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THE BEHAVIOR OF COPPER (II) CHELATES OF ALIPHATIC AMINOCARBOXYLIC ACIDS IN AQUEOUS SOLUTIONS

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ABSTRACT

The effective overall stability constants (log Ke values) and molar combining ratios for nine aliphatic aminocarboxylic acids (C_2 to C_6) with copper (II) ion were calculated from spectrophotometric and potentiometric data at pH 3.0, 4.0 and 5.0 and 25°C. In addition, calculated overall stability constants (log Kn values), using Bjerrum's method of analyzing potentiometric data, showed that chelates of alpha- and beta-aminocarboxylic acids with copper (II) were more stable than those of gamma- and epsilon-aminocarboxylic acids. Using Job's method of continuous variations for analyzing absorbance data, it was observed that while the alpha- and beta-aminocarboxylic acids formed both bicoordinated (1:1) and tetracoordinated (2:1) chelates with copper (II) ion at pH 4:0 and 5:0 and the alpha-aminocarboxylic acids formed only a (1:1) chelate at pH 3.0, the gamma and epsilon-aminocarboxylic acids showed only bicoordinated (1:1) chelate formation at pH 5.0.

The complexation of epsilon-aminocarboxylic acid ligand to copper (II) ions appears to be through both the amino and the carboxylate groups rather than through carboxylate ion, alone as previously suggested by other workers. The implication of this finding is that the formation of chelate ring structures greater than eight members

is a distinct possibility in the aliphatic aminocarboxylic acid series and further suggests the opportunity for more complex metallo-binding in proteins as well.

INTRODUCTION

In 1904, Ley was the first to study the interaction between cupric ion and simple aliphatic amino acids; such as glycine, alanine and aminobutyric acid in aqueous solution (1). He also recognized the special significance of the cyclic structure and electroneutrality of the bicoordinated, copper-glycinate chelate and based upon Werner's coordination theory, postulated that the deep blue color of these simple copper-amino acid chelates resembled that of Werner's copper-ammonium complexes (2). By means of differences in electrical conductance, Ley showed that copper chelates of glycine and alpha-alanine in aqueous solution were more stable than those of beta-alanine and gamma-aminobutyric acid.

At least two electronegative groups contributed by the ligand are involved in chelate or "claw complex" formation in solution. In the case of glycine and other simple aliphatic aminocarboxylic acids, the electronegative groups involved in complex formation are the amino nitrogen and the hydroxyl oxygen of the terminal carboxylic acid group. Chelation results in the formation of two displaced water molecules per proton from either the carboxylic acid group at low pH values or the protonated amine at acidic pH values.†

Copper (II)-glycine, chelation results in the formation of a stable 5-membered, bicoordinated (1:1) charged structure or a 5-membered, tetracoordinated (2:1) uncharged structure depicted as follows:

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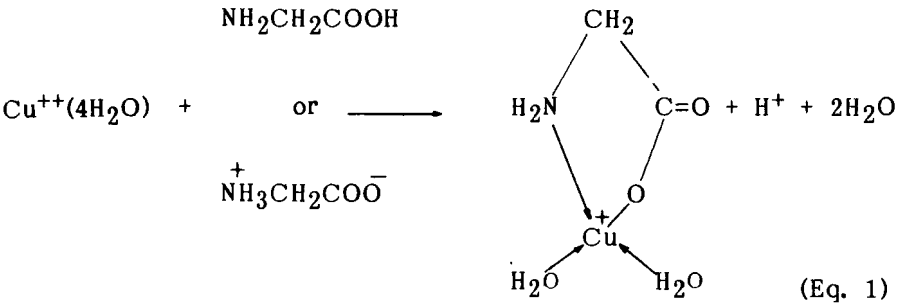
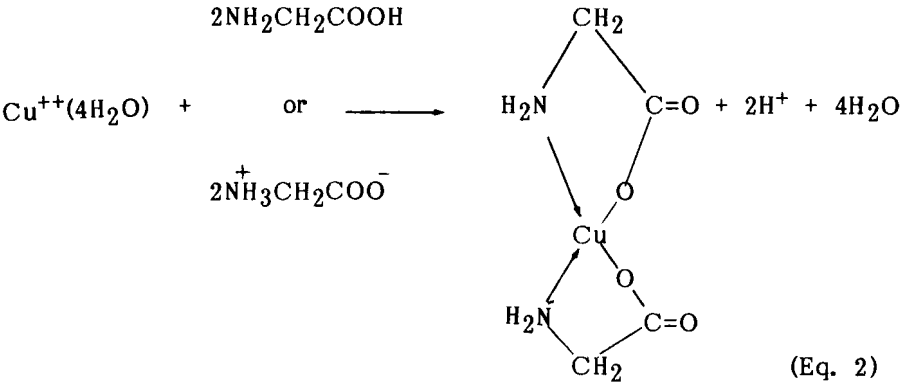
† Coordination complexes of cupric ion are formed by first promoting one d-orbital electron to the 4p level and thus leaving four dsp hybrid orbitals available for planar complex formation with electronegative atoms.



(Eq. 1)



(Eq. 2)

The neutral (2:1) glycine-copper chelate called bis(glycinato) aquo copper (II) was isolated and characterized by Tomita and Nitta and exists as two planar glycine rings attached to copper with cis configuration (3). Beta-alanine forms less stable six-membered (1:1 and 2:1) chelates in a similar fashion. However, little is known about the complexing tendency of copper for other simple, aliphatic aminocarboxylic acids in aqueous solution.

