

# Integrated Microanalytical System for Simultaneous Voltammetric Measurements of Free Metal Ion Concentrations in Natural Waters

Stéphane Noël,<sup>a</sup> Mary-Lou Tercier-Waeber,<sup>a\*</sup> Lin Lin,<sup>a</sup> Jacques Buffle,<sup>a</sup> Olivier Guenat,<sup>b</sup> Milena Koudelka-Hep<sup>b</sup>

<sup>a</sup> CABE, Department of Inorganic, Analytical and Applied Chemistry, University of Geneva, Sciences II, 30 Quai E.-Ansermet, 1211 Geneva 4, Switzerland

<sup>b</sup> Institute of Microtechnology, University of Neuchâtel, Jaquet-Droz 1, 2007 Neuchâtel, Switzerland

\*e-mail: Marie-Louise.Tercier@cabe.unige.ch

## Abstract

A complexing gel integrated microelectrode (CGIME) for direct measurements of free metal ion concentrations in natural waters has been developed. It is prepared by the successive deposition of microlayers of a chelating resin, an antifouling agarose gel and Hg on a 100-interconnected Ir-based microelectrode array. The trace metals of interest are in a first step accumulated on the chelating resin in proportion to their free ion concentration in solution, then released in acidic solution and detected simultaneously by using square wave anodic stripping voltammetry (SWASV). The reliability of this sensor for the simultaneous measurement of copper, lead and cadmium has been studied by a series of replicate laboratory tests. The proportionality between the voltammetric peak current intensity and the free metal ion concentrations in solution has been demonstrated by using malonate as a model ligand. Finally, the CGIME sensor was applied to the Cu and Pb free concentration measurement in sea water samples and the results compared to the free metal ion concentrations measured using hollow fiber based permeation liquid membrane (HF-PLM) coupled to inductively coupled plasma mass spectrophotometer (ICP-MS). Comparable concentration values were found for both metals with both techniques allowing to validate the CGIME measurements in complex media.

**Keywords:** Metal speciation, Free metal ions, Microsensor array, Microanalytical system, Square wave voltammetry, Environmental analysis, Natural waters

## 1. Introduction

Measurements of relevant fractions of trace metals in natural waters are essential to improve our understanding of their behavior and long-term impact [1, 2]. The individual physicochemical forms of metals in the natural waters may include particulate, colloidal and dissolved species such as simple inorganic and organic complexes [3]. For many aquatic organisms, the free hydrated ions of Cd(II), Cu(II) and Pb(II) and their mobile and labile complexes are the bioavailable and toxic species [4].

The determination of free metal ions is regarded as crucial to assess the capability or limitation of the free-ion activity model (FIAM) [4] or the biotic ligand model (BLM) [4–6] traditionally used to predict toxicological impact of trace metals in environmental systems. Very few analytical methods allow to measure selectively the free ions at ppt level in complex media. One of these methods is the permeation liquid membrane (PLM) technique, based on the diffusive transport of the target metal ions, by a complexing carrier, across a hydrophobic liquid membrane, from the sample source phase to a small volume of receiving solution. Under correctly chosen conditions, the free metals in the test solution can be determined from the metal measured in the receiving solution using FAAS, ICP-MS or

voltammetry [7, 8]. Alternative approaches for the free ion measurement are the Donnan Membrane technique (DMT) [9] and the ion selective electrodes (ISE) with improved sensitivity [10, 11]. All these techniques present some drawbacks for direct measurements of free metal ions in natural waters. Some lipophilic metal complexes may also be measured in PLM [12] in addition to the free ions. Very long accumulation time is required in DMT, to allow subnanomolar concentration measurements, i.e., deployment time  $\geq$  one day. ISE response time is rather slow at subnanomolar concentrations and their selectivity at very low concentration in complex media has not yet been studied in details. In addition, ISE do not allow multielement analysis.

