

A Potentiometric Study of the Copper(2+)–Aspartic Acid System

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The equilibria between Cu(II) and aspartic acid (H_2A) were studied at 25 °C by hydrogen ion determinations in 0.25M $NaNO_3$ medium. The measurements were carried out as potentiometric titrations using a glass electrode. The concentration ratio ligand/Cu(II) was varied from 9 to 1. The data can be explained by assuming the main reaction products to have the compositions CuA , CuA_2 , and $CuHA$. Evidence has also been obtained for the formation of small amounts of CuH_2A_2 and $CuHA_2$ at high ligand to metal ion ratios. Stereoselectivity is not present in the system.

Aspartic acid is an α -amino acid with an additional carboxylic acid group in the side chain. The acid, which has the formula $CH(NH_2)(COOH)-CH_2(COOH)$, will be denoted by H_2A . The complexation between Cu(2+) and aspartic acid has been repeatedly studied.^{1–6} Part of the interest in this system is due to the increased number of modes of attachment of the amino-acidate ligand to the copper ion made possible by the presence of a ligating group in the side chain. There is general agreement on the main bonding scheme, which involves the formation of CuA and CuA_2 (charges omitted) by glycine-like chelation. Spectroscopic⁷ and thermodynamic^{1,3} measurements indicate that the β -carboxylate group is bonded to an apical position in CuA . It is noteworthy that in CuA_2 only one of the β -carboxylate groups appears to be coordinated.

In the bis-complex, *cis-trans* isomerism is possible and has been observed in the solid state for some α -amino acids.⁸ When the ligands are of

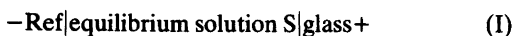
the same chirality, the side chains will be situated on the same side of the CuN_2O_2 -plane in the *trans* isomer but on opposite sides in the *cis* isomer. When the ligands are of opposite chirality the converse geometries will be found. If the isomers are of different stability the complexation reaction should show stereoselectivity implying a deviation of the ratio $[CuDL]/([CuD_2]+[CuL_2])$ from the statistical value of 1. D and L are used here to denote the two isomers of H_2A . The recently developed technique⁹ to study this phenomenon has now been applied to the Cu^{2+} –aspartate system.

The presence of the carboxylate group in the side chain might lead to the formation of an increased number of protonated species, CuA_pH_q , compared to systems with unsubstituted amino-acids. The presence of $CuAH$ has been recognized in most of the recent studies but there is disagreement on other protonated species. Brookes and Pettit³ found CuA_2H and CuA_2H_2 although the amounts were small at a ligand-to-metal ion ratio equal to 1. Other investigators did not find these species to be present in statistically significant amounts⁴ or did not include them in the calculations.^{1,6} Quite generally, little information is given on the concentration and pH ranges covered in studies of the complexation between Cu^{2+} and α -amino-acids. Therefore, the experimental conditions may not have been optimal for the detection of protonated species. Hence a reinvestigation of the Cu^{2+} –aspartic acid system, which covers a broad range in pH and in the quotient between the ligand and copper(2+) concentrations, has been made.

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METHOD

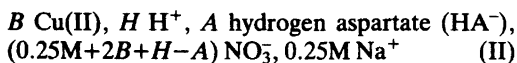
The experiments were carried out as potentiometric titrations in 0.25M NaNO₃ at 25 °C. The hydrogen ion concentration, h , of solutions of known total concentrations of H⁺, H ; copper(2+), B ; and L-aspartic acid (H₂A), A were measured with the glass electrode using the cell



$$E = E_0 + 59.158(\text{mV})\log(h/M) + E_j \quad (1)$$

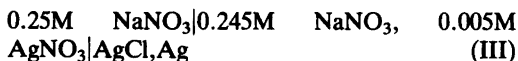
The liquid junction potential, E_j , was assumed to be a function of h only and determined as described by Biedermann and Sillén.¹⁰ The correction used was $E_j = -285h + 1460h^2$,

Solution S had the composition



HA⁻ has been chosen as component since it will generally be the dominant aspartate species. The analytical hydrogen ion concentration in S was increased by addition of HNO₃ and decreased by addition of NaOH or NaHCO₃. The latter reagent was preferred and can be used up to ph 5.5 although somewhat longer times to reach constant potentials (± 0.02 mV for at least 1 hour) were required than with OH⁻ as titrant.

