

Complex Formation with Ethyleneglycol-bis(β -aminoethylether)-Tetraacetic Acid. Polynuclear Chelate Species

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The reagent ethyleneglycol-bis(β -aminoethylether) tetraacetic acid forms 2:1 complexes with divalent copper. Depending on the pH of the solutions, three different complex species are formed. The stability constants are measured and the possible structures are formulated.

Ethyleneglycol-bis(β -aminoethylether) N,N,N',N'-tetraacetic acid (EGTA) forms stable complexes with many metal ions,¹⁻³ and is like ethylenediaminetetraacetic acid found valuable in complexometric titrations. In contrast to EDTA, EGTA forms much less stable complexes with magnesium than with calcium; this is utilized in determinations of the two metals.⁴⁻⁹ Many of the complexes are coloured, and this is used in the colourimetric methods of analysis.¹⁰⁻¹⁴ A polarographic study of EGTA complexes of copper(II) was recently published by the present author,¹⁵ in which two polarographic reduction waves in neutral and alkaline solutions with an *excess* of the ligand were observed. This was explained as due to two different kinds of complexes. The first wave results from a complex with four bonds from the ligand, and the second from a complex with more than four bonds from it (this might also be a polymeric compound). These effects could also be explained by assuming that the copper ions form distorted octahedral complexes as a result of the Jahn-Teller effect. The complexes were not studied with an excess of copper(II) ions to find polynuclear compounds. In a recent paper,¹⁶ Da Silva and Calado studied different chelates of EGTA by a pH-potentiometric method. They used a great excess of metal ions, and found no 2:1 complexes with Tl⁺, Ag⁺, Mn²⁺, and Co²⁺. The stability constants of the 1:1 complexes were determined. The data for copper did not yield consistent results, and they concluded that binuclear complexes are formed. This was also confirmed with spectrophotometry.

The existence of binuclear copper-EGTA complexes is not contradicted by polarographic studies¹⁵ as formulated by Da Silva and Calado.¹⁶ The present

author¹⁵ did not study polynuclear compounds as they could not exist under the experimental conditions used.

The present investigation is mainly concerned with the polynuclear compounds formed between excess copper and EGTA.

EXPERIMENTAL

Materials. *Purum p.a.* ethyleneglycol-bis(β -aminoethylether) N,N,N',N'-tetraacetic acid was obtained from Fluka A.G., Switzerland. Stock solutions of divalent copper were made by dissolving *p.a.* $\text{Cu}(\text{NO}_3)_2$ in water, and were standardized by EDTA titration. To determine the free acid content in the copper nitrate solutions, 1:1 Cu(II)-EDTA solutions were titrated with standard NaOH. A *p.a.* disodium EDTA was used for this purpose, and the free acid content was determined by subtracting two equivalents of base from the added amount of NaOH. (Direct titration of copper solutions will give erroneous results due to the formation of hydrolyzed copper(II) compounds and a precipitate).

All the remaining chemicals were of reagent grade.

Method. All the data were obtained at $25^\circ\text{C} \pm 0.1^\circ$ and the ionic strength was kept at 0.1 by additions of sodium perchlorate. The pH values were determined by means of a Radiometer pH-meter No. 4 (Copenhagen, Denmark) and were checked by repeating the experiments utilizing a Beckman Zeromatic pH-meter. To obtain the most reproducible results, the solutions were bubbled with oxygen-free nitrogen gas between the measurements.

In all the acid-base titrations, equilibrium was attained within one minute, the solutions having been stirred with a magnetic stirrer.

RESULTS

Existence and composition of the chelates, pH titration data

Titration data of EGTA in the presence of one equivalent and two equivalents of bivalent copper are indicated in Fig. 1.