J. Bjerrum, using a potentiometric technique, was the first to determine the magnitude of the equilibrium or stability constants for the copper-glycine complex (4). According to the Law of Mass Action, the simplified stepwise formation of metal chelate such as copper-glycinate is represented as follows, where M equals the cupric or metallic ion and L equals the ligand or organic complexing agent:

$$L + M^{++} = M^{+}L \qquad K_1 = \frac{[M^{+}L]}{[M]\,[L]} \text{ (1:1 chelate)} \qquad \text{(Eq. 3)}$$

$$L + M^{+}L = ML_2 \qquad K_2 = \frac{[ML_2]}{[M^{+}L]\,[L]} \text{ (2:1 chelate)} \qquad \text{(Eq. 4)}$$

The overall stability constant, Kn,† equals K_1,K_2. Higher order glycine complexes (for example 3:1) which are known for cobalt, nickel, zinc and cadmium have never been reported for copper. Since the magnitude of the individual stability constants is normally greater than 100, the values are usually reported in their logarithmic form. Thus log K_n equals log K_1 + log K_2. Ignoring thermodynamic considerations, that is, ionic strength and activity coefficients of the reacting species at ambient room temperature (24 ± 2°C), the approximate log K_1 for the (1:1) glycine-copper chelate is 8 and the approximate log K_2 for the (2:1) chelate is 7. Thus the overall log stability constant for the five-membered, glycine-copper complex in aqueous solution is 15. In a similar fashion, the overall log stability constant for the six-membered, copper-beta-alanine complex ranges between 12 and 13.

The stepwise stability or formation constant of Bjerrum neglects hydrogen and hydroxyl ion interactions in complex formation. For example, in the case of copper-glycinate at pH values greater than 5, there exists the possibility of ternary chelate formation between copper ion, the organic ligand and hydroxyl ion, while at even higher pH values the formation of insoluble cupric hydroxide inhibits the formation of water soluble chelates altogether. At low pH values, below a pH value of 3 or 4, the competition between hydrogen ion, a stronger acid in water, then cupric ion for the organic ligand base, favors the formation of the free acid, NH_2CH_2COOH and the protonated base, $\overset{+}{N}H_3CH_2COOH$ and so little or no complex formation between copper and glycine is observed with the usual concentration ratios of copper: ligand. According to modern acid-base theory, where hard acids such as hydrogen ion react with hard bases such as hydroxyl ion rather than weaker bases such as acetate or

† Kn is more appropriately referred to as the conditional overall stability constant.

glycine ions, complex formation is favored when the concentration of hydrogen and hydroxyl ions is decreased. In the case of copper-glycinate, this normally occurs in water at essentially neutral to slightly acid pH values.

Therefore, it is not the overall stability constant, K_n, but the effective overall stability constant, K_e that describes the true interaction between copper and glycine in aqueous solution. The latter term, K_e, depends upon the relative strength (pKa) of the organic ligand and the hydrogen ion concentration (pH) of the aqueous solution. Since the concentration and relative strengths of acids and bases influence complex formation, metallic ions and organic ligands can be displaced by stronger and more concentrated reacting species or can interact successfully with these species to form intermediate, ternary complexes (5).

The purpose of the present investigation was to study the chelation properties of copper ion for a series of structurally related simple, aliphatic amino (C_2 to C_6) carboxylic acids in aqueous solution in order to determine their combining ratio in acidic media (pH 3 to 5) and to describe their effective overall stability constants. Chelation and complex formation between metallic ions, such as copper, and organic ligands, such as the aliphatic aminocarboxylic acids, is an important consideration in both the biologic and physical sciences and thus an understanding of the shorter range interactions that take place between such reacting species in biologic systems and the longer range interactions in chemical solutions was considered worthy of more detailed study.

EXPERIMENTAL METHODS

1. Standardization and Purification of Reagents

All purified grades of amino acids used in the study were recrystallized from 50% aqueous ethanol. Carbonate-free 0.1 M sodium hydroxide solution was standarized using reagent grade potassium hydrogen phthalate and a 0.002 M reagent grade cupric nitrate solution was standardized by electrodeposition.

2. Determination of Stability Constants

a. Potentiometric Titration

A 0.01 M amino acid solution was prepared in a 0.1 M sodium perchlorate ($NaClO_4$) solution previously prepared with boiled-out, glass distilled water. Exactly 50 ml of amino acid solution was added to either 50 ml of 0.1 M sodium perchlorate solution or 50 ml of cupric nitrate solution in a jacketed beaker for a total volume of 100 ml. Titrations were carried out at 25 $\pm$ 0.5°C with the aid of a magmix stirrer and thermostated water bath. Solutions were purged during each titration with purified nitrogen to maintain a carbon dioxide-free atmosphere. Solutions were titrated with standard 0.1 M sodium hydroxide solution (0.50 ml increments) from a buret. The pH at each addition of base was determined with the aid of a Model 10 Corning pH meter equipped with a Ross combination glass electrode, that was previously calibrated against pH 7 and rechecked with pH 4 standard buffer solution. The precision of the pH measurements was $\pm$ 0.02 pH units.

b. Spectrophotometric Analysis

Various mixtures of 0.1 M amino acid (prepared with 0.1 M $NaClO_4$) in boiled-out glass, distilled water and standardized 0.1 M cupric nitrate solutions were prepared and adjusted to pH values of 3.0, 4.0, and 5.0 with standard 0.1 M sodium hydroxide solution and then diluted to a final volume of 10 ml in a volumetric flask using boiled-out glass, distilled water. The absorbances of the resulting solutions were determined at selected wavelengths of 615, 630, 650, 700 and 720 nm with the aid of a Perkin-Elmer, Model 552 spectrophotometer previously calibrated by performing Beer's Law plots with copper solutions. One cm quartz cells were used for all measurements. Absorbance corrections were made by using appropriate amino acid blank solutions.