The reference electrode was



and E_0 was determined by a measurement in a solution of known h in 0.25M NaNO₃.

During a titration in the alkaline direction A/B was kept constant and the quotient was varied between 1 and 9. B was 0.002 M or less in order to avoid precipitation at about ph 4.5. In the titrations concerned with the stereoselectivity of the complexation, the ratio $A/B=2.5$ was used and the initial value of \bar{n} was 1.8. All solutions in these experiments were prepared from the same stock solutions and the titrations were carried out with the same solution of HNO₃. This procedure ensures that the analytical compositions are the same in corresponding points in the titrations of the L, D, and (L+D) forms of the ligand.

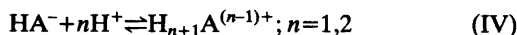
Chemicals and analysis. All chemicals were of *pro analysi* quality unless otherwise stated. Aspartic acid (Fluka), D and L forms, were recrystallized from water and dried at 105 °C. Nitric acid solutions were prepared from HNO₃ (Merck) by dilution and standardized against KHCO₃. Sodium hydroxide and hydrogen carbonate solutions were standardized against standard HNO₃ solution.

Copper nitrate stock solution was prepared from recrystallized Cu(NO₃)₂ (Merck). Some nitric acid was added in order to stabilize the solution. The amount of acid was determined by the method of standard additions using a dilute solution of HNO₃ of known concentration. The copper content was found by electrogravimetry. Sodium nitrate (Merck) was recrystallized before use.

Apparatus. The titrations were monitored with a home-built automatic titrator and the measurements were performed in an oil thermostat at 25 ± 0.1 °C. An Ingold 102 glass electrode was used and its potential measured to ± 0.01 mV with a digital voltmeter.

CALCULATIONS AND RESULTS

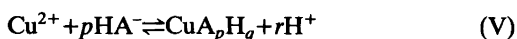
The protonation of the hydrogen aspartate ion will be described by



$$\beta_n^{\text{H}} = \frac{[\text{H}_{n+1}\text{A}]}{a h^n} \quad (2)$$

where $a = [\text{HA}^-]$. It is worth noting that the first protonation constant of the aspartate ion is not needed for the calculations of the speciation. This is a distinct experimental advantage since the value of this constant is such that its determination must be done in solutions with $ph > 9$. At $ph > 9$ the glass electrode usually deviates from Nernstian behaviour and the protonation constant should be determined from measurements with the hydrogen electrode for accurate results. The first and second protonation constants of HA⁻ were evaluated from experiments with $B=0$ in (II). The measurements yielded $\log \beta_1^{\text{H}} = 3.653$ and $\log \beta_2^{\text{H}} = 5.636$ and these values were used in all subsequent calculations

Since only mononuclear copper(II) complexes are formed in the concentration ranges studied, the equilibria will be written



with

$$\beta_{pq} = \frac{[\text{CuA}_p\text{H}_q]h^r}{ba^p} \quad (3)$$

where $b = [\text{Cu}^{2+}]$ and $r (= p - q)$ may be a negative number or zero. The mass balance equations with HA^- as reference level for H^+ , neglecting $[\text{OH}^-]$ and $[\text{A}^{2-}]$, are

$$B = b + \sum_{pq} \beta_{pq} ba^p h^{-r} \quad (4)$$

$$A = a + \sum_n \beta_n^{\text{H}} ah^n + \sum_{pq} p \beta_{pq} ba^p h^{-r} \quad (5)$$

$$H = h + \sum_n n \beta_n^{\text{H}} ah^n - \sum_{pq} r \beta_{pq} ba^p h^{-r} \quad (6)$$

The average number of A bound per B , \bar{n} , can be found from

$$B\bar{n} = A - a - \sum_n \beta_n^{\text{H}} ah^n \quad (7)$$

In order to calculate \bar{n} , a must be found. This can be done from eqn. (6) if $r=0$ or from eqns. (5) and (6) if p/r is a constant for all complexes. These conditions are fulfilled, and then only approximatively, for restricted concentration ranges in the present case.