Therefore, a Complexing Gel Integrated Microelectrode (CGIME) for the specific, simultaneous monitoring of Cu, Pb and Cd free ions present at subnanomolar levels in complex media was developed. This was achieved by integrating, between the gel and the sensor surface of a gel integrated microelectrode (GIME) [13, 14], a thin layer of a microsized cation exchange resin on which metals can be accumulated in proportion to their free concentrations in solution. The CGIME is thus an integrated microanalytical system coupling: on-line separation of colloidal/particulate fouling materials [13, 14], metal chemical accumulation,

sensitive voltammetric detection of the accumulated metals in a  $2 \text{ mm}^3$  volume (Fig. 1b). This paper reports: i) the CGIME preparation and measurement principle, ii) the optimization of the analytical procedure for reliable chemical preconcentration and square wave anodic stripping voltammetric (SWASV) detection of trace metal in non-complexing media, iii) the performance of the CGIME sensor for direct, simultaneous measurements of copper, lead and cadmium free ion concentrations at subnanomolar level in model complexing solution, and finally iv) the first applications of the CGIME for direct, simultaneous measurements of Cu and Pb free ion concentrations in real sea water samples.

## 2. Experimental

### 2.1. CGIME Preparation and Measurement Principle

The schematic diagram of the CGIME sensor is given in Figure 1a. The preparation of the CGIME involves the successive deposition of a complexing resin, an agarose gel and Hg semidrops on an Ir-based microelectrode array. The microelectrode array consists of  $5 \times 20$  interconnected iridium micro disks of  $5 \mu\text{m}$  in diameter and a center-to-center spacing distance of  $150 \mu\text{m}$  surrounded by a  $300 \mu\text{m}$  thick Epon SU8 containment ring (Fig. 1). These microelectrode arrays are produced by thin film technology on silicon/ $\text{Si}_3\text{N}_4$  substrates as described in [13, 15]. The complexing resin is a polystyrene immobilized iminodiacetate (IDA) resin with an average bead size of  $0.2 \mu\text{m}$  (Microchelex resin, CETAC Technologies-USA; see Sec. 2.2). This