3. Treatment of Data

Stability constants were initially calculated using the method of Bjerrum (4) as modified by Calvin and Melchior (6) where $\bar{n}$, equals the average number of ligand molecules bound to a single copper ion, which

may be determined from the horizontal distance between the titration curve of amino acid in the presence and absence of cupric ions (see Figures 1 and 2). This represents the exact amount of base consumed in the reactions (see equations 5 and 6) and equals the ligand bound in the complex.

$$M + LH^+ + OH^- = ML + H_2O \qquad \text{(Eq. 5)}$$

$$ML + LH^+ + OH^- = ML_2 + H_2O \qquad \text{(Eq. 6)}$$

The total moles of ligand, L, bound divided by the total concentration of metallic ion yields the value, $\bar{n}$ (see equation 7):

$$\bar{n} = \frac{\text{(ml of titrant to curve)} \times \text{(Molarity of titrant)}}{\text{(Molarity of } Cu(NO_3)_2 \text{ solution)} \times \text{(Total volume in ml to the curve)}} \qquad \text{(Eq. 7)}$$

When $\bar{n}$ becomes constant, the maximum for the average number of ligand molecules bound to a single metal ion is obtained. At any pH, the value of free ligand is calculated from the following relationship:

$$pL = -\log L = pKa - pH - \log [LH^+_{initial} - OH^-] \qquad \text{(Eq. 8)}$$

Thus when $\bar{n}$ is plotted on the y-axis versus pL on the x-axis of a coordinate graph paper, the extrapolated values on the x axis for $\bar{n}$ = 0.5, 1.0 and 1.5 represent log K_1, log $K_n/2$, log K_2, respectively (see figure 3). Only the complex formation curves for glycine and epsilon-aminocaproic acid have been presented here as they are representative of the types of curves we obtain for the other alpha-aminocarboxylic acids studied as well as the other amino acids, where the amino grouping was displaced further down the chain. The stability constants estimated by graphical means were confirmed by use of a computer program, which calulates stepwise formation constants for divalent metal ion-ligand complexes (7).

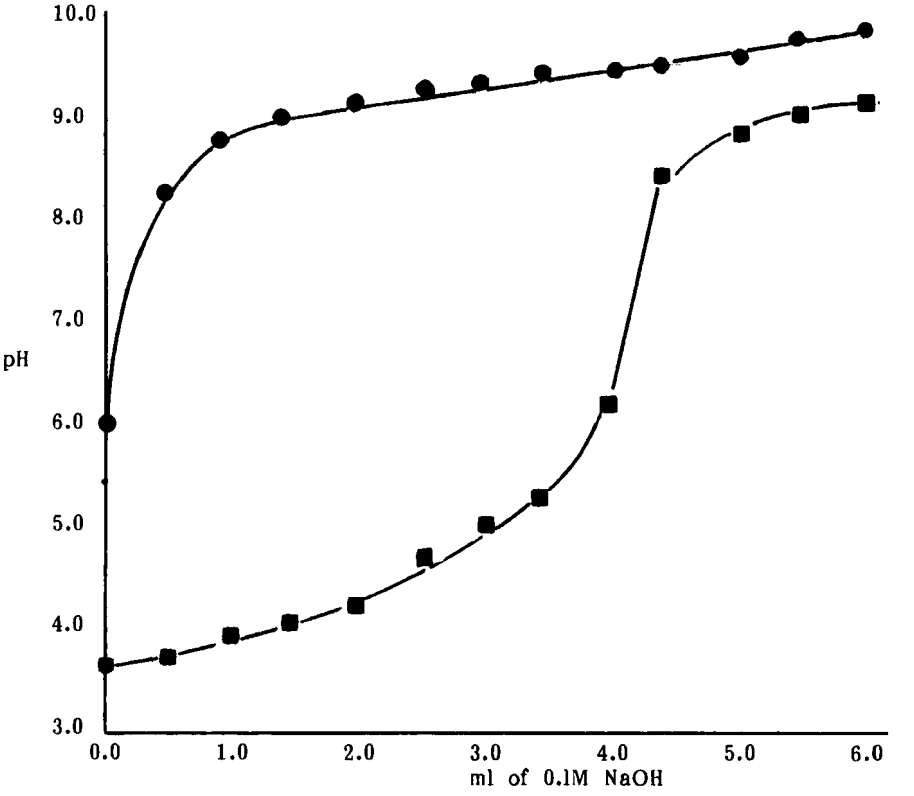


Figure 1: Titration Curve of Glycine in Presence and Absence of Cubric Ions at 25°C and Ionic Strength 0.1 M

Glycine alone ● Glycine + Cupric Ion ■

4. Calculation of Chelation Molar Ratios

Using the method of Job (8) as modified by Vosburgh and Cooper (9), a spectrophotometric method for determining the composition of a soluble metal complex as formed in solution between a metallic ion, M and a ligand, L, is possible if the wavelength of maximum absorbance for the complex differs from the wavelength of the metallic ion in solution. This is the case with respect to the formation of colored chelates between copper ion and aliphatic aminocarboxylic acid ligands at the pH values selected for study. The absorbance of solutions containing varying ratios of metallic ion to organic ligand are plotted versus the mole fraction of

