

Study of interactions of concentrated marine dissolved organic matter with copper and zinc by pseudopolarography

Rudy Nicolau, Yoann Louis, Dario Omanovic, Cédric Garnier, Stéphane Mounier, Ivanka Pizeta

► To cite this version:

Rudy Nicolau, Yoann Louis, Dario Omanovic, Cédric Garnier, Stéphane Mounier, et al.. Study of interactions of concentrated marine dissolved organic matter with copper and zinc by pseudopolarography. *Analytica Chimica Acta*, Elsevier Masson, 2008, 618, pp.35-42. <10.1016/j.aca.2008.04.038>. <hal-01096828>

HAL Id: hal-01096828

<https://hal-univ-tln.archives-ouvertes.fr/hal-01096828>

Submitted on 5 Jan 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/aca

Study of interactions of concentrated marine dissolved organic matter with copper and zinc by pseudopolarography

Rudy Nicolau^{a,d}, Yoann Louis^a, Dario Omanović^b, Cédric Garnier^{a,c}, Stéphane Mounier^a, Ivanka Pižeta^{b,*}

^a Laboratoire PROTEE, Université du Sud Toulon - Var - BP 132, 83957 La Garde, France

^b Center for Marine and Environmental Research, Ruđer Bošković Institute, P.O. Box 180, 10002 Zagreb, Croatia

^c Groupe de Physico Toxico Chimie des Systèmes Naturels, Institut des Sciences Moléculaires (ISM - UMR CNRS 5255), Université Bordeaux I, 33405 Talence, France

^d GRESE, ENSIL, Université de Limoges, 16 rue Atlantis, 87068 Limoges cedex, France

ARTICLE INFO

Article history:

Received 9 November 2007

Received in revised form

14 April 2008

Accepted 16 April 2008

Published on line 24 April 2008

Keywords:

Dissolved organic matter

Pseudopolarography

Copper

Zinc

Metal speciation

Sea water

ABSTRACT

The interaction of dissolved organic matter (DOM) with copper and zinc in a concentrated seawater sample was characterised by pseudopolarography. Measurements performed at increased concentrations of copper(II) ions showed successive saturation of active DOM sites which indicate possible partition of copper between (i) free or labile complexes, (ii) reduced and released within the potential window of the method, and (iii) electroinactive copper complexes. Pseudopolarograms measured at pH 4 indicate a release of copper which was bound to the active sites of DOM that formed non-labile complexes. Variation of the peak position and half-peak width along the scanned deposition potentials and with the increasing concentration of copper bear the information about the complex electrochemical processes at the electrode surface and in the bulk of the solution. Pseudopolarograms of zinc showed a strong dependence of the peak current and the peak position along the scanned deposition potentials on pH values, indicating preferential complexation of zinc with carboxylic-like active sites of DOM in the measured sample. Pseudopolarography is a valuable method in the trace metal complexation and speciation studies, serving as a fingerprint of the analysed sample.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Dissolved organic matter (DOM) in natural aquatic systems, such as marine waters and estuaries, is well known to interact with metals and organic pollutants, thus modifying their speciation and subsequently their transport, fate, and bioavailability towards micro- and macro-organisms [1–2]. Organic pollutants–DOM associations occur mostly due to the DOM hydrophobic nature, and are simply characterised by par-

titution coefficients (K_{DOC}), often correlated to the octanol/water partition coefficient (K_{ow}) [3]. In contrast, metal ions–DOM associations, although often of great intensity (e.g. more than 99% of Fe^{III} or Cu^{II} are strongly bound by organic ligands in surface seawaters [4–6]), are metal- and DOM-specific complexes, dependent on the physico-chemical conditions: ionic strength, pH, presence of other major and trace cations. Characterisation of the DOM binding properties has been a subject of numerous studies, leading to the development of analyti-

* Corresponding author. Tel.: +385 1 4680 231; fax: +385 1 4680 231.

E-mail addresses: rudy.nicolau@ensil.unilim.fr (R. Nicolau), louis@univ-tln.fr (Y. Louis), omanovic@irb.hr (D. Omanović), c.garnier@ism.u-bordeaux1.fr (C. Garnier), mounier@univ-tln.fr (S. Mounier), pizeta@irb.hr (I. Pižeta).

0003-2670/\$ – see front matter © 2008 Elsevier B.V. All rights reserved.

doi:10.1016/j.aca.2008.04.038

cal techniques and modelling tools (e.g. [7–9]). However, due to low organic carbon content ($0.1\text{--}1\text{ mgC L}^{-1}$) and trace levels of metals (rarely higher than several nmol L^{-1}), a study of DOM–metal binding properties in unpolluted marine waters still remains a challenge.

In order to understand the role of the marine DOM in metals speciation, various techniques have been used ([10], and references cited therein). Among them anodic stripping methods offer satisfactory sensitivity and selectivity and could provide useful information about the DOM–metal association (complexing parameters) required by the mathematical speciation programs [11–13]. Titration of the marine DOM with metals (preferably in a non-linear addition mode [14]) and/or methods based on ASV measurement at different deposition potentials (pseudopolarography, pseudovoltammetry or scanned (deposition potential) stripping technique) were widely used to accomplish this [15–28].

