

Significance of data treatment and experimental setup on the determination of copper complexing parameters by anodic stripping voltammetry

Dario Omanovic, Cédric Garnier, Yoann Louis, Véronique Lenoble, Stéphane Mounier, Ivanka Pižeta

► To cite this version:

Dario Omanovic, Cédric Garnier, Yoann Louis, Véronique Lenoble, Stéphane Mounier, et al.. Significance of data treatment and experimental setup on the determination of copper complexing parameters by anodic stripping voltammetry. *Analytica Chimica Acta*, Elsevier Masson, 2010, 664, pp.136 - 143. <10.1016/j.aca.2010.02.008>. <hal-01096839>

HAL Id: hal-01096839

<https://hal-univ-tln.archives-ouvertes.fr/hal-01096839>

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.



Significance of data treatment and experimental setup on the determination of copper complexing parameters by anodic stripping voltammetry

Dario Omanović^{a,*}, Cédric Garnier^{b,1}, Yoann Louis^{b,c,2}, Véronique Lenoble^b, Stéphane Mounier^b, Ivanka Pižeta^a

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

^b Laboratoire PROTEE, Université du Sud Toulon Var, BP 20132, 83957 La Garde, France

^c Laboratoire Eau Environnement et Systèmes Urbains (LEESU), Université Paris-Est AgroParisTech, 61 Avenue du Général de Gaulle, 94010 Créteil, France

ARTICLE INFO

Article history:

Received 10 December 2009

Received in revised form 11 February 2010

Accepted 15 February 2010

Available online 23 February 2010

Keywords:

Anodic stripping voltammetry

Metal speciation

Copper complexing capacity

Data treatment

Fitting

ABSTRACT

Different procedures of voltammetric peak intensities determination, as well as various experimental setups were systematically tested on simulated and real experimental data in order to identify critical points in the determination of copper complexation parameters (ligand concentration and conditional stability constant) by anodic stripping voltammetry (ASV). Varieties of titration data sets ($Cu_{measured}$ vs. Cu_{total}) were fitted by models encompassing discrete sites distribution of one-class and two-class of binding ligands (by PROSECE software). Examination of different procedures for peak intensities determination applied on voltammograms with known preset values revealed that tangent fit (TF) routine should be avoided, as for both simulated and experimental titration data it produced an additional class of strong ligand (actually not present). Peak intensities determination by fitting of the whole voltammogram was found to be the most appropriate, as it provided most reliable complexation parameters.

Tests performed on real seawater samples under different experimental conditions revealed that in addition to importance of proper peak intensities determination, an accumulation time (control of the sensitivity) and an equilibration time needed for complete complexation of added copper during titration (control of complexation kinetics) are the keypoints to obtain reliable results free of artefacts.

The consequence of overestimation and underestimation of complexing parameters is supported and illustrated by the example of free copper concentrations (the most bioavailable/toxic specie) calculated for all studied cases. Errors up to 80% of underestimation of free copper concentration and almost two orders of magnitude overestimation of conditional stability constant were registered for the simulated case with two ligands.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

In natural waters trace metals are associated with inorganic and organic ligands, forming complexes of various strengths [1,2]. Inorganic side of metal complexation is well known and easily predictable, but complexation with ligands from the natural organic matter (NOM) is still not completely known and is a subject of great scientific interest and challenge [3–5].

Continuous or discrete models of complexing sites distribution accountable for metal complexation are mostly used to characterize NOM–metal interactions [3,5–13]. It is shown that discrete models of one to three classes of natural ligands are often sufficient to fit

experimental data [3,5,13]. Among many, voltammetric techniques such as anodic stripping voltammetry (ASV) and competitive ligand exchange–adsorptive cathodic stripping voltammetry (CLE–AdCSV) are widely used because of their excellent sensitivity and selectivity in natural water samples [3,5,6,8–15]. Both techniques are based on sample titration by metal of interest (usually copper), followed by the mathematical treatment of measured data to obtain one or more conditional stability constants of formed metal complexes and concentrations of corresponding ligands. For this purpose linearization method proposed by Ružić and vandenBerg is widely used [16,17], although non-linear fitting approaches were practiced and shown to be superior [3,7,18]. Distinguishing of more complex and also more probable bi-ligand and mixed metal–ligand complexes than simple 1:1 metal–ligand systems is shown to be achievable too [19]. One of the major factors obstructing the calculation of more complex metal–ligand system is the precision of measurements.

For most of complexation studies, calculated complexation parameters are restricted to be used for modelling of metal spe-

* Corresponding author. Tel.: +385 1 4680 231; fax: +385 1 4680 231.
E-mail addresses: omanovic@irb.hr (D. Omanović), cgarnier@univ-tln.fr (C. Garnier), louis@univ-tln.fr (Y. Louis), lenoble@univ-tln.fr (V. Lenoble), mounier@univ-tln.fr (S. Mounier), pizeta@irb.hr (I. Pižeta).

¹ Tel.: +33 494142099; fax: +33 494142168.

² Tel.: +33 494142608.

ciation in a particular aquatic environment and are not considered to be applied on larger spatial scale. In dynamic environments such as estuaries, a large variety of complexing properties could be obtained, ensuring prediction of metal speciation only on limited part of a water body [8,14].

However, not only the diversity in water composition could generate large differences in complexing parameters, but the experimental setup, data treatment and calculations, systematic errors in the analytical method or interpretation of the data could result in variations of complexation properties for the same studied sample, even if the same technique is used.

Recently a discussion regarding equilibration time in CLE-AdCSV attracted attention of the scientific community, finishing finally as “unresolved issue” [15,20–22]. Equilibration time during titration is a critical part for the ASV, as well. Very large differences in equilibration time are reported in the literature, ranging from only few minutes to overnight equilibration [3,6,8,9,12–14,23,24]. Checking of signal stability during larger period of time (e.g. more than 1 h) is favoured [8,24], in order to ensure reliable determination of complexing parameters. Two hours of equilibrium time is found to be sufficient for copper to reach equilibrium higher than 95% in estuarine conditions [8].

The comparison of the complexation parameters found in the literature is a common practice in this type of studies, whether the same or different measurement technique is used. The two mostly used electrochemical techniques ASV and CLE-AdCSV operate at different “detection windows” [3,6,11,25]. The “detection window” of AdCSV could be adjusted by the concentration of added competing ligand. The conditional stability constants determined by this technique are generally higher than that of ASV [3]. However, for example, in a study of Bruland et al. [3], it is found that with ASV three different classes of binding ligands were distinguished and characterized, compare to the two-class obtained by CLE-CSV. Thus it is not straightforward to directly compare metal complexation parameters obtained by these two different techniques, and it should be avoided. However, a complementary study by both techniques is always advisable [3], regarding their “detection windows”.

The aim of speciation studies is the estimation of complexing parameters which could be used to predict the concentration of different metal species in a particular aquatic environment. The most important metal specie is a free metal ion, as it is confirmed to be the most bioavailable/toxic to biological organisms [26]. Thus, it should be expected that whichever the measurement technique used, the calculated free ion concentrations should not be in a high discrepancy for the same studied sample.