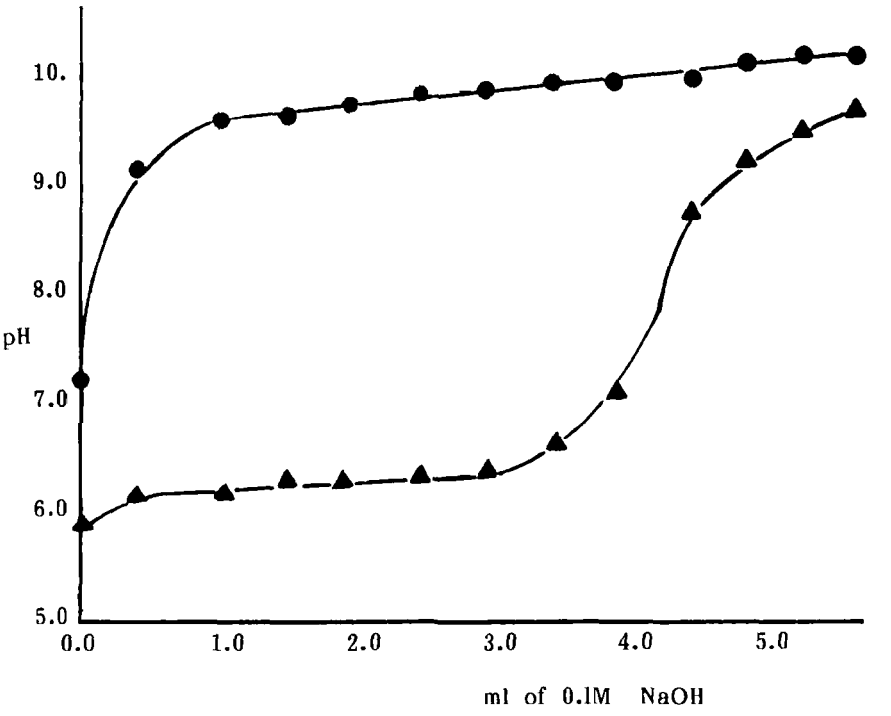


Figure 2: Titration Curve of 6-Aminocaproic Acid in Presence and Absence of Cupric Ions at 25°C and Ionic Strength 0.1 M ($NaClO_4$)

6-aminocaproic acid alone ● 6-aminocaproic acid + Cupric Ion ▲

the binary mixture of metallic ion and ligand at a fixed wavelength and the maximum absorbance obtained is associated with the molar ratio of the metallic ion and ligand in the complex (see Figures 4 and 5).

DISCUSSION OF RESULTS

The apparent ionization constants for nine C_2 to C_6 aliphatic aminocarboxylic acids determined at 25°C are summarized and listed in Table 1. An examination of these data reveals that when the amino group is attached to the alpha carbon adjacent to the carboxyl function, the acidity constants for the series of 2-amino aliphatic carboxylic acids are pKa_1 equals 2.36 $\pm$0.07 and pKa_2 equals 9.73 $\pm$ 0.08 with an isoelectric

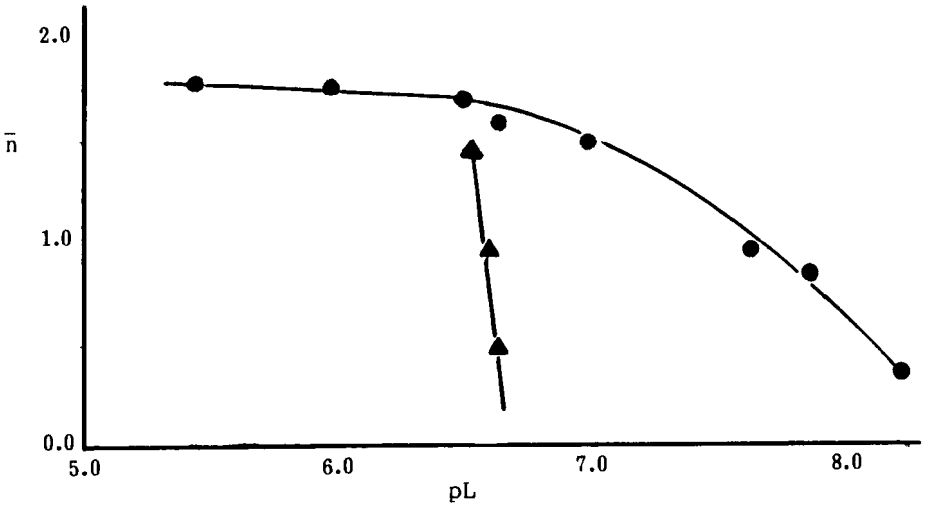


Figure 3: Complex Formation Curve of Glycine and 6-Aminocaproic Acid at 25°C and Ionic Strength 0.1 M ($NaClO_4$)

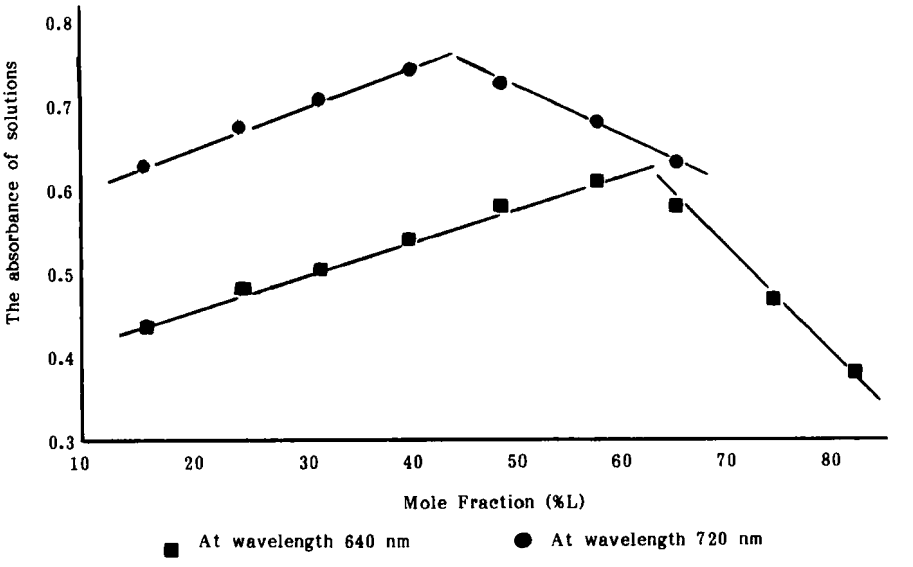


Figure 4: Continuous Variations Analysis Curves of 0.1 M $Cu(NO_3)_2.3H_2O$ Chelated with 0.1 M Glycine at pH 5.0, 25°C and Ionic Strength 0.1 M ($NaClO_4$)