The expression for a obtained from eqn. (6) is

$$a = \frac{H-h}{\beta_1^{\text{H}} h + 2\beta_2^{\text{H}} h^2} + \frac{\Sigma(p-q)[\text{CuA}_p\text{H}_q]}{\beta_1^{\text{H}} h + 2\beta_2^{\text{H}} h^2} \quad (8)$$

but only the first term in the right-hand member of this equation can be directly calculated. It will

be denoted by a^* . From eqn. (7) the expression for \bar{n} is found to be

$$\bar{n} = \frac{A}{B} - \frac{a^*}{B} (1 + \beta_1^{\text{H}} h + \beta_2^{\text{H}} h^2) - \quad (9)$$

$$\frac{(1 + \beta_1^{\text{H}} h + \beta_2^{\text{H}} h^2) \Sigma(p-q)[\text{CuA}_p\text{H}_q]}{(\beta_1^{\text{H}} h + 2\beta_2^{\text{H}} h^2) B}$$

where only

$$\bar{n}^* = \frac{A}{B} - \frac{a^*}{B} (1 + \beta_1^{\text{H}} h + \beta_2^{\text{H}} h^2) \quad (10)$$

can be found immediately from the experiment.

A pseudo Bjerrum plot of $\bar{n}_{\text{HA}} = f(\log[\text{HA}^-])$ was prepared by plotting \bar{n}^* as a function of $\log a^*$, Fig. 1. A separate curve is obtained for each ratio A/B and only at high total concentrations of aspartic acid and low ph , do the curves tend to merge. Estimates of β_{11} and β_{22} were obtained on the assumption that the lowest curve approaches the true Bjerrum plot for the formation of $\text{Cu}(\text{HA})_n$ complexes ($r=0$).

The presence of complexes of composition CuA_n will lead to the inequalities $a^* < a$ and $\bar{n}^* < \bar{n}$. Then the curves will spread out as observed in Fig. 1. Since the relative amount of CuA will be greatest at low A/B ratios, the corresponding curves were used to find a preliminary value of β_{10} . With β_{11} and β_{22} fixed and assuming values of β_{10} , theoretical $\bar{n}^* = f(\log a^*)$ were calculated from the analytical concentrations and volumes used in the titrations. The calculations were made

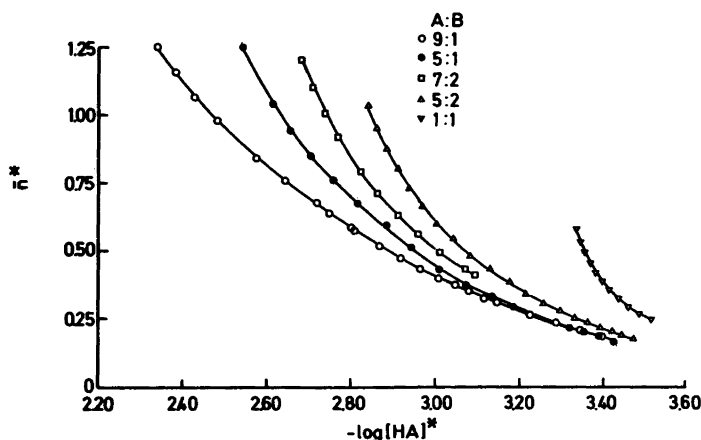


Fig. 1. Part of the experimental data plotted as $\bar{n}^* = f(\log[\text{HA}^-]^*)$, where \bar{n}^* and $[\text{HA}^-]^*$ have been calculated from eqns. (8) and (10). The drawn curves have been calculated with the constants in Table 1.

Table 1. Stability constants for the proposed complexes in the Cu(2+)-aspartic acid (H₂A) system. β_{pq}^{HA} refers to the reaction $\text{Cu}^{2+} + p\text{HA}^- \rightleftharpoons \text{CuA}_p\text{H}_q + r\text{H}^+$ and β_{pq}^{A} to the reaction $\text{Cu}^{2+} + p\text{A}^{2-} + q\text{H}^+ \rightleftharpoons \text{CuA}_p\text{H}_q$. In the calculation of β_{pq}^{A} from β_{pq}^{HA} the value $\log K_1=9.61$ taken from Ref. 12 was used for the protonation constant of the aspartate ion.

<i>p</i>	<i>q</i>	$\log \beta_{pq}^{\text{HA}}$	$\log \beta_{pq}^{\text{A}}$
1	1	2.753(7)	12.36
2	2	4.32(4)	23.54
1	0	-0.905(5)	8.70
2	0	-3.517(4)	15.70
2	1	0.65(6)	19.87

by the speciation program HALTAROLF for homogenous solution developed in this department. In this way, a reasonably good fit for the data displayed in Fig. 1 was obtained with the complexes Cu(HA), Cu(HA)₂, and CuA after adjustment of β_{11} and β_{22} .