For example, it is found that >99% of copper at its ambient concentration (few nM) in seawater is bound to complexing sites which correspond to stronger class of organic ligand (of lower concentration), while weaker class of ligands (of higher concentration) are considered as potential “buffering capacity” for increased copper concentration [3,8]. For natural waters with low dissolved organic carbon (DOC) and low copper concentration this type of binding ligands could be efficiently calculated only if the corresponding range of copper concentrations in titration curve is covered. Usually this range starts at the ambient concentration, which in turn requires sufficient sensitivity of the applied voltammetric technique (as the measurable copper concentration could be below nM level). Using inadequate measuring procedure (e.g. short accumulation time) this range could be missed, resulting in impossibility of the determination of stronger type of binding ligands.

The most noticed complication in ASV is found to be an adsorption of surface active substances (SAS) originating from NOM on the surface of the electrode [27–29]. Fortunately, such problems could be effectively eliminated by applying very negative “desorption potential” (e.g. -1.5 V) for a short period of time (1% of total accu-

mulation time) at the end of the accumulation period [10,29,30]. Without this desorption step, a broad and relatively high peak overlapping with the oxidation peak of copper appears, which is usually assigned to copper peak by most of the authors.

Taking all of the above-mentioned points into account, an over- and under-estimation of both conditional stability constants and ligand concentrations could occur, which in turn lead to incorrect metal speciation determination. To avoid this inaccuracy and to correctly predict metal speciation (as much as possible), not only the verified measurement procedures, but also the peak treatment procedures should be obeyed.

Following this idea, the purpose of this paper is to present on both model data and real samples an effect of different data treatment routines and experimental setups on the determination of copper complexing parameters. In addition, an implication on free copper concentration estimation at its ambient conditions is illustrated.

2. Experimental, simulation and fitting

2.1. Experimental

2.1.1. Sample description

Samples were collected by scuba-diving [31] (pre-cleaned 1 L FEP bottles, Nalgene) in March 2008 in the Krka River estuary (Croatia) along a depth profile near the scientific marine station “Martinska”, Ruđer Bošković Institute (GPS coordinates: $43^{\circ}44'07.92$ N, $15^{\circ}52'39.61$ E). These samples have already been the object of a previous study (for more details about the sampling zone, sampling protocol and analytical techniques, see [8]). The two samples, selected to be studied in more detail concerning experimental conditions (equilibration time, deposition time, addition of Triton-X-100), correspond to depths 1.2 m (sample 1) and 3.8 m (sample 2), respectively. Salinity, total dissolved Cu and dissolved organic matter concentrations are as follows: $31, 8.47 \pm 0.54$ nM, 0.97 ± 0.01 mg_C L⁻¹ (sample 1) and $38, 4.84 \pm 0.63$ nM, 0.89 ± 0.02 mg_C L⁻¹ (sample 2).

2.1.2. Instrumentation

All voltammetric measurements were carried out using an Autolab potentiostat (EcoChemie, Utrecht, The Netherlands) (PGSTAT12) equipped with a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland). The instrument was computer-controlled using GPES 4.9 control software. The working electrode was a static mercury drop electrode (size 1, 0.25 mm²), the counter electrode was a platinum wire, and the reference electrode was Ag|AgCl (sat. NaCl). Stirring of the solution was performed by built-in Teflon stirrer rotating at 3000 rpm. All measurements were performed at room temperature (22 ± 1 °C), in a PFA cell. pH was controlled by a Metrohm pH meter (Model 713) with a glass pH electrode. Three automatic burettes (Cavro XE 1000 syringe pumps, Tecan, Swiss) were used to automate copper titration in logarithmic mode. Differential pulse anodic stripping voltammetry (DPASV) was applied as adequate sensitive and robust measuring technique because it gives high signal to noise ratio and reasonable high scan rate. The following experimental parameters were used: pulse amplitude, $A = 20$ mV; potential step increment, $E_{\text{st inc}} = 2$ mV; time between pulses, $t_{\text{int}} = 0.1$ s; pulse duration, $t_{\text{mod}} = 0.04$ s.

2.2. Simulation of voltammograms

Simulated voltammograms of copper (from which peak area is used for the construction of titration curves) were obtained as follows: on smoothed voltammogram without copper signal, obtained in real seawater sample without accumulation (considered here as baseline current), simulated voltammograms of copper

(using $1/\cosh^2$ function) with increasing peak heights were added. Intensities of copper signals were calculated according to copper concentrations not bound to hypothetical organic ligands L_1 ($[L_1] = 3 \text{ nM}$, $\log K_1 = 10$) and L_2 ($[L_2] = 50 \text{ nM}$, $\log K_2 = 8$) assuming sensitivity of 0.2 nA nM^{-1} [32]. Simulated copper titration starting with concentration of 2.9 nM (i.e. matching the real concentrations in Šibenik bay) up to 600 nM was performed in 15 equal increments of copper additions in logarithmic scale ($\Delta p\text{Cu}$). Two sets of “pure” simulated data were constructed comprising one-ligand system (L_1) and two-ligand system (L_1 and L_2). In addition, other parallel sets of voltammograms were constructed with a random noise (ranged between $\pm 4 \times 10^{-11} \text{ A}$, estimated according to real measurements) added to the voltammetric curves in order to reproduce real measurement conditions, leading to two sets of “noised” simulated data.

2.3. Modes of peak determination

The area under the peak is used as a measure of the signal. The area is calculated by integration of baseline-subtracted voltammograms. For this purpose three different procedures for baseline approximation were tested:

- (1) TF – tangent fit – the baseline in the potential range of the peak is defined as linear (the most used procedure of peak determination).
- (2) SF – spline fit – the baseline in the potential range of the peak is defined as a spline function using points preceding and following the range of peak appearance. This function is automatically performed by home-written software.
- (3) VF – voltammetric fit – each voltammogram is fitted in the complete potential range scanned, by combination of polynomial ($I_{\text{baseline}}^{\text{poly}} = a \times E^3 + b \times E^2 + c \times E + d$), exponential ($I_{\text{baseline}}^{\text{exp}} = \alpha \times \exp(\beta \times E + \gamma)$) and up to two $1/\cosh^2$ functions [33]. All the parameters of these functions are optimized using the Solver subroutine of Excel to fit the complete voltammogram. The peak area is then determined by integration of the $1/\cosh^2$ functions which corresponds to copper peak. This peak determination procedure was already successively applied in previous studies [8,34,35].

For both simulated and experimental titration curves, voltammetric sensitivities (nA nM^{-1}) were determined by linearization of

the 3–5 last points (the range where organic ligands are already saturated by copper) [3,8,9]. Using corresponding sensitivities, the relationships of $[\text{Cu}]_{\text{measured}}$ vs. $[\text{Cu}]_{\text{total}}$ were constructed, and used for the further calculations and transformations. In total 30 titration data sets (12 simulated and 18 experimental) were generated for both simulated and experimental data.