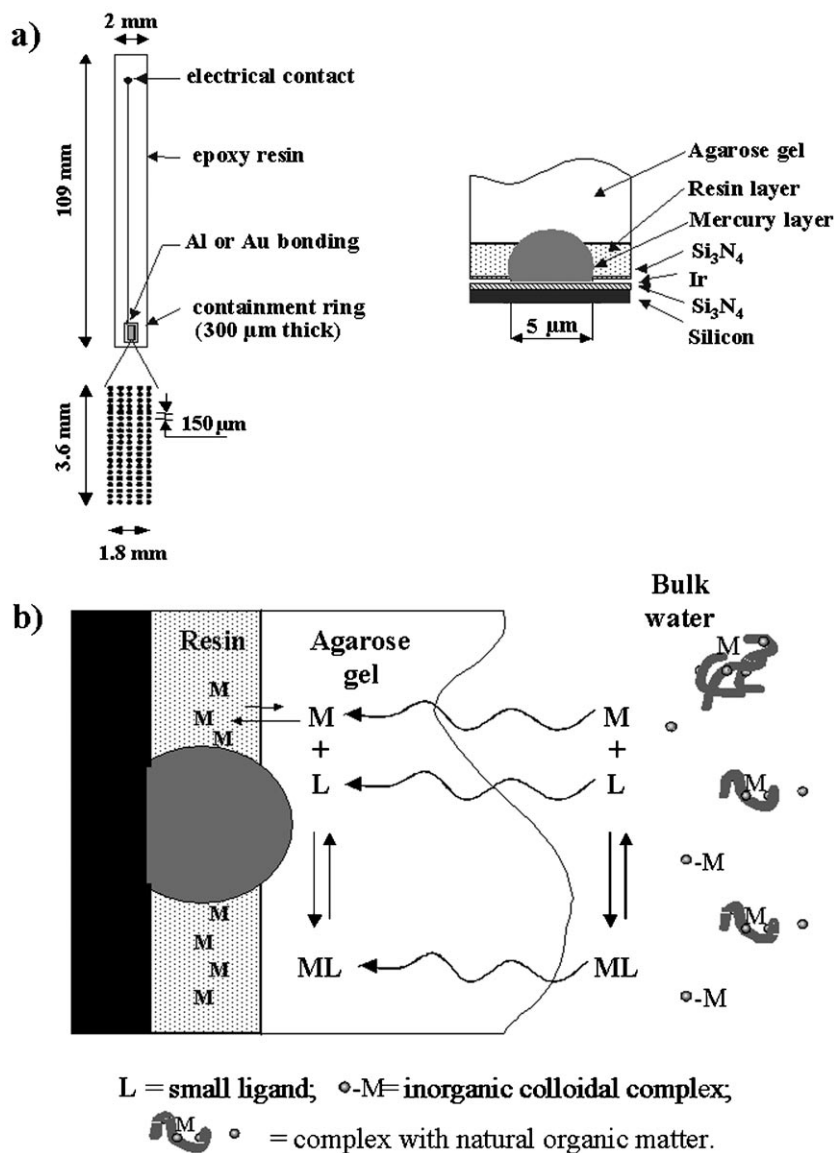


Fig. 1. Schematic diagrams of a) the CGIME and b) its equilibration with the test solution.

resin present several prerequisite characteristics for the development of the CGIME, namely: i) sufficiently large complexing capacity and stability constants for the trace metals of interest, ii) sufficient selectivity for the target metals towards  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , in particular in the pH range 6–9; iii) possible desorption of the test metals under not too drastic acidic conditions; iv) resin bead size small enough to allow the deposition of a complexing resin layer with a thickness  $< 10 \mu\text{m}$ . This is required to ensure that diffusion through this layer is not be too slow. The deposition of the Microchelex resin on the microelectrode array surface was performed as follows:  $1 \mu\text{L}$  of a 2.5% (w/v) Microchelex aqueous suspension stock solution was deposited in the containment ring surrounding the sensor chip by means of a micropipette. The sensor was then gently rotated by hand until water was evaporated resulting in a white deposit, covering the whole sensor surface. It was then stored in a horizontal position, in a closed plastic box, at least 12 hours to ensure complete drying of the resin. The theoretical thickness of the complexing resin, calculated by assuming a uniform deposit on the whole sensor surface and a porosity of 50% is  $7 \mu\text{m}$ . The agarose gel antifouling membrane was then deposited on the chelating resin by filling the containment ring with 1.5% LGL agarose gel at  $80^\circ\text{C}$  by means of a pipette. The gel was left to cool at room temperature for approximately one minute, then equilibrated for one hour in Milli-Q water and minimum 3 hours in  $0.1 \text{ M NaNO}_3$  before its first use. When not in use, the sensor was stored in  $0.1 \text{ M NaNO}_3$  to keep the agarose gel hydrated. More details on gel preparation and characteristics are given elsewhere [13, 16, 17]. The mercury semidrops were electrochemically deposited and reoxidized through the gel membrane and the chelating resin, under the conditions reported in Section 2.3.

Measurements with the CGIME sensor are performed in three steps: 1) equilibration of the sensor with the test sample (Fig. 1b) to accumulate the metals on the chelating resin; 2) exchange of the sample by an acidic solution and electroconcentration: the metals fixed on the resin are released by the acid and immediately electrochemically reduced and preconcentrated on the array of Hg semidrops; 3) detection of the electroconcentrated, accumulated metals by SWASV. The key features of the CGIME are the following. Since the number of complexing sites in the resin layer is very small compared to the metal reservoir in the sample solution, only a negligible amount of each metal accumulates on the resin during the step 1). Thus equilibrium between bound and free form in solution remains virtually undisturbed. Interference of the matrix on the metal accumulation, due in particular to the fouling of the chelating resin, does not occur as colloidal/particulate matters are efficiently excluded from the agarose gel [13, 16, 17]. Under these conditions, it is expected that: i) each metal will accumulate on the resin in proportion to their free ion concentrations in the sample; ii) the free ion concentrations of the target metals in solution can be determined from SWASV signal measured in step 3) using a calibration curve

established, at a fixed sensor/sample equilibration time, in pure electrolyte spiked with various amount of trace metals.