Pseudopolarography and analogues techniques have been used for speciation studies for the last 30 years [15–28]. But, as it is a time-consuming technique, it has seldom been used until the last decade, when new computer-controlled instruments offered the possibility to automate the whole process. The theory and experimental verification of pseudopolarography [15–23] and scanned stripping chrono-potentiometry [24,25] are well described. The main benefit of using pseudopolarography when compared to the classical polarography/voltammetry is the possibility to measure speciation at natural concentration level of trace metals ($<10^{-8}\text{ mol L}^{-1}$). This technique allows discrimination/separation of different fractions of metals, from the electrochemically labile to the inert ones, regarding the analytical window of the method. It is successfully applied for the speciation of metals in model solutions and in natural waters, both for the labile and for the inert metal complexes. According to the theory and to experimental evidences [15–17,22], one or more reduction waves could be obtained (similarly like in classical polarography) which correspond to one labile and/or to one or more inert metal complexes. The position of those waves (half-wave potentials) depends on the corresponding stability constants of the formed metal complexes. Although the theory for inert metal complexes is valid only for the standard electrode potentials [16], experimentally obtained dependence of the reduction half-wave potential on the stability constants (“chelate scale”) was successfully applied for the estimation of the stability constants of unknown inert metal complexes in natural waters [19–22]. Since the DOM is of complex and heterogeneous nature, complications such as its adsorption on electrode surface could occur and lead to erroneous results [29–31]. Thus, special attention should be given to the interpretation of obtained results.

In an unpolluted marine environment, average DOM concentration is about 1 mg L^{-1} . In such clean seawater systems the determination of complexing parameters by stripping voltammetry was often achieved near the detection limit or resolution capability of the method [14]. So, the opportunity has been taken to join the project which concentrated the DOM in a marine sample [32], and to analyse its interactions with metal by pseudopolarography, exploring and showing more clearly the possibilities of the method.

The objective of this work is to explore the pseudopolarographic behaviour of the copper and zinc complexes present in the seawater sample with high concentration of DOM. An attempt has been made to extract some quantitative data regarding the metal complexes in the sample and to point out certain features of the pseudopolarographic approach as a kind of “finger-print” method.

2. Experimental

2.1. Sample preparation

The seawater sample was taken in the coastal area of southern France. The sampling site (bay of Balaguier: $43^{\circ}05'51.70''\text{N}$ – $5^{\circ}54'28.5''\text{E}$), in the bay of Toulon, is interesting as a semi-enclosed area subjected to different anthropogenic activities: a military port, tourism and aquaculture activities. The sample was taken at 1-m depth in the middle of the bay with a Teflon membrane pump (RETSCH GMBH) connected to a N_2 bottle, filtered in-line through a $0.22\text{ }\mu\text{m}$ -filter Whatman, Polycap 150TF. 940 L of filtered sample was stored in Nalgene bottles (20 and 50 L) at 4°C in the dark before concentration.

Within the framework of a French research project (called MONALISA for Matière Organique Naturelle en milieu SALé), coordinated by IFREMER (French Research Institute for Exploitation of the Sea), a new methodology has been developed to concentrate marine DOM in large volumes (500–1000 L), using two subsequent concentrations steps: nanofiltration followed by reverse-osmosis [32]. The concentration step is necessary to obtain a sufficient quantity of DOM for the subsequent characterisation of its properties by other MONALISA participants by using NMR ^{13}C , NMR ^{113}Cd , pyrolysis–GC–MS, HPSEC and acido-basic titrations. The method was conducted in two steps: (1) with osmosis equipment (TIA) by nanofiltration (Osmonics DL2540) from 940 L of filtered sample of the salinity $S=37$ and $\text{DOC}=1.1\text{ mg L}^{-1}$, the sample reached 32 L with an addition of MilliQ water at the end to decrease the salinity to the value of $S=10.2$ and $\text{DOC}=10.8\text{ mg L}^{-1}$, and (2) with reverse-osmosis (Filmtec SW30–2540) the final 10.5 L of the sample with the salinity of $S=28$ and $\text{DOC}=30.4\text{ mg L}^{-1}$ was obtained. The obtained DOM concentrated sample, stored in the dark at 4°C after spiking with NaN_3 ($100\text{ }\mu\text{L}$ of 0.1 mol L^{-1}) to prevent any biological activity, was subsequently analysed with voltammetric methods by the addition of copper(II) ions and by changing the pH of the sample.

2.2. Chemicals, instrumentation and measurement protocol

A copper stock solution was prepared using a salt of copper nitrate trihydrate of reagent grade (MERCK, Darmstadt). Samples were acidified using 69% HNO_3 (J.T. Baker). All measurements were carried out with voltammetric analyser PGSTAT 12 with GPES 4.9 software (EcoChemie, Utrecht, The Netherlands) coupled to a three-electrode system of a Metrohm 663 VA Stand. The working electrode was a static mercury drop electrode (SMDE) (drop area of 0.25 mm^2). All potentials were referred to $\text{Ag|AgCl|KCl } 3\text{ mol L}^{-1}$ reference

electrode. A carbon rod was used as a counter electrode. The electrolyte solution was stirred at 3000 rpm using a Teflon rotating stirrer. To minimise adsorption of metal ions a Teflon electrochemical cell was used. Before each measurement all vessels, the cell and electrodes were cleaned with 10% (analytical grade) nitric acid and rinsed with ultra-pure MilliQ-water. pH was controlled by a pH meter (Metrohm 713) with a combined pH-micro-electrode (Mettler, Inlab422, reference: Ag|AgCl|KCl 3.0 mol L⁻¹).

