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ABSTRACT

A new procedure is presented for the determination of the ligands of copper(II) in natural waters, based on titration with the metal ion, monitored by measuring the concentration of copper(II) sorbed on the carboxylic resin Amberlite CG 50. The data are treated by the Ruzic linearization method to obtain the concentration of the ligands and the conditional stability constant of the complexes. Ligands with reaction coefficient α_M higher than 0.1 K^*w/V are detected, where K^* is the ratio of the concentration of sorbed metal to the concentration of free metal in solution, which can be evaluated from the sorption equilibria of copper(II) on Amberlite CG 50, w is the amount of water in the resin phase, and V the volume of the solution phase. Some natural waters at high and low salinity were examined. The ligand concentration determined in these samples ranged from around 50 to 2000 nM, while the original copper concentrations from 11 to 130 nM. The ligand concentration was always much higher than that of copper(II). The conditional stability constants were very high, particularly in low salinity waters, where values as high as $K' = 10^{15.7}$ were obtained. In high salinity waters values around 10^9 were found for the complex formation constant of the ligands titrated with copper(II). The investigation was also extended to a model solution, containing EDTA, obtaining $K' = 10^{15.5}$, in acceptable agreement with that evaluated from the literature values.

Keywords: ligands of copper(II) in natural waters, ligand titration with copper(II), ligand titration by sorption on carboxylic resin.

INTRODUCTION

The concentration and the strength of the metal ligands in waters of environmental interest are important for the distribution of metal ions in different physico-chemical species (speciation). Their solubility, mobility and toxicity strictly depend on the ligands present. The investigation is preferably carried out by titrating the ligands directly in the sample, with the appropriate metal ion, and monitored by different experimental methods. The ligands are often titrated with copper(II), determining the complexation capacity towards this particular metal (Gardner *et al.*, 2000; van den Berg *et al.*, 1992), since it is widely diffused in the environment, and it is able to combine with most of the ligands, inorganic and organic, which can be present in natural and contaminated waters (Gardner *et al.*, 2000).

The voltammetric methods, ASV and CSV, are widely employed for the detection, because they have a low detection limit, which makes possible the investigation of ligands at very low concentration and with high complexation constant. These methods have been widely applied to many different environmental waters, at high (van den Berg *et al.*, 1992; Miller *et al.*, 1997; Scarponi *et al.*, 1996; Donat *et al.*, 1994) and low salinity (Gardner *et al.*, 2000), but they have some drawbacks, consisting mainly into the fact that interferences in the

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amperometric detection must be expected when dealing with natural matrices, and that the "labile metal" concentration obtained experimentally is not exactly defined, and strongly depends on the procedure.

Methods based on competition with solid phases have also been used (van den Berg *et al.*, 1997), employing manganese dioxide as sorbing solid competing with the ligands in solution, but they are much less common than the solution competition methods (Miller *et al.*, 1997). In the present paper the adsorption of copper(II) on a complexing resin was used for monitoring the titration of ligands in some natural waters, and also in a sample containing EDTA.

Some synthetic solutions containing known ligands at known concentration were previously studied (Pesavento *et al.*, 2000) by the method here proposed, using the two resins Chelex 100 and Amberlite CG 50 for monitoring, obtaining results in good agreement with the expected values, not only for the ligand concentration, but also for the conditional complexation constants.

In the present investigation Amberlite CG 50, a complexing resin containing carboxylic groups, was used. Its sorbing properties for copper(II) are known (Pesavento *et al.*, 1994; Biesuz *et al.*, 2001). The resin Amberlite CG 50 has relatively low sorbing properties in comparison with other complexing resins, as for instance Chelex 100, and it has been used for the titration in order to detect ligands forming with copper(II) complexes of high and intermediate stability.

An important point of the method here used is that the concentration of the free metal ion in solution in the presence of the resin can be evaluated from that of the labile metal, which is experimentally determined (Pesavento *et al.*, 2000) as is described below, under "Methodology". In principle, this allows the real complexation constant to be evaluated from the experimental data. In some way the calculation method is similar to that used in CSV ligand titration, in which the analytical ligand is soluble.

An interesting difference from the ligand titration methods based on voltammetric monitoring is that complexes with lower formation rate can also be detected, because the sorption on resin requires a long equilibration, so that the titrant metal has time to equilibrate also with the ligands in solution. In contrast, in the ligand titrations based on voltammetric monitoring the titrant metal has a shorter time, sometimes only a few minutes, for equilibration with the solution ligands after its addition to the sample.