1:1 data. A steep inflection occurs when base has been added corresponding to four equivalents of the EGTA acid. This indicates formation of a 1:1 compound according to ($\text{H}_4\text{X} = \text{EGTA}$):

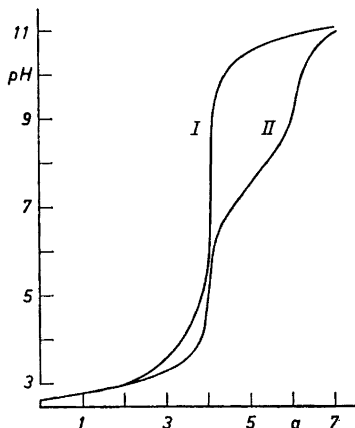
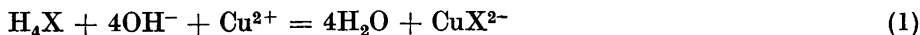


Fig. 1. Titration of 1.00×10^{-3} M EGTA in the presence of I) equimolar amounts of Cu(II), II) two equivalents of Cu(II) with NaOH. Abscissa α = moles of NaOH per mole of amino acid (25°C and with 0.1 M NaClO_4).



No further inflections appear when more base is added, and therefore no compounds of the kind $\text{Cu}(\text{OH})\text{X}^{3-}$ seem to be formed in appreciable quantities.

With 3 equivalents of base, no well-developed inflection occurs, but a well-developed buffer region is observed in the pH range corresponding to between 3 and 4 equivalents of base. This points to the formation of a protonized species:

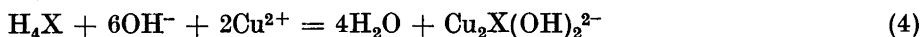


The existence of higher protonated complexes seems improbable.

2 : 1 d a t a. Entirely different titration curves are obtained from solutions with two equivalents of copper per equivalent of EGTA, as indicated in Fig. 1. A steep inflection is observed at four equivalents of base:



At pH values exceeding 6 the buffering capacity increases at first, but then decreases to an inflection corresponding to 6 equivalents of base. This might be explained by the formation of a hydroxyl complex:



No inflection is observed when 5 equivalents of base are added, but as there is buffering capacity, albeit low, between 4 and 5 equivalents of base, the following step is assumed:



Alternative reactions

1 : 1 d a t a. Eqns. 1 and 2 give only a formal description of the reactions. Owing to the very high stability constant, even in a very acid solution the complex CuHX^- (or CuX^{2-}) does exist, and inflections resulting from, for example:



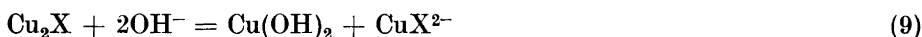
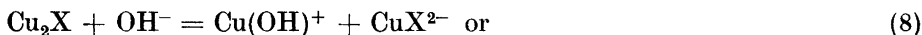
are insignificant because of negligible quantities of free ligand. This would also make it impossible to determine the stability constant of the complex by a direct pH-titration method. However, it would be possible to determine it by other indirect methods, among them the »tren exchange method», exchange with the mercury complex or measurement of the shift-to-negative values of the polarographic half-wave potential from the reduction of the copper complex. As Holloway and Reilley,² measuring with a mercury electrode, reported this stability constant to be $10^{17.8}$, it was not determined in the present investigation.

2 : 1 d a t a. If the excess of copper ions does not react with the EGTA, inflection would according to eqn. 1, also appear at four equivalents of base

added. This would provide no evidence of the formation of a binuclear chelate, but the 2:1 curve yields very much lower pH values than those observed for the 1:1 curve before 4 equivalents of base are added. This may be due to the reaction:



When 5 and 6 equivalents of base are added, possible reactions are hydrolysis and precipitation:

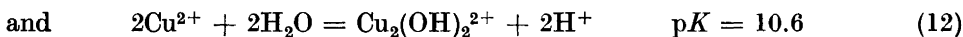


No precipitation corresponding to eqn. 9 was observed before pH exceeded 10. Above pH = 10 the displacement:



takes place.

Hydrolysis of the copper (II) ion has been extensively studied by Biedermann.¹⁷ The hydrolysis constants are reported to be



This hydrolysis (and also eqn. 8) was taken into account in the calculation of the stability constants.

