

VOLTAMMETRIC MEASUREMENT OF COPPER(II)/ORGANIC INTERACTIONS IN ESTUARINE WATERS

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(Received 2nd August 1984)

SUMMARY

The voltammetry of copper in organic ligand/chloride media is dominated by the formation of CuCl_2^- species and by induced adsorption of Cu(I) in organic coatings on the electrodes. These phenomena are utilised in a novel method for evaluating Cu(II)/organic ligand interactions, based on the principle of ligand exchange. The Cu(II)/organic species competes with glycine which forms copper glycinate. These two complexes can be distinguished voltammetrically: copper glycinate gives a higher surface excess of copper at a gelatin-coated hanging mercury drop electrode, partly because of the increased production of CuCl_2^- from copper glycinate at the electrode surface. The method proved satisfactory for pure ligand/surfactant/chloride media and for estuarine waters. It is shown that there are two types of Cu(II)-binding ligand in estuarine waters: humic material ($>10^{-6}$ mol l^{-1} , assuming 1:1 site binding) with polyelectrolyte-type binding, and discrete ligands ($\leq 10^{-6}$ M) with stability constants around 10^9 . The extent of Cu(II) binding by the humic material decreases down the estuary because of dilution and increased salinity.

The measurement of trace metal interaction with organic matter in natural waters is a subject of great importance. Such studies provide a logical link between the geochemical cycling and biological uptake of trace metals. Copper is often chosen for study because it has a high affinity for organic matter [1] and because its toxicological properties are well characterised. Many methods have been used in studies of Cu(II)/organic interaction. Some controversy has arisen: many of the measuring procedures have no well defined scientific basis, and part of the natural organic material is in the form of polyelectrolyte macromolecules [2] so that its association with trace metals is itself of a complex nature [3].

Polyelectrolytes are characterised by their surface activity and large number of functional groups [4]. Their metal binding is influenced by conformational and electrostatic effects and the number of coordinating groups is ill-defined. The binding constants are often a function of the fraction of metal sequestered by the polymer. Metal/polyelectrolyte binding is very different from chelation with monomeric ligands such as EDTA, NTA and simple amino acids.

Electrochemical measurements have often been used for the determination

of copper speciation in natural waters but there has been much ambiguity and misinterpretation [5] because of the complexity of the electrochemistry and the sensitivity of electrodes to adsorption effects of natural polyelectrolytes. In addition, many copper complexes are labile to the sensing technique and are not readily determined by conventional electrochemical methods. The electrochemistry of copper in estuarine waters has recently been clarified [6–8]. Many reactions are involved but all hinge around the CuCl_2^- intermediate. On the basis of these results, a technique has been developed for evaluation of the interaction of copper(II) with organic matter in estuarine waters. The method utilises the importance of the CuCl_2^- intermediate in chloride media and the adsorption of organic material on the hanging mercury drop electrode (HMDE).

The method is based on the induced adsorption of CuCl_2^- from Cu(II) species in solution on an organically-coated, positively-charged HMDE. It was observed from pseudopolarograms of Cu(II) in u.v.-irradiated estuarine water that the Cu(II) -NTA complex gave a diffusion-controlled current identical to the uncomplexed Cu(II) . There was only a small shift in the half-wave potential (20–30 mV) on formation of Cu-NTA . However, the CuCl_2^- adsorption at a gelatin-coated HMDE was depressed by more than half. This example served to exemplify that many Cu(II) complexes may provide a labile response in differential-pulse anodic stripping voltammetry (d.p.a.s.v.) but cause decreased CuCl_2^- adsorption on the HMDE in chloride media.

Induced copper adsorption at the HMDE

In previous papers [6–8], adsorption processes were shown to be important in the reduction of copper in estuarine waters. The conclusions may be summarised as follows: (i) both organic complexes of Cu(II) and CuCl_2^- are reduced in part via an adsorption mechanism; (ii) the adsorption of organic material on the mercury electrode enhances the adsorption of CuCl_2^- ; (iii) the most electroactive form of copper adsorbed on the electrode is CuCl_2^- .

When copper(II) is added to an organic ligand/chloride medium containing gelatin, the copper(II) will be complexed by the organic ligand (Y). If a mercury electrode in this solution is maintained at a potential between the reduction potential to Cu(Hg) and the potential of the mercury dissolution wave, then the following reactions will occur at the interface: $\text{Cu(II)Y} \rightarrow \text{Cu(II)Y}_{\text{ads}}$, $\text{Cu(II)Y}_{\text{ads}} \rightleftharpoons \text{CuCl}_{2\text{ads}}^-$, and $\text{Cu(II)Y} \rightleftharpoons \text{CuCl}_{2\text{ads}}^-$, where the subscript ads refers to the adsorbed species. The Cu(II) complexes will co-adsorb with the gelatin coating on the electrode. If the same system but with sufficient glycine added to convert all Cu(II)Y to copper glycinate (CuGl_2) is considered, then the reactions at the interface will be: $\text{CuGl}_2 \rightarrow \text{Cu(II)Y}_{\text{ads}}$, $\text{Cu(II)Y}_{\text{ads}} \rightleftharpoons \text{CuCl}_{2\text{ads}}^-$, and $\text{CuGl}_2 \rightleftharpoons \text{CuCl}_{2\text{ads}}^-$. The copper glycinate will exchange glycine for Y already adsorbed on the electrode because Cu(II)Y complexes are more stable on the electrode owing to the stereochemical environment and reduction of Cu(II) within the complex [9].