In the present paper, some natural waters have been examined by the proposed procedure. The same samples have also been studied by other laboratories, using other titration methods, mainly cathodic and anodic stripping voltammetry (van Veen *et al.*, 2002). These results will be compared with those obtained by the procedure proposed here, in order to understand what is obtained by the different procedures.

METHODOLOGY

The ligand titration here proposed is based on the determination of the metal ion adsorbed in the resin phase, c, which is the "labile metal", in this context. It has been shown elsewhere (Pesavento *et al.*, 2000) that in the case of sorption on complexing resins the concentration of the free metal ion in the solution phase $[M]$, can be evaluated from the concentration of metal ion sorbed on the resin (c), which is determined experimentally. The relationship is

$$K^* = \frac{c \cdot V}{[M] \cdot w} \qquad (1)$$

where w is the amount of water in the resin phase, V the volume of the solution phase, and K^* represents the ratio of the concentration of metal sorbed in the resin phase to the free metal in solution. K^* can be calculated from the sorption equilibria of the metal on the resin, as extensively reported in previous papers, for instance in (Pesavento and Alberti, 2000). The values of K^* used in this investigation for each of the samples examined are reported in Table 1. They have been evaluated from the known sorption equilibria of copper(II) on

Table 1 K^* and limiting reaction coefficient α_M for adsorption of copper(II) on Amberlite CG 50, at the conditions of the ligand titration.

Sample	pH[a]	K^{*}[b]	Low limiting α_M for detectable ligands[c]
A *(1st repl.)*	3.48	5.32×10^1	1.06×10^{-2}
A *(2nd repl.)*	3.50	5.76×10^1	1.15×10^{-2}
B *(1st repl.)*	7.96	2.72×10^{10}	5.44×10^6
B *(2nd repl.)*	8.12	5.08×10^{10}	1.02×10^7
C *(1st repl.)*	7.97	2.45×10^{10}	4.90×10^6
C *(2nd repl.)*	8.16	4.94×10^{10}	9.89×10^6
D *(1st repl.)*	7.26	2.65×10^5	1.43×10^1
D *(2nd repl.)*	7.67	2.67×10^5	1.44×10^1
E *(1st repl.)*	7.23	1.67×10^5	9.02×10^0
E *(2nd repl.)*	7.63	1.68×10^5	9.03×10^0
F *(1st repl.)*	6.79	7.15×10^4	1.43×10^1
F *(2nd repl.)*	7.54	7.30×10^4	1.46×10^1
G *(1st repl.)*	7.92	3.86×10^{10}	7.72×10^6
G *(2nd repl.)*	8.10	8.61×10^{10}	1.72×10^7

[a] pH after equilibration with the resin
[b] K^* evaluated at the pH of the ligand titration, and for the ionic composition of the sample in (van Veen *et al.* 2002) (samples indicated by the same letter). Values obtained from the intrinsic complexation constants of copper(II) in resin phase (Pesavento *et al.*, 1994; Pesavento *et al.*, 2000).
[c] Only complexes with α_M higher than the value reported are detected.

Amberlite CG 50 (Pesavento *et al.*, 1994, Biesuz *et al.*, 2001). The $K*$ values strongly depend on the conditions, in particular the acidity of the solution, and its ionic composition. For example, they are much higher in the case of low salinity waters, containing a lower concentration of metal ions, in particular alkaline earth metals, which compete with copper(II) for the sorption on the resin.

It is well known that, for each adsorbing solid, a detection window exists for which the determination of the ligand concentration and of the conditional stability constant is possible (Miller *et al.*, 1997, Donat *et al.*, 1994). This is mainly determined by the sorbing properties of the solid towards the considered metal at the considered conditions. In the case of complexing resins, it has been shown that ligands forming complexes with reaction coefficient from 0.1 $K*$ w/V to 10 $K*$ w/V can be fully investigated.

The lowest reaction coefficient, ($\alpha_M = 0.1$ $K*$ w/V) which can be evaluated by the proposed method is reported in the last column of Table 1, for the experimental conditions under which the different samples were examined. Weaker complexes, with reaction coefficients lower than those reported, are totally dissociated in the presence of the resin, meaning that more that 90% of the metal ion is sorbed on the resin, as it has been previously discussed (Pesavento *et al.*, 2000; Pesavento and Alberti, 2000). In this case the evaluation of the complexation parameters is not sufficiently accurate (Miller *et al.*, 1997). The ligands forming complexes that are too strong to be dissociated in the presence of the resin, having a too high reaction coefficient ($\alpha_M \geq 10$ $K*$ w/V), are detected, and their concentration is determined, but their complexation constant cannot be evaluated. Only in the case of complexes partially dissociated in the presence of the resin, both the concentration of the ligand and the conditional complexation constant can be determined.