For each experiment, 20 mL of sample was placed in the voltammetric cell. Dissolved oxygen was removed from the solution prior to the measurements by purging with water-saturated nitrogen for 15 min and a nitrogen blanket was maintained above the sample surface during the measurement. Measurement protocol: total metal concentrations were determined by standard additions of Cu and Zn ions by differential pulse anodic stripping voltammetry (DPASV) after UV-irradiation (Hanovia, 450 W medium Hg pressure, 12 h) at acidic pH. Voltammograms for pseudopolarography were recorded using DPASV with the following measurement parameters: deposition potential $E_{\text{dep}} = -1.6$ to -0.020 V in steps of 0.020 V (altogether 80 scans), deposition time $t_{\text{dep}} = 360$ s, pulse amplitude $A = 25$ mV, interval time $t_{\text{int}} = 0.15$ s, modulation time $t_{\text{mod}} = 0.04$ s, step increment $E_{\text{st}} = 0.002$ V, initial potential $E_i = -1.2$ V, final potential $E_f = 0.01$ V. An equilibration at -1.2 V for 15 s was applied at the end of the accumulation period. During this period, desorption of adsorbed surface active substances on the mercury surface occurs, preventing negative effects on the copper anodic peak [31]. A home-written program incorporated in the Project mode of the GPES software was used to automate pseudopolarographic measurements. The voltammetric parameters (peak height, peak position and half-peak width) were automatically determined by the home-written software, as well.

3. Results and discussion

The purpose of this work was a pseudopolarographic study of copper complexes in concentrated seawater sample. As the procedure of voltammetric measurements included a zinc oxidation peak as well, it was found very interesting to present and to try to interpret behaviour of zinc pseudopolarograms in addition to the copper ones. The formation of CuZn intermetallic compound is not likely to occur, because of much lower concentrations of copper and zinc in our experiments when compared to those reported in literature, which should be in a milli-molar range [33,34].

While in the original sample total copper(II) concentration was found to be $(1.48 \pm 0.12) \times 10^{-8}$ mol L⁻¹, the concentration was 100 times higher $(1.43 \pm 0.17) \times 10^{-6}$ mol L⁻¹ in the concentrated sample. Total zinc concentration in the concentrated sample was $(3.9 \pm 0.3) \times 10^{-7}$ mol L⁻¹. Dissolved organic carbon (DOC) before and after pre-concentration was 1.1 and 30.4 mg L⁻¹, respectively. This showed that during the concentration process ratios of analytes were changed, but it is not the subject of this study.

A set of anodic stripping voltammograms obtained in a concentrated seawater sample without metal additions

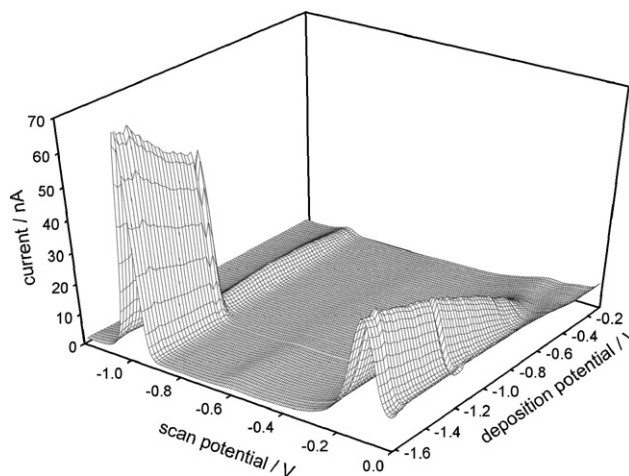


Fig. 1 – Set of voltammograms of pure concentrated sample of pH 8.3. Measurement parameters: DPASV method; E_{dep} interval: from -1.6 to -0.02 V in steps of 20 mV, $t_{\text{dep}} = 360$ s, $A = 25$ mV, $t_{\text{int}} = 0.15$ s, $t_{\text{mod}} = 0.04$ s, $E_{\text{st}} = 0.002$ V, $E_i = -1.2$ V, $E_f = 0.01$ V.

appears in Fig. 1. Two well separated and expressed peaks related to zinc (~ -1.0 V) and copper (~ -0.18 V) are evident, ensuring the accurate and reliable peak parameters read outs needed for the construction of corresponding pseudopolarograms. It is interesting to note that although total copper concentration was 3.5 times higher than that of zinc, the obtained anodic peak of copper (at $E_{\text{dep}} = -1.6$ V) was about half of that of zinc. Taking into account that copper in chloride solutions undergoes one-electron charge transfer [35] and that the sensitivity is half of that required for two-electron charge transfer, still remains the factor of 3.5 to be explained. Such a smaller copper peak could be attributed to the presence of its organic complexes which are either (i) non-electroactive at this potential, (ii) have much lower diffusion coefficient from that of free/labile copper, or both of this. This example simply illustrates that experiments performed in natural samples should be very carefully analysed in respect to the expected theoretical behaviour.