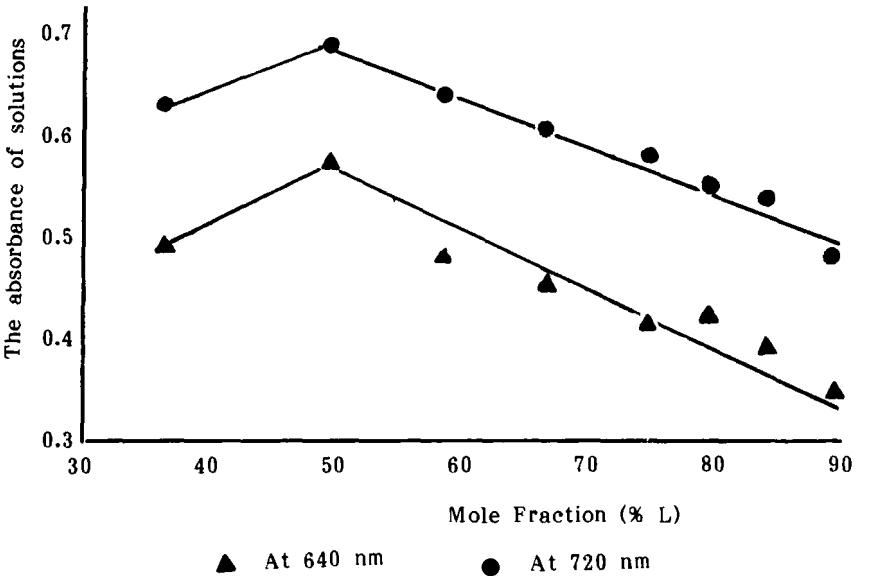


Figure 5: Continuous Variations Analysis Curve of 0.1 M $Cu(NO_3)_2.3H_2O$ Chelated with 0.3M 6-Aminocaproic Acid at pH 5.0, 25°C and Ionic Strength 0.1 M ($NaClO_4$)

point for the zwitterion, pI equal to 6.05. On the other hand, when the amino group moves down the aliphatic carbon chain, the acid strength of both groups decreases as expressed by an increase in the resulting pKa_1, pKa_2 and pI values. If the pKa_1 curve is extrapolated to a C_8 carbon chain, the resulting pKa_1 for say 8-aminocaprylic acid would appear to approximate the ionization constant of acetic acid (pKa equals 4.76). Thus the influence of the terminal amino group on the ionization of the carboxylic acid function is diminished. (see Figure 6).

Monk (10) and Albert (11) have each shown separately that among the common divalent cations, copper II forms the strongest chelates with glycine. The tendency to form strong complexes with this particular amino acid is given in the following decreasing order:

$$Cu^{II} > Ni^{II} > Zn^{II} > Co^{II} > Fe^{II} > Mn^{II} > Mg^{II}$$

This relationship appears to holds for many other organic ligands as well.

Table 1: Apparent Acidity Constants of Aliphatic Aminocarboxylic Acids at 25°C.

Amino Acid	pKa_1 (R-COOH)	pKa_2 + ($R-NH_3$)	$pI = \frac{pK_1 + pKa_2}{2}$
2-aminoacetic	2.35[a], 2.34[c]	9.78[a], 9.60[c]	6.02
2-aminopropionic	2.35[a], 2.34[c]	9.87[a], 9.69[c], 9.78[d]	6.06
3-aminopropionic	3.55[a], 3.60[c]	10.24[a], 10.19[c]	6.90
2-aminobutyric	2.28[a], 2.55[c], 2.60[d]	9.83[a], 9.68[b], 9.60[c]	6.06
4-aminobutyric	4.03[a], 4.23[c]	10.56[a], 10.43[c], 10.00[d]	7.23
2-aminovaleric	2.32[a], 2.30[b]	9.78[b], 9.70[a]	6.03
5-aminovaleric	4.20[d], 4.50[d]	10.77[c], 10.40[d]	7.39
2-aminocaproic	2.39[c]	9.76[c]	6.08
6-aminocaproic	4.37[a], 4.50[d]	10.80[a]	7.50

a. J.T. Edsall and J. Wyman, Biophysical Chemistry, vol. I, Academic Press, New York, 1958.

b. D.D. Perrin, Dissociation constants of organic bases in aqueous solution, Butterworths, London, 1965.

c. Dawson, et al., Data for Biochemical Research, Clarendon, Oxford, 1959.

d. Determined in our laboratory using the method of T.V. Parke and W.W. Davis, Anal. Chem., 26, 642, 1954.