The complexing capacity of the sample determined by the ligand titration using Amberlite CG 50, includes ligands forming strong and intermediate complexes with copper(II). The weakest complexes, i.e. those with α_M lower than the values reported for each sample in Table 1, are not detected at all.

The reaction coefficient of a metal ion in the aqueous phase in the presence of a complexing resin can be evaluated from the concentration of metal ion sorbed in the resin phase, considering eq. (1), as here reported:

$$\alpha_M = \frac{c_M - c}{[M]} = \frac{(c_M - c) \cdot K* \cdot w}{c \cdot V} \qquad (2)$$

For simplicity it is assumed that the complexation reaction in the solution phase is

$$M + I \rightleftarrows MI \qquad K' = \frac{[MI]}{[M][I]} \qquad (3)$$
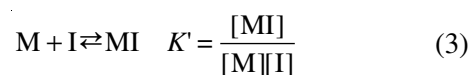
The mass equation of the ligand I and of the metal ion M are

$$c_I = [I] + [MI] \qquad (4)$$

$$c_M = c + [MI] + [M] \qquad (5)$$

The reaction coefficient is

$$\alpha_M = 1 + K'[I]$$

The concentration of the ligand I (c_I), and the conditional stability constant are evaluated from the relationship

$$\frac{c}{c_M - c} = \frac{K* \cdot w}{K' c_I \cdot V} + \frac{c}{c_I} \qquad (6)$$

which is obtained from the Ruzic linearization (Ruzic, 1982), assuming that the concentration of free metal ion [M] is negligible with respect to the total copper concentration. K' can be evaluated only if $K*$ is known, and its accuracy depends on the accuracy of $K*$.

All the equations reported above hold only at equilibrium conditions. So the resin must always be equilibrated with the solution phase for a sufficiently long time.

EXPERIMENTAL

Reagents and materials

Amberlite CG 50 (Aldrich Chemie) was obtained in the H+ form, and it was washed in acid as previously described (Pesavento *et al.*, 2000). It was converted in the ammonium form by treatment in concentrated (1.0 M) ammonia solution. All the equipment was washed with acid, and rinsed as previously described (Pesavento *et al.*, 2000). All the reagents were of analytical or pure grade.

Experimental procedure

Eight subsamples for each titration were considered, each of 50 ml, and containing different concentrations of added copper(II). Each subsample was contacted with 0.1 g of dry resin for 16 hours at room temperature, under stirring, in 100 ml polyethylene bottles. In the case of high salinity estuarine waters 0.027 g of dry resin were used, in order to maintain the concentration of alkaline earth metals in solution unchanged, after sorption of the alkaline earth metal ions on the resin. The liquid phase was separated by suction, and the resin

in the bottle rapidly washed with 5 ml of Milli-Q water. Then it was contacted with 10 ml of 1 M HNO_3 for 2 hours to elute the sorbed metal ion. This was determined in the solution by Atomic Absorption Spectroscopy (flame or graphite furnace atomization).

The conditions under which each ligand titration was performed are reported in Table 2. Not any buffer was added to the samples, but in the case of the estuarine and marine water (2nd replicate) some pure NH_3 was added, since the pH variation after equilibration with the resin was higher than in freshwater samples. Each sample was titrated twice, the first one immediately after opening the bottle containing the sample, and the second one 48 hours later. During this time the samples were kept at 4°C, in the same half empty bottle.

The titration curve was obtained by plotting c vs c_M, (the added copper plus the copper initially present). The titrant (metal ion) additions ranged from a concentration approximately equal to that present in the sample, and up to at least 200 times higher (see Table 2). If the last three points of the titration curve (c vs c_M) were on a straight line with slope equal to the predicted one (Pesavento *et al.*, 2000), the titration was considered as finished. If not, other additions were made until the predicted slope of the straight line was obtained. This is based on the assumption that the ligands are completely combined with the metal, and a metal excess is added.

Treatment of the ligand titration data

The concentration of the ligands can be evaluated directly from the ligand titration curve, as previously shown (Pesavento *et al.*, 2000), and from the Ruzic linearization (Ruzic, 1982), which was used as reported in eq. (6). The conditional complexation constant K', defined as in eq. (3) was evaluated using $K*$ reported

in Table 1. Only the points for which the fraction of sorbed metal was lower than 0.80 were used for the Ruzic linearization, in order to reduce the experimental uncertainty. In general five titration points for each titration were considered.

