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Copper complexation by thiol compounds in estuarine waters

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Abstract

The stability of copper complexes with thiol substances in estuarine waters was determined for the first time using a new procedure based on cathodic stripping voltammetry (CSV). The free thiol concentration was monitored during titrations with copper in the presence of a competing ligand salicylaldoxime (SA); concentrations of copper-complexing ligands and conditional stability constants were determined simultaneously but independently. The decrease in the free thiol concentration with increasing copper concentration was used as an independent measure of the thiol-complex stability. The conditional stability constant of the thiol complexes (log $K'_{CuThiol}$) was between 12.3 and 14.1, and decreased with increasing salinity. The copper complexing titrations were found to fit to two complexing ligands: L_1 with concentrations between 10 and 33 nM, and L_2 between 14 and 300 nM. The complex stability of most of the thiols was similar to that of CuL_2. Titrations at different detection windows showed a shift in the thiol complex stability suggesting that a second thiol species was present. It is therefore possible that L_1 is also a thiol species. The estimated thiol concentrations can account for up to half of the total ligand concentration at low to intermediate salinities and for all of the ligands at high salinities.
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Keywords: Copper; Thiol; Salicylaldoxime; Metal speciation; Estuarine waters

1. Introduction

Complexation by organic matter dominates the chemical speciation of the biogenic metals copper, zinc, cobalt and iron in sea water (Ellwood and van den Berg, 2001; Gledhill and van den Berg, 1994; Moffett, 1995; Saito and Moffett, 2001; van den Berg et al., 1987). This complexation is important because it affects the availability of metals to organisms, for instance by facilitating the uptake of iron (Maldonado and Price, 1999); there is some evidence that the Cu^{2+} ion induces the release of complexing ligands by sensitive phytoplankton like cyanobacteria (Moffett and Brand, 1996) and several other species in sea water (Croot et al., 2000; Leal et al., 1999) as well as freshwater (van den Berg et al., 1979) probably in order to ameliorate toxic effects of copper. Organic complexation may also control the geochemistry of metals by reducing the rate of scavenging; a relationship between the concentrations of dissolved copper and zinc with that of their respective ligand concentrations in the Scheldt estuary (van den Berg et al., 1987) has suggested that interactions between

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sediments, suspended particles and dissolved organic complexation controlled the residual metal concentrations in estuarine waters with a high supply of metals.

Little is known about the source of the ligands in estuarine waters, and the studies into this have been sparse. There is some evidence that the ligands may be sulphide species (Rozan et al., 2000), or thiol compounds (specifically for copper) (Leal and van den Berg, 1998), but also colloidal (Tang et al., 2001); the ligands may originate from the sediments (Skrabal et al., 1997) but also from the freshwater end of the estuary (Gerringa et al., 1996). The presence of specific thiols like glutathione in ocean waters (Le Gall and van den Berg, 1998) and estuarine waters (Tang et al., 2000), and the high stability of thiol complexes specifically with copper(I) (Leal and van den Berg, 1998) suggest that these are candidates for the identity of the copper-complexing ligands. Humic and fulvic acids can also function as copper complexing ligands forming quite stable complexes (Kogut and Voelker, 2001; Xue and Sigg, 1999).

In this work, we have determined the chemical speciation of copper in the estuary of the river Scheldt by cathodic stripping voltammetry (CSV) with ligand competition, and at the same time determined the complex stability with thiols present in these waters using a novel, independent, voltammetric procedure. The copper-thiol complex stability was determined by following the voltammetric peak height of the natural thiols during titrations with copper, whilst at the same time monitoring the copper speciation from the CSV peak height for copper complexed with salicylaldoxime (SA). The method and the study of the Scheldt estuary are presented here.

2. Materials and methods

2.1. Instrumentation and reagents

Voltammetric equipment consisted of an Autolab PGStat 10 voltammeter connected to a Metrohm VA 663 electrode stand, and controlled by a computer (PC). The reference electrode was double junction, Ag/AgCl, KCl (3 M), saturated AgCl, with a salt-bridge filled with 3 M KCl, and the counter electrode was a glassy carbon rod. pH measurements were with a Metrohm 605 pH meter. For pH monitoring experiments, a narrow-neck pH electrode was inserted in the voltammetric cell for the duration of the experiment. Calibrations were against NBS pH buffers.

Water used for dilutions was Milli-Q (MQ); methanol and HCl were purified by sub-boiling distillation on a quartz condenser. A stock solution of 0.01 M SA (BDH) was prepared in 0.1 M HCl. Metal contamination of SA was removed by recrystallisation from methanol into HCl followed by MQ-water addition. The pH buffer contained 1 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, Aristar grade) and 0.55 M NaOH. The addition of 100 μl of the buffer solution to the samples yielded a pH of 7.8 (NBS scale). Standard copper solutions were prepared by dilution of an atomic absorption standard solution (Spectrosol, BDH) with MQ, and acidified to pH 2.2 using 6 M HCl.

Stock solutions of thioacetamide, glutathione, thiourea, cysteine (all from BDH) and 3-mercaptopropionic acid (Fluka) were prepared freshly before use by dissolution in MQ water.

2.2. Sample collection

Samples for this study were collected during a cruise with RS Navicula (April 2001) on the estuary of the river Scheldt in the Netherlands. Water was continuously pumped on-board ship from a depth of ~1 m using a peristaltic pump via a 20-m poly(vinyl chloride) (PVC) hose, which was attached to an epoxy-coated, iron, ‘‘fish’’. The water was passed through two filtration cartridges, one containing two filters, of 0.8 and 0.4 μm pore-size, and a second with a 0.2 μm pore-size filter. Samples were collected in 500-ml acid-cleaned polyethylene bottles and then immediately frozen until the day before analysis. Samples were thawed overnight at 4° and carefully swirled to ensure dissolution of any particulates that might have formed by the freezing process.

The study area was the estuary of the river Scheldt, which was sampled at 13 stations covering the entire salinity range, the final sample being

taken in the North Sea (salinity 33), close to the coast, north of the mouth of the Scheldt estuary (Fig. 1).

2.3. Copper determinations

The concentration of dissolved copper was determined using a procedure similar to that described before (Campos and van den Berg, 1994). Seawater was UV-digested (1 h) after acidification to pH 2.2 by addition of 10 μl 6 M HCl per 10 ml of sample in acid cleaned silica tubes. A 10-ml sample aliquot was pipetted into the voltammetric cell and ammonia was used to approximately neutralise the pH; HEPES buffer (100 μl, final concentration 0.01 M) and SA (30 μl, final concentration 30 μM SA) were added. The solution was deaerated by purging (5 min) with nitrogen. Voltammetric parameters were: deposition 30 s at −1.1 V whilst stirring; 8 s quiescence at −0.1 V; potential scan using the square-wave modulation: 10 Hz, step height 2.5 mV, pulse height 25 mV, from 0 to −0.8 V. The sensitivity was calibrated by standard copper additions to each sample.

2.4. Copper complexing ligand titrations

The procedure used for the copper titrations was similar to that described before (Campos and van den Berg, 1994). A 130-ml thawed sample was transferred to a Teflon bottle, and HEPES buffer (0.01 M) and SA (usually 10 μM, except when the detection window was varied) were added. The 10-ml aliquots were pipetted into 12, 30 ml, polystyrene vials (Bibby, Sterilin), previously spiked with copper to give a concentration range between 0 and up to 300 nM added copper (actual range depending on the initial copper concentration and the determined ligand concentration), and allowed to equilibrate overnight at room temperature. Prior to the first titration the tubes were conditioned twice with water containing the same range of copper

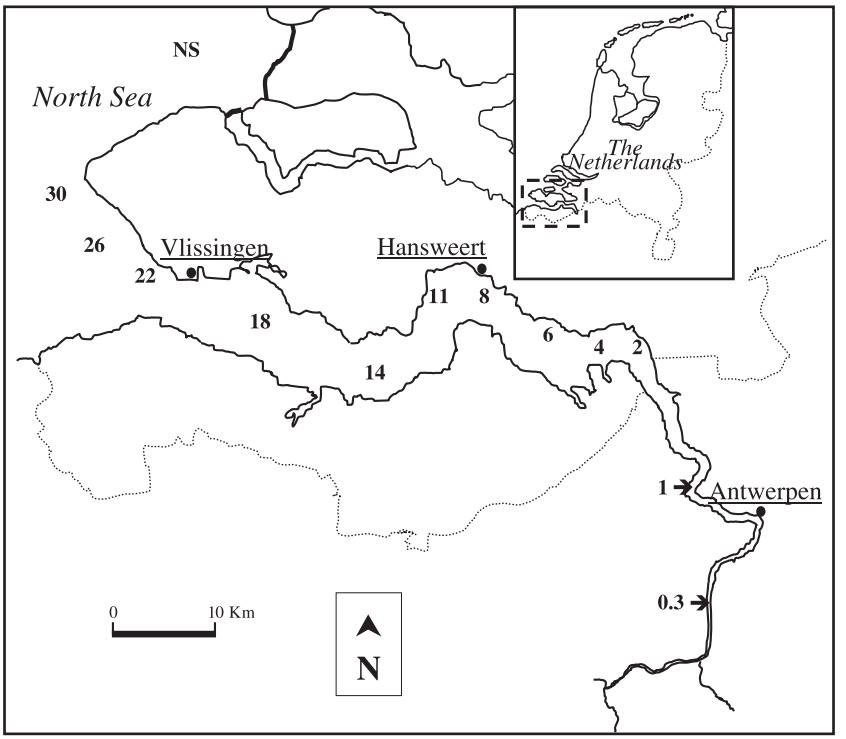

Fig. 1. Map showing the estuary of the Scheldt and the sample locations.

concentrations. The equilibrium concentration of copper bound by SA was determined by CSV using an adsorption potential of −0.1 V and a deposition time of 60 s. The sensitivity was calibrated using copper additions (two or three depending on the relative peak heights) to the voltammetric cell containing the aliquot with the highest copper concentration where the copper concentration>ligand concentration; the peak height was recorded one minute later, thus allowing for equilibration with SA, which is present at great excess, but minimizing equilibration with the unknown ligands L. The labile copper concentration at the start of the calibrating additions typically was 100–150 nM.