Copper glycinate will also be reduced to CuCl_2^- adsorbed in the organic coating on the electrode. Copper glycinate dissociates quite readily and has a faster diffusion rate than the bulkier Cu(II)Y , thus a higher total surface excess of copper (Cu_{se}) will be accumulated with a higher fraction of adsorbed CuCl_2^- . As a result, by monitoring the surface film of copper after a fixed accumulation period in a stirred solution, information can be obtained about the speciation of copper. The increase in Cu_{se} on addition of glycine will be proportional to the copper glycinate formed and so the complexing capacity of the organic ligand for Cu(II) can be measured. This can be done quite easily by ligand competition because the complexing properties of glycine with Cu(II) are well established. Figure 1 outlines the mechanism of adsorption from a solution containing surface-active (Y) and surface-inactive (X) ligands, with and without addition of glycine. The effect of ligands, X, on the adsorption of copper from solution is described below. In general terms, all organic ligands in solution will be referred to as Y.

EXPERIMENTAL

The apparatus, materials, sample collection and treatment were as described previously [6–8]. In all cases, 1 mg l^{-1} of gelatin was added to the electrolyte immediately before determinations to aid the development of a surface film on the electrode. The surface excess of copper (Cu_{se}) was measured after accumulation for 5 min in stirred solution by potential-step reduction to -1000 mV and d.p.a.s.v. of Cu(Hg) .

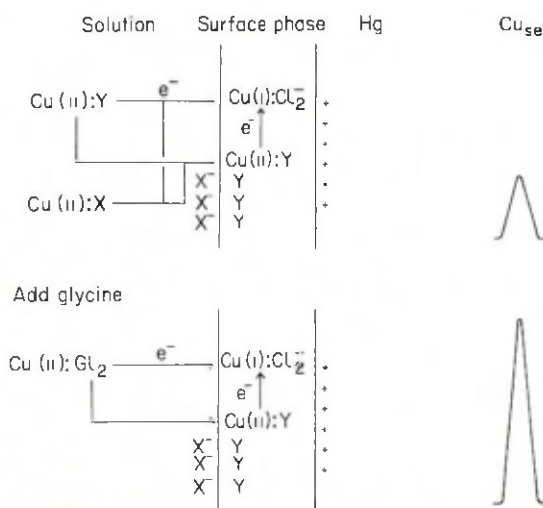


Fig. 1. Mechanism of adsorption of copper on organically coated mercury from organic complexes of copper(II) with and without glycine added: Y, surface-active ligand; X, surface-inactive ligand.

Measurement of Cu(II) interaction with organic ligands

In order to determine the extent of interaction of Cu(II) with organic ligands in solution, the method of standard additions was utilised. For every sample electrolyte, aliquots with successive additions of Cu(II), ($0-5 \times 10^{-6}$ mol l⁻¹) were prepared. Each aliquot was divided into several sub-aliquots to which were added increasing concentrations of glycine ($0-10^{-3.5}$ mol l⁻¹). Thus, for a total of eight Cu(II) and five glycine additions (including zero addition of either and both solutions), forty sub-aliquots were examined. Solutions were allowed to equilibrate for 12 h prior to d.p.a.s.v.

In all cases, ΔCu_{se} refers to the difference in adsorbed copper from the solution in the presence and absence of glycine. For each glycine addition, the measured Cu_{se} is plotted against added Cu(II) (Cu_T). From this ΔCu_{se} is estimated and plotted against Cu_T . An increase in ΔCu_{se} with Cu_T indicates gradual filling of the ligand binding sites with Cu(II); when all complexing sites are filled, the slope of the plot decreases and at higher Cu_T there is often a decrease in ΔCu_{se} [8]. Figure 2(a, b) shows how such a binding curve is derived for an estuarine water sample. The details and interpretation of this are discussed later. Briefly, for a given low Cu_T a plot of ΔCu_{se} against glycine concentration represents a ligand competition curve if it is assumed that ΔCu_{se} is proportional to the copper glycinate formed. Indeed, each glycine addition can be graphically depicted as a side-reaction coefficient for Cu(II) complexing (Fig. 2c) and at any point in the curve

$$\begin{aligned} \Delta Cu_{se} / \Delta Cu_{se}(\max) &= [CuGl_2] / [CuGl_2]_{\max} = [CuGl_2] / Cu_T \\ &= \alpha_{Gl} / (\alpha_{Gl} + [Y]\beta_{CuY}) \end{aligned} \quad (1)$$