Metal titration data

Solutions of EGTA and 3.5 or 4.5 equivalents of NaOH were added to a standard copper(II) solution. The titration curves are indicated in Fig. 2.

With 3.5 equivalents of base, a great lowering of the pH of the solution is observed when one equivalent of metal ions is added, corresponding to the formation of CuHX^- and CuX^{2-} and the liberation of protons. With two equivalents of copper added, further lowering of the pH is observed probably due to reaction 7. As no 3:1 complex is formed, the pH of the solution is independent of the concentration of copper exceeding two equivalents.

With 4.5 equivalents of base, the EGTA is almost completely dissociated and no lowering of the pH is observed when one equivalent of copper ions is added. Further addition of copper ions results in the lowering of the pH of the solution, because of the formation of binuclear hydroxyl complexes. In contrast to the titration curve with 3.5 equivalents of base, the pH varies on addition of more than 2 equivalents of copper ions. This might be due to hydrolysis, eqns. 11 and 12.

Determination of the stability constants

1:1 complexes. As the stability constant of the 1:1 complex CuX^{2-} is very high ($10^{17.8}$), the dissociation of the complex to metal and free ligand is negligible. Thus it is possible to determine the stability constant of the

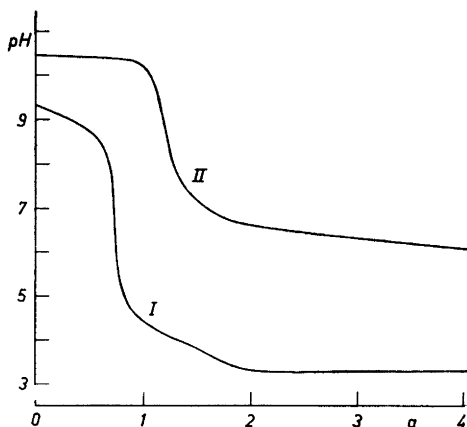


Fig. 2. Titration of 1.00×10^{-3} M EGTA in the presence of I) 3.50 equivalents of NaOH, II) 4.50 equivalents of NaOH with $\text{Cu}(\text{NO}_3)_2$. Abscissa a = moles of $\text{Cu}(\text{II})$ per mole of amino acid (25°C and with 0.1 M NaClO_4).

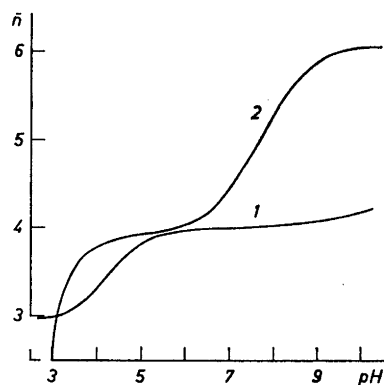
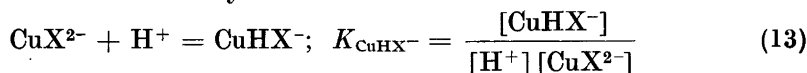


Fig. 3. Formation function of 1) 1:1 and 2) 2:1 copper(II)-EGTA chelates with hydroxyl. (25°C , with 0.1 M NaClO_4 and 1.00×10^{-3} M EGTA) \bar{n} is the average number of hydroxyl bound per mole of the chelates.

hydrogen complex by Bjerrum's method¹⁸ from the titration data. This stability constant is defined by:



The hydrogen ions are regarded as ligands which react with the complex CuX^{2-} . The stability constant was thus found to be

$$K_{\text{CuHX}^-} = 10^{4.28} \quad (14)$$

As the \bar{n} plot (Fig. 3) shows that no competitive reactions occur, the constant is equal to the pH of the solution at $\bar{n} = 3.50$, and no correction was necessary. Existence of hydroxyl mononuclear chelates was not indicated by these experiments.

2:1 complexes. The calculation of the stability constant of the binuclear compound Cu_2X is based on the following assumptions (from the 2:1 curve):

1. No hydrogen compound of the binuclear complex exists (*e.g.* no Cu_2HX^+ compound). This is entirely reasonable, as the tendency to form hydrogen complexes decreases with the number of metal ions bound to the ligand.