Determination of the original concentration of copper in the considered samples

In the freshwater samples (sample A, B, C, G), the determination was made directly in the sample, after acidification with 0.5% HNO_3, (BDH Aristar, cat n° 450046R), by AAS-EA with a Shimadzu AA6601G atomic spectrometer with autosampler Shimadzu ASC 6100. The graphite tube was pyrolytically coated, the self reversal background correction (10–500 mA), slit width of 0.5 nm at λ= 324.8 nm were applied. The furnace program was as follows:

Temperature (°C)	Time (s)	Mode	Argon (l/min)
150	20	Ramp	0.1
250	10	Ramp	0.1
800	10	Ramp	1.0
800	10	Step	1.0
800	3	Step	–
2300	2	Step	–
2500	2	Step	1.0

The determination was done with a calibration curve obtained with three standards, respectively 15.7, 39.4, 78.7 nM. The limit of detection, LOD, determined with 10 replicates with a 4.7 nM standard and 10 replicates of a blank (Milli-Q water), was 3.1 nM (2 times the background variation).

In the high salinity samples (sample D, E, F), the total concentration was determined by Differential Pulse

Table 2 Conditions of the ligand titration by copper(II) with Amberlite CG 50 of the natural water samples. The volume of the sub-samples was always 50 ml

Sample	Description	Original pH of the sample	Dissolved copper conc. (nM)	Amount of dry resin (g)	Form of the resin	Useful points for the Ruzic linearization	Concentration of added copper(II) (nM)
A	Upland river water, soft, coloured	3.78	39.2	0.10	H^+	0	39 ÷ 11376 *30 ÷ 180000 (2nd repl.)*
B	Lowland river water, unpolluted	8.10	11.5	0.10	NH_4^+	4	11.5 ÷ 5729
C	Lowland river water, hard, polluted	8.13	36.0	0.10	NH_4^+	5	36 ÷ 11145
D	Estuarine water	7.89	129	0.027	NH_4^+ *(+ NH₃ in 2nd repl.)*	5	139 ÷ 21020
E	Estuarine water	7.86	112	0.027	NH_4^+ *(+ NH₃ in 2nd repl.)*	5	146 ÷ 21005
F	Seawater	7.76	32.9	0.10	NH_4^+ *(+ NH₃ in 2nd repl.)*	6 *(0 in 2nd repl.)*	32.9 ÷ 11075 *56.9 ÷ 10969 (2nd repl.)*
G	EDTA	7.76	<LOD (2.5×10^{-9} M)	0.10	NH_4^+	4	8.6 ÷ 5719

Anodic Stripping Voltammetry (DPSAV), using the operative method here described.

The sample solution (50 ml) was added with 1 ml suprapure HCl (BDH Aristar, cat n° 450020H), and kept for 24 h at room temperature, in a Teflon container. Aliquots of 10 ml were taken for the determination by DPASV using the Amel Polarographic Analyzer mod. 433 equipped with a three electrodes Teflon cell: working electrode: HDME, drop size 40 u.a.; reference electrode: Ag/AgCl, KCl satured; counter electrode: Platinum. The deposition potential was -500 mV, with purge and stirring time 600 s, deposition time 600 s for sample F, 240 s for samples D and E, delay period before stripping 10 s, with pulse amplitude 50 mV and scan rate 20 mV/s.

The cell was repeatedly cleaned with ultrapure 1M HCl solutions.

Before each measurement a blank control (10 ml Milli-Q water and 200 µl suprapure HCl) was performed under the same operative conditions used for the samples. The procedure was repeated till the blank showed no peaks and the baseline was satisfactory.

Determinations were done by standard additions (minimum 4) from an acidified standard solution of copper(II) (1.0 mg l^{-1}) daily prepared.

The determination was repeated three times for each sample.

RESULTS AND DISCUSSION

Six waters of environmental interest at different salinity were examined, together with a synthetic sample containing EDTA at unknown concentration. All the samples were obtained in the context of an interlaboratory test whose purpose was to compare different titration methods of the copper(II) ligands in natural waters. The results of the test have been published by van Veen *et al.* (2002).