where α_{Gl} is the side-reaction coefficient for copper glycinate formation (including $CuGl_2$ and $CuGl^+$ species, both of which are represented in the text as $CuGl_2$); $[Y]$ is the concentration of other complexing ligand(s); β_{CuY} is the conditional stability constant for the copper/ligand complex assuming 1:1 stoichiometry. The value of α_{Gl} is calculated from the stability constants, pH and the glycine concentration [6]. When $\Delta Cu_{se} / \Delta Cu_{se}(\max) = 0.5$, $\alpha_{Gl} = [Y]\beta_{CuY}$. Because $[Y]$ can be approximated from the addition curve (Fig. 2a), β_{CuY} can be calculated. For the estuarine sample shown in Fig. 2, with an estimated complexing capacity of 10×10^{-7} mol l⁻¹, a value for $\log \beta_{CuY}$ of 8.7 was obtained.

RESULTS AND DISCUSSION

Copper(II)/NTA/glycine system

Some preliminary tests were done with a solution containing gelatin (1 mg l^{-1}), pyrocatechol (10^{-5} mol l⁻¹), potassium chloride (0.51 mol l^{-1}), magnesium chloride (10^{-2} mol l⁻¹) and sodium hydrogencarbonate ($0.0037 \text{ mol l}^{-1}$). The ligand studied was NTA (10^{-6} mol l⁻¹). Pyrocatechol and gelatin

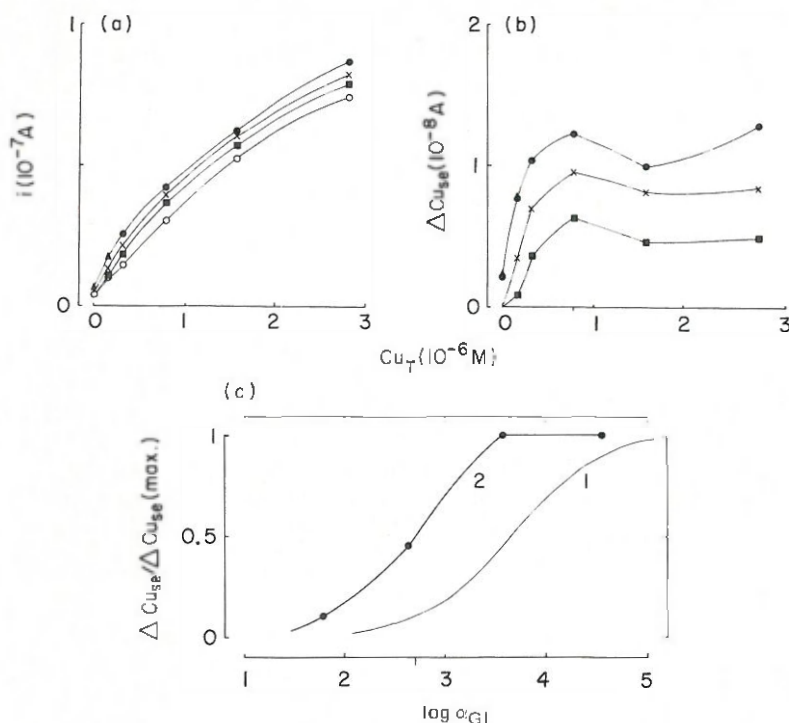


Fig. 2. Representative tests on estuarine water. (Salinity, 18‰; DOC, 3.43 mg l $^{-1}$; pH 7.91.) Copper accumulation at -34 mV. (a) Current (i) corresponding to Cu_{se} vs. Cu_T : (o) without glycine; (■) 10^{-5} mol l $^{-1}$ glycine; (x) $10^{-4.5}$ mol l $^{-1}$ glycine; (●) 10^{-4} mol l $^{-1}$ glycine; (▲) $10^{-3.5}$ mol l $^{-1}$ glycine. (b) ΔCu_{se} vs. Cu_T ; symbols as in (a); inflexion marked on Cu_T scale for calculation of complexation capacity. (c) $\Delta Cu_{se}/\Delta Cu_{se}(\max.)$ vs. $\log \alpha_{GI}$: (1) idealised 1:1 stoichiometric ligand competition curve; (2) experimental curve for sample with $Cu_T = 1.6 \times 10^{-7}$ mol l $^{-1}$. Value of $\log \alpha_{GI}$ marked on $\log \alpha_{GI}$ scale for calculation of stability constant.

were used to assist adsorption of Cu(I) [8] in the surface layer on the electrode and were added immediately prior to d.p.a.s.v. The inorganic salts were added to simulate sea water. Taking account of side-reactions of NTA with magnesium ions and pyrocatechol with Cu(II) in solution, the side-reaction coefficient of NTA with Cu(II) is $10^{3.5}$ (stability constants were taken from Sillen and Martell [10]).