2. No hydroxyl binuclear compound is formed when less than 4 equivalents of NaOH is added. This is equally reasonable because of the pronounced inflection on the curve at 4 equivalents.

3. No hydrolysis of uncomplexed copper ions takes place. This follows from the reported hydrolysis constants¹⁷ (eqns. 11 and 12).

4. The concentration of free ligands is negligible, owing to the very high stability constant of the complex CuX^{2-} .

If these assumptions are correct, the following relations completely define the system:

$$C_M = [\text{Cu}^{2+}] + [\text{CuX}^{2-}] + [\text{CuHX}^-] + 2[\text{Cu}_2\text{X}] \quad (15)$$

$$C_X = [\text{CuHX}^-] + [\text{CuX}^{2-}] + [\text{Cu}_2\text{X}] \quad (16)$$

$$(4-a)C_X - [\text{H}^+] + [\text{OH}^-] = [\text{CuHX}^-] \quad (17)$$

(Here C_M and C_X are total concentrations of metal ions and ligand and the symbol a represents the number of equivalents of base added). This makes it possible to calculate the stability constant:

$$\text{CuX}^{2-} + \text{Cu}^{2+} = \text{Cu}_2\text{X}; K_{\text{Cu}_2\text{X}} = \frac{[\text{Cu}_2\text{X}]}{[\text{CuX}^{2-}][\text{Cu}^{2+}]} \quad (18)$$

The value found was $K_{\text{Cu}_2\text{X}} = 10^{4.31}$

In the case of the hydroxyl binuclear chelates $\text{Cu}_2\text{X}(\text{OH})^-$ and $\text{Cu}_2\text{X}(\text{OH})_2^{2-}$, equations very similar to those above might be used, but hydrolysis of the copper ions which do not take part in the complex formation, has to be considered. The easiest interpretation of the data is as a first approximation to assume the concentration of uncomplexed copper and the products of hydrolysis to be zero. It is possible to consider OH^- as ligands reacting with the complex Cu_2X , and Bjerrum's method may be employed.

The equilibrium constants are defined as

$$K_{\text{Cu}(\text{OH})\text{X}^-} = \frac{[\text{Cu}_2(\text{OH})\text{X}^-]}{[\text{OH}^-][\text{Cu}_2\text{X}]} \quad (19a)$$

and

$$K_{\text{Cu}_2(\text{OH})_2\text{X}^{2-}} = \frac{[\text{Cu}_2(\text{OH})_2\text{X}^{2-}]}{[\text{OH}^-][\text{Cu}_2(\text{OH})\text{X}^-]} \quad (19b)$$

The preliminary logarithmic values of the constants, determined from \bar{n} plots as equal to $-\text{pOH}$ at $\bar{n} = 4.5$ and 5.5 , respectively, (Fig. 3), are 6.95 and 5.75. These values might be corrected by successive approximations as outlined by Carlson *et al.*¹⁹ by means of the convergence formulas

$$k_1 = \frac{1}{[\text{A}]_{\bar{n}=1/2}} \times \frac{1}{1 + 3k_2[\text{A}]_{\bar{n}=1/2}} \quad (20a)$$

and

$$k_2 = \frac{1}{[\text{A}]_{\bar{n}=3/4}} \left(1 + \frac{3}{k_1[\text{A}]_{\bar{n}=3/4}} \right) \quad (20b)$$

starting with k_1° and k_2° as $[\text{A}]_{\bar{n}=1/2}^{-1}$ and $[\text{A}]_{\bar{n}=3/4}^{-1}$

However, successive approximation should be unnecessary, as with the elimination of k_1 in eqn. 20, the following direct expressions for the stability constants are obtained:

$$k_1 = \frac{[A]_{\bar{n}=3/2} - 3[A]_{\bar{n}=1/2}}{[A]_{\bar{n}=1/2} \times [A]_{\bar{n}=3/2}} \quad (21)$$

and

$$k_2 = \frac{1}{[A]_{\bar{n}=3/2} - 3[A]_{\bar{n}=1/2}} \quad (22)$$

If more than two complexes appear, similar formulas for the stability constants are easily arrived at.