Titration were carried out to *ph* 5.8. The data so far discussed cover the interval $2.4 < \text{ph} < 3.5$. Pseudo Bjerrum plots of $\bar{n}_A = f(\log[A^{2-}])$ were prepared from data collected at higher *ph* using $[\text{HA}^-]h^{-1}$, which is proportional to $[A^{2-}]$, as master variable. Since CuA and CuA₂ are expected to predominate, the equation for the calculation of *a* should not contain these species, cf. eqn. (8). By combining eqns. (5) and (6), [CuA] and [CuA₂] are eliminated and the following expression for *a* is found

$$a = \frac{A + H - h}{1 + 2\beta_1^{\text{H}}h + 3\beta_2^{\text{H}}h^2} - \frac{\Sigma q\beta_{pq}ba^p h^{-r}}{1 + 2\beta_1^{\text{H}}h + 3\beta_2^{\text{H}}h^2} \quad (11)$$

Table 2. Compilation of stability constants for the reaction $s\text{Cu}^{2+} + p\text{A}^{2-} + q\text{H}^+ \rightleftharpoons \text{Cu}_s\text{A}_p\text{H}_q$ at 25 °C. A²⁻=aspartate ion. References within parentheses.

<i>s</i>	<i>p</i>	<i>q</i>	0.1M KNO ₃ (3)	0.1M KNO ₃ (6)	0.1M KNO ₃ (2)	0.1M KNO ₃ (4)	0.2M KCl (1)
0	1	1	9.83	9.71		9.77	9.63
0	1	2	13.60	13.42		13.55	13.31
0	1	3	15.69	15.12			15.43
1	1	1	12.82	12.70	12.59	12.71	12.48
1	2	2	25.15				
1	2	1	21.21				
1	1	0	9.08	8.84	8.94	8.97	8.80
1	2	0	16.25	15.24	15.89	15.83	15.76

where, as before, only the first term denoted by *a** can be calculated directly. The equations for \bar{n} and \bar{n}^* , respectively, are analogous to eqns. (9) and (10). Plots of $\bar{n}^* = f(\log a^* h^{-1})$ were used to find β_{20} . However, a good fit could not be obtained by the addition of CuA₂ only and it was necessary to include CuA₂H to reach a satisfactory agreement with the experimental data.

The final refinement of the experimental data was made by the least squares program MINQUAD.¹¹ The results are presented in Table 1. The table also contains the values of the stability constants written with Cu²⁺, H⁺ and A²⁻ as reactants. The recalculation was done with $\log K_1=9.61$ for the first protonation constant of the aspartate ion. This value was obtained with the hydrogen electrode in 0.25M NaCl.¹² Since the values of the second and the third protonation constants determined by us in 0.25M NaNO₃ agree with those determined in 0.25M NaCl¹² within 0.01 logarithmic units, the uncertainty introduced should be small. The deviations between measured and calculated potentials were in general less than 0.15 mV. At *ph*>5 the deviations were somewhat larger, 0.3 mV, and appeared to be systematic. It was not possible to remove this discrepancy by introducing additional complexes.

The same set of species has been found in this study as was proposed by Brookes and Pettit.³ Only CuAH₂ is missing from the list of complexes most likely to be present. Refinements including this species failed to detect statistically significant amounts of it. Since the first dissociation constant of aspartic acid is about 0.01 M and non-chelated copper(2+)-complexes with carboxylic acids are fairly weak, the value of the formation constant