In the ligand-exchange process with glycine, mixed ligand species such as $(Cu-NTA-glycine)^{2-}$ can be formed, but the ligand exchange to give copper glycinate is energetically the most favourable. The adsorption/standard addition curve is shown in Fig. 3. Understanding the form of variation of ΔCu_{se} with copper concentration needs knowledge of the adsorption mechanism. The production and adsorption of $CuCl_2$ have been described in detail [6–8], but a further feature is significant here. The ligand NTA is concentrated in the diffusion layer speciated as $MgNTA^-$ and so is attracted to the positively

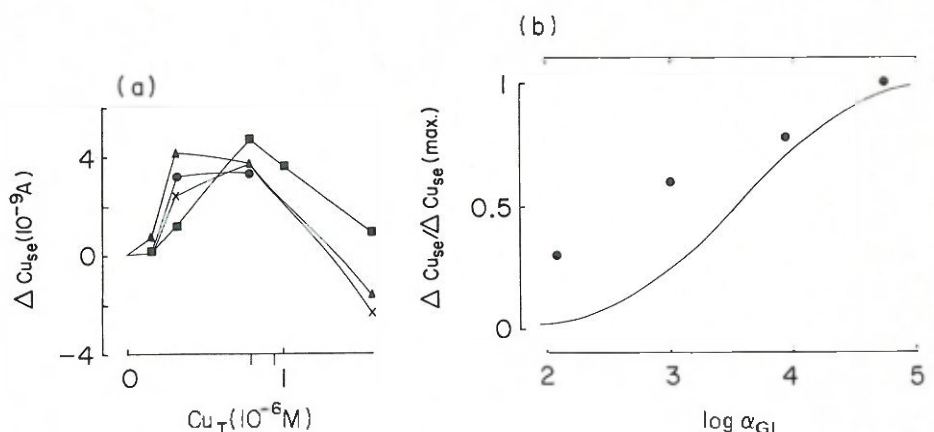


Fig. 3. Evaluation of Cu(II)-NTA interaction. Solution prepared as in text; NTA, $1 \times 10^{-6} \text{ mol l}^{-1}$; residual Cu, $6 \times 10^{-8} \text{ mol l}^{-1}$. Equilibration at pH 8.90; d.p.a.s.v. at pH 8.10; accumulation at -53 mV . (a) ΔCu_{se} vs. Cu_T ; symbols as in Fig. 2. (b) $\Delta Cu_{se}/\Delta Cu_{se(max)}$ vs. $\log \alpha_{GI}$; $Cu_T = 3.2 \times 10^{-6} \text{ mol l}^{-1}$; the full line is the idealised 1:1 stoichiometric ligand-competition curve.

charged HMDE. This hinders the transfer of Cu^{2+} from copper glycinate into the surface film for reduction to $CuCl_2^-$. When Cu_T is much less than the total NTA concentration in solution, the production of adsorbed $CuCl_2^-$ from all fractions of copper glycinate during ligand competition will be equally suppressed; the extra NTA released on formation of copper glycinate is insignificant. However, when Cu_T corresponds to a significant fraction of the total NTA, the NTA released on formation of copper glycinate will increasingly suppress the production of adsorbed $CuCl_2^-$. Thus addition of glycine will first give an increase in Cu(I) adsorption; further additions will cause more copper glycinate to form, releasing NTA which inhibits Cu(I) adsorption.

In the ΔCu_{se} vs. Cu_T plots, ΔCu_{se} for all glycine additions first increases with Cu_T . At higher copper contents, the slope of the plot for the highest glycine addition ($10^{-3.5} \text{ mol l}^{-1}$) begins to curve; with more Cu_T , the plot curves for the next glycine addition ($10^{-4} \text{ mol l}^{-1}$). Finally, at 80% of saturation of the NTA, the plot for $10^{-5} \text{ mol l}^{-1}$ glycine reaches a maximum and then decreases. The form of these plots is significant because they serve to quantify and characterise any simple Cu(II)-binding ligand in a chloride/surfactant medium.

For the Cu/NTA/glycine system, values of ΔCu_{se} were too small for a meaningful ligand competition binding curve to be derived at high NTA: Cu_T ratios. With higher Cu_T contents, the distortion of the plot in Fig. 3(b) compared to the ideal curve is evident.

Copper(II)/glycine/polyelectrolyte/chloride system

The ΔCu_{se} vs. Cu_T curves for a "blank" KCl/ $NaHCO_3$ solution are shown in Fig. 4. There is a complexation capacity of about $3 \times 10^{-7} \text{ mol l}^{-1}$, which

can be attributed to residual organic material in the distilled water and the instability of dissolved inorganic Cu(II) at pH 8.90. The blank is not due to gelatin addition because there is no detectable ΔCu_{se} when the copper is added to the electrolyte at the same time as gelatin with no equilibration time.