One liter of each sample was provided for the investigation, each denoted by a capital letter, the same by which the samples are here indicated. The main components of the samples are also reported in the same paper. The knowledge of the concentration of alkaline and alkaline earth metals is important for the titration procedure proposed in the present paper, since this makes it possible the evaluation of $K*$ (Pesavento and Alberti, 2000, Pesavento *et al.*, 1995). The $K*$ values calculated according to the previously described method are reported in Table 1. The conditions under which the different samples were examined are listed in Table 2. The concentration of copper(II) in each sample is also reported in Table 2, determined as described in the experimental part. AAS-EA cannot be used for the direct determination in the high salinity waters, so that an amperometric determination was here employed. By comparing the copper(II) concentrations

here obtained, reported in Table 2, and those obtained by the other laboratories participating to the inter-comparison, reported in (van Veen *et al.*, 2002) an acceptable agreement is observed, except in the case of the seawater (sample F) in which the concentration here found was five times higher than that obtained by the other laboratories. The results of the ligand titration of sample F are presented as well, considering that the use of different original concentrations does not introduce any significant variation in the in the final results obtained by the ligand titration procedure.

The method for the determination of the copper(II) concentration in the 1 M HNO$_3$ eluates was always AAS-EA, as described in the experimental part. Only the eluates with copper concentration higher than the detection limit were considered for the evaluation.

All the samples were titrated twice, one immediately after opening the sample, and the other 48 hours after the first titration. The samples were stored at 4°C, in the same plastic bottles in which they were mailed.

The original acidity of the samples is reported in Table 2, and that after equilibration with the resin in the specified form, is reported in Table 3.

Amberlite CG 50 in the ammonium form was used for monitoring the titration, as reported in the experimental part. This seems to preserve well the pH to a value near to the original one in the case of the low salinity waters, while not so well in the case of high salinity waters, due to the fact that probably in this case much more ammonium is exchanged for other ions. In the case of high salinity waters the second replicate was made after addition of a small amount of ammonia solution, in order to increase the final pH to a value near to that of the original sample.

Concentration of Cu(II) ligands and conditional stability constants in natural waters.

As an example, the titration curves of sample C (a river water, low salinity water) and sample F (seawater) are reported in Figures 1 and 2 respectively. The two repeated titration curves are similar for sample C, and different for sample F. The last points of the titrations are on a straight line with slope 0.94(0.02) and 1.09 respectively, which is acceptably near to that expected (Pesavento *et al.*, 2000). The possibility of predicting the slope of the titration curve when a titrant excess is present is interesting, since it is a good check that the titration is really finished (Miller *et al.*, 1997).

The ligand concentration obtained directly from the titration curve (Pesavento *et al.*, 2000), *i.e.* from the last three points, is respectively 95 (77) nM and 528 (419) nM, which are affected by the large uncertainty reported in parenthesis. For this reason the Ruzic linearization was used for the evaluation of the ligand concentration. Moreover in order to reduce the uncertainty only the points with the fraction of metal ion

Table 3 Ligands of copper(II) in natural waters at high and low salinity investigated by ligand titration with copper(II), and adsorption on Amberlite CG 50

Sample	pH of the replicates[a]		1st repl.		2nd repl.	
	1st repl.	2st repl.	Ligand conc. (nM)	log K'	Ligand conc. (nM)	log K'
A	3.48	3.50	//[b]		//[b]	
B	7.96	8.12	53.1 (24.0)	15.32 (0.23)	42.2 (4.6)	15.82 (0.08)
C	7.97	8.16	62.0 (4.5)	15.72 (0.18)	95.1 (2.7)	15.74 (0.05)
D	7.26	7.67	1688 (172)	8.35 (0.15)	1117 (36)	8.29 (0.41)
E	7.23	7.63	2038 (467)	8.48 (0.18)	1367 (417)	7.88 (0.26)
F	6.79	7.54	118 (9)	9.48 (0.11)	~0[c]	α_M <15
G	7.92	8.10	411 (13)	15.51 (0.30)	416 (8)	15.77 (0.16)