3.1. Pseudopolarograms of copper

Experimental conditions and labelling of the results are summarised in Table 1 and are valid for Figs. 2–4. Fig. 2a shows untreated pseudopolarograms obtained without added copper and with additions of 4×10^{-6} and 8×10^{-6} mol L⁻¹ of copper(II) ions at natural pH 8.3 and 4. In addition, two pseudopolarograms at pH 2 were also recorded for the two additions of copper, but they were practically the same as the pseudopolarograms obtained at pH 4 (data not shown). The three dotted lines correspond to the peak currents anticipated for reduction of only free/labile inorganic copper complexes (in DOM-free sample). These values were obtained according to the sensitivity obtained in UV-digested sample at natural pH. To get better overview and relations between pseudopolarograms, each of them was normalised to the above-mentioned projected currents in non-complexing media (i_{NC}) (Fig. 2b).

Table 1 – Additions of copper to the sample containing $(1.43 \pm 0.17) \times 10^{-6} \text{ mol L}^{-1}$ copper (II) ions

Cu(II) added (mol L^{-1})	pH	Symbols	Curve labelling
–	8.3	○	1a
–	4	●	1b
4×10^{-6}	8.3	□	2a
4×10^{-6}	4	■	2b
4×10^{-6}	2	⊠	2c
8×10^{-6}	8.3	◇	3a
8×10^{-6}	4	◆	3b
8×10^{-6}	2	⬠	3c

Conditions and labelling of the curves.

The shapes of the copper pseudopolarogram without addition and for the first addition of copper (curves 1a and 2a) clearly show that the wave which corresponds to the reduction of the free/labile copper species has not appeared. Only continuous increase of the peak current starting from $E_{\text{acc}} = -0.25 \text{ V}$ was evolved. However, for second addition of copper (curve 3a) an evolution of the wave at $E_{\text{acc}} = -0.2 \text{ V}$ occurred. This indicates a marked increase in concentration of free copper ion and its labile complexes comparing to copper-organic complexes. Several interpretations could be given for the continuous increase of the peak current at the accumulation potentials more negative than -0.3 V (for all three pseudopolarograms at pH 8.3). Many authors reported that an increase of the peak current more negative than

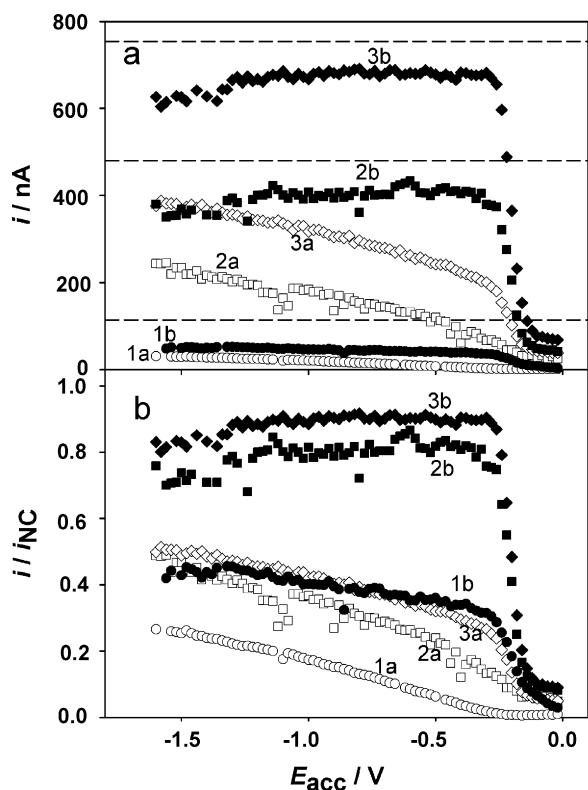


Fig. 2 – Peak current pseudopolarograms of copper (a) row data and (b) data normalised to current expected for non-complexed copper (i_{NC}). Conditions and labelling of the curves are given in Table 1.