For system with $k_1^{\circ}/k_2^{\circ} \gg 10^4$, Bjerrum's method may be used without correction. That is also in accordance with eqns. 21 and 22. When the stability constants become more nearly equal, corrections have to be made. Sometimes the convergence formulas 19 and 20 cannot be used owing to divergence. From formulas 21 and 22 is seen that the condition for convergence is that

$$[A]_{\bar{n}=3/2} > 3 [A]_{\bar{n}=1/2} \quad (23)$$

or that the difference between the two preliminary constants exceeds 0.48 pK units. A more detailed discussion of this method will be published soon. The corrected constants in the case of the binuclear copper(II) hydroxyl EGTA chelates are (logarithmic) 6.86 and 5.84. These constants are valid if the hydrolysis was negligible. As that is probable not the case, composite equilibria have to be taken into consideration. With the species CuX^{2-} , Cu_2X , and Cu^{2+} together considered as the central ion and hydroxyl ions as the ligand, the following pH dependent expressions are valid:

$$(k_1)_{\text{pH}=7.05} = 10^{6.86} = \frac{[\text{Cu}_2\text{X}(\text{OH})^-] + [\text{Cu}(\text{OH})^+] + 2[\text{Cu}_2(\text{OH})_2^{2+}]}{[\text{OH}^-] ([\text{CuX}^{2-}] + [\text{Cu}^{2+}] + [\text{Cu}_2\text{X}])} \quad (24)$$

$$(k_2)_{\text{pH}=8.25} = 10^{5.84} = \frac{[\text{Cu}_2\text{X}(\text{OH})_2^{2-}]}{[\text{OH}^-] ([\text{Cu}_2\text{X}(\text{OH})^-] + [\text{Cu}(\text{OH})^+] + 2[\text{Cu}_2(\text{OH})_2^{2+}])} \quad (25)$$

The compound $\text{Cu}_2(\text{OH})_2^{2+}$ will as found from the dimerization constant, always appear in very low and negligible concentrations under these experimental conditions. It is also a reasonable approximation to assume $[\text{CuX}]$ to be zero. With a solution of $\text{pH} = 7.05$, the ratio $[\text{Cu}(\text{OH})^+]/[\text{Cu}^{2+}]$ is approximately 0.11; *i.e.* only 11 % of the uncomplexed copper ions is hydrolyzed. Thus, without the highest accuracy, $K_{\text{Cu}_2(\text{OH})\text{X}^-} = 10^{6.9}$.

Similar considerations with eqn. 25, taking into account that compared to $\text{Cu}_2\text{X}(\text{OH})^-$, $\text{Cu}(\text{OH})^+$ is almost negligible, yield

$$K_{\text{Cu}_2(\text{OH})_2\text{X}^{2-}} = 10^{5.8}$$

Formation of other polynuclear complex species

By analogy to the reaction



the chelate dimerization



is possible. Recently, many diol-chelate compounds have been reported (*e.g.* the ferric 1:1 chelates of EDTA, *etc.*²⁰), and such compounds are even more to be expected with 2:1 compounds, for example with copper. Therefore it was found necessary to ascertain that such compounds do not exist in measurable quantities under the present experimental conditions, as the presence of diol compounds would make the calculations presented above unreliable.

A solution of 2:1 copper(II) and EGTA at high concentration (10^{-2} M) containing 4.5 equivalents of NaOH and 0.1 M NaClO₄ was "titrated" with a solution of 0.1 M NaClO₄. This dilution was continued until the concentration of the chelate was approximately 10^{-4} M. The \bar{n} was calculated and found to be independent of the dilution. Considering the diol formation from eqn. 27, this results in removing Cu₂X(OH)⁻ from the mixture. When 4.5 equivalents of base are added and the expected chelates are Cu₂X and Cu₂X(OH)⁻, the reaction



proceeds approximately half to the right, but as a diol formation is equivalent to the removal of Cu₂X from the solution, a further formation of hydroxyl chelates occurs. With an increasing concentration of chelate, polymerization of this kind is indicated by an increase of the \bar{n} value and the lowering of the pH in this buffer region.