2.5. Thiols

Thiols were determined by CSV using a method (Leal et al., 1999) adapted by measuring in the presence of SA as this lowers the free copper concentration thus releasing thiols making them available for detection. The voltammetric conditions were the same as for labile copper: adsorption at −0.1 V, followed by a scan from that potential. The thiol peak was located at approximately −0.5 V (this peak potential varied with the copper concentration).

The stability of thiol–copper complexes was determined from measurement of the thiol peak height as a function of the copper concentration. These titrations were in the presence of SA so the copper-SA peak height could be used to calculate the pCu in each aliquot, whereas the ratio of the (peak current in the presence/peak current in the absence of added copper) was used as a measure of the ratio of the free/total thiol concentration.

3. Theory

3.1. Evaluation of complexing capacity titrations

The procedure to evaluate complexing ligand concentrations and conditional stability constants from titrations with copper has been described before (Ruzic, 1982; van den Berg, 1982; van den Berg and Kramer, 1979). The following relationship was used to test the data for the presence of a single complexing ligand:

$$\frac{[\mathrm{Cu}_{\mathrm{labile}}]}{[\mathrm{CuL}]} = \frac{[\mathrm{Cu}_{\mathrm{labile}}]}{[C_{\mathrm{L}}]} + \frac{\alpha_{\mathrm{Cu}}}{K'_{\mathrm{CuL}} C_{\mathrm{L}}} \quad (1)$$

where [CuL] is the concentration of copper complexed by natural ligands L, $[\mathrm{Cu}_{\mathrm{labile}}]$ is the labile copper concentration, C_{L} is the concentration of natural copper ligands, α_{Cu} is the α-coefficient of Cu^{2+} with inorganic complexes and SA, and K'_{CuL} is the conditional stability constant for the formation of CuL. This constant is dependent not only on the affinity of the ligands for copper, but also on the medium composition (salinity, pH, etc.).

A plot of $[\mathrm{Cu}_{\mathrm{labile}}]$ versus $[\mathrm{Cu}_{\mathrm{labile}}]/[\mathrm{CuL}]$ is straight in case a single ligand is present, and it is curved when a second or more ligands are detected with different complex stability. In case of linearity, the ligand concentration and the conditional stability constant can be calculated from the slope and *Y*-axis intercept of the plot using a linear least squares regression of $[\mathrm{Cu}_{\mathrm{labile}}]$ versus $[\mathrm{Cu}_{\mathrm{labile}}]/[\mathrm{CuL}]$; however, all samples showed curvature and an iterative fitting procedure was used to fit the data to two ligands.

The labile copper concentration is defined by:

$$[\mathrm{Cu}_{\mathrm{labile}}] = \Sigma[Cu(\mathrm{SA})_x] + [\mathrm{Cu}'] \quad (2)$$

where $\Sigma[\mathrm{Cu(SA)}_x]$ is the concentration of copper complexed by SA and [Cu′] is the concentration of inorganic copper (free ionic and complexed by inorganic ligands). The labile copper concentration was calculated by dividing the copper peak height (nA) by the sensitivity S (nA/nM). The sensitivity was calculated from a linear least squares regression of the in-cell copper additions (at copper concentrations>ligand concentration) made to the final aliquot of the titration.

The ionic copper concentration is related to the labile copper concentration through

$$[\mathrm{Cu}^{2+}] = [\mathrm{Cu}_{\mathrm{labile}}]/\alpha_{\mathrm{Cu}}$$

where α_{Cu} is the overall α-coefficient of copper:

$$\alpha_{\mathrm{Cu}} = \alpha_{\mathrm{Cu}'} + \alpha_{\mathrm{CuSA}} \quad (3)$$

where $\alpha_{Cu'}$ is the α-coefficient for complexation of Cu^{2+} with the major anions and α_{CuSA} that for complexation of copper by SA:

$$\alpha_{CuSA} = K'_{CuSA}[SA'] + \beta'_{Cu(SA)_2}[SA']^2 \quad (4)$$

where K'_{CuSA} and $\beta'_{Cu(SA)_2}$ are the conditional stability constants for the formation of CuSA and $Cu(SA)_2$, respectively, and [SA′] is the concentration of SA not complexed by copper. Values for the Cu–SA stability constants were calculated for each salinity using the following relationships (Campos and van den Berg, 1994):

$$\log K'_{CuSA} = (10.12 \pm 0.03) - (0.37 \pm 0.02)\log S \quad (5)$$

$$\log \beta'_{Cu(SA)_2} = (15.78 \pm 0.08) - (0.53 \pm 0.07)\log S. \quad (6)$$

3.2. Iterative data fitting to two ligands

The plots of most copper titrations showed curvature, indicating the presence of more than one type of ligands with a different affinity for copper. In these cases, ligand concentrations and stability constants were obtained by an iterative calculating method (van den Berg, 1984). Initial estimates for K'_1 and C_{L_1} (the stronger ligands) were obtained through linearization of the first few data points. Then the contribution of L_1 to the complexation was subtracted for the remainder of the titration data, and K'_2 and C_{L_2} were calculated using a second linear least squares regression of these data. The process was repeated for the first part of the data but now subtracting the contribution of L_2 to these data, and this method was iterated. The first (low copper) part of the data was fitted to a Scatchard equation in case of non-convergence to L_1 (Laglera-Baquer et al., 2001).

The free, ionic, copper concentration in the original seawater (in the absence of SA) was calculated from the following quadratic equation:

$$\begin{aligned}&[Cu^{2+}]^2(K'_{CuL_1}(\alpha_{Cu'} + \alpha_2) + K'_{CuL_2}(\alpha_{Cu'} + \alpha_1)) \\ &\quad + [Cu^{2+}](K'_{CuL_1}(C_{L_1} - Cu_{TOT}) \\ &\quad + K'_{CuL_2}(C_{L_2} - Cu_{TOT}) + \alpha_{Cu'}) - Cu_{TOT} = 0\end{aligned} \quad (7)$$

where α_1 and α_2 are the α-coefficients for the complexation of the stronger and weaker copper ligands. Thus, the overall α-coefficient for the complexation of copper with both natural organic ligands (α_{CuL}) is:

$$\alpha_{CuL} = \alpha_1 + \alpha_2 = ([CuL_1] + [CuL_2])/[Cu^{2+}] \quad (8)$$

A first estimate of the free ion concentration was obtained by setting α_1 and α_2 to zero. This value was then used to calculate the concentrations of CuL_1 and CuL_2 in the original seawater, which were then used to obtain estimates for α_1 and α_2. These values were refined by iteration.

3.3. Determination of the stability of the copper–thiol complexes

The conditional stability constants of the copper–thiol complexes were determined by detection of the labile thiol concentration by CSV during titrations with copper of the samples. The presence of thiols was apparent from a peak at −0.5 V. This peak is due to complexation of the thio-group with mercury from the electrode and adsorption of the complex species: (1) adsorption step at −0.1 V: Thiol-SH + Hg(0) → Thiol-S-Hg(II)$_{adsorbed}$ + $2e^-$ + H^+. During the voltammetric scan, the mercury(II) in the complex is reduced: (2) Thiol-S-Hg(II)$_{adsorbed}$ + H^+ + $2e^-$ → Thiol-SH + Hg(0).

Binding with copper caused the CSV peak to decrease as its complexation with the thiol compound competes with the formation of the mercury species. The underlying principle is therefore one of competition between copper and mercury for complexation with the thiol compound, and the effect of this that the mercury-thiol peak can be used as a measure of the reactive thiol concentration. The decrease of the reactive thiol concentration upon copper additions was used as a measure of the complex stability. This method is analogous to that used before to determine the stability constants of sulphide with trace metals in seawater (Al-Farawati and van den Berg, 1999; Zhang and Millero, 1994), except that in this work the measurements were done in the presence of 10^{-5} M SA. The SA was added to lower the initial free copper concentration so as to release any thiols which were bound by the copper initially present in the water, and at the same time the CSV peak height for CuSA could

be used as a measure of the Cu^{2+} concentration. SA additions above 10^{-5} M did not cause a further increase in the thiol peak suggesting that approximately all thiol had been released from the copper and that the initial voltammetric signal corresponded with the total thiol concentration.

The term [thiol′] denotes the labile thiol concentration, i.e. that not-complexed by copper in the samples, comprising the free thiols and those complexed by the major cations (the S-group on the thiols is omitted in the equations). Without copper additions and in the presence of SA the thiol concentration approximately equals the reactive thiol concentration:

$$C_{\text{thiol}} = [\text{thiol}'] \tag{9}$$

Copper additions caused the thiol peak to decrease as a result of metal–thiol complex formation. This reaction is expressed as:

$$Cu^{2+} + \text{Thiol}' \leftrightarrow \text{CuThiol} \tag{10}$$

The conditional stability constant of this reaction is

$$K'_{\text{CuThiol}} = \frac{[\text{CuThiol}]}{[Cu^{2+}][\text{thiol}']} \tag{11}$$

It is assumed for now that the thiol–copper complexes are of the type 1:1. The use of a model based on 2:1 thiol–copper complexes, or on both types, did not improve the data fitting. Without being able to vary the thiol concentration in a particular sample it is not possible to verify whether the complexes are 1:1 or 2:1.