## 2.2. Reagents and Solutions

Water purified with a Milli-Q system (Millipore,  $18 \mu\text{S cm}^{-1}$ ) was used for the preparation of all solutions.

$\text{Hg}(\text{CH}_3\text{COO})_2$ , KSCN,  $\text{NaNO}_3$ ,  $\text{HNO}_3$  and malonic acid were purchased from Merck. Dichloroacetate and 2-[*N*-morpholino]ethanesulfonic acid (MES) buffers were purchased from Sigma.  $\text{NaNO}_3$ ,  $\text{HNO}_3$  were of suprapur-reagent grade while the other chemicals were of analytical-reagent grade. Standard stock solutions of  $10 \mu\text{M Cu(II)}$ ,  $5 \mu\text{M Pb(II)}$  and  $6 \mu\text{M Cd(II)}$  were prepared from atomic absorption spectroscopy  $1 \text{ g L}^{-1}$  metal standard solutions (Merck) and acidified to pH 2 using suprapur concentrated  $\text{HNO}_3$ . The LGL agarose was purchased from Biofinex-Switzerland.

The 2.5% (w/v) Microchelex suspension stock solution was prepared by dilution of the 10% (w/v) suspension of Microchelex in deionized water purchased from CETAC Technologies-USA (ref. SP5627). The Microchelex resin is the only commercially available resin which fits the requirements mentioned in section 2.1. Unfortunately, precise information of the supplier on the physicochemical properties of this resin is rather poor. The core of the resin is made of polystyrene on which iminodiacetate (IDA) groups are attached by chemical bonds which are kept secret. The complexing capacity and average size are given as ca.  $2.5 \text{ mmol/g}$  and ca.  $200 \text{ nm}$  respectively; the provided acid-base and the metal complex stability constants are those for iminodiacetate reported in [18]. These values however are probably not reliable since the binding of IDA group is expected to affect somewhat the stability of complexes. Much more reliable data have been published in [19] for complexing latex particles which appear to be very similar to the Microchelex. These latex particles include a polystyrene core with IDA groups attached by glycidyl-methacrylate bonds. The complexing capacity may vary from 1.2 to  $2.5 \text{ mmol/g}$ , depending on the polystyrene proportion, but the corresponding change of their physicochemical characteristics is small. Well defined size distributions with average size of  $100\text{--}120 \text{ nm}$  are described. An important result is that  $K$  values for acid-base and complexation reactions are 1 to 3 orders of magnitudes larger than those with unbound IDA. For the latex with  $1.16 \text{ mmol/g}$  of IDA, the values of the first and second protonation constant and of the Cu complexation constant are  $10^{10.25}$ ,  $10^{4.68}$  and  $10^{11.3}$  respectively. Interestingly, it is also reported that most sites are located on the particle surface, which may significantly influence the stability of complexes by neighbour interactions. We have briefly compared these data with those of our CETAC Microchelex, by performing acid-base titrations and Cu complexation experiments using a Cu ion selective electrode. Based on acid-base titration, the number of IDA sites of Microchelex was found to be  $1.3 \text{ mmol/g}$  and  $\log K$  values of ca. 10 and 5.1 were found for the first and second

protonation at 0.1 M ionic strength. In the pH range 6–8, it was found that 1 proton was exchanged per Cu(II) fixed on the resin, and that the value of the constant for the reaction  $\text{Cu}^{++} + \text{R-SH} \rightleftharpoons \text{R-SCu} + \text{H}^+$  was  $10^{1.5}$ , in reasonable agreement with the results of [19] (R = core of the resin, S = complexing site of the resin).

