve 1, 0.01 *M* glycylglycinate, *p*H 4.44; curve 2, 0.02 *M* glycylglycinate, *p*H 5.47; curve 3, 0.03 *M* glycylglycinate, *p*H 6.67.

Figure 5, curves 1, 2 and 3, are plots of Y *vs.* wave lengths of three different copper serinate mixtures in which the mole ratio of copper to serinate is 1:1, 1:2, 1:3, respectively. The absorption maxima for curves 2 and 3 are at the same wave length, 625 mμ, indicating that the same complex, presumably CuA_2, is present in the two solutions. Curve 1 has a maximum at 680 mμ, indicating that a different complex is present, presumably CuA.

Although Figs. 4 and 5 refer to copper–serinate mixtures, very much the same behavior is shown by copper(II) complexes with ions of other α-amino acids, namely, glycinate, alaninate, glutamate, aspartate and arginine. These others are too numerous to include here. The following is noted: Whenever copper(II) and ions of α-amino acids are mixed in the mole ratio of 1:2, absorption maxima occur in the region 615 to 650 mμ; whenever the mole ratio in the mixture is 1:1, maxima occur at higher wave lengths, around 700 mμ. We may conclude then that (a) an absorption maximum in the region 615 to 650 mμ indicates the presence of a 1:2 complex, where two bonds are of the amine type



and (b) an absorption maximum around 700 mμ indicates a 1:1 complex. Our conclusion may be compared to that of Klotz, *et al.*,[5] who state that the absorption peak of copper(II) ion in complexes shifts toward shorter wave lengths, from 700 to 600 mμ, as coördination number is increased.

Figure 6, curves 1, 2 and 3 are similar to Fig. 5, except that here glycylglycinate is the ligand. Here all three curves have the same peak at 630 mμ. Therefore, from what has been said above, only one complex is present, namely, CuA_2.

It must be noted that in Figs. 4, 5, and 6 the *p*H varies from mixture to mixture, depending on the ratio of potassium serinate or potassium glycylglycinate to copper(II) nitrate in the mixture. This variation, however, has no effect on the conclusions obtained on the formulas of the complexes. It is seen in Table IV that when an alkali is added to a given copper–serine mixture for the purpose of varying the *p*H, the values of λ_{max} fall in the region 635 to 617 mμ over a *p*H range of 5.15 to 10.50. This wave length region of absorption maxima is, as noted above, an indication of the presence of a 1:2 complex. In addition it is noted in Table IV that over a *p*H range of 6.46 to 10.50 for the particular copper–serine mixture, the value of the molar extinction coefficient at the wave length of maximum absorption is almost constant. For these reasons we are confident that the spectrophotometric results are valid in spite of the variation in *p*H.

Summary on Number and Formulas of Complexes.—Tables I, II, III, and Fig. 4 show that the polarographic, potentiometric, conductometric and spectrophotometric methods are in agreement with respect to the existence of the 1:2 complex. The conductometric and spectrophotometric methods reveal an additional 1:1 complex whenever the ligand is an α-amino acid ion, but fail to show this when the ligand is the anion of glycylglycine.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUQUESNE UNIVERSITY]

Stability of Zinc Complexes with Glutathione and Oxidized Glutathione[1]

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Several acid dissociation constants of glutathione and oxidized glutathione and the stability of zinc complexes with glutathione and with oxidized glutathione were determined at 25°. Equations for calculation of the several acid dissociation constants by the Bjerrum method have been deduced and equilibrium formation constants for the 1:1 complexes, *i.e.*, 1 mole of zinc ion to 1 mole of chelating agent, are reported. It is suggested that in the zinc–glutathione complex, zinc is probably coördinated through the sulfur atom and the amino group, and that in the zinc–oxidized glutathione complex, the coördination is probably through the amino and α-carboxylate groups.

Because of current biochemical interest in glutathione and oxidized glutathione, this paper reports the determination of the formation constants of zinc complexes with glutathione and oxidized glutathione, by an adaptation of Bjerrum's method.[2] This method, which involves measurement of the *p*H of solutions containing known amounts of the metal ion, the peptide and a base, was carried out at a constant ionic strength of 0.15. This value of the ionic strength was chosen so as to be the same as the ionic strength used in zinc–albumin studies, inasmuch as the purpose of this study is to furnish background information on metal–protein complexes.

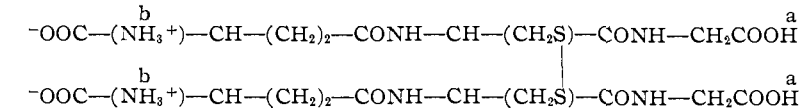
In order to obtain the concentration of the ligand, taken to be the anion of the peptide, it is necessary to determine the acid dissociation constants of the peptides. Values for pK_2 (COOH), 3.53, pK_3 (NH_3^+), 8.66, and pK_4(SH), 9.12, of glutathione have been listed by Cohn and Edsall.[3] Their pK_4 however, is in serious disagreement with the value, 9.62, reported by Pirie and Pinhey.[4] Because of this large discrepancy we have redetermined these values. The pK's of oxidized glutathione have not been reported.

Calculation of Constants

(A) Dissociation Constants of Oxidized Glutathione and Glutathione.—The dissociation constants for oxidized glutathione which are necessary for calculating formation constants are determined by the following equilibria present in aqueous solutions

$$H_4A = H_3A^- + H^+;\ K_3 = (H_3A^-)(H^+)/(H_4A) \quad (1)$$
$$H_3A^- = H_2A^{-2} + H^+;\ K_4 = (H_2A^{-2})(H^+)/(H_3A^-) \quad (2)$$
$$H_2A^{-2} = HA^{-3} + H^+;\ K_5 = (HA^{-3})(H^+)/(H_2A^{-2}) \quad (3)$$
$$HA^{-3} = A^{-4} + H^+;\ K_6 = (A^{-4})(H^+)/(HA^{-3}) \quad (4)$$

where ( ) represent molar concentration and H_4A is



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Removal of protons in the successive ionizations are assumed to take place in the order a,a,b,b for equations 1, 2, 3, 4, respectively.

(1) With the support of Grant No. 1496 from the Penrose Fund of the American Philosophical Society.

(2) J. Bjerrum, "Metal Ammine Formation in Aqueous Solutions," P. Haas & Sons, Copenhagen, 1941.

(3) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 85.

(4) N. W. Pirie and K. G. Pinhey, *J. Biol. Chem.*, **84**, 321 (1929).