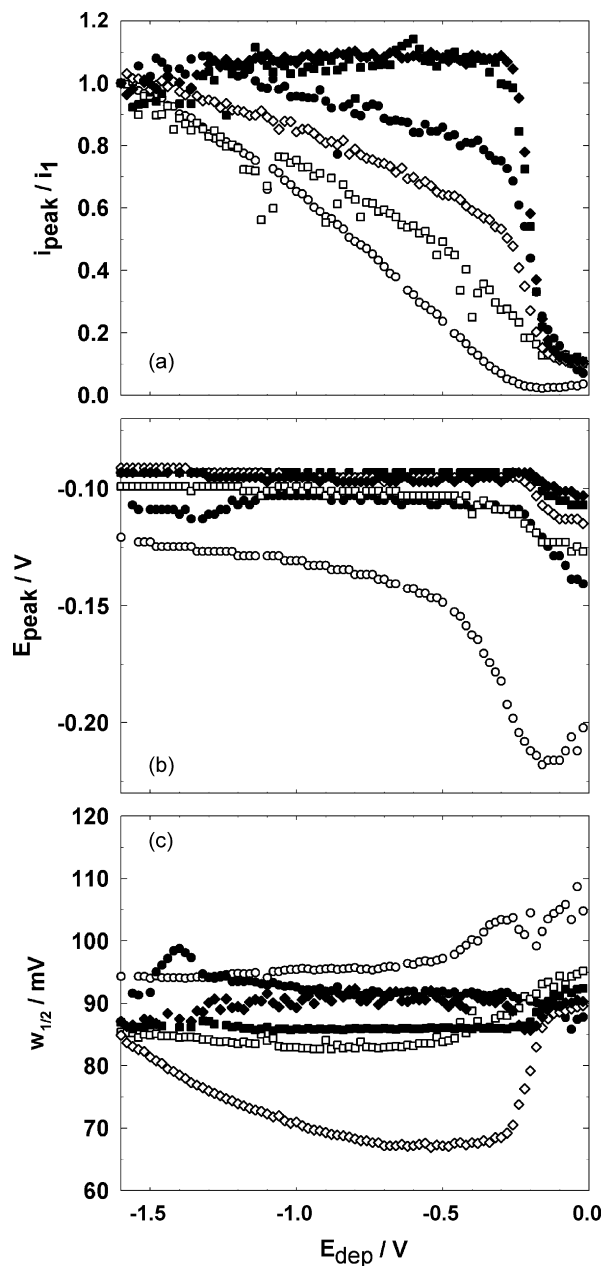


Fig. 3 – (a) Pseudopolarograms from Fig. 2 with the current normalised to the first point; (b) peak position vs. deposition potential curves of copper; (c) half-peak width vs. deposition potential curves of copper. Conditions and labelling of the curves are given in Table 1.

reduction potential of free/labile copper corresponds to the reduction of various copper organic complexes [16–22]. Two or more well separated waves could be obtained depending on metal complexes having different stability constants [16,19–22,36]. Several papers by the group of Luther III showed that thermodynamic stability constants of inert metal complexes could be estimated according to the formed “chelate scale” [19–22].

However, there are two completely distinct approaches concerning both, determination of complexing capacity and

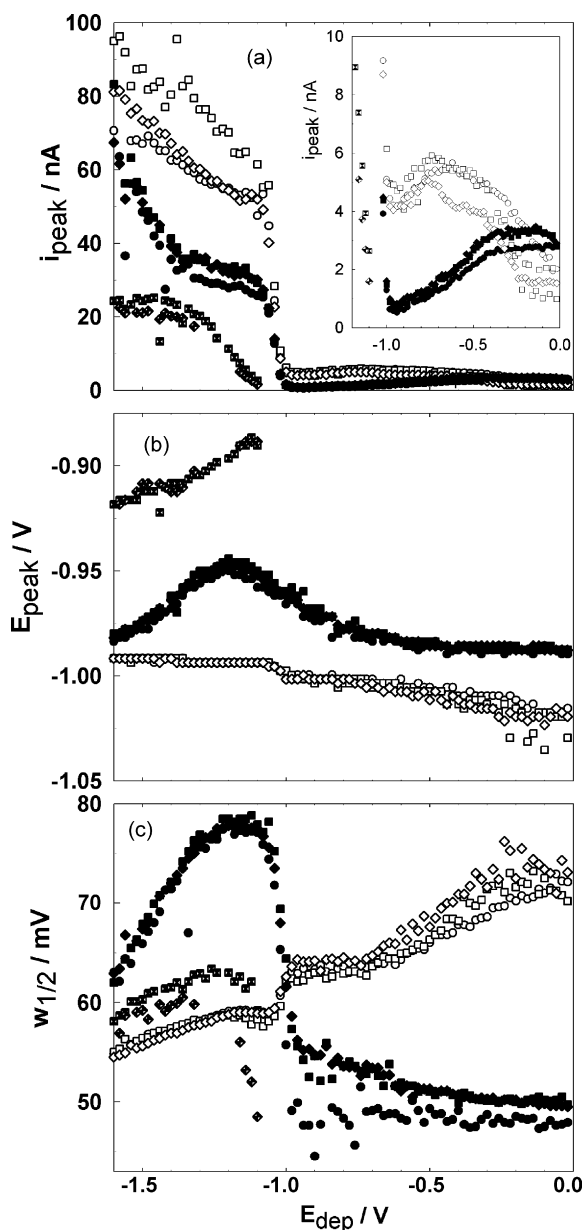


Fig. 4 – (a) Peak current pseudopolarograms of zinc. *Inset:* detail of Fig. 4a; (b) peak position vs. deposition potential curves of zinc; (c) half-peak width vs. deposition potential curves of zinc. Conditions and labelling of the curves are given in Table 1.

pseudopolarography. The first approach considers the difference in diffusion coefficients of labile complexes as minor, while the direct reduction of inert metal complexes takes place at potentials more negative than labile metal complexes [16,19–22,36]. In this case two or more reduction pseudopolarographic waves could be obtained. This approach is most widely used in the literature. The second approach assumes that all complexes are labile, and that there is only one pseudopolarographic wave having a flat limiting current controlled by the mean diffusion coefficient of both weak and strong metal complexes [17,28,37]. However, many authors recorded

pseudopolarograms in a relatively narrow range of deposition potentials and there was no opportunity to see possible evaluation of pseudopolarographic waves of other directly reduced inert metal complexes [17,28].

Both approaches are very strict and often do not consider other possible options. Both approaches should be respected in the interpretation of the voltammetric data obtained in natural water samples. Taking everything mentioned above into consideration, there are no severe anchorages to apply one of those two approaches to our results to be able to extract some quantitative information at natural pH. The main unknown parameter is the diffusion coefficient which cannot be determined from this set of data. However, despite the lack of quantitative data, some relations could be assigned.

The shapes of all three pseudopolarograms at pH 8.3 showed increasing peak current trend towards negative potentials, without reaching a plateau in the analytical window of the method. It is reasonable to assume that this trend would continue for more negative potential which were not scanned in our experiments.