CGIME characterisation/optimisation in non complexing media was performed in a 0.1 M  $\text{NaNO}_3$  suprapur solution, at pH 6.5, spiked with various concentrations of the target metals. CGIME measurements in model complexing media was performed using malonic acid in solutions buffered with MES at pH 6.3. The release and the detection of the accumulated metals were studied in the pH range 1 to 2 using  $\text{HNO}_3 + 0.1 \text{ M NaNO}_3$  solutions non-buffered or buffered with dichloroacetate. Nitrogen (purity 5.0; Pangas Switzerland) was used for deoxygenation of the metal test solutions.

### 2.3. Material and Methods

For the preliminary tests of the CGIME, batch electrochemical measurements were performed by using a computer controlled Amel 433 potentiostat (Milan-Italy) and a Metrohm cell based on a three-electrode configuration. The auxiliary electrode was a Metrohm platinum rod, and the reference electrode a Metrohm Ag/AgCl/KCl(sat) with an additional bridge of suprapur 0.1 M  $\text{NaNO}_3$ . This additional bridge serves to avoid contamination of the test solution by metal ions present in the saturated KCl electrolyte, as well as the diffusion of  $\text{Cl}^-$  in the test solution. A homemade preamplifier, based on the system developed by Faulkner [20], with a  $100\times$  amplification was used. For systematic laboratory and field tests, the CGIME was installed in a voltammetric in situ profiling system (VIP system) [21] which allow well controlled on-line solution exchange and start of the voltammetric detection. pH measurements were carried out by using a Metrohm model 744 pH meter. Total metal concentrations in the test solutions were measured using ICP-MS (Hewlett-Packard model 4500 series).

Low density polyethylene (LDPE, Nalgene, Fisher Scientific, Switzerland) reagent and sample bottles and polypropylene volumetric flasks (PP, Nalgene, Fisher Scientific, Switzerland) for solution preparations were cleaned by soaking in suprapur 0.1 M  $\text{HNO}_3$  for 24 hours, Milli-Q water for 12 hours, then two times successively in suprapur  $10^{-2}$  M  $\text{HNO}_3$  and Milli-Q water for 12 hours. After the cleaning steps, the bottles and flasks were filled with suprapur  $10^{-2}$  M  $\text{HNO}_3$  and stored in plastic bags. Before use, they were thoroughly rinsed and then filled with Milli-Q water and left to equilibrate for at least 1 hour. The sampling bottles were rinsed three times with the water sample before filling up.

Hg deposition on the Ir-microdisks was performed by applying a constant potential of  $-400 \text{ mV}$  (vs. Ag/AgCl/KCl<sub>sat</sub>//0.1 M  $\text{NaNO}_3$ //reference electrode) in a deoxygenated solution of 5 mM  $\text{Hg}(\text{CH}_3\text{COO})_2$  and 0.1 M  $\text{HClO}_4$  [22] for 8 min. Before each mercury electroplating, the sensor was left to equilibrate for 30 min in the Hg(II) solution.

Under these conditions, the average radii of each Hg semidrop, determined from the deposition charges,  $Q_{\text{red}}$ , were in a range of 4.8 to 5.6  $\mu\text{m}$ . Reoxidation of mercury was carried out at the end of each experiment which lasts typically from 2 to 7 days. This was done by scanning the potential linearly from  $-300$  to  $+300 \text{ mV}$  at  $5 \text{ mV/s}$  in a deoxygenated 1 M KSCN solution [22]. It has been observed that complexation of the oxidized mercury by  $\text{SCN}^-$  prevents the formation and deposition of mercuric oxide on the Ir substrates [22]. After the deposition of the Hg semidrops, the complexing resin on the sensor was cleaned to release metal impurities fixed on it. To do this, the CGIME sensor was dipped in a 0.1 M  $\text{HNO}_3 + 0.1 \text{ M NaNO}_3$  solution and replicate SWASV measurements were performed until a flat base line was observed.