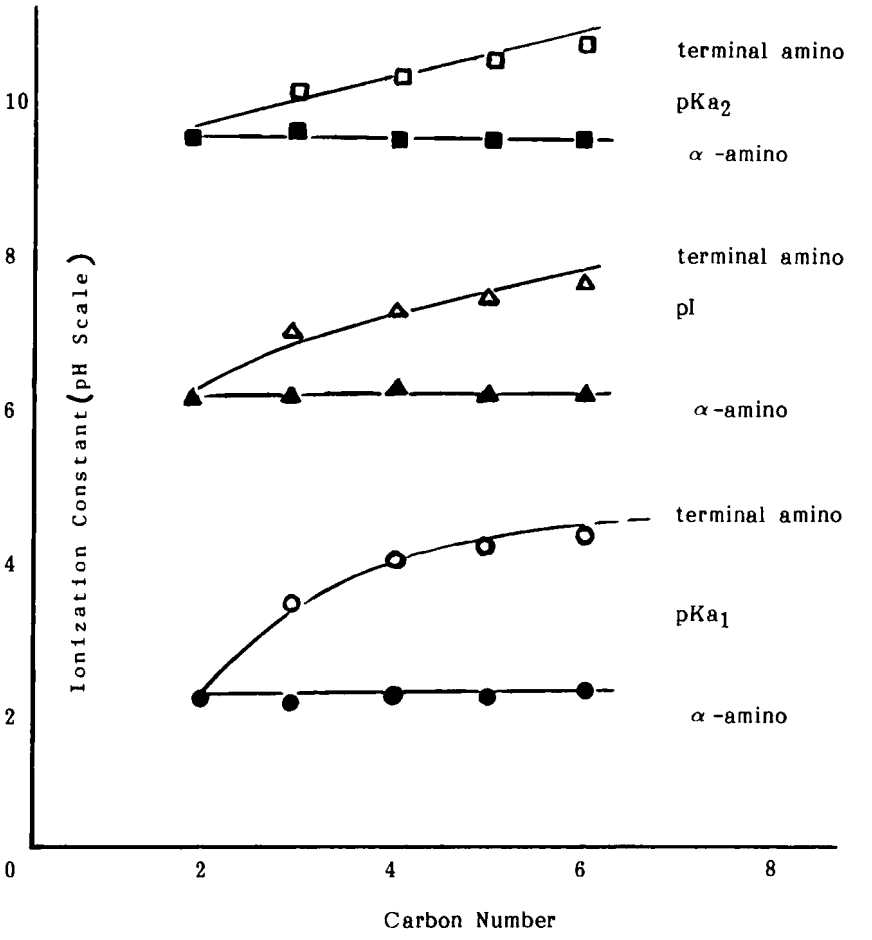


Figure 6: Relationship Between Ionization Constant at 25°C and Carbon Chain Length in the Aliphatic Amino Carboxylic Acid Series

Using the method previously described, the pL and $\bar{n}$ values were computed. The resulting binding or stability constants thus obtained for nine copper (II) aliphatic aminocarboxylic acids are presented in Table 2. Our values appear to be in reasonably good agreement with data previously reported by other workers. It is observed that for those amino acids where the amino group is attached to the alpha carbon adjacent to the carboxylate function,ignoring ionic strength considerations for these five 2-amino aliphatic carboxylic acids, the log K_1 was calculated to be equal to 8.20 $\pm$ 0.24 and log K_2 equals 6.81 $\pm$ 0.16 with an overall stability

Table 2: Stability Constants of Copper (II) Chelates of Simple Aliphatic Aminocarboxylic Acids at 25°C.

Amino Acid	Log K_1	Log K_2
2-aminoacetic	8.62 (0.02)*	6.97 (0.02)[a]
	8.22 (0.03)	6.90 (0.03)[h]
	8.10 (0.1)	7.00 (0.1)[b]
	8.00 (0.1)	6.70 (0.1)[h]
	8.07 (0.2)	6.77 (0.2)[c]
2-aminopropionic	8.51 (0.02)	6.86 (0.02)[a]
	8.30 (0.03)	6.70 (0.03)[d]
	8.10 (0.1)	6.60 (0.1)[e]
	8.07 (0.2)	6.72 (0.2)[c]
	8.20 (0.1)	6.95 (0.1)[h]
3-aminopropionic	7.10 (0.03)	5.30 (0.03)[d]
	7.15 (0.1)	5.74 (0.1)[f]
	7.15 (0.1)	6.33 (0.1)[h]
2-aminobutyric	8.01 (0.2)	6.70 (0.2)[c]
	7.76 (0.1)	6.5 (0.1)[h]
4-aminobutyric	6.30 (0.1)	- [h]
2-aminovaleric	8.68 (0.02)	7.10 (0.02)[g]
	8.07 (0.02)	6.75 (0.2)[c]
	7.90 (0.1)	6.86 (0.1)[h]
5-aminovaleric	6.58 (0.1)	- [h]
2-aminocaproic	8.2 -	6.9 - [f]
6-aminocaproic	6.22 (0.02)	- [h]
	6.60 (0.1)	- [h]

* Ionic strengths listed in parentheses.

a. C.B. Monk, Trans. Faraday Soc., 47, 285 - 291 (1951).
b. Anderegg, Helv. Chim. Acta, 44, 1673 (1961).
c. A. Gergely and I. Sovago, J. Inorg. Nucl. Chem., 35, 4355 (1973).
d. H.A. Platt and A. N. Martin, J. Pharm. Sci., 49, 518 (1960).
e. Smith, Taylor and Smith, J. Biol. Chem., 122, 109 (1937).
f. N.C. Li and E. Doody, J. Am. Chem. Soc., 76, 221 (1954).
g. R.E. Rebertus, Ph. D Dissertation, Univ. Illinois (1954).
h. This work.