2.4. Tool for fitting—PROSECE

For each data set, the concentration of complexing ligands ($[L]_{\text{T}}$) and corresponding conditional stability constants (K'_i) were determined by fitting software named PROSECE [7]. Data are fitted assuming models comprising one- and two-class of binding ligands (L_1 and L_2). The confidence interval is calculated by PROSECE as the maximal absolute variation (in percentage) of the parameter value corresponding to a 10% increase of the fitting error (sum of the difference between measured and calculated concentrations expressed in log) [8,35]. For the experimental titration data sets, the inorganic copper speciation is taken into account in the model fitted by PROSECE, and accordingly, the conditional stability constant are given in respect to free Cu^{2+} . It is assumed that the electro-labile copper measured by DPASV mostly corresponds to the free and inorganic copper complexes. For simulated titration data sets copper speciation is restricted to its free (Cu^{2+}) and organic (CuL_1 , CuL_2) fractions.

The following parameters were used in the calculations of inorganic copper speciation: pH, major inorganic cations and anions concentrations (calculated from the salinity). Stability constants used for inorganic copper speciation were those provided by MINEQL [36] and MINTEQA2 databases.

3. Results and discussion

3.1. Effect of data treatment procedures (simulated titrations)

Four cases of simulated voltammetric titrations were generated defining (1) one-ligand system ($[L_1]_{\text{T}} = 50 \text{ nM}$, $\log K_1 = 8$), (2) two-ligand system ($[L_1]_{\text{T}} = 3 \text{ nM}$, $\log K_1 = 10$, $[L_2]_{\text{T}} = 50 \text{ nM}$, $\log K_2 = 8$), and in addition, (3) and (4) the same as (1) and (2) with the random noise added to simulated voltammograms. Each titration curve was fitted by means of discrete model of site distribution assuming (i) one-class ligand model and (ii) two-class ligand model. An analysis of variances is used as criterium for decision of acceptance of the correct model (F -test).

Table 1
Copper complexing capacity parameters as results of different procedures for simulated one-class ligand model^a.

	1st ligand				2nd ligand				Σ ligand		s	F-Test
	L_1 (nM)	+/-	$\log K\text{CuL}_1$	+/-	L_2 (nM)	+/-	$\log K\text{CuL}_2$	+/-	L_{T} (nM)	+/-		
S1P-TF-1L	46.8	3.9	8.37	0.04					46.8	3.9	0.102	
S1P-TF-2L	3.4	0.0	10.03	0.03	50.4	0.4	8.08	0.01	53.9	0.4	0.009	S
S1P-SF-1L	53.3	0.7	7.97	0.00					53.3	0.7	0.014	
S1P-SF-2L	31.8	0.7	7.98	0.02	21.6	0.7	8.01	0.03	53.4	0.9	0.011	NS
S1P-VF-1L	49.3	0.1	8.01	0.00					49.3	0.1	0.001	
S1P-VF-2L	13.2	0.1	8.00	0.01	36.2	0.1	8.00	0.01	49.5	0.1	0.001	NS
S1N-TF-1L	46.9	3.7	8.37	0.04					46.9	3.7	0.088	
S1N-TF-2L	4.6	0.0	9.67	0.01	49.6	0.3	8.03	0.01	54.2	0.3	0.007	S
S1N-SF-1L	50.2	1.2	7.99	0.02					50.2	1.2	0.032	
S1N-SF-2L	1.8	0.2	8.88	0.11	49.7	0.8	7.93	0.02	51.5	0.8	0.032	NS
S1N-VF-1L	49.4	0.4	8.01	0.00					49.4	0.4	0.014	
S1N-VF-2L	4.0	0.2	8.00	0.04	45.4	0.2	8.00	0.01	49.4	0.3	0.007	S
Expected	50.0		8.0						50.0	0.0		

^a S1 = simulated one-class ligand model; P, N = pure, noised data; TF, SF, VF = tangent, spline, voltammogram fit for baseline correction; 1L, 2L = fit to one-class and two-class ligand model; s = standard deviation of fit; F-test ($\alpha = 0.05$) S = significant difference, NS = not significant difference. The best fitting is highlighted in bold.

3.1.1. One-class ligand (L_1) simulated titrations

In Table 1 copper complexing parameters obtained by PROSECE fitting to titration data constructed by three different modes of peak intensities determination (TF, SF and VF) are presented, for both pure and noised voltammograms.

For both pure and noised data sets, the PROSECE fitting of titration curve produced an additional “false” class of ligand in the case of TF mode of peak determination, while the two other modes (SF, VF) gave accurate and expected results (only one-class of ligand). It is important to point out that PROSECE fitting of the two-ligand model to simulated one-ligand titration data produced two conditional stability constants which were not significantly different (see Table 1), which in turn suggests that fitting data to two-ligand model is not reasonable. This is clearly illustrated in Table 1 for the complexing parameters obtained by fitting of titration data obtained by SF and VF and additionally supported by the not significant (NS) difference in variances. In Fig. 1 titration data obtained for all three modes of peak determination are illustrated, where an effect of underestimation of peak intensities by TF mode is obvious. In addition, for TF mode two inflection points are clearly noticeable, indicating virtual existence of two classes of binding ligands.

The appearance of the additional “false” class of ligand in the case of TF is caused by the non-ideal approximation of voltammogram baseline, which is a drawback of this method [37]. Namely, the baseline under the peak is approximated by the straight line. If the position of the peak is on the flat part of the voltammogram, this will not cause significant error in peak height (area) determination. However, if the peak position is on the rising or descending part of the voltammogram (as frequently observed for Zn and Cu in ASV measurements with Hg electrode), the peak height (area) could be extremely underestimated (>50%). Inaccuracy in peak determination is more pronounced at small peak intensities, leading to higher relative errors at lower concentrations. The effect of smaller peak intensity determination at starting part of the titration curve (at low total copper concentrations) caused the appearance of this additional “false” class of ligand in the case of TF.