As the present investigation yielded results with \bar{n} independent of dilution, it can be concluded that no diol compounds actually exist in the concentration range used. (Similar results were obtained with 1:1 chelates as also was expected.)

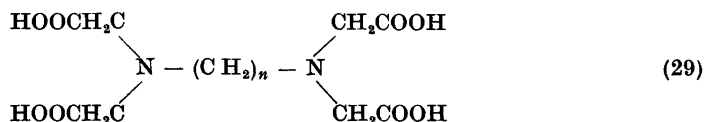
Table 1. Summary of stability constants of the copper complexes. The EGTA acid is denoted H₄X. (25°C.).

Reaction	log K	Ref.
Cu ²⁺ + H ₂ O = Cu(OH) ⁺ + H ⁺	- 8.0	17
2Cu ²⁺ + 2H ₂ O = Cu ₂ (OH) ₂ ²⁺ + 2H ⁺	- 10.6	17
Cu ²⁺ + X ⁴⁻ = CuX ²⁻	17.8	2
CuX ²⁻ + H ⁺ = CuHX ⁻	4.28	
CuX ²⁻ + Cu ²⁺ = Cu ₂ X	4.31	
Cu ₂ X + OH ⁻ = Cu ₂ X(OH) ⁻	6.9	
Cu ₂ X(OH) ⁻ + OH ⁻ = Cu ₂ X(OH) ₂ ²⁻	5.8	
CuX ²⁻ + OH ⁻ = CuX(OH) ³⁻	negligible	
2CuX(OH) ³⁻ = Cu(OH)X ₂ (OH)Cu ⁰	negligible	
2Cu ₂ X(OH) ⁻ = Cu ₂ (OH)X ₂ (OH)Cu ₂ ²⁻	negligible	

DISCUSSION

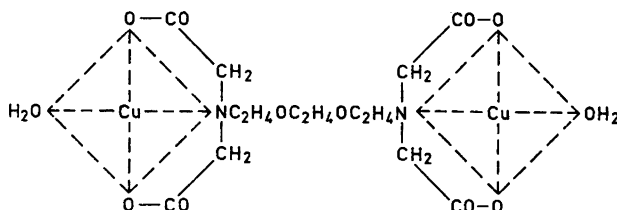
Different chelate species do exist in solution under the above experimental conditions. (The equilibrium constants are summarized in Table 1.) The most interesting feature is the existence of binuclear species. In the case of EDTA complexes, such compounds have not been reported.

An interesting study of Schwarzenbach *et al.*¹ of the calcium chelates of the aminocarboxylic acids



shows that as the length of the carbon chain increases, the stability constants of the 1:1 chelates decrease, while those of the 2:1 chelates increase. Similar tendencies as for the 2:1 compounds are valid for the formation of the 1:1:1 hydrogen chelates.

Many structural formulas are possible for the binuclear copper chelate. The increasing tendency to form 2:1 compounds with calcium with an increase in the length of the carbon chain might indicate that when the two imidodiacetate groups are sufficiently far apart, they tend to act independently of each other, each of them then as a maximum tridentate ligand. By analogy a possible structure of the 2:1 copper (II) EGTA chelate is:



The ether oxygen atoms are assumed not to take part in the complex formation. Only four of the coordination sites of copper are assumed to take part in the complex formation. The proposed structure results in a chelate with the imidodiacetate groups sufficiently far apart and corresponds to approximately $n = 7$ in the aminocarboxylic acid, formula (29). The lower coordination number of copper compared with that of calcium also favours the formation of binuclear compounds.

The formation of hydroxyl 2:1 chelates would take place through ionization of water hydrogen in the above structure. The hydrolytic tendency increases as expected with the number of metal ions in the molecule because more coordination sites of the metal ions will be available to the hydroxyl ligands.

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