For analysis of trace metals, the CGIME prepared as mentioned above is placed in the test sample which was either a model solution, with or without  $\text{N}_2$  deoxygenation, spiked with known concentrations of Cu(II), Pb(II) and/or Cd(II), or a nondegassed freshly collected natural sample. The CGIME is left to equilibrate in the test solution, for various amounts of time ranging from typically 30 minutes to few hours. The sample is then exchanged with the 0.1 M or  $10^{-2}$  M  $\text{HNO}_3 + 0.1 \text{ M NaNO}_3$  acidic solution and the released metal detected by SWASV. SWASV conditions used for both the blank and metal measurements were as follows, unless otherwise stated: deposition potential =  $-800$  to  $-850 \text{ mV}$ ; deposition time = 5 min; final scanning potential =  $+100 \text{ mV}$ ; frequency = 50 Hz (Amel 433A) and 200 Hz (VIP); wave amplitude = 25 mV; step amplitude = 8 mV.

## 3. Results and Discussion

### 3.1. Characterization and Optimisation in Noncomplexing Media

#### 3.1.1. Influence of pH on the Release of Trace Metals Accumulated on the Resin

A complete release of the accumulated target metals during the measurement step 2 is a prerequisite condition to avoid memory effect and thus to achieve reproducible and reliable measurements. The influence of the pH of the acidic solution used in this step on the metal peak current intensities recorded in step 3 was thus examined. For this purpose, the CGIME was first equilibrated for 1 h 30 in 0.1 M  $\text{NaNO}_3$  spiked with 10 nM Cu(II), Pb(II) and Cd(II). Peak current intensities of the metals were then recorded as a function of time by performing replicate SWASV measurement in buffered (dichloroacetate) and nonbuffered  $\text{HNO}_3$ , 0.1 M  $\text{NaNO}_3$  electrolyte at pH 1, 1.5 and 2. Typical results are shown in Figure 2.

At pH 2, desorption time of Pb(II) and Cd(II) were found to be  $\geq 2 \text{ h}$  and  $\leq 40 \text{ min}$  respectively, while no signal was obtained for copper (Fig. 2a). This suggests that a pH of 2 is insufficient to release the most strongly complexed metal,

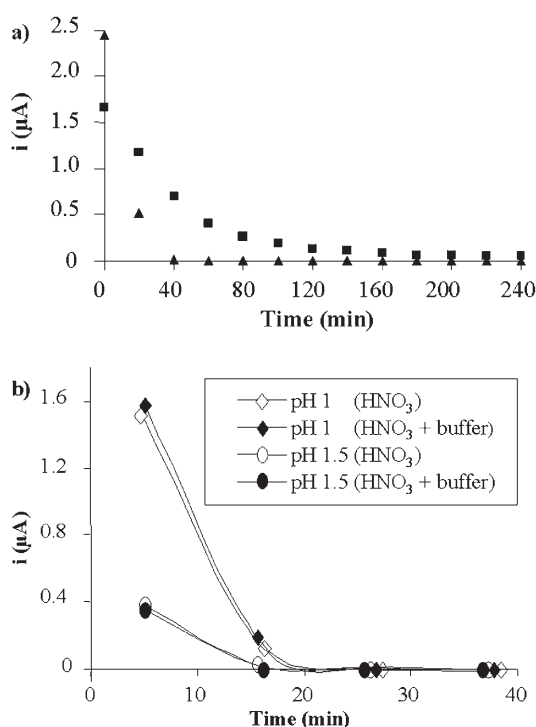


Fig. 2. Examples of Cu(II) [ $\blacklozenge$ ,  $\diamond$ ,  $\bullet$ ,  $\circ$ ], Pb(II) [ $\blacksquare$ ] and Cd(II) [ $\blacktriangle$ ] desorption kinetic curves measured in a) nonbuffered pH 2, and b) nonbuffered and buffered (chloroacetate) pH 1 and 1.5, acidified 0.1 M  $\text{NaNO}_3$  electrolyte after a chemical accumulation of 1 h 30 in 0.1 M  $\text{NaNO}_3$  solution at pH 6.5 spiked with 10 nM Cu(II), Pb(II), Cd(II). A time delay of 5 min was set between the replicate SWASV measurements. SWASV conditions used:  $E_{\text{dep}} = -900$  mV;  $t_{\text{dep}} = 5$  min;  $E_{\text{r}} = +100$  mV; wave amplitude = 25 mV; step amplitude = 2 mV; frequency = 50 Hz.