3.1.2. Two-class ligand (L_1 and L_2) simulated titrations

Results of PROSECE fitting of simulated titration data constructed as two-class ligand model are presented in Table 2. Similarly to previous case, TF mode produced unsatisfactory results regarding the accuracy and the precision. As a first, PROSECE fitting with one-class ligand model resulted in underestimation of total ligand concentration (23%), while the fitting with two-class ligand model (the expected one) lead to the overestimation of the con-

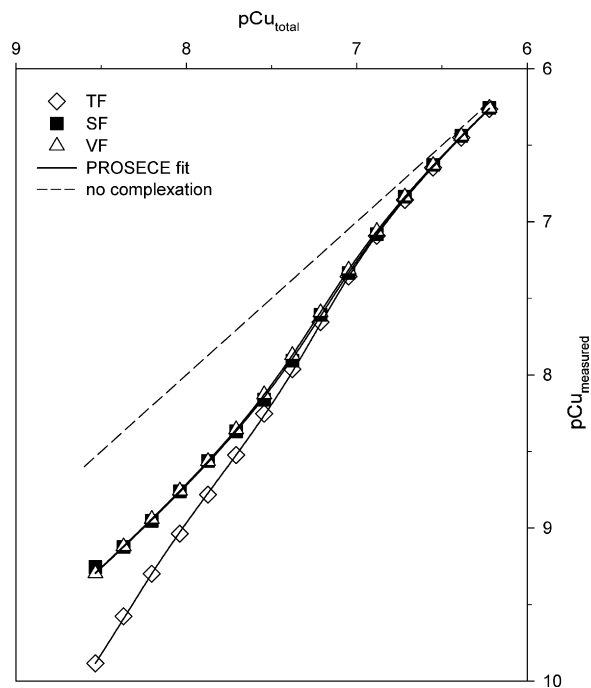


Fig. 1. Influence of different modes of peak intensities determination (TF, SF, VF) on the titration data for simulated one-class ligand model. Solid lines represent PROSECE fit based on F -Test significant model.

ditional stability constant by almost two orders of magnitude. The same is obtained for both pure and noised data sets. Other two modes of peak determination (SF, VF) provided correct results, i.e. the variances of fit for two-class ligand model were significantly smaller than those for one-class ligand model, regardless of pure or noised data. Consequently the one-class ligand model is considered as less probable and thus discarded.

3.2. Variation of experimental parameters (real samples)

According to our previous study [8], two samples were selected to examine the variation of different experimental parameters, as well as different modes of peak intensities determination, on the determination of copper complexing parameters: S1—sample 1 (salinity 31) and S2—sample 2 (salinity 38). These two samples were selected since in S1 two-class and in S2 one-class of binding

Table 2

Copper complexing capacity parameters as results of different procedures for simulated two-class ligand model^a.

	1st ligand				2nd ligand				Σ ligand		s	F-Test
	L_1 (nM)	+/-	$\log K_{CuL_1}$	+/-	L_2 (nM)	+/-	$\log K_{CuL_2}$	+/-	L_T (nM)	+/-		
S2P-TF-1L	38.4	2.8	8.75	0.00					38.4	2.8	0.388	
S2P-TF-2L	3.9	0.0	11.54	0.02	46.9	0.6	8.28	0.01	50.7	0.6	0.037	S
S2P-SF-1L	50.2	3.9	8.26	0.03					50.2	3.9	0.082	
S2P-SF-2L	3.7	0.0	9.71	0.01	56.0	0.3	7.94	0.01	59.7	0.3	0.006	S
S2P-VF-1L	47.0	3.2	8.24	0.04					47.0	3.2	0.098	
S2P-VF-2L	2.9	0.0	10.00	0.01	49.5	0.1	8.00	0.01	52.4	0.1	0.001	S
S2N-TF-1L	38.3	6.2	8.76	0.12					38.3	6.2	0.224	
S2N-TF-2L	6.2	0.1	10.40	0.03	49.8	1.1	8.08	0.02	56.0	1.1	0.054	S
S2N-SF-1L	52.1	2.3	8.20	0.01					52.1	2.3	0.050	
S2N-SF-2L	6.2	0.3	9.00	0.06	54.9	1.0	7.89	0.02	61.1	1.1	0.029	S
S2N-VF-1L	47.1	3.2	8.24	0.04					47.1	3.2	0.104	
S2N-VF-2L	2.8	0.0	10.26	0.04	49.4	0.3	8.02	0.01	52.2	0.3	0.024	S
Expected	3.0		10.0		50.0		8.0		53.0	0.0		

^a S2 = simulated two-class ligand model; P, N = pure, noised data; TF, SF, VF = tangent, spline, voltammogram fit for baseline correction; 1L, 2L = fit to one-class and two-class ligand model; s = standard deviation of fit; F-test ($\alpha = 0.05$) S = significant difference, NS = not significant difference. The best fitting is highlighted in bold.

Table 3
Copper complexing capacity parameters as results of different procedures for sample 1 of salinity 31^a.

Case		1st ligand				2nd ligand				Σ ligand		s	F-Test
		L ₁ (nM)	+/-	log KCuL ₁	+/-	L ₂ (nM)	+/-	log KCuL ₂	+/-	L _T (nM)	+/-		
A	Eq5m td 60 s TF-1L	25.5	3.0	11.73	0.04					25.5	3.0	0.217	S
	Eq5m td 60 s TF-2L	13.1	0.6	12.31	0.15	30.4	2.4	10.26	0.07	43.5	2.5	0.128	
	Eq5m td 60 s SF-1L	36.7	2.9	10.68	0.05					36.7	2.9	0.132	
	Eq5m td 60 s SF-2L	8.2	0.1	12.63	0.11	35.4	1.1	10.01	0.03	43.6	1.1	0.028	
	Eq5m td 60 s VF-1L	34.9	1.0	10.57	0.02					34.9	1.0	0.043	
	Eq5m td 60 s VF-2L	33.4	0.8	10.60	0.03	2.2	2.1	9.79	0.30	35.6	2.3	0.043	
B	Eq5mn td 300 s TF-1L	18.4	0.6	11.00	0.02					18.4	0.6	0.041	S
	Eq5mn td 300 s TF-2L	14.0	0.2	11.23	0.02	51.2	7.9	8.81	0.08	65.2	7.9	0.024	
	Eq5mn td 300 s SF-1L	18.3	0.6	10.75	0.02					18.3	0.6	0.033	
	Eq5mn td 300 s SF-2L	16.2	0.3	10.83	0.03	68.4	14.2	8.34	0.10	84.7	14.2	0.026	
	Eq5mn td 300 s VF-1L	17.0	0.6	10.73	0.02					17.0	0.6	0.042	
	Eq5mn td 300 s VF-2L	15.9	0.5	10.77	0.04	70.0	49.2	8.03	0.36	85.9	49.2	0.041	
C	Eq2h td 300 s TF-1L	26.6	3.0	10.95	0.04					26.6	3.0	0.100	S
	Eq2h td 300 s TF-2L	11.1	0.4	11.85	0.12	65.7	3.1	9.45	0.03	76.8	3.1	0.029	
	Eq2h td 300 s SF-1L	35.0	3.5	10.37	0.03					35.0	3.5	0.093	
	Eq2h td 300 s SF-2L	7.5	0.4	12.06	0.52	83.0	4.8	9.31	0.04	90.5	4.8	0.054	
	Eq2h td 300 s VF-1L	74.2	5.9	9.84	0.03					74.2	5.9	0.085	
	Eq2h td 300 s VF-2L	6.7	0.4	11.91	0.39	88.8	3.6	9.40	0.03	95.6	3.6	0.039	

^a TF, SF, VF = tangent, spline, voltamogram fit for baseline correction; 1L, 2L = fit to one-class and two-class ligand model; s = standard deviation of fit; F-test ($\alpha = 0.05$) S = significant difference, NS = not significant difference. The best fitting is highlighted in bold.

ligands were identified in the previous study. Titration by copper was performed in the range from 1 nM to 335 nM of added copper.