constant log K_n of 15.0 $\pm$ 0.37. Thus by potentiometric analysis, aliphatic 2-amino carboxylic acids appear to form both bicoordinated (1:1) and tetracoordinated (2:1) 5-membered chelates with cupric ion in aqueous solution with an overall apparent affinity constant at 25°C of 1 X 10^{15} liters/mole. In contrast, beta-alanine (3-aminopropionic acid) forms both bicoordinated (1:1) and tetracoordinated (2:1) 6-membered copper chelates with an average log k_1 equals 7.13 and an average log K_2 equals 5.79 for an overall log K_n equal to 12.9. On the other hand, 4-aminobutyric, 5-aminovaleric and 6-aminocaproic acids exhibit only bicoordinated (1:1) copper chelate formation with an average log K_1 equal to 6.42. Osterberg and Toftgard have provided substantiation that 6-aminocaproic acid only forms bicoordinated complexes with copper (II) (12). It is only reasonable to expect that both 4-aminobutyric and 5-aminovaleric acids would do likewise.

The method of continuous variations is a simple and effective procedure for determining the combining ratios or formula and also the stability of a chelate provided that only a single compound is formed at a given wavelength from the components and that the absorbance of the resulting complex is measurable by spectrophotometric means. In the case of the deep blue copper-amino acid complex, its absorbance is measured between wavelengths 600 and 740 nm in the visible region of the spectrum. It is only necessary to make measurements with a variety of wavelengths, covering the region of the spectrum absorbed. If all wavelengths lead to the same result it may be concluded that only a single complex is formed. On the other hand, if there is a shift in combining ratios at a different wavelength, a second or possibly a third component may be detected as is the case in Figures 4 and 5. Li and Doody reported that when alpha-amino acids are mixed with cupric ion in the mole ratio of 2:1, absorbance maxima occur in the region 615 - 650 nm and whenever the mole ratio is 1:1, maxima occur at higher wavelengths, around 700 nm (13). Thus 640 and 720 nm were selected as wavelengths for the detection of complex formation. The combining ratios for nine copper-aliphatic aminocarboxylic acid chelates were determined at pH values of 3.0, 4.0 and 5.0. These data are reported in Table 3.

Table 3: Chelation Ratios of Copper Aliphatic Aminocarboxylic Acids At Various pH Values and Wavelengths as Determined by Spectrophotometric Analysis in Accordance with the Method of Continuous Variations

Amino Acid	pH 3.0		pH 4.0		pH 5.0	
	640nm	720nm	640nm	720nm	640nm	720nm
2-aminoacetic	1:1	NC*	2:1	1:1	2:1	1:1
2-aminopropionic	1:1	NC	2:1	1:1	2:1	1:1
3-aminopropionic	NC	NC	1:1	NC	2:1	1:1
2-aminobutyric	1:1	NC	2:1	1:1	2:1	1:1
4-aminobutyric	NC	NC	NC	NC	1:1	1:1
2-aminovaleric	1:1	NC	2:1	1:1	2:1	1:1
5-aminovaleric	NC	NC	NC	NC	1:1	1:1
2-aminocaproic	1:1	NC	2:1	1:1	2:1	1:1
6-aminocaproic	NC	NC	NC	NC	1:1	1:1

* NC equals no complex formed at this pH value or none detected at this wavelength.

The molar ratios reported at pH 5.0 are consistent with stability constants determined and reported in Table 2 for the nine amino acids of interest. The complexes listed at pH 3.0 and 4.0 have been interpreted in terms of the effective stability constant concept, first proposed by Reilley, Schmid and Sadek (14). According to these workers, the hydrogen ion competes with the metallic ion for the ligand base (amino acid) and this competition may be expressed by the pH-dependent term, α. The effective overall stability constant, Ke, is thus the effective tendency for reaction between the metallic ion and the ligand base at any particular pH value and may be represented by the following expression:

$$\log Ke = \log Kn - \log \alpha \quad \text{(Eq. 9)}$$

The value α for amino acids may be calculated using the following equation:

Table 4: Overall Effective Stability Constants for Copper Aliphatic Aminocarboxylic Acid Chelates in Acidic Aqueous Solution at 25°C.

Amino Acid	pH	Log α †	Log $K_1.K_2$ - Log α = Log Ke	Chelates Observed (L:M)
2-aminocarboxylic acids	5.0	4.73	15.0 - 4.73 = 10.3	(2:1) and (1:1)
	4.0	5.74	15.0 - 5.74 = 9.3	(2:1) and (1:1)
	3.0	6.82	15.0 - 6.82 = 8.2	(1:1)
3-aminopropionic	5.0	5.23	12.9 - 5.23 = 7.7	(2:1) and (1:1)
	4.0	6.35	12.9 - 6.35 = 6.6	(1:1)
	3.0	7.89	12.9 - 7.89 = 5.0	NC
4-aminobutyric	5.0	5.38	6.3 - 5.38 = 0.9	(1:1)
	4.0	6.35	6.3 - 6.70 = -0.4	NC
	3.0	7.89	6.3 - 8.49 = -2.2	NC
5-aminovaleric	5.0	5.65	6.6 - 5.65 = 0.9	(1:1)
	4.0	7.00	6.6 - 7.00 = -0.4	NC
	3.0	8.81	6.6 - 8.81 = -2.2	NC
6-aminocaproic	5.0	5.89	6.4 - 5.89 = 0.5	(1:1)
	4.0	7.32	6.4 - 7.32 = -0.9	NC
	3.0	9.19	6.4 - 9.19 = -2.8	NC

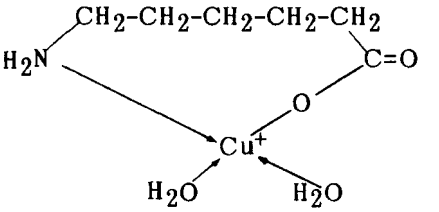
† Calculated from (Eq. 10) using data reported in Table 1.

$$\alpha = 1 + \frac{[H^+]}{Ka_2} + \frac{[H^+]}{Ka_1.Ka_2} \qquad \text{(Eq. 10)}$$

where Ka_1 and Ka_2 represents the acidity constants of the organic ligand. Log Kn values for the nine copper amino acid chelates from data presented in Table 2 and log α values for the amino acid ligands were calculated at pH 3.0, 4.0 and 5.0 using equation 10 in order to determine the effective overall stability constants, log Ke values, for the nine copper (II) chelates in acidic aqueous solution at 25°C. These data and the molar ratios of the copper complexes observed at these three pH values were tabulated and presented in Table 4.