The parameters of the plateau could only be explained by speculations, because there are two unknown terms which were discussed above (diffusion coefficients and electroactivity and (non)lability of copper complexes). Gradual increase of peak current at potentials more negative than -0.3V reflects the loss of lability of copper heterogeneous complexes [17] and could be assigned to the gradual reduction of numerous copper (inert) organic complexes having increasing stability constants [20]. In such a system the calculation of any binding parameter is precluded even for known sample composition [17]. However, as the diffusion coefficients and the electro(in)activity of all strong inert copper complexes in the sample are unknown it is not possible to declare the quantity of copper species. The absence of the wave of free/labile copper on the pseudopolarogram obtained without the copper addition implies that copper in this concentrated sample exists mainly in a form of relatively strong inert complexes. By gradual addition of copper, the distribution of copper species was changed thus forming more labile complexes, finally for the second addition of copper producing an evident wave. The position of this wave (Fig. 2, curve 3a) matched those obtained in the acidified sample (Fig. 2, curves 1b, 2b and 3b). Normalised pseudopolarograms at pH 8.3 show this evident increase of labile copper complexes with increasing concentrations of copper (Figs. 2b and 3a).

The acidification of the sample leads to the redistribution of copper complexes. The redistribution of the organic fraction of copper complexes to inorganic forms during the acidification is a consequence of competition between Cu^{2+} and H^+ for DOM binding sites that regards a part of carboxylic-like sites. The part of phenolic-like DOM sites with strong affinities towards proton was not importantly influenced as shown by Lu and Allen on concentrated freshwater DOM [8] (presuming the general distribution of DOM binding sites onto carboxylic-like sites (average pK_a of 4.5) and the phenolic-like ones (average pK_a of 10) [38]). Three pseudopolarograms measured at pH 4 show that a large part of copper was released from its inert complexes. Limiting currents of pseudopolarograms for two copper additions approached the projected level

for non-complexed copper. The difference between expected and obtained limiting current for all three pseudopolarograms remained the same, implying that not all of strong copper inert complexes (reduced within the scanned deposition potentials) were destroyed at pH 4. The portion of these complexes became smaller with a higher copper concentration which is illustrated in the normalised plot (Fig. 2b). These relations indicate that at such relatively weak acidic conditions (pH 4) the proton is a strong competitor to copper for these active sites of DOM, implying existence of weaker copper complexes in the sample solution. Additional experiments in the same concentrated sample involving competition with proton and calcium were already done and the paper is under submission.

It is evident from this entire consideration that numerous different experiments should be performed in the same sample to be able to correctly interpret obtained data in a manner of trace metal speciation of a particular natural water sample. Such an analysis can serve operationally to characterise a sample and to compare the sample with another one. In this way, pseudopolarograms of trace metals could be considered as a “fingerprint” of the sample.

In Fig. 3a pseudopolarograms are normalised to the most negative point. This transformation graphically illustrates the ability of the sample to complex copper(II) ions by either, the addition of copper or by changing of pH. The relationships of the peak potential and the half-peak width vs. deposition potential curves could provide additional information about electrochemical reactions (Fig. 3b and c). There is a clear distinction of the peak potentials of the copper anodic peak potentials obtained in samples with and without copper additions. This might indicate the existence of a fraction of labile copper(II) complexes which were concealed with higher copper(II) additions, this resulted in a peak shift towards more positive potentials. Shift towards more negative potentials when the deposition potential approaches peak potential has already been noticed [17,26,39–42] and was ascribed to the existence of strong complexing ligands concentrated at the electrode surface. Half-peak width bears also some information. Its accurate determination is subjected to disturbances like humps affecting a baseline subtraction procedure and should be taken with attention. However, it should be considered of great interest as it could indicate, for example, the existence of two closely overlapped peaks that might come from adsorption processes, and could be a motivation for further investigation. The half-peak width of copper oxidation is about 90 mV that corresponds to one-electron charge transfer, as expected in a seawater sample [35].

3.2. Pseudopolarograms of zinc

Eight pseudopolarograms of zinc, analogous to those of copper, were drawn by tracing the peak of oxidation of zinc in the same experiments as those described for copper. Similarly shaped pseudopolarograms were obtained in natural, non-concentrated sea water sample taken in the same bay of Toulon, France (data not shown). It is a good argument for accepting the proposed concentration procedure for the DOM investigation. In Fig. 4a the zinc peak current pseudopolarograms are shown in absolute values. Fig. 4b and c show the

peak potential and the half-peak width vs. deposition potential curves of zinc. There is a strong dependence on pH that affects peak height, peak position and half-peak width, and groups the signals accordingly.

Pseudopolarograms of the samples acidified to pH 2 have a particular shape. Their amplitude is lower (indicating possible blocking of the electrode by adsorption of protonated DOM) and they shifted negatively (variation about 200 mV), with a remarkable change of slope (indicating a quasi-reversible or irreversible cathodic reaction [23,36]). At the same time, peak potentials are about 60–70 mV more positive than for other conditions (pH 8.3 and 4), which might indicate the missing of some labile complexes that would shift the peaks negatively ([43], p. 49). The half-peak widths of the single curves indicate a simple reversible two-electron reaction ([44], p. 129).