Figure 5 shows the plots obtained with added polyacrylic acid (PAA; m.w. 250 000, 5.6×10^{-6} mol l⁻¹ carboxyl group equivalent) in the same base electrolyte at the same pH values. Polyacrylic acid is a Cu(II)-complexing polyelectrolyte [11]; its carboxyl groups are completely dissociated at pH > 8. There is continual complexation of Cu(II) throughout the range of additions as shown in Fig. 5. Final saturation of sites is indicated by the maxima of the curves. Media spiked with aged (2 weeks) PAA solutions did not show entirely similar Cu(II)-binding behaviour; the polymer appeared to aggregate at high Cu_T ($> 4 \times 10^{-6}$ M). The form of the adsorption/standard addition curve differs from that with the simple ligand, NTA. The rise and fall of ΔCu_{se} with varying additions of glycine is not significant because the PAA does not oppose the copper adsorption mechanism as with NTA, but rather assists the adsorption.

Figure 6 shows the curves for the competitive binding of Cu(II) by glycine and PAA; these are not subject to the same distortion as the Cu/NTA/glycine curves. It is assumed that in all cases $\Delta Cu_{se}/\Delta Cu_{se}(\max)$ is proportional to the formation of copper glycinate from copper polyacrylate. The insignificance of the "blank" Cu(II) complexation relative to the PAA-Cu(II) binding is clearly seen. The slope of the experimental Cu(II)-PAA ligand-competition curve is significantly less than that of the ideal curve, probably because the

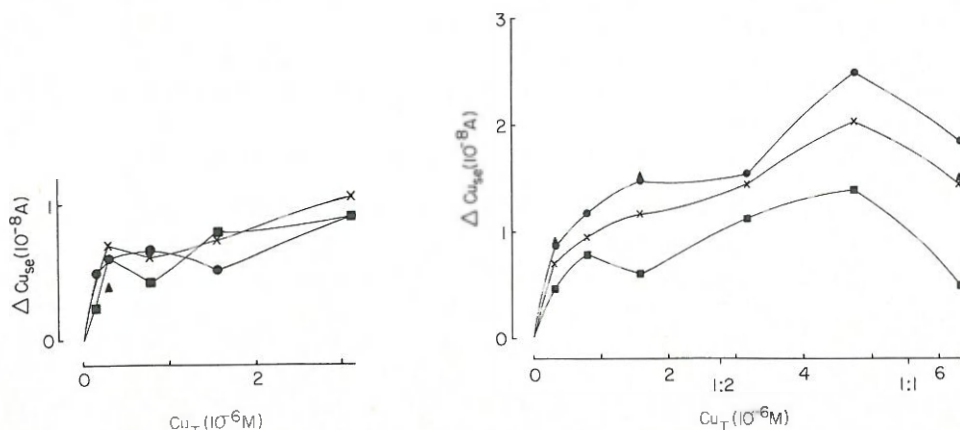


Fig. 4. Plots of ΔCu_{se} vs. Cu_T for a "blank" solution (0.55 mol l⁻¹ KCl, 0.0037 mol l⁻¹ NaHCO₃) with glycine added (symbols as in Fig. 2). Equilibration at pH 8.90; d.p.a.s.v. at pH 8.10; 1 mg l⁻¹ gelatin added; accumulation at -53 mV.

Fig. 5. Evaluation of Cu(II)-PAA interaction; ΔCu_{se} vs. Cu_T plots. All conditions as for Fig. 4, except that PAA (5.6×10^{-6} mol l⁻¹) was also added.

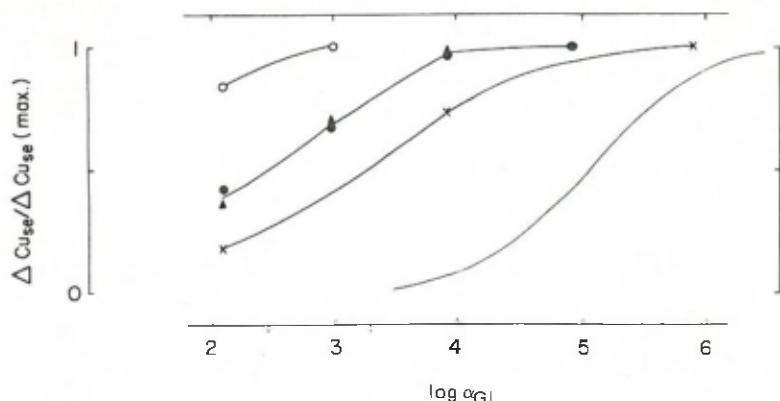


Fig. 6. Evaluation of Cu(II)-PAA interaction; $\Delta Cu_{se}/\Delta Cu_{se}(\max)$ vs. $\log \alpha_{GI}$ plots. Conditions: KCl, $NaHCO_3$, gelatin, pH values, accumulation potential as in Fig. 4. Curves: (o) no PAA; $Cu_T = 3.2 \times 10^{-7} \text{ mol l}^{-1}$; (●) PAA = $5.6 \times 10^{-6} \text{ mol l}^{-1}$; $Cu_T = 3.2 \times 10^{-7} \text{ mol l}^{-1}$; (▲) PAA = $5.6 \times 10^{-6} \text{ mol l}^{-1}$; $Cu_T = 1.6 \times 10^{-6} \text{ mol l}^{-1}$; (x) PAA = $5.6 \times 10^{-5} \text{ mol l}^{-1}$; $Cu_T = 1.6 \times 10^{-6} \text{ mol l}^{-1}$; (—) idealised 1:1 stoichiometric ligand-competition curve.