The mass balance for the thiols is:

$$C_{\text{thiol}} = [\text{Thiol}'] + [\text{CuThiol}] \tag{12}$$

Combination of Eqs. (11) and (12) yields a ratio, R, of free over total thiol:

$$R = \frac{[\text{thiol}']}{C_{\text{Thiol}}} = \frac{1}{1 + K_{\text{CuThiol}}[Cu^{2+}]} \tag{13}$$

The free metal ion concentration was obtained from the height of the copper–SA peak and the sensitivity:

$$[Cu^{2+}] = i_{\text{p,CuSA}}(S\alpha_{Cu})^{-1} \tag{14}$$

Values for the conditional stability constants K'_{CuThiol} were calculated by non-linear least-squares regression of Eq. (13) as a function of $[Cu^{2+}]$ using Sigma Plot 2000 (SPSS).

As we did not know the identity of the thiol compound(s), we could not calibrate the measurements in terms of specific thiol concentrations. The ratio R was nevertheless accessible by CSV as it could be obtained from the ratio of the thiol peak height in the presence of copper (i_{thiol}) over that in the absence of copper ($i_{\max}$):

$$R = \frac{[\text{thiol}']}{C_{\text{Thiol}}} = \frac{i_{\text{thiol}}}{i_{\max}}. \tag{15}$$

3.4. Estimation of an upper limit for the thiol concentration

Although it was not possible to calculate the thiol concentration from the titrations, an estimate of their concentration could be made. During the copper titration, increasing copper additions raised the free copper ion concentration in the sample. When this concentration equalled the inverse of the copper–thiols stability constant:

$$[Cu^{2+}] = K'_{\text{CuThiol}}{}^{-1} \tag{16}$$

Eq. (11) was simplified to

$$[\text{Cu(thiol)}] = [\text{thiol}'] \tag{17}$$

From the mass balance of the thiol (Eq. (12)), we can infer that at this stage of the titration half the thiol concentration is complexed by copper, i.e.:

$$C_{\text{thiol}} = 2[\text{CuThiol}] \tag{18}$$

By assuming that at this point of the titration ($[Cu^{2+}] = 0^{-1}$) all copper complexed by ligands of the type of L_2 is complexed by thiols, we can get an upper limit for the thiol concentration:

$$C_{\text{Thiol}} \leq [\text{CuL}_2]*2 \ (\text{when} [Cu^{2+}] = K'_{\text{CuThiol}}{}^{-1}) \tag{19}$$

It is an upper limit (and may be an overestimate) as L_2 may also consist of ligands other than thiols. Copper complexed by L_1-type ligands was excluded as their complex stability was greater. The copper

speciation where Eq. (18) was valid was calculated from the known complexing parameters ([Cu^{2+}], C_{L_1}, C_{L_2}, K'_{CuL_1}, K'_{CuL_2}).

4. Results

4.1. Dissolved copper

The dissolved copper concentration was between 9 nM at the high salinity end and 23 nM at the low salinity end of the estuary (Fig. 2 and Table 1). A plot of copper as a function of salinity (Fig. 2) was curved suggesting that copper was released from the sediments in the upper estuary, at salinities up to 20. At salinities greater than about 22, the copper decreased relatively steeply from 20 to 9 nM. The copper concentration across the estuary (10–30 nM) was similar to that found in other recent studies (Baeyens et al., 1998; Paucot and Wollast, 1997; Zwolsman et al., 1997), but much less than found in 1987 (van den Berg et al., 1987) when low salinity copper levels were as high as 150 nM. This drop in the copper concentrations from high levels in the 1970s and 1980s has been attributed to higher oxygen concentrations in the estuary in the 1990s (Nolting et al., 1999): apparently copper was mobilised from sediments due to anoxic conditions in the upper estuary, and improvements in the upper estuary have much reduced this anoxia, or the riverine copper inputs have been reduced. An important feature in the cross-estuarine copper distribution is the apparent mobilisation of copper at intermediate salinities, which was apparent in several previous studies (e.g., Paucot and Wollast, 1997; Zwolsman et al., 1997) (clearly apparent also in our data as a broad bulge in the dissolved copper concentration on Fig. 2) which has been ascribed to releases from the particulate phase (Baeyens et al., 1998; Paucot and Wollast, 1997) but which can also be due to remobilization from the sediments as has been demonstrated for arsenic in estuarine conditions (Knox et al., 1984) and for various metals on the continental shelf (Kremling, 1983; Muller et al., 1994; Zhang et al., 1995).

4.2. Organic complexation of copper

Ligand concentrations and conditional stability constants for copper complexes in the estuary are summarised in Table 1 and Fig. 2. Initial complexing capacity titrations were carried out at a lower detection window using 2 μM SA, causing the labile copper concentration to be immeasurably low until 10–20 nM copper additions. For this reason, the detection window was raised by using a higher concentration of 10 μM SA. The plots according to Eq. (1) were curved for all samples indicating that two or more ligands controlled the copper speciation in the range of copper concentrations tested. A change in the slope of these plots indicates that the speciation is

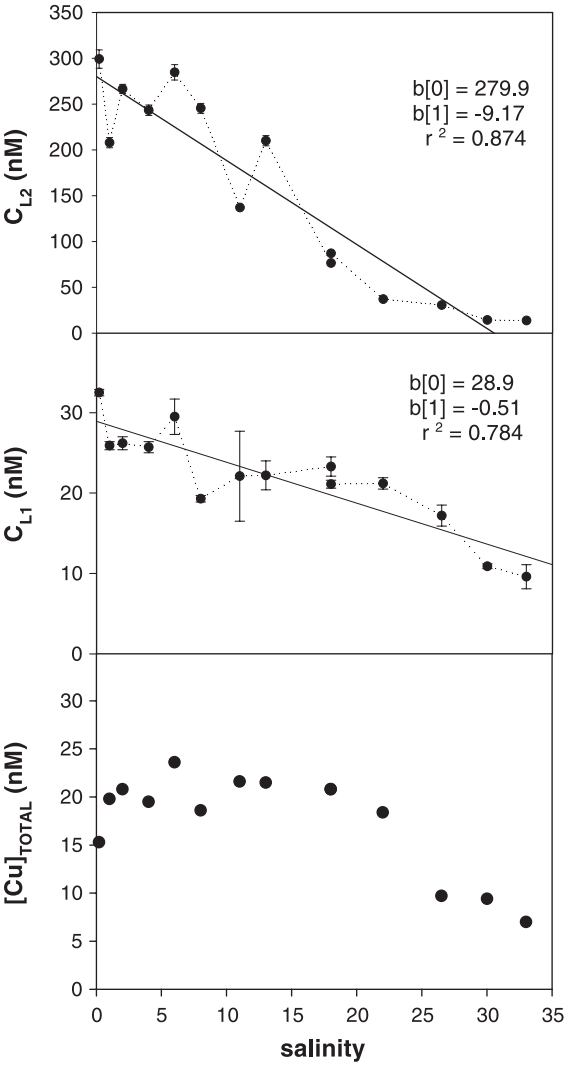

Fig. 2. Concentrations of the copper complexing ligands (L_1 and L_2) and dissolved copper in the Scheldt estuary.

Table 1
Dissolved copper and ligand concentrations (nM), conditional stability constants, pCu values, the α-coefficient for the SA-copper complex in the analysis conditions (SA concentration and salinity), and the overall α-coefficient for the natural ligands in the Scheldt estuary and North Sea

Salinity	C_{CU}	C_{L1}	log K_1	C_{L2}	log K_2	pCu	log α_{CuSA}	log α_{CuL}
0.2	15.3	32.5 ± 0.4	15.85 ± 0.14	299 ± 10	13.46 ± 0.04	15.96	6.13	8.14
1	19.8	25.9 ± 0.5	16.14 ± 0.20	208 ± 6	13.13 ± 0.04	15.67	5.87	7.96
2	20.8	26.2 ± 0.8	15.75 ± 0.43	266 ± 5	13.14 ± 0.03	15.32	5.72	7.64
4	19.5	25.7 ± 0.7	15.74 ± 0.19	243 ± 6	12.96 ± 0.03	15.33	5.57	7.62
6	23.6	29.5 ± 2.2	15.01 ± 0.42	285 ± 9	12.80 ± 0.04	14.69	5.48	7.07
8	18.6	19.3 ± 0.4	15.28 ± 0.05	245 ± 5	12.72 ± 0.02	14.64	5.42	6.91
11	21.6	22.1 ± 5.6	14.71 ± 0.33	137 ± 2	12.83 ± 0.03	14.24	5.35	6.58
13	21.5	22.2 ± 1.8	14.91 ± 0.23	210 ± 6	12.70 ± 0.03	14.37	5.31	6.71
18	20.8	23.3 ± 1.2	14.68 ± 0.29	76 ± 3	12.79 ± 0.08	14.21	5.24	6.48
18	20.8	21.1 ± 0.5	15.01 ± 0.08	87 ± 2	12.89 ± 0.04	14.21	5.24	6.56
22	18.4	21.2 ± 0.7	14.80 ± 0.34	37 ± 2	13.18 ± 0.18	14.32	5.20	6.58
26.5	9.7	12.9 ± 1.7	14.84 ± 0.37	35 ± 0.8	13.20 ± 0.07	14.58	5.16	6.57
30	9.4	10.9 ± 0.3	14.86 ± 0.09	14.3 ± 1.4	13.00 ± 0.18	14.27	5.14	6.24
North Sea	7.0	9.6 ± 1.5	14.51 ± 0.36	13.7 ± 0.8	13.24 ± 0.11	14.30	5.12	6.15

The complexation parameters were calculated by iterative linearization.

predominantly controlled by one (relatively strong binding) ligand (L_1) at low copper concentrations (including the original ambient concentration), changing to a different, weaker binding, ligand (L_2) when the copper concentration was increased during the titration. The change in slope occurred when L_1 was saturated with added copper, so at higher than the normal ambient copper concentration. The change of slope occurred at pCu values between [13.7–13.9] at high salinity and [13.9–14.4] at low salinity.

The speciation of copper was predominantly controlled by L_1 at the ambient copper concentration, whereas L_2 would be important in natural conditions only if L_1 were to become saturated for instance by copper released from the sediments; during this study L_1 was not saturated and its concentration was always greater than that of copper (Fig. 3).