even though this should be the case with unbound IDA [18]. This result confirms the stronger complexing ability of microchelex compared to unbound IDA (see Sec. 2.2). Similar kinetic curves were obtained in buffered and nonbuffered media (see example for Cu given in Fig. 2b). This suggests that the different behavior of the microchelex, compared to the expected one for unbound IDA, is not due to a high negative surface charge of the resin, leading to a large difference in pH between the resin and the bulk solution, but more likely to a slow kinetic effect (see Sec. 3.2). No significant differences were observed on the peak current intensities and the desorption rate using pH lower than 1. Thus the acidic solution used for all further tests was a 0.1 M  $\text{HNO}_3$  + 0.1 M  $\text{NaNO}_3$  nonbuffered solution.

### 3.1.2. Influence of the Chemical Accumulation and Electrochemical Preconcentration Times on the Voltammetric Signal

The SWASV signals measured with CGIME sensors largely depend on two factors: i) the chemical accumulation time on the resin and ii) the SWASV preconcentration time on the electrode. Systematic tests have been performed to study the influence of these parameters. Concerning point ii),

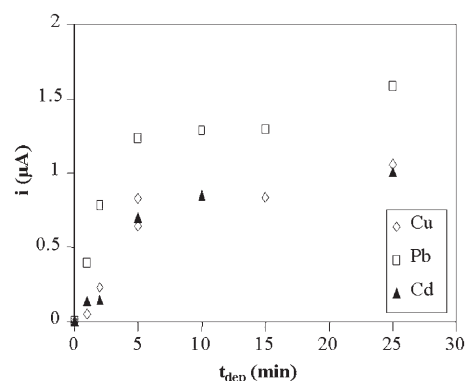


Fig. 3. Typical example of CGIME metal peak current intensities as a function of the SWASV preconcentration time obtained using the VIP system. Chemical accumulation time: 1 h. SWASV parameters:  $E_{\text{dep}} = -850$  mV;  $E_{\text{r}} = +150$  mV; frequency = 200 Hz; wave amplitude = 25 mV; step amplitude = 8 mV. Test solution: 0.1 M  $\text{NaNO}_3$  at pH 6.5 spiked with 45 nM Cu(II), 27 nM Pb(II) and 100 nM Cd(II).

SWASV measurements using preconcentration times between 1 and 30 min were investigated after a chemical accumulation step of 1 hour in solutions containing the metals in the concentration range 10–170 nM. Typical example of results are given in Figure 3. The metal peak currents were found to first increase with deposition time and then to level off. This is due to the fact that the amount of metal electrochemically reduced and preconcentrated on the electrode results from an integration over time of the pulse of metal released from the resin layer by the acidic solution. As shown in Figure 3, the maximum preconcentration of accumulated metal is achieved at  $t \approx 5$  min. This preconcentration time was thus used for all further measurements.

To test the role of the chemical accumulation time, the CGIME was left to equilibrate in freshly prepared 0.1 M  $\text{NaNO}_3$  solutions spiked with 10 nM of each metal for a duration of 1 hour up to 2 days. As shown in Figure 4, the

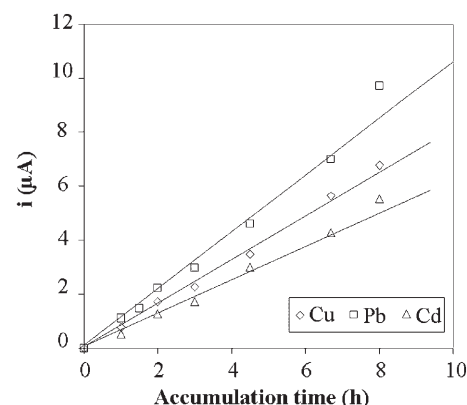


Fig. 4. Curves of peak current intensities as a function of the chemical accumulation time obtained for CGIME measurements in 0.1 M  $\text{NaNO}_3$  at pH 6.5 spiked with 10 nM of the three target metals. SWASV conditions as in Figure 2.