[a]pH after equilibration with the resin.
[b]Copper(II) is not sorbed on the resin at this acidity.
[c]In this titration the fraction of sorbed metal was always higher than 0.8, thus it was not useful for calculation.
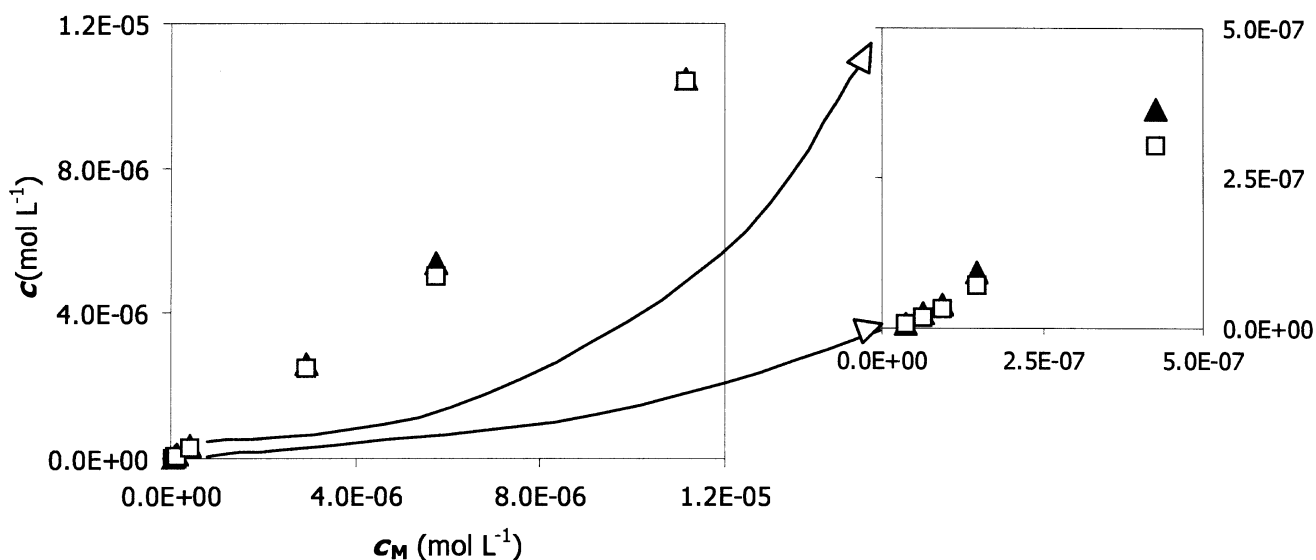

Figure 1 *Titration curves of sample C (river water). (▲) first replicate; (□) second replicate.*

sorbed in the resin from 0.1 to 0.8, were considered. The results obtained for the ligand concentration c_I, and the corresponding standard deviation, are reported in Table 3, for all the samples. The ligand concentration from the Ruzic linearization is affected by a much lower uncertainty than those directly obtained from the titration curve. The standard deviation is still considerably high in some titrations, while lower in others. Probably this is partly due to the low number of titration points, which are finally considered, so that an error in one point may have a large influence in the final result.

The results of the two replicates are generally different, a difference of 30% in the ligand concentration between the replicates being observed. This can be due to the irreproducibility of the method, or to the fact that a certain time elapsed between the first and second replicate. In the case of the high salinity waters, in which a systematically lower concentration was obtained in the second replicate, the effect could be due also to the different pH at which the titration replicates were carried out. In the case of seawater (sample F) not any ligand was titrated in the second replicate. This is well seen in the titration curve of Figure 2.

The reproducibility between the replicates is much better in the case of sample G, which contains EDTA. Evidently this ligand does not change in any way during the storage between the two replicates.

Sample A was originally at low pH (see Table 2). It can be evaluated from the sorption equilibria of copper(II) on Amberlite CG 50 that the fraction of copper adsorbed in these conditions is as low as 0.09, also considering a side reaction coefficient equal to one. This is seen also from the limiting value of α_M reported in Table 1, which is lower than 1, evidently a non-sense. Actually it was experimentally found that only a very
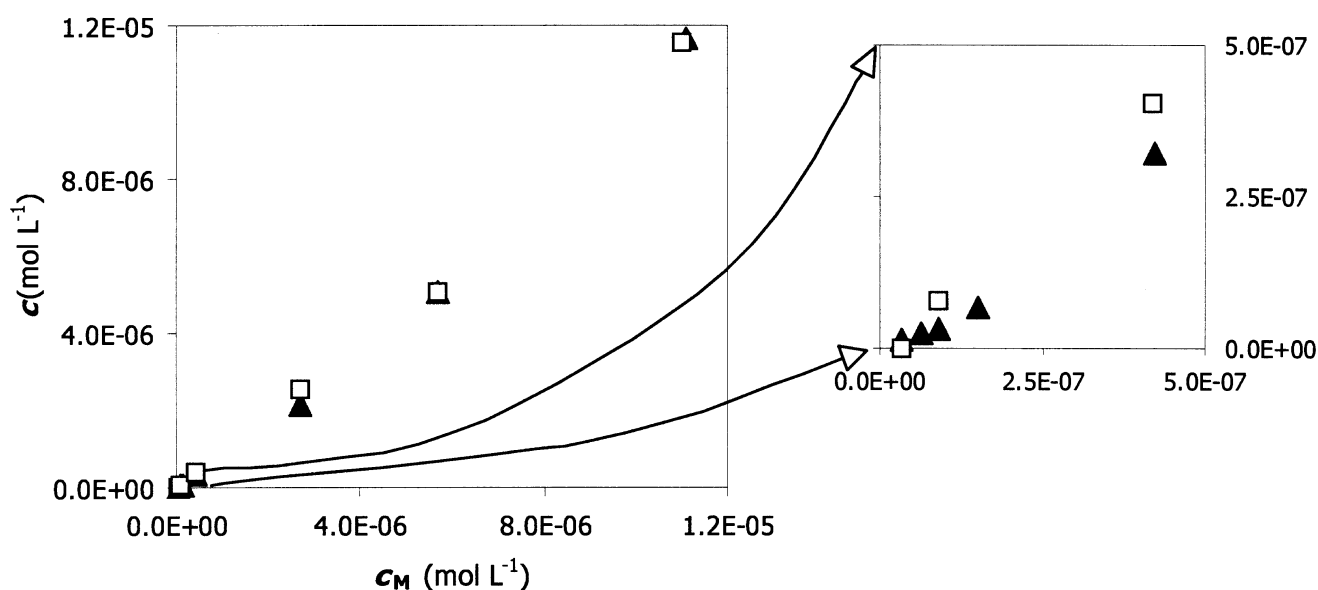
Figure 2 *Titration curves of sample F (seawater). (▲) first replicate; (□) second replicate.*