The concentrations of both L_1 and L_2 decreased with increasing salinity, L_1 from 33 to 10 nM and L_2 from 300 to 14 nM (Table 1 and Fig. 2). The decrease with increasing salinity suggests a predominantly low salinity origin of both ligands, which were diluted by seawater containing lower ligand concentrations. Both ligands showed a mid-estuarine bulge in their concentration, suggesting sedimentary releases such as due to bacterial break-down processes, but L_2 showed this increase at lower salinity.

The complex stability decreased with increasing salinity: log K'_{CuL_1} decreased from 15.8 to 14.8 and log K'_{CuL_2} from 13.5 to 13. These constants are conditional upon the water composition and a decrease with increasing salinity is in line with expectation due to the increased major ion competition; this decrease does not provide evidence for the presence of different ligands at higher salinity.

The overall organic complexing of copper, as expressed by α_{CuL}, decreased with increasing salinity (Fig. 4) from 10^8 to $10^{6.5}$: most of this change occurred at salinities below 8 and is mostly due to

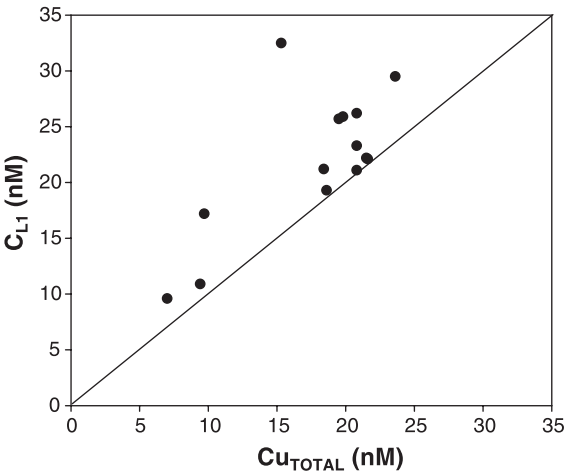

Fig. 3. Plot of the concentration of strong copper binding ligands (L_1) as a function of the copper concentration in the estuary showing that the trend in the ligands was similar to that of copper but the concentration of copper was always less than that of the ligands.

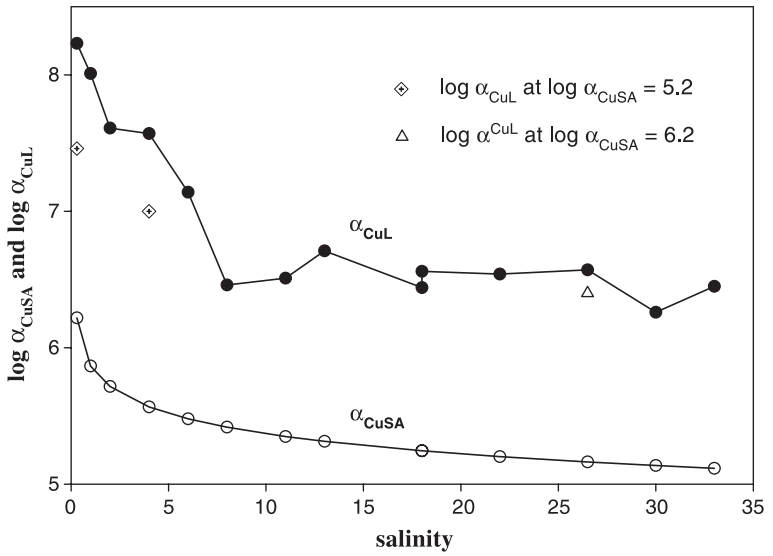

Fig. 4. Variation of the α-coefficients with salinity for the copper-complexation of SA and natural ligands in the Scheldt estuary, determined using the standard CSV condition of 10 μM SA. The symbol ◇ shows results for samples of salinities 0.3, 4 and 26.5 analysed at different SA concentrations.

the reduced complex stability as the ligand concentrations do not show a large change over this salinity range. A practical consideration is that the stability of the copper complex of salicylaldoxime (α_{CuSA}) also decreases (from $10^{6.3}$ to $10^{5.5}$) in the same salinity range, which would have shifted the detection window.

4.3. Effect of variation of the detection window

A shift in the detection window can cause a shift in the detected complex complexing ligands, and therefore in the apparent stability of the complexes and the ligand concentration, if the water contains several complexing ligands or complexing sites (van den Berg and Donat, 1992). By keeping the analytical conditions, such as the pH and the concentration of SA, constant, the detection window is constant and the analyses are internally consistent. However, in estuarine waters, the detection window varies due to changes in the salinity also at a constant SA concentration. At the concentration of SA used here (10 μM), the centre of the detection window (which equals α_{CuSA}, the α-coefficient for copper complexation by SA) moved from $10^{6.2}$ at a salinity of 0.2–$10^{5.1}$ at a salinity of 30, i.e. a shift of an order of magnitude. The apparent increase in the degree of copper complexation with decreasing salinity (Fig. 4) might therefore have been caused, fully or in part, by the shift in the detection window.

The possibility of a detection window effect was investigated by varying the SA concentration to maintain the detection window approximately constant at different salinities, and the copper speciation was evaluated at two detection windows. It was found (Table 2) that also at a constant detection window the concentrations of L_1 and L_2 in the estuary decreased with increasing salinity, whereas variation of the detection window in given samples caused comparatively little change in the detected ligand concentrations and complex stabilities: at a detection window of log $\alpha_{CuSA} = 5.2$, the concentration of L_1 decreased from 43 nM at a salinity of 0.2–11 nM at a salinity of 30. This showed that most of the change was due to ligand dilution with seawater containing a lower concentration of complexing ligands rather than due to the change in the detection window.

The detection window effect was tested furthermore by repeating the determination at a different concentration of SA in several estuarine samples. The detected ligand concentration varied by a comparatively small amount when the detection window

Table 2
Complexing ligand concentrations (nM) at two detection windows in samples from various salinities

Salinity	[SA]	log α_{CuSA}	C_{L1}	log K'_1	C_{L2}	Log K'_2	log α_{CuL}	pCu
0.2	2.5×10^{-6}	5.17	43.4 ± 0.5	15.2 ± 0.2	333 ± 11	12.4 ± 0.1	7.66	15.5
4.0	6×10^{-6}	5.18	22.6 ± 0.3	15.5 ± 0.1	268 ± 8	12.4 ± 0.1	7.14	14.8
22.0	10^{-5}	5.20	21.2 ± 0.7	14.8 ± 0.3	37 ± 2	13.2 ± 0.2	6.58	14.3
26.5	10^{-5}	5.16	12.9 ± 1.7	14.8 ± 0.4	35 ± 1	13.2 ± 0.1	6.58	14.6
30.0	10^{-5}	5.14	10.9 ± 0.3	14.9 ± 0.1	14 ± 1.4	13.0 ± 0.2	6.24	14.3
0.2	10^{-5}	6.22	32.5 ± 0.4	15.8 ± 0.2	299 ± 10	13.5 ± 0.1	8.14	16.0
26.5	3.7×10^{-5}	6.22	12.5 ± 0.5	14.9 ± 0.6	–	–	6.40	14.4

The SA concentration was varied to maintain the value of log α_{CuSA} approximately constant at either ~ 5.17 or at 6.2.

was altered: increasing the detection window from (log values) 5.2 to 6.2 caused the concentration of L_1 to drop from 43 to 33 nM, and of L_2 from 330 to 300 nM, at a salinity of 0.2; and L_1 dropped from 17 to 13 nM, L_2 from 30 nM to undetectable, at a salinity of 26. At the same time, the value for log α_{CuL} went up by 0.5 (salinities of 0.2 and 4) when the detection window was raised by 1 log unit: on the whole therefore the change in the detected complex stability was much less than that in the detection window indicating that although more ligands may be present this number is limited and there was only a partial co-variation of α_{CuL} with α_{CuSA}. The value for α_{CuL} at a salinity of 26 apparently decreased when the detection was increased (Table 2), but this was due to L_2 becoming undetectable at the higher detection window so this point was not fully comparable.

4.4. Comparison with other studies

The ligand concentrations and conditional stability constants found in this study are broadly similar to those found in previous studies of the same estuary: in 1986, ligand concentrations were found between 26 and 206 nM with log K'_{CuL} values of 11.7–13 (van den Berg et al., 1987), whilst in 1992/3 ligand concentrations between 19 and 300 nM were found (Gerringa et al., 1998). Our values for log K'_{CuL_1} tend to be greater than those found before. The differences may be due to a greater predominance of L_2 type ligands in the 1986 study which took place in the presence of much higher copper concentrations; the 1992/3 data did not reach the same low salinity as this study and the data were fitted to a single ligand so would tend to be a weighted average of several ligands.

4.5. Are the natural ligands thiols?

The peak used to detect thiol-like substances was located between -0.5 and -0.6 V, and occurred in all samples. Several compounds are known to produce a CSV peak in this potential range, notably thiol compounds but also sulphide and thiourea (Forsman, 1984; Leal and van den Berg, 1998; Luther and Church, 1988). The peak of these compounds is similar as it is due to the reduction of mercury (originating from the electrode) complexed with the SH-group. The peak in the Scheldt estuary samples was stable indicating that it was not due to sulphide or bisulphide (Al-Farawati and van den Berg, 1997) (the peak was also less narrow than that produced by sulphide) so it was suspected that its origin was a thiol compound or thiourea. Thiol compounds are known to form very stable complexes with copper (Leal and van den Berg, 1998) so it is likely (in view of their abundance) that the thiol compounds are part of the natural complexing ligands. Specific thiol compounds like glutathione and phytochelatin have been shown to occur in estuarine waters (Le Gall and van den Berg, 1993; Tang et al., 2000) so these compounds are candidates for the composition of the unknown estuarine complexing ligands; however, the peak location and its electrochemistry, indicate that glutathione was not significant in these estuarine waters.