polyelectrolytic nature of the PAA provides electrostatic contributions to the copper binding. As Cu-PAA is lost to form copper glycinate, the formation of the glycinate is retarded, and the slope of the ligand-competition curve is depressed. Thus the $\log \beta$ value estimated at 0.5 on the vertical axis (Fig. 6) is a conditional value which takes account of the electrostatic contribution. As can be seen in Fig. 6, there is little change in the shape of the binding curve with increase in Cu_T up to $1.6 \times 10^{-6} \text{ mol l}^{-1}$. If 1:1 complexation of Cu(II) with each functional group is assumed, then for the addition of $5.6 \times 10^{-6} \text{ mol l}^{-1}$ PAA, a $\log \beta$ value of 7.7 can be estimated for each site complexation. When the concentration of PAA is increased ten-fold, the binding curve is shifted to the right as expected. The strength of binding increases by a factor of 7.

Copper(II)/glycine system in estuarine waters

Figure 7 depicts the results obtained for estuarine water samples from a summer profile during a high production period in the Tamar Estuary, South West England. Potassium chloride was added to samples with salinity less than 13‰ to increase the chloride content to 0.20 mol l^{-1} , and help stabilise $CuCl_2$ species during adsorptive accumulation. Initial interpretation of these results is best done visually. In the low salinity region, the Cu(II) complexation is dominantly of a polyelectrolytic nature (cf. PAA) with continuing complexation of Cu(II) to high values of Cu_T . This is to be expected with the bulk of natural organic material being of humic character. With increase in salinity, the polyelectrolyte nature of the Cu(II) complexation remains significant but decreases, both because of dilution of dissolved organic carbon from the river and increase in the competition for binding sites from

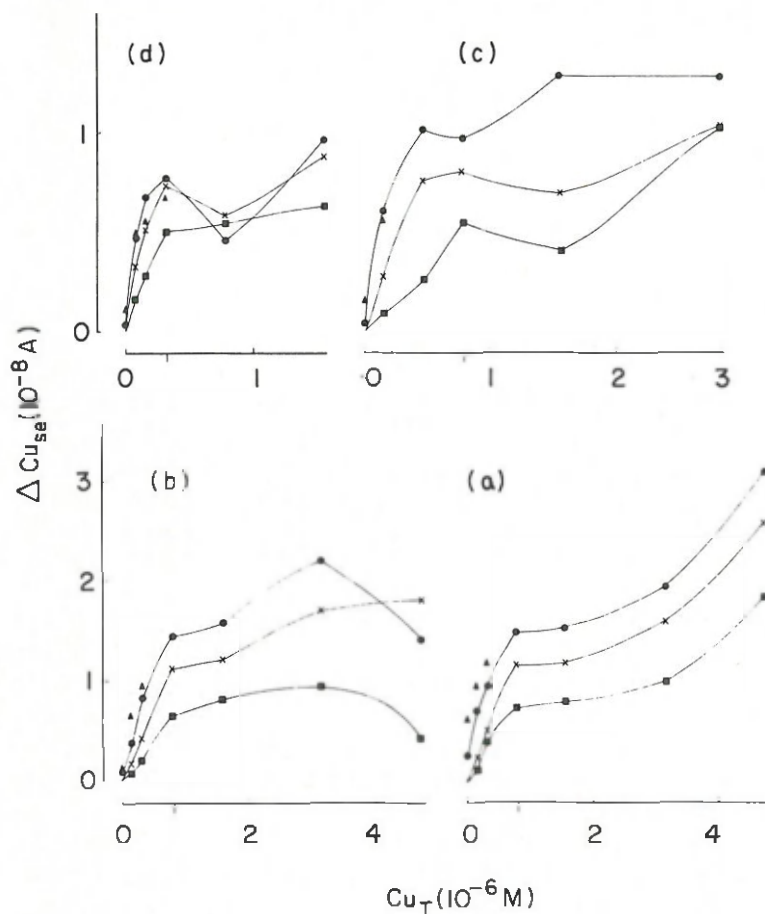


Fig. 7. Evaluation of Cu(II)-organic interactions in estuarine waters; ΔCu_{se} vs. Cu_T plots. (a) Salinity, 0‰; DOC, 2.46 mg l⁻¹; pH 7.56. (b) Salinity, 3.3‰; DOC, 3.03 mg l⁻¹; pH 7.62. (c) Salinity, 13‰; DOC, 2.97 mg l⁻¹; pH 7.77. (d) Salinity 32‰; DOC, 1.98 mg l⁻¹; pH 7.94. Accumulation potential: (a, b, c) -29 mV; (d) -59 mV. Glycine addition (mol l⁻¹): (■) 10^{-5} ; (x) $10^{-4.5}$; (●) 10^{-4} ; (▲) $10^{-3.5}$. Samples for (a, b) were boosted to 0.20 mol l⁻¹ chloride with KCl.