Regarding the complexation with DOM “the tails” of the pseudopolarograms representing the peaks of oxidation of zinc appeared to be of interest, while the deposition potential was more positive than the reduction potential, shown in detail in the inset of Fig. 4a. The maximum value of the tail moves from about -0.7 V for the natural pH to about -0.2 V for the pH 4. The tail does not exist at pH 2. At that pH most of the DOM is protonated and is not capable of complexing with zinc. Either adsorbed at the electrode or not, it does not help the accumulation of zinc at the electrode either during the deposition time or during the scanning time. As the tail exists at pH 4, one might conclude that the competition effect between Zn^{2+} and H^+ towards the DOM binding sites in favour of H^+ prevalently occurs at the pH below 4, in contrast to Cu (see Section 3.1). It points out the important quantity of complexing sites of carboxylic type [8,45–47]. It also reveals that Zn–DOM binding sites and Cu–DOM ones are not identical. As the strength of Cu–DOM interactions is generally larger from that of Zn–DOM ones [6] and the total Cu concentration is increased, it is probable that Zn is not bound by the Cu-specific DOM sites, which are assumed to be of phenolic-like type (see Section 3.1), but by less strong sites with acidic properties supposed to be of carboxylic-like type that cover the pK_a range of 1–7 [8,38]. This hypothesis has already been expressed to explain the weak competition effect between Cu and major divalent cations towards DOM binding sites [8].

A closer insight in the peak position and half-peak width vs. deposition potential curves (Fig. 4b and c) shows the redistribution of labile zinc species which was reflected in the peak position shift correlated with changing of pH, particularly complex shifting and width changing of the peak at pH 4. As mentioned previously, the presence of labile complexes is manifested by the shifting of the peaks in negative direction. However, the possible causes of the shifting of peaks vs. deposition potential are manifold. Taking into account the entire effects one might speculate about the adsorption of neutral organic matter: it is favoured at the potential of zero charge of the mercury drop, or the adsorption of charged species would influence the signal at the more extreme negative or positive potentials [48]. However, these effects appear to be interesting for further qualification of organic matter and for differentiation of the various dissociation constants of the numerous groups of organic ligands

that could be traced by the combination of pH titration and pseudopolarography.

4. Conclusions

The ability of DOM to complex copper has been tested on a seawater sample concentrated by nanofiltration and reverse osmosis by titration with up to 8×10^{-6} mol L⁻¹ copper(II) ions applying pseudopolarography. The results obtained for the analysed sample clearly evidenced a distribution of copper in three categories: (i) free or labile (deposition potential at -0.3 V), (ii) complexes that are reduced and released within the analytical window of the method (deposition potential from -0.3 to -1.6 V), and (iii) electroinactive complexes. Their ratios change with the addition of copper(II) ions and with changing pH. Such an analysis can serve operationally to characterise a sample and to compare the sample to other ones.

Zinc pseudopolarograms showed significant dependence on pH value. A large difference in pseudopolarograms of zinc between pH 4 and 2 reveals the existence of organic matter with protonation constant pK_a between 2 and 4. Those analyses also indicate the differences of DOM sites for copper and zinc complexation regarding their stability and acidity constants. Additional experiments are necessary for more detailed DOM characterisation regarding zinc and possible zinc–copper competition. Experiments with the addition of other cations are planned for they might cause different reactions and characterisation of DOM, which would be reflected in the shapes of pseudopolarograms.

The complex structure of the obtained pseudopolarograms reflects and indicates composite structure of the ligands making complexes with metals. This raises a challenge to construct the models with such behaviour, and to match the curves with the theory of pseudopolarography for retrieving quantitative information out of the signals regarding the speciation in the bulk of the solution and regarding the electrode processes.

Acknowledgements

This study was supported by the French MONALISA research project coordinated by the IFREMER (Grant No. 03/1214910/T). The grant of the University of Sud Toulon and Var for Ph.D. and researcher exchanges between France and Croatia, as well as financial support of the Ministry of Science, Education and Sports of the Republic of Croatia (through Grant No. 098-0982934-2720) are gratefully acknowledged. We thank Marta Plavšić and Damir Krznarić for their precious discussions. We also thank to the anonymous reviewers, especially to one of them.