4.6. Determination of the copper–thiol complex stability

The conditional stability constants of the copper–thiol complexes were calculated from the labile thiol concentration, determined by CSV during titrations

with copper of the complexing ligands in the samples. It is likely that the dissolved thiol–Cu species is a 2:1 ($thiol_2Cu$) species, like that formed with glutathione (Leal and van den Berg, 1998) but for now a 1:1 composition was assumed as the identity of the thiol was not known.

The thiol peak did not increase further when the SA concentration was increased above that (10 μM SA) used for the copper complexing capacity titrations indicating that this caused nearly all thiols to be released from the copper originally present in the sample. This SA concentration was used therefore to simultaneously determine the thiol complex stability and the copper complexing capacity, in a single titration with copper. However, the determination of the complex stability of the Cu–thiol species was independently from that of the CuL species because the former was calculated from the free thiol peak, and the latter from the CuSA peak.

The voltammetric scans showing the decreasing thiol peak during a titration of Scheldt water are shown in Fig. 5; the shoulder of the increasing copper-SA peak is shown on the left at −0.3 V. It can be seen that the decreasing thiol peak shifts to more negative potentials with increasing copper concentration; this shift is opposite to the shift seen in the sulphide peak when this is titrated with metals (Luther et al., 1996) and it is probably caused by conversion of the mercuric–thiol species to a copper–thiol species at high copper concentrations; this is known to happen for glutathione (Le Gall and van den Berg, 1993). This can happen on the electrode surface during the scan:

$$\mathrm{HgThiol_{ads}} + \mathrm{CuSA} + 2e^- \rightarrow \mathrm{CuThiol_{ads}} + \mathrm{Hg(0)} + \mathrm{SAH}$$

or in solution. The progressive thiol peak shift to more negative potentials during the titration could be related to the presence of more thiols complexing copper with different stability. Copper(I) is known to form very stable complexes with several thiol compounds (Leal and van den Berg, 1998) so this mechanism is possible. The behaviour of these peaks is complicated because it is due to the reduction of the metal species (mercury or copper) rather than of the thiol itself. The shift in the thiol peak is not related to an interaction with SA or mixed species between copper, SA and thiol, because the same effects (peak lowering and shift) occurred for measurements with copper additions without SA.

The decrease in the peak height as a function of pCu is shown for each sample in Fig. 6, and the values for the conditional stability constants are presented in

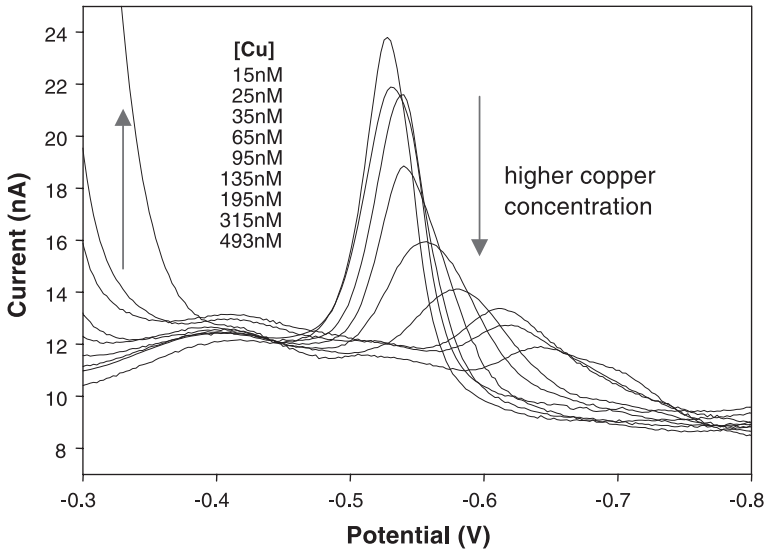

Fig. 5. Voltammetric scans showing the effect of copper additions on the thiol peak in a sample (salinity 2, 10 μM SA, pH 7.8) of the Scheldt estuary.

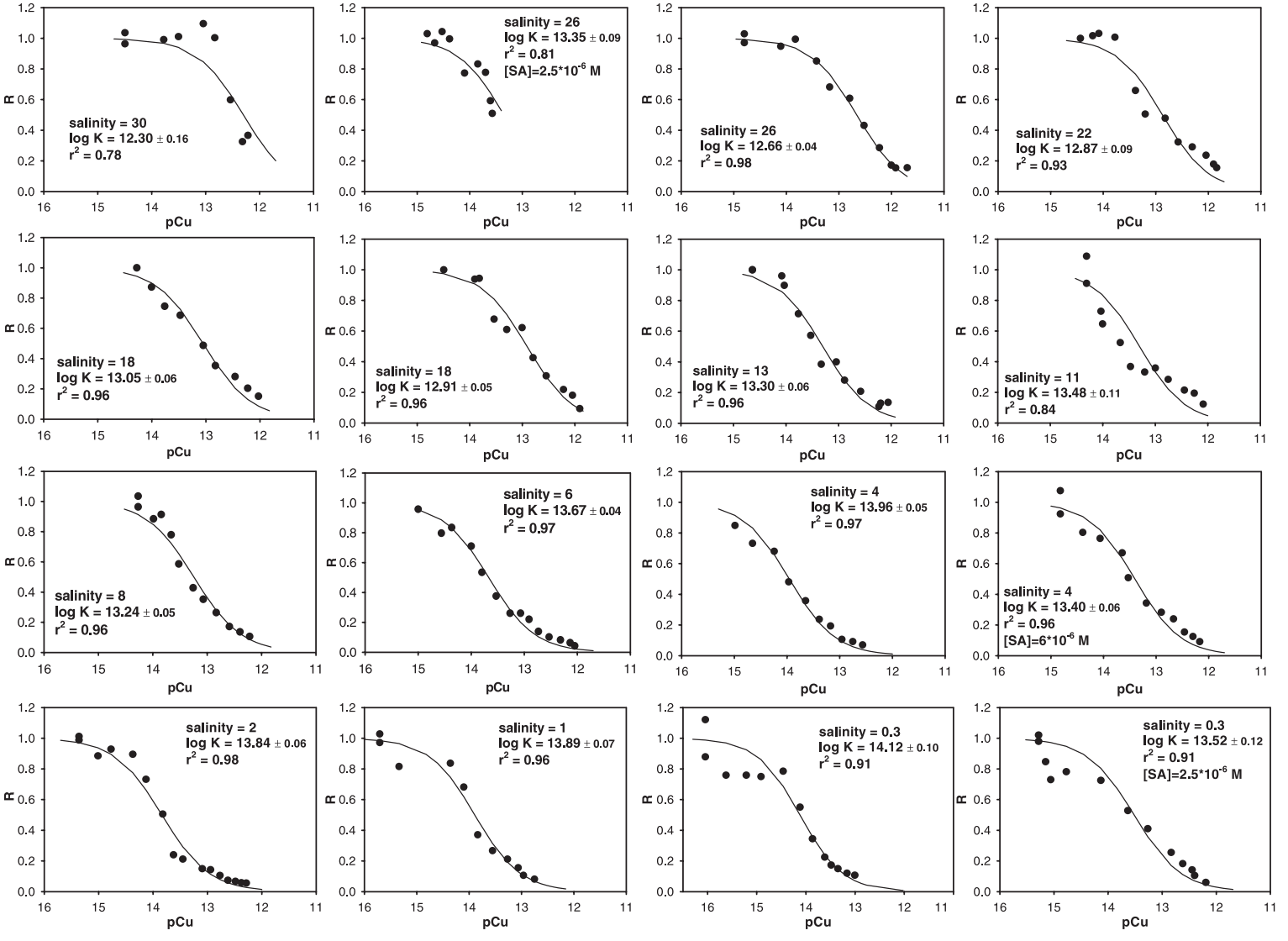

Fig. 6. Determination of the stability of copper complexes with thiol-like substances in the Scheldt estuary: each plot shows the decrease in the free thiol peak height with increased copper concentration for each sample. The curved line shows the model fit to the data by non-linear least-squares regression.

Table 3. It can be seen (Fig. 6) that the thiols become complexed with copper at pCu values between 13 and 14: at Cu^{2+} concentrations $>10^{-13}$ the thiols tend to be saturated with copper, whilst at concentrations $<10^{-14}$ the thiols tend to be "free" (not complexed with copper but they could be complexed with a major cation). The log $K'_{CuThiol}$ values are in the 12.3–14.1 range (13.35 ± 0.5), which is much less than found for the L_1 type ligands (15.1 ± 0.5) but more similar to that found for the L_2 ligands (13.0 ± 0.2) (Table 3): the thiol complex stability was greater than the CuL_2 species at salinities below 20, whilst it was smaller at higher salinities. These data suggests the dominant thiols (greatest contribution to the thiol peak height) are similar to the L_2-type ligands.

Three samples were titrated at two detection windows (at different concentrations of SA), and in each case the value for log $K'_{CuThiol}$ was 0.6 log units higher at the higher detection window (Table 3). The calculation of the complex stability was based on a single complex, and the variation in the complex stability with the detection window indicates that a second thiol species is present (or more), similar to the detection of two ligands from the complexing ligand titrations. This suggests that the same thiol peak was produced by at least two different thiol compounds. Because the determination of the thiol–Cu complex stability is based on the decrease of the combined, free, thiol concentration, the higher concentration of the weaker binding thiol tends to mask the second thiol even though it may form a more stable complex with copper. The shift in the detected Cu–thiol complex stability was much smaller than the difference between that for CuL_1 and CuL_2, but this is to be expected as the calculation of CuL_1 was dominated by the bottom part of the copper titration, whereas the Cu–thiol complex stability was calculated by curve fitting of all the data: due to the much higher concentration of L_2 its influence on the thiol peak-height tended to swamp the decrease in the peak height for L_1, making the presence of a L_1-type thiol apparent only from the shift in the complex stability.