The influence of deposition (accumulation) time and equilibration time after addition of copper into the sample were tested on sample 1. Three different analytical conditions were adjusted: (A) 5 min of equilibration time between copper additions and 60 s of deposition time, (B) the same as (A) but with 300 s of deposition time and (C) the same as (B) but with 2 h of equilibration time between copper additions. For all three cases the deposition potential of -0.5 V was used, with a short “desorption step” (3 s) at -1.5 V at the end of accumulation step [3,8,10,35], in order to remove adsorbed organic matter from the mercury electrode surface. The deposition potential of -0.5 V was selected according to pseudopolarograms (stripping current vs. deposition potential curves) [8,10], and corresponds to potential where reduction of only reactive/labile copper (inorganic and kinetically labile weak organic complexes) takes place, while strong inert organic complexes are electroinactive. Sixty seconds of deposition time was selected as it is deposition time often used for such kind of measurements [12,38].

In Table 3 complexing parameters obtained by PROSECE fitting are presented. Since for natural samples the number of different classes of binding ligands is not known, the reasonable approach in complexing parameters estimation is to follow guidelines obtained on model titrations. In this manner, complexing parameters obtained by using VF (voltammetric fit) of peak intensities determination were considered as accurate values. In addition, the fitting results for the other two modes of peak intensities determination were provided for comparison and further speciation analysis.

Three different experimental complexation results were obtained from the different experimental procedures. According to F-test, one-class ligand model is assigned to experimental procedures in which equilibration time after copper addition was only 5 min (cases A and B), while two classes of ligands were assigned to data set obtained using 2 h of equilibration time (case C). Taking into account total ligand concentrations assigned to all three cases, the highest value is obtained for the case C (95.6 nM), while only 17.0 nM for the case B. Ligand concentration for case A (34.9 nM) is in-between. Comparing cases A and B (60 s and 300 s accumulation time), an overestimation of the ligand concentration for the case A is a consequence of the poor sensitivity obtained with only 60 s

of accumulation time. Although we used the most precise mode of peak intensities determination (VF), underestimation of very low copper signals occurred (for the first few points, the copper peak is not registered at all), which is finally recognized as complexation and resulted in higher ligand concentration. Although one-class of ligand is assigned for the case B, evidently different conditional stability constants for two-class ligand model were obtained (SF and VF), which in turn could serve as an alert for additional systematic analysis of data and/or “request” for repeated measurement.

As in natural samples initial concentration of free/labile copper complexes might be very small, it is important to adjust the measuring procedure in order to accurately determine that value. Higher sensitivity is accomplished by longer deposition time, which should be adjusted according to previously measured total concentration, which should always be known for the studied sample. Those three cases are illustrated in Fig. 2, where relationship of negative logarithms of measured vs. total copper concentrations (pCu_{labile} vs. pCu_T) and corresponding fitting lines are plotted.

Kinetics of metal complexation with natural ligands is controlled by competitive reaction with other divalent cations in the sample [35,39,40]. Although the natural systems are in so-called “dynamic equilibrium”, the prediction of metal speciation is performed according to complexation parameters determined assuming equilibrium conditions during measurement. However, in the literature authors reported wide varieties of equilibration time, ranging from only several minutes to overnight equilibration, which subsequently implied suspicion on “at equilibrium” conditions in many studies. In our previous paper, we showed that 2 h is sufficient to reach at least 95% equilibrium after each copper addition [8]. Case C corresponds to this experimental procedure. Following this approach, two-class of binding ligands were determined for the case C, and finally assigned to sample S1. Longer time needed to reach equilibrium is a consequence of slow kinetics of copper complexation which is more pronounced at the middle range of titration curve (see Fig. 2). The highest total ligand concentration (95.6 nM) is determined by this experimental approach.

The variation of experimental procedures is further extended on sample S2 (salinity 38). Again the effect of equilibration time is tested, while in addition, the influence of surface active substance (SAS, 2 mg L⁻¹ of Triton-X-100) is inspected, based on its positive effect registered in solutions with model ligands (EDTA) [41]. For all three procedures, 300 s of accumulation time is applied. Cases D and

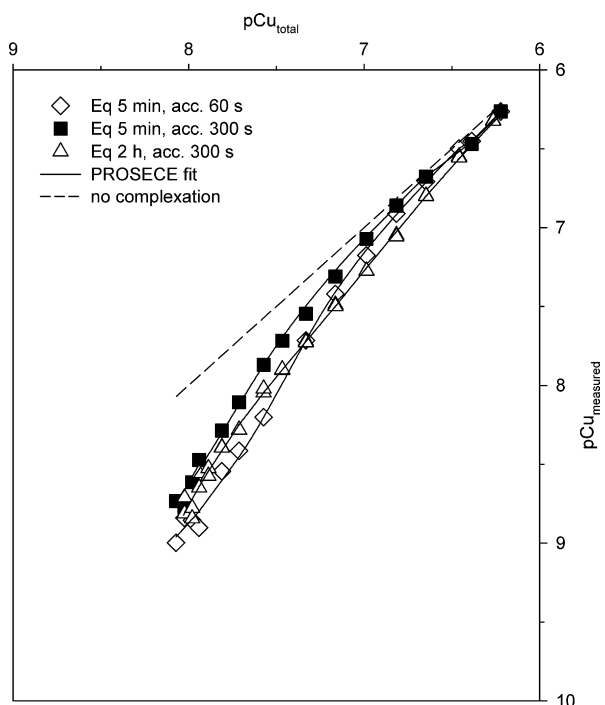


Fig. 2. Titration data for sample S1 obtained for different experimental setup using VF as a mode of peak intensities determination. Solid lines represent PROSECE fit based on *F*-Test significant model.

E correspond to 5 min of equilibration time, while for case F, 2 h is left for copper equilibration (the same protocol as case C, for sample S1). Respecting voltammetric fit mode of peak determination and experimental procedure with 2 h of equilibration time as referent rules, one-class of binding ligand is assigned to sample S2 (decision based on *F*-test value).

Similarly as for sample S1, lower total ligand is determined for shorter equilibration time (27.2 nM, case D; comparing to 107.7 nM, case F), which is in line with previous elaboration regarding the kinetics of copper complexation. Addition of T-X-100 to the sample (case E) yielded about 20–30% higher total ligand concentrations comparing to case D. However, to our present knowledge, “3 s desorption step” procedure [10] is enough for reliable results, so for the sake of simplicity in a final procedure it should have the advantage over surfactant addition.