The data reported in Table 4 are consistent with the observations made concerning these nine copper-amino acid chelates. In the case of the 2-amino acids (alpha-amino acids), the calculated log Ke values would support the formation of both the 2:1 and 1:1 chelates at pH values 4.0 and 5.0. In the case of beta-alanine, chelates are not possible at pH 3:0. In the case of 4-aminobutyric, 5-aminovaleric and 6-aminocaproic acids, only a feeble 1:1 chelate is possible at pH 5.0. However, for alpha-amino acids, the formation of a 1:1 chelate was observed at pH 3.0. In addition, there is the formation of both 1:1 and 2:1 chelates at pH 4.0 and 5.0. For beta-amino acid, there was no chelate formation at pH 3.0 but there existed both 1:1 and 2:1 chelates at pH 4.0 and 5.0.

Schwarzenbach and Ackermann (15) have postulated the possiblity of the existence of rings containing more than six members in the series of aminopolycarboxylic acids. These investigators reported the formation of an eight membered ring for a calcium chelate of ethylenediamine tetraacetic acid homolog. Thus the possible existence of a copper (II) chelate of 6-aminocaproic acid with the following structure is being proposed in the present work:



(1:1) Chelate of Copper-6-Aminocaproic Acid at pH 5.0

However, Osterberg and Toftgard (12) have suggested that 6-aminocaproic acid forms bicoordinated complexes in water with copper (II) through its carboxylate group rather than its amino group and have proposed the following structure based upon the dimerization of 6-aminocaproic acid in solution:

$$Cu_2(HL)_3^{4+}$$

Potentiometric and spectrophotometric techniques cannot be used to follow the chelation of copper (II) ions to amino acids above pH 5.0 because of the hydrolysis of cupric ions in solution and the formation of significant amounts of insoluble cupric hydroxide. However, using equations derived by Reilley, Schmid and Sadek (14) the possibility of complex formation between copper (II) and alpha-amino acids in solution between pH 5.0 and 8.0 has been indicated.

SUMMARY AND CONCLUSIONS

This study presents some new and interesting facts concerning the behavior of copper (II) chelates of aliphatic aminocarboxylic acids in aqueous solutions. Stability constants of nine copper (II)-amino acid complexes at 25°C were determined by potentiometric technique. These data show that the stability constants for copper (II) chelates of alpha-amino acids are essentially the same. This finding was not entirely unexpected, since it was known that the acidity constants of these very same amino acids are also similar.

Determination of the molar ratios of the deep blue copper (II) chelates of alpha-amino acids by spectrophotometric means showed that both 1:1 and 2:1, 5 membered chelates were formed at pH 4.0 and 5.0, but that only the 1:1 chelate existed at pH 3.0. Calculation of the effective overall stability constant, Ke, predicted this result. The present study reconfirmed a previous finding of Ley and others (1, 4, 10 and 11) that copper (II) has a stronger avidity for glycine and other alpha-amino acids than it does for beta-alanine.

However, the most interesting finding was the discovery of a 1:1 copper (II) complex for 4-aminobutyric, 5-aminovaleric and 6-aminocaproic acids in a pH 5.0 aqueous solution. Again, calculation of effective stability constants for these 3 ligands showed the possiblity of weak complex formation at this pH value. This has raised the distinct possibility that 4, 5 or 6 carbon chain, amino acids are capable of forming 7, 8 and 9 membered bicoordinated chelates with copper (II) in aqueous solution at room temperature.

The strongest argument for the formation of aminocarboxylic acid complexes in solution at pH 5.0 is that the wavelength of maximum absorbance of these (1:1) bicoordinated complexes does not change as the amino function is displaced further down the carbon chain away from the carboxylate group. However, it was also noted that a much greater concentration of 6-aminocaproic acid was required in order to carry out analysis by the methods of continuous variations than was required for comparable alpha-amino acids.

As further support for the hypothesis that chelation with copper (II) takes place through both the amino and carboxyate function of 6-aminocaproic acid, ninhydrin, a non-specific, colorimetric reagent for alpha -amino acids, peptides, amines, amino alcohols and ammonia, was reacted separately with a known amount of glycine and 6-aminocaproic acid at pH 5.0 in the absence and presence of a 1:1 molar ratio of cupric ion (16). A decrease in the absorbance reading at 570 nm for both the ninhydrin-glycine and the ninhydrin-6-aminocaproic acid reaction products in the presence of cupric ion, in each case, was taken as additional proof that the amino group of 6-aminocaproic acid was involved in complex formation with cupric ion.

In addition, preliminary results, not reported here, for related amino acids appear to show that the complexing tendency of 5-aminocaproic acids is similar to that of 5-aminovaleric acid and 3-aminobutyric acid (beta-aminobutyric acid) is similar to that of 3-aminopropionic acid (or beta-alanine).

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