calcium and magnesium. At higher salinities, a second group of ligands becomes significant at lower concentrations; these ligands show similarities to the NTA binding of Cu(II). These ligands dominate the Cu(II) complexation at the seaward end of the estuary (Fig. 8). Thus the adsorption/standard addition curves suggest that there are two separate Cu(II)-binding systems; but it is to the lower concentration of discrete group of ligands that quantitative estimates of Cu(II)-organic binding have been made.

Complexation capacities are estimated from the first inflexion on the ΔCu_{se} vs. Cu plot for 10^{-5} mol l⁻¹ glycine additions. This is taken to repre-

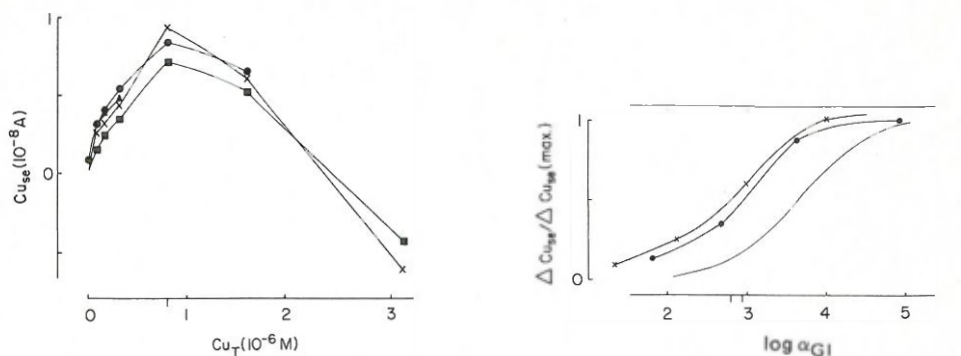


Fig. 8. Evaluation of Cu(II)-organic interaction in sea water; ΔCu_{se} vs. Cu_T plots. Salinity, 34‰; pH 8.02. Accumulation at -59 mV. Glycine addition as in Fig. 7.

Fig. 9. Evaluation of Cu(II)-organic interactions in estuarine waters; $\Delta Cu_{se}/\Delta Cu_{se(max)}$ vs. $\log \alpha_{Gl}$ plots. (x) Salinity, 3.3‰; DOC, 3.03 mg l⁻¹; pH 7.62; Cl⁻ increased to 0.2 mol l⁻¹ with KCl; accumulation at -29 mV. (•) Salinity, 23‰; DOC, 2.6 mg l⁻¹; pH 7.91; accumulation at -35 mV. (—) Idealised 1:1 stoichiometric ligand-competition curve.

sent 80% saturation of ligand sites and the total ligand is calculated on the assumption of 1:1 complexation. Figure 9 shows representative ligand-competition binding curves for copper in estuarine waters. These were derived for low $Cu_T:[Y]$ ratios and display little distortion compared to the ideal binding curve; distortion is readily recognised at higher $Cu_T:[Y]$ ratios. Binding constants were therefore estimated by using the above complexing capacities and assuming 1:1 binding stoichiometry. Table 1 lists the $\log \beta$ values calculated for the various samples, together with the binding capacity and other features. The metal-organic binding constants show some variation. There is some correlation between the stability constants and production levels (chlorophyll- α), although other factors also control Cu(II)-organic

TABLE 1

Copper(II)-binding ligands in estuarine waters (Tamar Estuary, South West England, July 1983)

Salinity (‰)	DOC (mg l ⁻¹)	Chlorophyll- α (mg l ⁻¹)	Binding capacity (10 ⁻⁷ mol l ⁻¹)	$\log \beta_{CuY}$ (mol ⁻¹)
32	2	6.9	3.9	8.6
29	2	12.2	3.9	9.4
23	2	8.0	3.9	9.1
18	3.4	14.3	10	8.7
13	3.0	37.8	10	8.5
3.3	3.3	99.7	10	8.8
0	2.5	23.0	10	8.5

interactions. The binding capacity follows the DOC. The main effect of salinity is on the humic complexation.