In addition to the presented experimental parameters variation, the influence of three different modes of peak intensities determination for the case F is illustrated in Fig. 3. Although the total concentration of complexing ligands estimated by the tangent fit (TF) and voltammetric fit (VF) is almost equal (108 nM), TF mode produced appearance of additional strong ligand. This anomaly is evident in Fig. 3, where almost one order of magnitude lower free/labile copper concentration is quantified by TF at actual total copper concentration in the sample S2 (4.84 ± 0.63 nM).

3.3. Estimation of free copper concentration

The purpose of the complexation studies is the determination of trace metal speciation in natural aquatic systems. Obtained complexation parameters are subsequently used to predict metal speciation for studied aquatic environment using mathematical modelling software [36,42–45]. The concentration of free metal ion is the most examined parameter in toxicological studies, as well as studies regarding bioavailability. Thus, calculation of free copper was performed using complexation parameters given in Tables 1–4, in order to show variations in its concentration regarding different

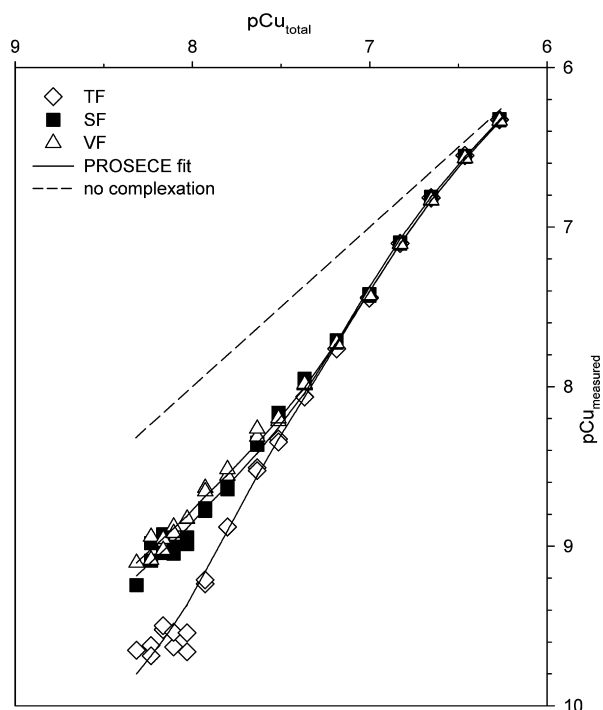


Fig. 3. Titration data for sample S2 obtained using different modes of peak intensities determination (TF, SF, VF) for the same experimental setup (2 h of equilibration time and 300 s of accumulation time). Solid lines represent PROSECE fit based on *F*-Test significant model.

mode of peak intensities determination and alterations of experimental setup. MINEQL [36] was used for this purpose. Two levels of total copper (5 nM and 25 nM) were selected, which relate to low level of total copper in examined estuary during non-touristic seasons [8,46], and increased level occurring during summer season as a result of copper leaching from large number of touristic boats coated by antifouling paint.

In Fig. 4 free copper concentration for simulated titration data sets are presented. Dashed lines represent expected values of free copper for the two simulated cases: one-class of binding ligands and two-class of binding ligands. As a result of appearance of addi-

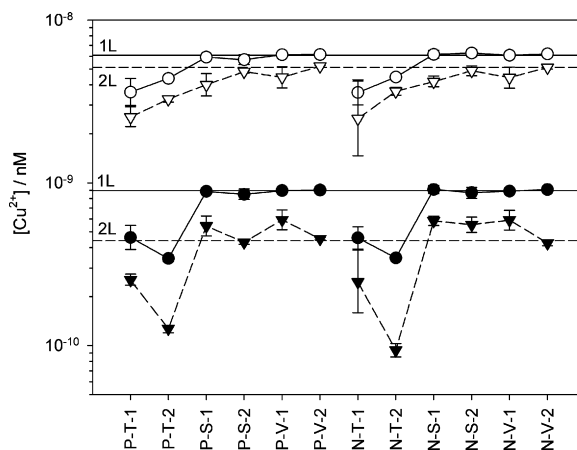


Fig. 4. Free copper concentrations calculated by MINEQL for simulated cases for 5 nM of total copper in the solution (filled symbols) and 25 nM (hollow symbols); circles, triangles – one-class ligand and two-class ligand model simulated titration; P, N – pure and noised data; T, S, V – tangent, spline and voltamogram fit for baseline correction; 1, 2 – PROSECE fit to one-class ligand and two-class ligand model. Dashed lines: expected free copper concentrations for both total copper concentrations and both simulated titrations.

Table 4
Copper complexing capacity parameters as results of different procedures for sample 2 of salinity 38^a.

Case		1st ligand				2nd ligand				Σ ligand		s	F-Test	
		L ₁ (nM)	+/-	log KCuL ₁	+/-	L ₂ (nM)	+/-	log KCuL ₂	+/-	L _T (nM)	+/-			
D	Eq5m td 300 s TF-1L	14.8	0.7	11.16	0.04					14.8	0.7	0.059	S	
	Eq5m td 300 s TF-2L	10.2	0.1	11.49	0.02	25.4	1.1	9.55	0.03	35.6	1.1	0.024		
	Eq5m td 300 s SF-1L	16.8	0.3	10.74	0.01					16.8	0.3	0.020		
	Eq5m td 300 s SF-2L	15.1	0.2	10.82	0.02	95.2	33.1	7.96	0.18	110.3	33.1	0.017		NS
	Eq5m td 300 s VF-1L	20.7	0.8	10.60	0.02					20.7	0.8	0.044		
	Eq5m td 300 s VF-2L	9.2	0.2	11.15	0.04	18.1	1.0	9.85	0.05	27.2	1.0	0.022		S
E	TX Eq5m td 300 s TF-1L	20.7	0.9	11.17	0.03					20.7	0.9	0.069	S	
	TX Eq5m td 300 s TF-2L	9.9	0.1	11.84	0.02	25.1	0.5	9.99	0.02	35.0	0.5	0.017		
	TX Eq5m td 300 s SF-1L	19.3	0.8	10.94	0.02					19.3	0.8	0.050		
	TX Eq5m td 300 s SF-2L	9.5	0.1	11.54	0.03	26.0	0.9	9.75	0.02	35.6	0.9	0.015		S
	TX Eq5m td 300 s VF-1L	19.6	0.8	11.03	0.02					19.6	0.8	0.056		
	TX Eq5m td 300 s VF-2L	11.5	0.3	11.53	0.06	26.1	1.8	9.69	0.04	37.6	1.8	0.036		NS
F	Eq2h td 300 s TF-1L	69.7	7.9	10.84	0.04					69.7	7.9	0.185	S	
	Eq2h td 300 s TF-2L	16.8	1.3	11.67	0.11	91.1	7.1	9.91	0.06	107.9	7.2	0.098		
	Eq2h td 300 s SF-1L	92.5	4.9	10.18	0.02					92.5	4.9	0.065		
	Eq2h td 300 s SF-2L	17.3	1.3	10.86	0.07	95.2	6.2	9.76	0.04	112.6	6.4	0.059		NS
	Eq2h td 300 s VF-1L	107.7	3.3	10.02	0.01					107.7	3.3	0.041		
	Eq2h td 300 s VF-2L	21.6	3.3	10.01	0.12	86.1	3.3	10.02	0.04	107.7	4.6	0.041		NS

^a TF, SF, VF = tangent, spline, voltammogram fit for baseline correction; 1L, 2L = fit to one-class and two-class ligand model; s = standard deviation of fit; F-test ($\alpha = 0.05$) S = significant difference, NS = not significant difference. The best fitting is highlighted in bold.