REFERENCES

- [1] J. Buffle, *Complexation Reactions in Aquatic Systems, Analytical Chemistry*, Ellis Horwood, Chichester, 1988, p. 692.
- [2] R.H. Byrne, *Pure Appl. Chem.* 68 (1996) 1639.
- [3] J. Akkanen, J.V.K. Kukkonen, *Environ. Toxicol. Chem.* 22 (2003) 518.
- [4] E.L. Rue, K.W. Bruland, *Mar. Chem.* 50 (1995) 117.
- [5] J.W. Moffett, L.E. Brand, R.G. Zika, *Deep-Sea Res.* 37 (1990) 27.
- [6] K. Hirose, *Anal. Sci.* 22 (2006) 1055.
- [7] E. Tipping, M.A. Hurley, *Geochim. Cosmochim. Acta* 56 (1992) 3627.
- [8] Y. Lu, H.E. Allen, *Water Res.* 36 (2002) 5083.
- [9] K.W. Bruland, E.L. Rue, J.R. Donat, S.A. Skrabal, J.W. Moffett, *Anal. Chim. Acta* 405 (2000) 99.
- [10] G.E. Batley, S.C. Apte, J.L. Stauber, *Aust. J. Chem.* 57 (2004) 903.
- [11] K.H. Coale, K.W. Bruland, *Deep-Sea Res.* 37 (1990) 317.
- [12] F.L.L. Muller, *Mar. Chem.* 67 (1999) 43.
- [13] C. Garnier, S. Mounier, J.Y. Benaïm, *Environ. Technol.* 25 (2004) 589.
- [14] C. Garnier, I. Pižeta, S. Mounier, J.Y. Benaïm, M. Branica, *Anal. Chim. Acta* 505 (2004) 263.
- [15] S. Bubić, M. Branica, *Thalassia Jugoslav.* 9 (1973) 47.
- [16] G. Branica, M. Lovrić, *Electrochim. Acta* 42 (1996) 1247.
- [17] R.M. Town, M. Fillela, *J. Electroanal. Chem.* 488 (2000) 1.
- [18] D. Omanović, M. Branica, *Croat. Chem. Acta* 71 (1998) 421.
- [19] B.L. Lewis, G.W. Luther III, H. Lane, T.M. Church, *Electroanalysis* 7 (1995) 166.
- [20] P. Croot, J.W. Moffett, G.W. Luther III, *Mar. Chem.* 67 (1999) 219.
- [21] T.F. Rozan, G.W. Luther III, D. Ridge, S. Robinson, *Environ. Sci. Technol.* 37 (2003) 3845.
- [22] J.J. Tsang, T.F. Rozan, H. Hsu-Kim, K.M. Mullaugh, G.W. Luther III, *Environ. Sci. Technol.* 40 (2006) 5388.
- [23] D. Omanović, *Croat. Chem. Acta* 79 (2006) 67.
- [24] H.P. van Leeuwen, R.M. Town, *J. Electroanal. Chem.* 536 (2002) 129.
- [25] R.M. Town, H.P. van Leeuwen, *Croat. Chem. Acta* 79 (2006) 15.
- [26] I. Pižeta, G. Billon, D. Omanović, V. Cuculić, C. Garnier, J.-C. Fischer, *Anal. Chim. Acta* 551 (2005) 65.
- [27] M.P. Hurst, K.W. Bruland, *Anal. Chim. Acta* 546 (2005) 68.
- [28] P. Chakraborty, I.I. Fasfous, J. Murimboh, C.L. Chakrabarti, *Anal. Bioanal. Chem.* 388 (2007) 463.
- [29] M.G. Bugarin, A.M. Mota, J.P. Pinheiro, M.L.S. Gonçalves, *Anal. Chim. Acta* 294 (1994) 271.
- [30] A.E. Witter, S.A. Mabury, A.D. Jones, *Sci. Total Environ.* 212 (1998) 21.
- [31] Y. Louis, P. Cmok, D. Omanović, C. Garnier, V. Lenoble, S. Mounier, I. Pižeta, *Anal. Chim. Acta* 606 (2008) 37.
- [32] A. Huguet, C. Innocent, H. Roux De Balman, E. Parlanti, in: F.H. Frimmel, G. Abbt-Braun (Eds.), *Universität Karlsruhe, Proceedings of the 13th Meeting of the International Humic Substances Society, July 30–August 4, Karlsruhe, Germany, 2006*, p. 373.
- [33] J.A. Wise, D.A. Roston, W.R. Heineman, *Anal. Chim. Acta* 154 (1983) 95.
- [34] E. Sahlin, D. Jagner, *Electroanalysis* 10 (1998) 532.
- [35] A. Nelson, R.F.C. Mantoura, *J. Electroanal. Chem.* 164 (1984) 237.
- [36] D. Omanović, M. Branica, *J. Electroanal. Chem.* 565 (2004) 37.
- [37] T. Cheng, H.E. Allen, *J. Environ. Manage.* 80 (2006) 222.
- [38] E.M. Perdue, *Acidic functional groups of humic substances*, in: G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. MacCarthy (Eds.), *Humic Substances in Soil, Sediment and Water*, 1, Wiley, New York, 1985 (Chapter 20).
- [39] J. Buffle, J.J. Vuilleumier, M.L. Tercier, N. Parthasarathy, *Sci. Total Environ.* 60 (1987) 75.
- [40] R.M. Town, H.P. van Leeuwen, *J. Electroanal. Chem.* 541 (2003) 51.
- [41] R.M. Town, H.P. van Leeuwen, *J. Electroanal. Chem.* 535 (2002) 11.
- [42] A.M.A. Mota, J. Buffle, S.P. Kounaves, M.L.S. Goncalves, *Anal. Chim. Acta* 172 (1985) 13.
- [43] D.R. Crow, *Polarography of Metal Complexes*, Academic Press, London, 1969.

- [44] J. Heyrovský, J. Kůta, Principles of Polarography, Publishing House of the Czechoslovak Academy of Science/Academic Press, Prague/New York, 1966.
- [45] J. Masini, G. Abate, E. Lima, L. Hahn, M. Nakamura, J. Lichtig, H. Nagatomy, *Anal. Chim. Acta* 364 (1998) 223.
- [46] J.D. Ritchie, J.M. Perdue, *Geochim. Cosmochim. Acta* 67 (2003) 85.
- [47] C. Garnier, S. Mounier, J.Y. Benaïm, *Water Res.* 38 (2004) 3685.
- [48] M. Plavšić, B. Čosović, *Anal. Chim. Acta* 284 (1994) 539.