To date, there have been no comparable studies on copper-organic associations in estuarine waters. However, the stability constants given by van den Berg [12] for copper-organic binding in sea water are higher ($\log \beta = 9.8$). In his work, a manganese dioxide adsorption technique was used but this method does not take into account the polyelectrolytic and inhomogeneous nature of copper-organic associations. In addition, the high Cu(II)-binding affinity of the manganese dioxide adsorbent will not allow weaker complexes to be detected. An electrochemical method [13] which measures free Cu^{2+} by constant-potential coulometric reduction to CuCl_2 , gives $\log \beta$ values for Cu-organic interaction in sea water of a similar magnitude to those estimated here ($\log \beta = 8.8$). In that study, however, measured ligand concentrations were lower.

All the samples assayed here were frozen after collection. To assess the effect of freezing the sample, the adsorption/standard additions tests were done both before and after the freezing-thawing process. The copper binding curve is similar at low Cu_T but is altered at higher Cu_T in the thawed frozen sample (Fig. 10). Both the initial complexation and the humic complexation

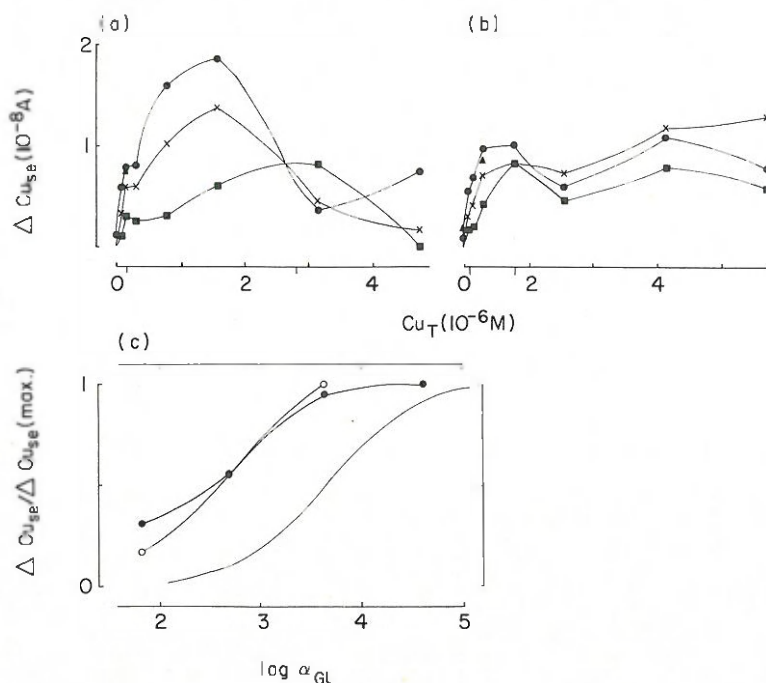


Fig. 10. Evaluation of estuarine waters (salinity 34‰, pH 8.02; accumulation at -59 mV). (a) ΔCu_{se} vs. Cu_T ; sample tested 24 h after collection. (b) ΔCu_{se} vs. Cu_T ; sample frozen after collection then thawed before testing. Glycine addition as in Fig. 7 for (a) and (b). (c) $\Delta \text{Cu}_{se} / \Delta \text{Cu}_{se}(\text{max.})$ vs. $\log \alpha_{GL}$: (○) unfrozen sample; (●) frozen/thawed sample; (—) idealized 1:1 stoichiometric ligand-competition curve.

in the thawed frozen sample are decreased. However, the ligand-competition binding curves are similar at low Cu_T . The effect of freezing on polyelectrolyte macromolecules and their binding and surfactant behaviour must be significant. That the initial ligand binding is relatively unaffected is to be expected. But the humic complexation, which is analogous to surface interaction between Cu(II) and polyelectrolytes, will be influenced if the size and conformation of the macromolecules are altered. Nonetheless, although done on frozen/thawed samples with changes to the copper-binding properties of the organic ligands, the estuarine profiles described here do provide insights into the underlying concepts of copper-organic binding in estuarine waters of varying salinity.

Conclusions

The approach to the investigation of Cu(II) -organic binding in estuarine waters described above takes advantage of the anomalies of copper interfacial electrochemistry. The method provides a meaningful response when tested on pure ligands in surfactant/chloride media and on estuarine waters. The technique gives both a diagnostic criterion on the nature of the Cu(II) -binding ligands and also an estimate of the complexation capacity and binding constants.

Estuarine waters appear to contain two populations of Cu(II) -binding ligands: a high concentration of humic material ($>10^{-6} \text{ mol l}^{-1}$ assuming 1:1 site binding) with polyelectrolyte-type binding, and a lower level of discrete ligands forming copper complexes with stability constants around 10^9 . The extent of Cu(II) binding by the humic material decreases down the estuary because of dilution and increased competition from metal ions. The possibility is not excluded that the lower concentration of ligands might represent separate binding sites for copper on the humic macromolecules. These ligands dominate copper(II) complexation at the seaward end of the estuary.

Thanks are due to M. Woodward and C. Llewellyn for the DOC and chlorophyll measurements.

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