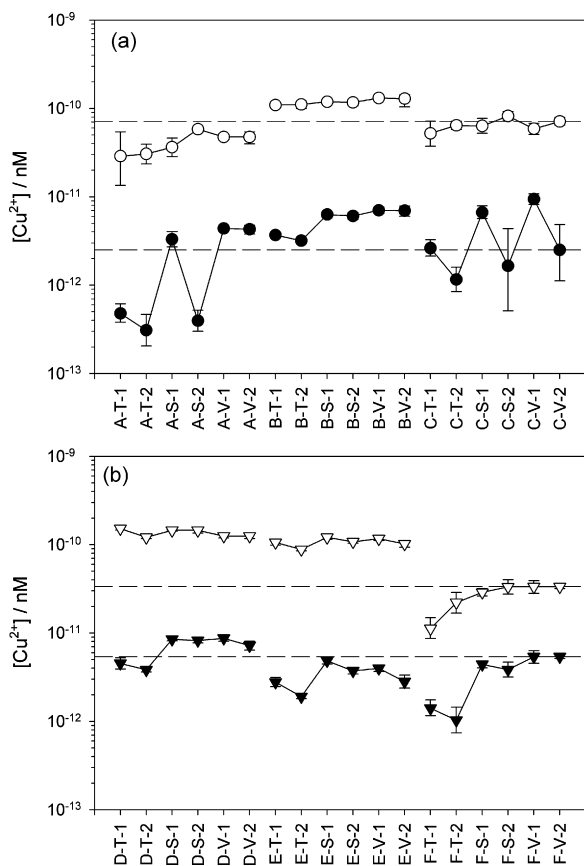


Fig. 5. Free copper concentrations calculated by MINEQL for sample S1 (a) and sample S2 (b) for 5 nM of total copper in the solution (filled symbols) and 25 nM (hollow symbols); Experimental setup: A – eq. 5 min td. 60 s, B – eq. 5 min td. 300 s, C – eq. 2 h td. 300 s, D – eq. 5 min td. 300 s, E – TX eq. 5 min td. 300 s, F – eq. 2 h td. 300 s; T, S, V – tangent, spline and voltammogram fit for baseline correction; 1, 2 – PROSECE fit to one-class ligand and two-class ligand model. Dashed lines: expected free copper concentrations for both total copper concentrations and both samples.

tional class of binding ligand (for one-ligand model) and estimation of higher conditional stability constants (for two-ligand model), free copper concentration calculated by parameters provided by TF mode are strongly underestimated. As expected, a lower relative error in free copper concentration is obtained at higher total copper concentration (25 nM, TF results), because active binding sites at this concentration are partly saturated. Thus, effects of inadequate peak determination are more pronounced at lower copper concentrations. Better results in free copper concentration prediction are obtained by other two modes of peak determination (SF and VF). Generally, a slightly lesser precision and accuracy were obtained for noised simulated data sets. However, free copper concentration predicted by VF mode favourably agreed with expected values.

Alterations in predicted free copper concentration for real samples are presented in Fig. 5a and b. Contrary to simulated case where exact values of free copper concentrations are known, for real samples the most-probable values were considered to be those calculated by using complexation parameters obtained by VF mode and 2-h of equilibrium time. Regarding the different experimental setup, more than one order of magnitude difference in free copper concentration could be calculated. We should highlight again here, that divergences obtained at low level of total copper are not followed at higher copper concentrations. For example for sample S2, for the two modes of peak intensities determination (D and E), very high overestimation of free copper is predicted for 25 nM of total copper due to underestimation of the total ligand concentration, while for 5 nM level, predicted free copper concentrations were around expected (case D) or underestimated (case E) due to overestimation of the conditional stability constants. To keep the consistency of the text detailed elaboration of each separate case is avoided.

4. Conclusions

Overestimation or underestimation of metal complexation parameters (ligand concentrations and conditional stability constants) is likely to occur if proper data treatment routines and experimental parameters were not respected. It is well demonstrated that fitting of copper titration data (both simulated and experimental) obtained by the tangent fit (TF) mode of peak intensi-

ties determination (the most commonly used method) revealed an existence of additional virtual class of strong binding ligand, which actually does not exist. More sophisticated modes of peak intensities determination which take into account curvature behaviour of baseline in the potential range of copper peak appearance (denoted here as spline fit (SF) and voltammetric fit (VF)) are proposed for reliable determination of complexation parameters. The SF procedure has the advantage to be less time consuming than the VF one, but could lead to incorrect results if performed automatically on a set of voltammograms presenting baseline and/or peak shape/position variations, so need to be carefully used. As a supplementary method of signal processing, a first or second derivative could be used, as they are less dependent on voltammogram baseline shape [47]. However, in simple model systems we found that intensities obtained by derivative methods are strongly dependent on half-peak width (which variation is frequent for Cu peak). As the estimation of the signal intensities is a very important subject in voltammetric data treatment and handling, it is a concern of our interest of both theoretical and experimental verification.

In addition to correct data treatment, appropriate experimental parameters which will take into account kinetics of the copper chemistry in real water systems should be strictly followed. Having this as an imperative, accumulation time should be adopted to be sufficient for proper determination of copper electroactive concentrations at its ambient level, while in addition, adequate time for attainment of equilibrium after each copper addition during titration should be secured. An unwanted side effect of inadequate determination of complexation parameters is incorrect prediction of copper speciation, which is more pronounced at lower total copper concentrations.

The following parameters are recommended in order to obtain more reliable copper complexation parameters in estuarine/marine systems using ASV: deposition potential of -0.5 ± 0.1 V, at least 300 s of deposition time including “desorption step” at more negative potential for a short period of time (e.g. 3 s at -1.5 V), at least 2 h of equilibration time after each copper addition, and finally proper peak intensity determination which include the best available method of baseline approximation (voltammetric fit in our case). Unfortunately, this procedure is time consuming (around 20–30 h per sample), and could be partly shortened if aliquots of sample with increasing copper additions were already separately prepared and left to equilibrate overnight. However, the later procedure is more complicated for handling and measurement, with higher contamination risk.

Acknowledgements

The financial support of the Ministry of Science, Education and Sports of the Republic of Croatia, under the Project “Interactions of trace metals in aquatic environment” (098-0982934-2720), is gratefully acknowledged. Financial support obtained in the framework of a PHC ECONET (18842QB) program gave the opportunity to organize joined meeting, associated to funds from the ANR MARSECO project used for the acquisition of a part of the used voltammetric equipment, as well as funds from the French Ministry of Education and Research for Y. Louis PhD support are acknowledged.