Table 3
Conditional stability constants of copper-thiol substances in the Scheldt estuary. Values of K'_{CuL2} (Table 1) are shown for comparison. Thiol concentrations (C_{Thiol}) (nM) were estimated using Eq. (19) (see text)

Salinity	[SA] (M)	log $K'_{Cu\text{-}thiol}$	log K'_{CuL2}	estimated C_{Thiol}
0.2	10^{-5}	14.1 ± 0.1	13.5 ± 0.1	107
0.2	2.5×10^{-6}	13.5 ± 0.1	12.3 ± 0.1	47
1	10^{-5}	13.9 ± 0.1	13.1 ± 0.1	62
2	10^{-5}	13.8 ± 0.1	13.1 ± 0.1	89
4	10^{-5}	14.0 ± 0.1	13.0 ± 0.1	44
4	6×10^{-6}	13.4 ± 0.1	12.4 ± 0.1	49
6	10^{-5}	13.7 ± 0.1	12.8 ± 0.1	68
8	10^{-5}	13.2 ± 0.1	12.7 ± 0.1	114
11	10^{-5}	13.5 ± 0.1	12.8 ± 0.1	50
13	10^{-5}	13.3 ± 0.1	12.7 ± 0.1	84
18	10^{-5}	12.9 ± 0.1	12.8 ± 0.1	66
18	10^{-5}	13.1 ± 0.1	12.9 ± 0.1	71
22	10^{-5}	12.9 ± 0.1	13.2 ± 0.2	50
26.5	10^{-5}	12.7 ± 0.1	13.2 ± 0.1	54
26.5	3.75×10^{-5}	13.3 ± 0.1	a	24
30	10^{-5}	12.3 ± 0.2	13.0 ± 0.2	107

The values for log $K'_{Cu\text{-}thiol}$ were calculated by curve-fitting, those for log K'_{CuL2} by iterative linearization. The standard errors were calculated from the individual titrations.

[a] For this sample only one type of ligand (probably L_1) was detected.

Unfortunately, it was not possible to accurately calculate the thiol concentration (total or free) of the thiol species from the titrations with copper, and their concentration could also not be evaluated from calibration of the thiol peak height against known thiol compounds because of large differences in response between compounds and with small variations in the electrochemical parameters such as adsorption potential and the pH. The peak height for the thiols in the Scheldt estuary was between 2 and 15 nA, and, for reference purposes, a peak height of 10 nA would be equivalent to 70 nM thioacetamide or 35 nM thiourea. Until the composition of the thiol compound(s) has been clarified it is not possible to calibrate the voltammetry response accurately in terms of thiol concentrations, or even on SH-group concentrations, which would make it possible to compare the ligand concentrations obtained from the CuSA peak height to those from the thiol peak height to corroborate whether they are indeed the same.

However, using the assumptions and Eqs. (16)–(19) outlined above, it was possible to obtain estimates for the upper limit of the thiol concentrations. These (Table 3 and Fig. 7) were lower than the concentration of L_2 (about a third) at low salinities increasing to similar to L_2 at high salinities.

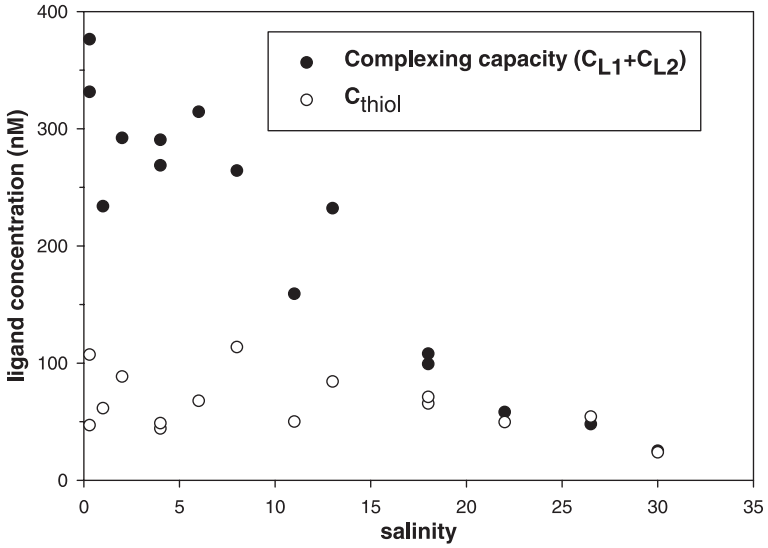

Fig. 7. Comparison of the concentration of thiol-ligands and the copper complexing capacity (sum of L_1- and L_2-type ligands) along the Scheldt estuary.

4.7. Nature and distribution of the thiol-like compound

The stability of the Scheldt copper-thiol species at the high salinity end (log $K'_{CuThiol} = 12.3-12.7$) is very similar to that of glutathione (12.2) and cysteine (12.7) in seawater (Leal and van den Berg, 1998) suggesting that thiols are indeed a good candidate for the composition of this ligand. Several thiol compounds and thiourea were tested to see whether they could be candidates for the unknown thiol compounds. Visual comparison of the voltammetric scans for cysteine, glutathione, 3-mercaptopropionic acid, thioacetamide and thiourea (Fig. 8) shows that there are large differences with glutathione and cysteine, indicating that these are not good model compounds for the compounds in the Scheldt estuary. Thioacetamide and thiourea come quite close in terms of the peak shape and potential.

However, there are systematic differences in the response of thioacetamide, thiourea, glutathione and the natural thiol-like substances, to changes in electrochemical parameters, such as the adsorption potential: when the adsorption potential was raised from -0.2 V to 0 V, the response for thioacetamide and thiourea *increased* by a factor of 10, and that of the natural compound by a factor of 2.5. In the same conditions, the response for glutathione *decreased* by $\sim$ 70% (Fig. 9B). We ascribe the opposite behaviour of glutathione to a systematically different electrode reaction: whilst thioacetamide, thiourea and the natural thiol compound, adsorb as Hg(II)-species (even though in solution they may occur bound to Cu(I)), the glutathione adsorbs as a Cu(I)-species (Leal and van den Berg, 1998); apparently, the glutathione is bound stronger by copper than by mercury relative to the other thiol compounds. Selection of a more positive adsorption potential, closer to 0 V, causes the concentration of Hg(II) at the electrode surface to increase as the mercury of the electrode is beginning to become oxidized, apparently increasing the formation of adsorbed Hg(II) species of thioacetamide and the natural thiol. An analogous effect tends to decrease the formation of adsorptive Cu(I) species at more positive adsorption potentials, as Cu(II) would tend to be favoured at potentials > -0.15 V and Cu(I) at more negative potentials; increased competition by Hg(II) would also tend to decrease formation of the copper(I) species at more positive potentials. Either way, the systematically different behaviour from glutathione confirms that the natural thiol tends to adsorb as a Hg(II)-species.

The differing rate of increased response with more positive adsorption potential suggests that

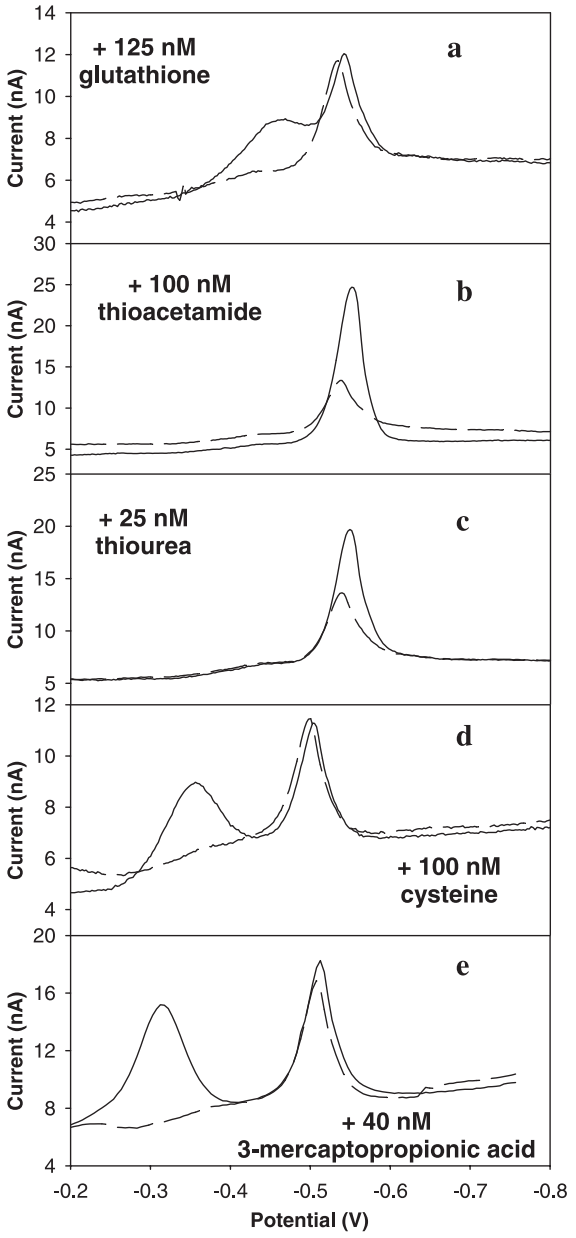

Fig. 8. Comparison of the voltammetric response for thiols in Scheldt water (salinity of 8) and for various thiol additions. EDTA (10^{-4} M) was added to bind excess copper and maximise the peak height for the thiols. The deposition potential was −0.1 V for (a), (d) and (e), and 0 V for (b) and (d). The continuous lines correspond to the model thiol additions.

thioacetamide and thiourea are also not good candidates compounds for the Scheldt "thiols". So, although we now know the complex stability of the copper-"thiol" species, we still know little about their composition.