low fraction of copper(II) was sorbed from sample A. Thus the ligand titration with copper(II) can not be monitored with Amberlite CG 50 in this sample at a pH near to the original one. Either a more strongly sorbing resin should be used, or different conditions. In any case it is interesting that the negligible sorption predicted from the known sorption equilibria, is actually experimentally found.

In all the samples the concentration of the ligands titrated by the suggested procedure, c_I , is higher than the concentration of copper(II) originally present. These ligands can be considered to be strong being K' c_I always higher than 10^2, and sometimes much higher, as in the case of the two freshwater samples B and C. It must be further underlined that the ligands titrated by the proposed procedure are those forming complexes with reaction coefficients higher than the limiting value reported in Table 2. It must also be clearly realized that only the conditional constant of complexes with reaction coefficient lower than $10 K^* w/V$ can be evaluated. Thus the values reported in Table 3 are referred to this kind of ligands. Stronger complexes may well be titrated but the corresponding conditional complexation constants can not be determined.

The ligands titrated are at low concentration in all the samples, but much higher in the two estuarine waters, in which the ligand concentration is near to 10^{-6} M. This seems to be peculiar for this particular class of samples. Only for such samples a large difference was found with the results obtained by the other laboratories participating to the intercomparison (van Veen *et al.*, 2002).

The chemical nature of the ligands cannot be assessed by the ligand titration, however they are certainly not the usual inorganic ligands, which are present as main components at much higher concentration. Also the ligands here titrated are not related to the organic matter content, since DOC is not very different in the different samples here considered, as reported in (van Veen *et al.*, 2002). Not any obvious correlation between the complexing capacity for copper(II) and the principal characteristics of the waters is observed, as it was previously reported in the case of English rivers waters, investigated by CSV (Gardner *et al.*, 2000).

The conditional complexation constants were obtained by Ruzic linearization as reported in eq. (6), using K^* given in Table 1. In the case of the two waters at low salinity (samples B and C) very high conditional complexation constants are evaluated, much higher than those in the high salinity waters. This could indicate that the ligands of copper(II) are completely different in samples of different salinity, but this could also be due, at least in part, to the fact that the alkaline earth metals, which are at high concentration in waters at high salinity, effectively compete with copper(II) for the combination with the ligands, lowering the conditional complexation constant of Cu(II). It must be observed that the alkaline earth metals are adsorbed on Amberlite CG 50 at the acidity here considered. This has been taken into account in the evaluation of K^*, reported in Table 2, according to the evaluation method previously described (Pesavento *et al.*, 1995). The fact that K^* is considerably lower in high salinity waters is due exactly to the competition by alkaline earth metals for the sorption on the complexing resin. Copper(II) is sorbed also from high salinity waters, but with a lower sorption coefficient K^*. This is also the reason why less strong ligands are titrated in the high salinity waters than in freshwaters.

The conditional constants determined in high salinity waters is high enough to completely bind copper(II) originally present.

The conditional constants in the low salinity waters here evaluated are much higher than those obtained by all other laboratories participating to the intercomparison test (van Veen *et al.,* 2002), while those in the high salinity waters are somewhat lower than those evaluated by two of the laboratories, and higher than those evaluated by the others, using ASV techniques.