References

- [1] K.W. Bruland, M.C. Lohan, in: H.D. Holland, K.K. Turekian (Eds.), *Treatise on Geochemistry*, Pergamon, Oxford, 2004, p. 23.
- [2] J. Buffle, *Complexation Reactions in Aquatic Systems: An Analytical Approach*, Ellis Horwood Ltd., Chichester, 1988.
- [3] K.W. Bruland, E.L. Rue, J.R. Donat, S.A. Skrabal, J.W. Moffett, *Anal. Chim. Acta* 405 (2000) 99.
- [4] K. Hirose, *Appl. Geochem.* 22 (2007) 1636.
- [5] R.M. Town, M. Filella, *Limnol. Oceanogr.* 45 (2000) 1341.
- [6] J.R. Donat, C.M.G. Vandenberg, *Mar. Chem.* 38 (1992) 69.
- [7] C. Garnier, I. Pižeta, S. Mounier, J.Y. Benaïm, M. Branica, *Anal. Chim. Acta* 505 (2004) 263.
- [8] Y. Louis, C. Garnier, V. Lenoble, S. Mounier, N. Cukrov, D. Omanović, I. Pižeta, *Mar. Chem.* 114 (2009) 110.
- [9] G. Capodaglio, G. Scarponi, G. Toscano, C. Barbante, P. Cescon, *Fresenius J. Anal. Chem.* 351 (1995) 386.
- [10] Y. Louis, P. Cmok, D. Omanović, C. Garnier, V. Lenoble, S. Mounier, I. Pižeta, *Anal. Chim. Acta* 606 (2008) 37.
- [11] M. Pesavento, G. Alberti, R. Biesuz, *Anal. Chim. Acta* 631 (2009) 129.
- [12] M. Plavšić, Ž. Kwokal, S. Strmečki, Ž. Peharec, D. Omanović, M. Branica, *Fresenius Environ. Bull.* 18 (2009) 327.
- [13] M. Scoullou, M. Plavšić, S. Karavoltsov, *Mar. Chem.* 86 (2004) 51.
- [14] M.P. Hurst, K.W. Bruland, *Anal. Chim. Acta* 546 (2005) 68.
- [15] R.M. Town, H.P. van Leeuwen, *Environ. Chem.* 2 (2005) 80.
- [16] I. Ružičić, *Anal. Chim. Acta* 140 (1982) 99.
- [17] C.M.G. Vandenberg, *Mar. Chem.* 11 (1982) 307.
- [18] L.J.A. Gerringa, P.M.J. Herman, T.C.W. Poortvliet, *Mar. Chem.* 48 (1995) 131.
- [19] C. Garnier, I. Pižeta, S. Mounier, V. Cuculjić, J.Y. Benaïm, *Anal. Chim. Acta* 538 (2005) 263.
- [20] K.A. Hunter, *Environ. Chem.* 2 (2005) 85.
- [21] R.M. Town, H.P. van Leeuwen, *Environ. Chem.* 2 (2005) 90.
- [22] C.M.G. van den Berg, *Environ. Chem.* 2 (2005) 88.
- [23] J.I. Lorenzo, M. Nieto-Cid, X.A. Alvarez-Salgado, P. Perez, R. Beiras, *Mar. Chem.* 103 (2007) 61.
- [24] A.E. Witter, S.A. Mabury, A.D. Jones, *Sci. Total Environ.* 212 (1998) 21.
- [25] R.M. Town, M. Filella, *Anal. Chim. Acta* 466 (2002) 285.
- [26] W.G. Sunda, P.A. Tester, S.A. Huntsman, *Mar. Biol.* 94 (1987) 203.
- [27] M. Boussemart, L. Menargues, J.Y. Benaïm, *Electroanalysis* 5 (1993) 125.
- [28] M. Plavšić, D. Krznarić, B. Čosović, *Electroanalysis* 6 (1994) 469.
- [29] G. Scarano, E. Bramanti, *Anal. Chim. Acta* 277 (1993) 137.
- [30] P.B. Kozelka, K.W. Bruland, *Mar. Chem.* 60 (1998) 267.
- [31] G. Kniewald, Ž. Kwokal, M. Branica, *Mar. Chem.* 22 (1987) 343.
- [32] I. Pižeta, M. Branica, *Anal. Chim. Acta* 351 (1997) 73.
- [33] A.J. Bard, L.R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd edition, John Wiley & Sons, New York, 2001.
- [34] V. Lenoble, C. Garnier, A. Masion, F. Ziarelli, J.M. Garnier, *Anal. Bioanal. Chem.* 390 (2008) 749.
- [35] Y. Louis, C. Garnier, V. Lenoble, D. Omanović, S. Mounier, I. Pižeta, *Mar. Environ. Res.* 67 (2009) 100.
- [36] J.C. Westall, J.L. Zachary, F. Morel, *MINEQL: A Program for the Calculation of Chemical Equilibrium Composition of Aqueous Systems*. Tech. Note, No. 18., Massachusetts Institute of Technology, Cambridge, MA, 1976.
- [37] I. Pižeta, D. Omanović, M. Branica, *Anal. Chim. Acta* 401 (1999) 163.
- [38] M. Plavšić, B. Gašparović, S. Strmečki, V. Vojvodić, N. Tepić, *Estuar. Coast. Shelf S.* 85 (2009) 299.
- [39] B. Raspor, H.W. Nürnberg, P. Valenta, M. Branica, *J. Electroanal. Chem.* 115 (1980) 293.
- [40] A. Iglesias, R. Lopez, S. Fiol, J.M. Antelo, F. Arce, *Water Res.* 37 (2003) 3749.
- [41] D. Omanović, I. Pižeta, Ž. Peharec, M. Branica, *Mar. Chem.* 53 (1996) 121.
- [42] A.L. Herbelin, J.C. Westall, *FITEQL 4.0: A Computer Program for Determination of Chemical Equilibrium Constants from Experimental Data*, Department of Chemistry, Oregon State University, Corvallis, 1999.
- [43] E. Tipping, *Comput. Geosci. (UK)* 20 (1994) 973.
- [44] J. Van der Lee, L. De Windt, *CHESS Tutorial and Cookbook. User's Guide Nr. LHM/RD/99/05*, CIG-École des Mines de Paris, Fontainebleau, France, 2000.
- [45] W. Verweij, *CHEAQS Pro, Program for Calculating Chemical Equilibria in Aquatic Systems*, 2007.
- [46] D. Omanović, Ž. Kwokal, A. Goodwin, A. Lawrence, C.E. Banks, R.G. Compton, S. Komorsky-Lovrić, *J. Iran. Chem. Soc.* 3 (2006) 128.
- [47] P. Salaun, B. Planer-Friedrich, C.M.G. van den Berg, *Anal. Chim. Acta* 585 (2007) 312.