4.8. Reversibility of the copper–thiol complex formation

As copper is bound as Cu(I) by the thiol-S-group (Leal et al., 1999), it might be possible that the reduction of Cu(II) to Cu(I) causes oxidation of part or all of the thiol compounds. If so, this could potentially cause the thiol concentration to be underestimated. Previous measurements of the copper binding capacity have indicated that the glutathione concentration is detected correctly using complexing capacity titrations (Leal and van den Berg, 1998), indicating that other ambient redox compounds (such as oxygen, or hydrogen peroxide which are amply available) were responsible for the redox chemistry of copper. The same might not be true for the natural thiols, although the reaction is likely to be the same.

Confirmation of an analogous reaction was obtained from reversible behaviour of the thiol peak when the free copper concentration was first increased, and subsequently decreased by the addition of EDTA, in a seawater containing known, added, thiol compounds and in seawater containing the natural thiol compounds. The thioacetamide peak, and the natural thiol peak, decreased as before with the increasing copper concentration, due to competitive complexation of these thiol compounds with Cu(I) causing the Hg(II)-S-thiol peak to decrease; however, with decreasing free copper levels in the same solution (by the EDTA addition), the peak for thioacetamide, and the natural thiol peak, recovered (Fig. 9A) showing that the reaction was reversible. The glutathione peak on the other hand showed little change with the change in the copper concentration, as this peak was caused by Cu(I)-S-glutathione adsorption (at copper concentrations similar to or greater than the glutathione concentration the Cu(I)-S-glutathione species is adsorbed on the mercury drop (Le Gall and van den Berg, 1993)). The recovery of the thiol peaks by the EDTA addition indicated that the thioacetamide and the natural thiol had not become

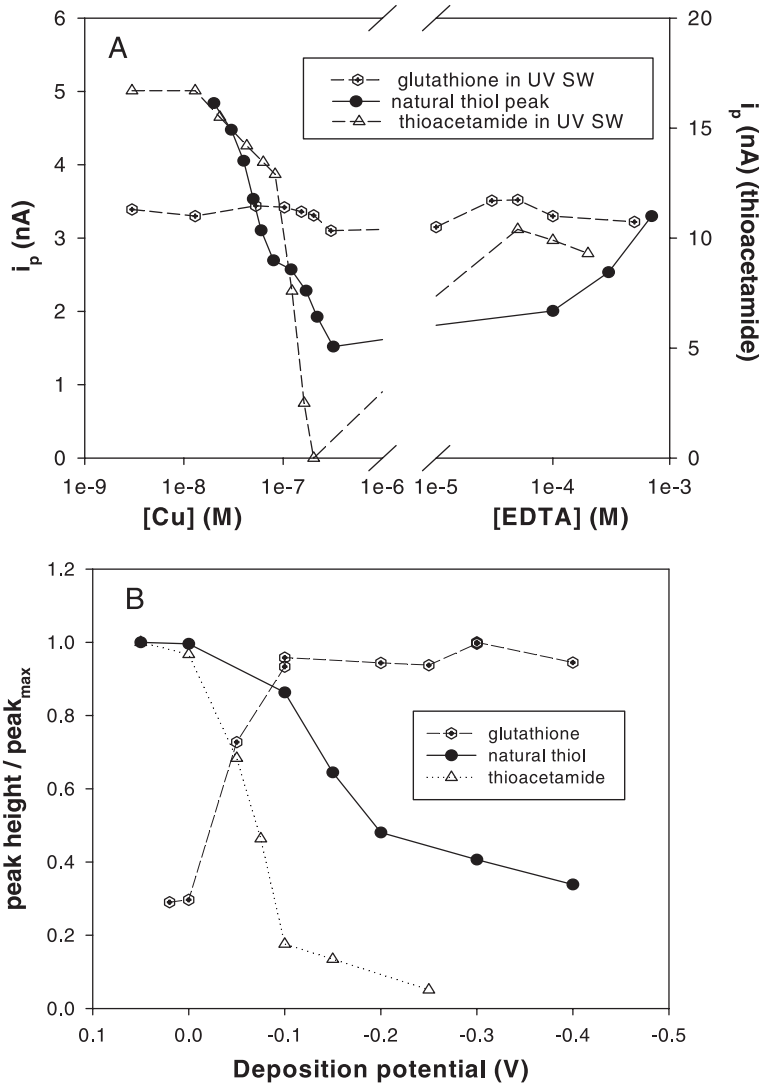

Fig. 9. Comparison of the voltammetric response for 10 nM glutathione and 10 nM thioacetamide, in UV SW, and the natural thiols in a Scheldt sample of intermediate salinity (salinity 8). (A) Effect on the peak height of increasing and subsequently decreasing (by adding EDTA) the free copper concentration, showing reversibility of the thiol peaks. (B) Effect of varying the adsorption potential on the thiol peak height in the presence of 2×10^{-4} M EDTA to maximise the peak height, showing opposite behaviour of glutathione.

oxidized by the copper addition, and confirmed again the opposite behaviour of glutathione.

5. Discussion

The speciation measurements using ligand competition against SA were interpreted by fitting the data to a two-ligand model. This is to some extent a subjective choice as it is likely that natural waters contain more complexing ligands with low to high complex stabilities as suggested for instance by (Filella et al., 1990); also, there is evidence that part of the complexing matter may be of colloidal nature (Mackey and Zirino, 1994; Muller, 1999; Wells et al., 1998) and this could be a mixture of organic matter stabilized by

fulvic acids (Buffle et al., 1998). Titrations with copper would tend to fill strong complexing ligands first followed by weaker ligands. Changes in the structure of polyelectrolytic ligands at increasing copper concentrations are a possibility too (Gregor et al., 1955) although this is perhaps not likely in the presence of high concentrations of calcium and magnesium in estuarine waters (even at a salinity of 1 there is more than 1 mM Ca^{2+} and 20 mM Na^{+}), which would tend to compete for empty binding sites and dominate the configuration.

The two-ligand fit was selected for practical reasons: curvature in the linearised plots of the data at low copper concentrations indicated the presence of at least two, maybe more, ligands. We used titrations of between 10 and 15 steps (the titrations were extended to 15 aliquots for low salinity samples due to their higher complexing capacity), and it is statistically dubious to fit more than two ligands as each ligand requires fitting two parameters, the ligand concentration and the conditional stability constant. Additionally, the data gave a good fit to the two-ligand model and the titration curve was well reproduced, indicating that additional ligands would have a negligible effect on the calculated metal speciation. There was therefore no need to try to fit more ligands to the data. However, the possibility exists that other ligands would be detected if the detection window were to be shifted significantly for instance by varying the concentration of the added competing ligand (SA).

There are other reasons why some ligands cannot be detected with current techniques based on metal titrations: modelling has shown (van den Berg, 1995) that a ligand is not detected if its concentration is much smaller than the metal concentration even if it forms a very stable copper complex; at the other end of the spectrum a very weak complexing ligand would not be detected (even when present at a concentration much greater than that of copper) if it binds copper much more weakly than the added ligand. A third possibility is the presence of ligands with very similar complex stabilities: ligands differing by 0.1 or 0.2 in the value for log K'_{CuL} would be detected as a single ligand (with a concentration equal to the sum of the individual ligands) as the effect on the *Y*-axis intercept would be negligible in the presence of the usual analytical variability.

Therefore, whilst the calculated ligand concentrations and conditional stability constants data have a specific use for the accurate estimation of metal speciation, it is possible that individual ligands of similar complexing stability are detected as a single ligand, and that very weak ligands, and ligands at very low concentration (below that of copper), have been missed altogether. On the other hand, the apparent ligand concentrations have specific importance if their concentration or stability constant can be compared to that of specific compounds detected using complimentary analytical techniques. This was attempted here by determination of the copper complexation of the thiol-like substances in the Scheldt estuary.

The decrease of the free thiol peak during the copper titrations indicated that thiols are at least one of the important complexing ligands. The shift in the Cu-thiol stability with the detection window suggests that at least two thiol species are present, and the similarity of the copper-thiol complex stability to that of the L_2-ligands agrees with the general observation that these account for the bulk of the complexing ligands (the concentration of L_2 is greater than that of L_1). The greater stability of L_1-species (calculated from the CuSA peak) is not evidence that L_1 is not a thiol as its lower concentration could have masked a small decrease in the thiol-peak-height at low copper concentrations.

The L_2-type ligands bind 3–23% of copper in these estuarine waters, and are therefore less important for copper speciation than the L_1 ligands. The known induction of thiols in cultures of several algae species including *Emiliania huxleyi* and cyanobacteria in response to copper addition (Croot et al., 2000; Leal et al., 1999; Moffett and Brand, 1996) is unlikely to greatly affect the copper speciation unless they are of the L_1 variety, as produced by cyanobacteria for instance (Croot et al., 2000) unless the copper concentration were to rise above the concentration of L_1. This suggests that the motivation for those apparent ligand releases is more that copper is expelled as copper–thiol species than a release of free thiols to change the external environment, although the effects of the thiols could be more important in oceanic waters.

The distribution of L_2 and the thiol-like substances (as quantified from their peak height in the presence of SA) is one of general dilution of high thiol

concentrations in the river end-member with lower concentrations in seawater (Fig. 7), indicating a freshwater source such as due to leaching out of the sediments in the more anoxic low salinity region of the estuary. The L_1 ligands shows a broad mid-estuarine source, similar to copper, superimposed on the dilution pattern (Fig. 2), suggesting either a possible biological response to increased water column copper levels or releases from sediments deposited at low to intermediate salinity. Another possible ligand source, originating from freshwaters, is humic and fulvic acids. The complex stability of these has recently been determined (Kogut and Voelker, 2001) with values for log K'_{CuL} of between 10 (weak complexes) and 12 (strong complexes). The strong species of that study have similar complex stability to the weaker end of the spectrum in the Scheldt estuary, suggesting that they could potentially be part of the L_2-type ligands.