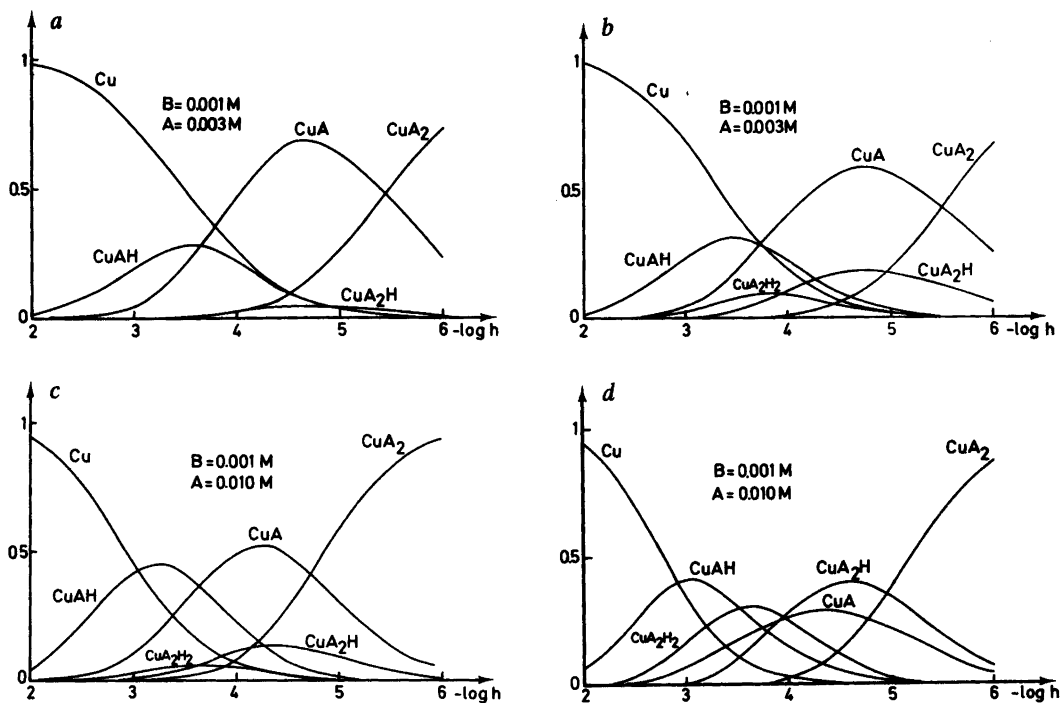


Fig. 2. Distribution of copper(2+) amongst the proposed species expressed as fractions of the total amount of metal. Diagrams *a* and *c* have been calculated with the present constants, and diagrams *b* and *d* with the constants found by Brookes and Pettit.³

of CuAH_2 should be very small. Recently determined stability constants have been compiled in Table 2. The agreement between the stability constants for the three predominating species with previously published values (Tables 1 and 2) is good after correction for the differences in ionic strength.¹ On the other hand, the values of the constants for the two minor species show poor agreement with the previous values. The discrepancies can not be explained by differences in the ionic media and refinements with starting values for the constants taken from Ref. 3 converged to the values in Table 1.

The present constants and those from Ref. 3 lead to a pronounced difference in the distribution of the species at high ligand concentrations as illustrated in Fig. 2. Whereas with our data, CuA_2H_2 and CuA_2H are always minor species, they even become predominant species with the constants from Ref. 3. The reason for the different results is difficult to ascertain since no experimental details are presented there. Work

on copper(2+)–amino acid complexation is, however, often carried out with limited concentration ranges. The data are collected from titrations with OH^- of mixtures of metal and ligand in the ratios 1:1 and 1:2. Whereas such experiments yield sufficient data in simple systems, where only CuA and CuA_2 are formed, they are not well designed for a study of more complicated systems with, for instance, formation of several protonated complexes. Hence the difference may arise from the use of a too restricted concentration range of the ligand by Brookes and Pettit.³

Since the numerical values of the formation constants and their information on the bonding scheme have been amply discussed in the cited literature^{1–4,6} and Ref. 13, this subject matter will be omitted here.

The search for stereoselectivity was done by comparing values of $E' = E - E^\circ$ [see eqn. (1)] from titrations carried out with solutions of identical analytical compositions except for the

optical form of the ligand.⁹ No significant difference (> 0.25 mV), $\Delta E'$, was found between the measurements with optically pure ligands and the racemic mixture. From the equation⁹

$$\Delta E' = \frac{RT}{F} \ln \frac{2 + \sqrt{K}}{4} \quad (12)$$

the limits for the equilibrium constant of the reaction $\text{CuD}_2 + \text{CuL}_2 \rightleftharpoons 2\text{CuDL}$, K , were found to be 4 ± 0.15 . The ratio $[\text{CuDL}] / ([\text{CuD}_2] + [\text{CuL}_2])$ is then 1 ± 0.02 . Ritsma² found this ratio to be 0.9, whereas no stereoselectivity was observed by Brookes and Pettit.³ We may conclude that the copper(2+)–aspartic acid system does not exhibit stereoselectivity.

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