Of course, the accuracy of the evaluation depends on the accuracy in the determination of the concentration of free metal ion concentration. This is evaluated from the concentration of metal ion sorbed on the resin, determined experimentally, according to eq. (1). Thus the accuracy depends also on the accuracy of $K*$. The accuracy of the determination can be demonstrated by examining model solutions, containing known ligands at known concentration, as reported in the next paragraph.

Ligand concentration and conditional complexation constant for Cu(II) of a model solution containing EDTA

The ligand titration method proposed here has also been used for examining sample G, containing the known ligand EDTA at unknown concentration. The total ligand concentration here found was very near to that determined by the other laboratories participating to the intercomparison (van Veen *et al.*, 2002). So all the methods used seem to be equally accurate as far as the determination of the EDTA concentration is concerned. The differences actually found by different methods in the natural waters examined (van Veen *et al.*, 2002) must be ascribed either to a different accuracy in the real samples, for instance to the presence of interfering substances, or to the different detection windows of the methods. It could also be due to kinetic reasons. In the ligand titration by sorption on complexing resin the equilibration time is long, 16 hours , as required to reach the equilibrium in the sorption process, much longer than in ligand titration monitored by voltammetric methods. For this reason complexes with a low formation rate, which can not be detected by more rapid methods, for instance the voltammetric ones, can be determined by the method here used. Evidently this is not the case for EDTA, but it could be in the case of natural ligands.

The standard deviation of the ligand concentration (c_I) is good, as is also the reproducibility between the replicates (see Table 2). The precision obtained in the case of the synthetic sample is much better than that of the natural waters, probably because in natural waters different ligands are present with somewhat different constants, and the sum of the concentration of all the ligands, with somewhat different complexation constants, is actually evaluated.

The conditional constant Cu/EDTA here evaluated is reported in Table 2 (sample G), and is acceptably near to that obtained in the previous literature (Martell *et al.*, 1974). This is very important in order to strengthen the confidence in the results obtained by the proposed procedure, even if the accuracy in the real samples can be well different from the synthetic solution here examined, containing only one ligand, of low molecular mass and well defined chemical structure. It is interesting to observe that the value obtained for this conditional complexation constant by the other methods used in the interlaboratory test (van Veen *et al.*, 2002) are often orders of magnitude lower than the literature value.

CONCLUSIONS

The ligand titration monitored by sorption of copper(II) on Amberlite CG 50 can be done for determining the concentration of ligands and the conditional stability constant with copper(II) in natural waters at high and low salinity. It is possible to predict that the ligands forming with copper(II) complexes with α_M higher than $0.1\, K*\, w\, /V$ are titrated, and only the conditional constant of the complexes with α_M between $10\, K*\, w\, /V$ and $0.1\, K*\, w\, /V$ can be determined. This cannot be done for complexes with α_M higher than $10 \cdot K*\, w\, /V$ since the metal ion linked to such complexes is not sorbed by Amberlite CG 50 under the considered conditions.

The main drawback of the ligand titration here presented is that it is time consuming, even if the equilibration with the resin does not require the active presence of the operator and can be carried out simultaneously for a large number of subsamples. On the other hand the long time required to reach the equilibrium in the metal sorption on the resin, allows the metal ion to equilibrate also with the ligands in solution phase, even if the complexation rate is low.

Other negative points can be that a large sample volume is required, for instance a total of around 500 ml for the titrations here described, and that the reproducibility is not good. This is in part due to the low number of points used for the evaluation, but probably more to the fact that the only seldom the ligands titrated are exactly homogeneous.

A positive aspect is that a separation of the considered metal from the original matrix and a preconcentration is achieved, so reducing the matrix interferences in the final detection method, which was here EA-AAS. Another positive aspect is that, at least in principle, the free metal ion concentration can be actually evaluated according to eq. (1), and used for the conditional stability constant evaluation, instead of the operationally defined "labile metal", and this allows an accurate determination of the conditional constant. This was demonstrated by the correct determination of the con-

ditional stability constant of the complex Cu(II)/EDTA, at low EDTA concentration, carried out in this investigation.

In all the natural waters here considered ligands with α_M higher than $0.1 \cdot K^* w / V$ have been found to be present, at concentration higher than that of copper(II) originally present, and the conditional constant was always high enough to substantially decrease the concentration of free copper(II) and its toxicity (Gardner *et al.*, 2000). On the other hand, the presence of ligands can increase the mobility of the metal ion, so contributing to its diffusion in the environment. Moreover it can not be excluded that also ligands at lower concentration, but forming stronger complexes, are present. The corresponding stability constant can not be determined, being to high for the method here proposed. To investigate them a stronger resin should be employed.

ACKNOWLEDGEMENTS

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