This work confirms that thiols are ligands for copper in estuarine waters, and may constitute a major proportion of the L_2-type ligands. Our data do not exclude the possibility that they are part of the L_1-type fraction too, The identity of the thiol compounds is still unclear.

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References

Al-Farawati, R., van den Berg, C.M.G., 1997. The determination of sulfide in seawater by flow-analysis with voltammetric detection. Marine Chemistry 57, 277–286.

Al-Farawati, R., van den Berg, C.M.G., 1999. Metal-sulfide complexation in seawater. Marine Chemistry 63 (3–4), 331–352.

Baeyens, W., Elskens, M., Gillain, G., Goeyens, L., 1998. Biogeochemical behaviour of Cd, Cu, Pb and Zn in the Scheldt estuary during the period 1981–1983. Hydrobiologia 366, 15–44.

Buffle, J., Wilkinson, K.J., Stoll, S., Filella, M., Zhang, J.W., 1998. A generalized description of aquatic colloidal interactions: the three-colloidal component approach. Environmental Science and Technology 32 (19), 2887–2899.

Campos, M.L.A.M., van den Berg, C.M.G., 1994. Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldoxime. Analytica Chimica Acta 284, 481–496.

Croot, P.L., Moffett, J.W., Brand, L.E., 2000. Production of extracellular Cu complexing ligands by eucaryotic phytoplankton in response to Cu stress. Limnology and Oceanography 45 (3), 619–627.

Ellwood, M.J., van den Berg, C.M.G., 2001. Determination of organic complexation of cobalt in seawater by cathodic stripping voltammetry. Marine Chemistry 75 (1–2), 33–47.

Filella, M., Buffle, J., van Leeuwen, H.P., 1990. Effect of physicochemical heterogeneity of natural complexants: Part I. Voltammetry of labile metal-fulvic complexes. Analytica Chimica Acta 232, 209–223.

Forsman, U., 1984. Stripping voltammetric determination of traces of peptides and proteins containing disulphide linkages. Analytica Chimica Acta 166, 141–151.

Gerringa, L.J.A., Poortvliet, T.C.W., Hummel, H., 1996. Comparison of chemical speciation of copper in the Oosterschelde and Westerschelde estuaries, The Netherlands. Estuarine Coastal and Shelf Science 42 (5), 629–643.

Gerringa, L.J.A., Hummel, H., Moerdijk-Poortvliet, T.C.W., 1998. Relations between free copper and salinity, dissolved and particulate organic carbon in the Oosterschelde and Westerschelde, Netherlands. Journal of Sea Research 40 (3–4), 193–203.

Gledhill, M., van den Berg, C.M.G., 1994. Determination of complexation of iron(III) with natural organic complexing ligands in sea water using cathodic stripping voltammetry. Marine Chemistry 47, 41–54.

Gregor, H.P., Luttinger, L.B., Loebl, E.M., 1955. Metal–polyelectrolyte complexes: I. The polyacrylic acid–copper complex. Journal of Physical Chemistry 59, 34–39.

Knox, S., Langston, W.J., Whitfield, M., Turner, D.R., Liddicoat, M.I., 1984. Statistical analysis of estuarine profiles: II. Application to arsenic in the Tamar estuary (S.W. England). Estuarine, Coastal and Shelf Science 18, 623–641.

Kogut, M.B., Voelker, B.M., 2001. Strong copper-binding behavior of terrestrial humic substances in seawater. Environmental Science and Technology 35 (6), 1149–1156.

Kremling, K., 1983. Trace metal fronts in European shelf waters. Nature 303, 225–227.

Laglera-Baquer, L.M., Gonzalez-Davila, M., Santana-Casiano, J.M., 2001. Determination of metallic complexing capacities of the dissolved organic material in seawater. Scientia Marina 65, 33–40.

Le Gall, A.-C., van den Berg, C.M.G., 1993. Cathodic stripping voltammetry of glutathione in natural waters. Analyst 118, 1411–1415.

Le Gall, A.-C., van den Berg, C.M.G., 1998. Folic acid and glutathione in the water column of the North East Atlantic. Deep-Sea Research: Part I. Oceanographic Research Papers 45, 1903–1918.

Leal, M.F.C., van den Berg, C.M.G., 1998. Evidence for strong copper(I) complexation by organic ligands in seawater. Aquatic Geochemistry 4 (1), 49–75.

Leal, M.F.C., Vasconcelos, M.T.S.D., van den Berg, C.M.G., 1999. Copper-induced release of complexing ligands similar to thiols by Emiliania huxleyi in seawater cultures. Limnology and Oceanography 44 (7), 1750–1762.

Luther III, G.W., Church, T.M., 1988. Seasonal cycling of sulfur and iron in porewaters of a Delaware salt marsh. Marine Chemistry 23, 295–309.

Luther III, G.W., Rickard, D.T., Theberge, S., Olroyd, A., 1996. Determination of metal (bi)sulfide stability constants of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} by voltammetric methods. Environmental Science and Technology 30, 671–679.

Mackey, D.J., Zirino, A., 1994. Comments on trace-metal speciation in seawater or do onions grow in the sea. Analytica Chimica Acta 284 (3), 635–647.

Maldonado, M.T., Price, N.M., 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. Deep-Sea Research: Part II. Topical Studies in Oceanography 46 (11–12), 2447–2473.

Moffett, J.W., 1995. Temporal and spatial variability of copper complexation by strong chelators in the Sargasso Sea. Deep-Sea Research: Part I. Oceanographic Research Papers 42, 1273–1295.

Moffett, J.W., Brand, L.E., 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. Limnology and Oceanography 41 (3), 388–395.

Muller, F.L.L., 1999. Evaluation of the effects of natural dissolved and colloidal organic ligands on the electrochemical lability of Cu, Pb and Cd in the Arran Deep, Scotland. Marine Chemistry 67 (1–2), 43–60.

Muller, F.L.L., Tappin, A.D., Statham, P.J., Burton, J.D., Hydes, D.J., 1994. Trace-metal fronts in waters of the Celtic Sea. Oceanologica Acta 17 (4), 383–396.

Nolting, R.E., Helder, W., de Baar, H.J.W., Gerringa, L.J.A., 1999. Contrasting behaviour of trace metals in the Scheldt estuary in 1978 compared to recent years. Journal of Sea Research 42 (4), 275–290.

Paucot, H., Wollast, R., 1997. Transport and transformation of trace metals in the Scheldt estuary. Marine Chemistry 58 (1–2), 229–244.

Rozan, T.F., Lassman, M.E., Ridge, D.P., Luther III, G.W., 2000. Evidence for iron, copper and zinc complexation as multinuclear sulphide clusters in oxic rivers. Nature 406 (6798), 879–882.

Ruzic, I., 1982. Theoretical aspects of the direct titration of natural waters and its information yield for trace metal speciation. Analytica Chimica Acta 140, 99–113.

Saito, M.A., Moffett, J.W., 2001. Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. Marine Chemistry 75 (1–2), 49–68.

Skrabal, S.A., Donat, J.R., Burdige, D.J., 1997. Fluxes of copper-complexing ligands from estuarine sediments. Limnology and Oceanography 42 (5), 992–996.

Tang, D.G., Hung, C.C., Warnken, K.W., Santschi, P.H., 2000. The distribution of biogenic thiols in surface waters of Galveston Bay. Limnology and Oceanography 45 (6), 1289–1297.

Tang, D.G., Warnken, K.W., Santschi, P.H., 2001. Organic complexation of copper in surface waters of Galveston Bay. Limnology and Oceanography 46 (2), 321–330.

van den Berg, C.M.G., 1982. Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2: I. Theory. Marine Chemistry 11, 307–322.

van den Berg, C.M.G., 1984. Determination of the complexing capacity and conditional stability constants of complexes of copper(II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper-catechol complex ions. Marine Chemistry 15, 1–18.

van den Berg, C.M.G., 1995. Evidence for organic complexation of iron in seawater. Marine Chemistry 50, 139–157.

van den Berg, C.M.G., Donat, J.R., 1992. Determination and data evaluation of copper complexation by organic ligands in sea water using cathodic stripping voltammetry at varying detection windows. Analytica Chimica Acta 257, 281–291.

van den Berg, C.M.G., Kramer, J.R., 1979. Determination of complexing capacities and conditional stability constants for copper in natural waters using MnO2. Analytica Chimica Acta 106, 113–120.

van den Berg, C.M.G., Wong, P.T.S., Chau, Y.K., 1979. Measurement of complexing materials excreted from algae and their ability to ameliorate copper toxicity. Journal of the Fisheries Research Board of Canada 36, 901–905.

van den Berg, C.M.G., Merks, A.G., Duursma, E.K., 1987. Organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt estuary. Estuarine, Coastal and Shelf Science 24, 785–797.

Wells, M.L., Kozelka, P.B., Bruland, K.W., 1998. The complexation of 'dissolved' Cu, Zn, Cd and Pb by soluble and colloidal organic matter in Narragansett Bay, RI. Marine Chemistry 62 (3–4), 203–217.

Xue, H.B., Sigg, L., 1999. Comparison of the complexation of Cu and Cd by humic or fulvic acids and by ligands observed in lake waters. Aquatic Geochemistry 5 (4), 313–335.

Zhang, J.-Z., Millero, F.J., 1994. Investigation of metal sulfide complexes in sea water using cathodic stripping square wave voltammetry. Analytica Chimica Acta 284, 497–504.

Zhang, H., Davison, W., Miller, S., Tych, W., 1995. In-situ high-resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. Geochimica et Cosmochimica Acta 59 (20), 4181–4192.

Zwolsman, J.J.G., VanEck, B.T.M., VanderWeijden, C.H., 1997. Geochemistry of dissolved trace metals (cadmium, copper, zinc) in the Scheldt estuary, southwestern Netherlands: impact of seasonal variability. Geochimica et Cosmochimica Acta 61 (8), 1635–1652.