peak currents were found to increase linearly with time over very long periods of time, at least up to 10 hours. Under this condition, the CGIME signal can be related to the metal concentration by an equation of the type :

$$i_p = \kappa [M^{n+}] t_{acc} \quad (1)$$

where  $i_p$  is the peak current,  $\kappa$  is a constant,  $t_{acc}$  is the accumulation time and  $[M^{n+}]$  is the metal concentration in solution. Deviation from linearity was observed for times  $\geq 15$  h. This shows that within typical experimental conditions (accumulation time  $\leq 2$  h), the resin is not in equilibrium with the test solution. The linear increase of the peak current over such extended periods of time also suggests that the accumulation of metal on the resin is controlled by a very slow process which will be discussed in Section 3.2.

### 3.1.3. Reproducibility, Sensitivity and Reliability of Trace Metal Measurements

The reproducibility of CGIME trace metal measurements was studied by performing replicate calibrations of the target metals in standard solution. The CGIME was left to equilibrate for a constant time (typically 1 or 2 h) in a 0.1 M  $\text{NaNO}_3$  solution spiked with various concentrations of a single metal or of a mixture of the three metals (concentration range: 2 to 170 nM). Typical examples of voltammograms and resulting calibration curves are given in Figure 5. A good linearity was observed between the peak current intensities and the metal concentrations ( $R^2 \geq 0.990$ ) for the three metals, and the resolution of the voltammograms (inset of Fig. 5) was found to be similar to the one obtained previously with the GIME sensors, i.e., in absence of the resin layer [23].

For a constant electrochemical preconcentration time, the sensitivity, expressed in  $\text{nA/nM } t_{acc}$ , is given by the slopes of the calibration curves normalized by the chemical accumulation time,  $t_{acc}$ , expressed in h, or by the slope of the graphs  $i_p = f(t_{acc})$  normalized by the metal concentrations. In both cases, the values of sensitivity were found to be similar and this for measurements performed either in solutions containing a single metal or a mixture of the three metals. These results show that there is no competitive interaction between the metal ions for the Microchelex adsorption sites

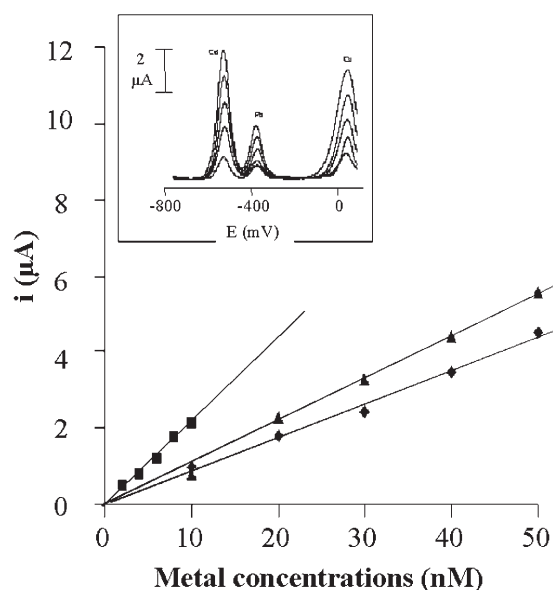


Fig. 5. Typical examples of SWASV voltammograms (inset) and calibration curves obtained for CGIME measurements in 0.1 M  $\text{NaNO}_3$  spiked with various concentrations of (◆) Cu, (■) Pb, (▲) Cd. Chemical accumulation time = 2 h at pH 6.5. SWASV conditions as in Figure 2. Concentrations of Cu(II), Pb(II) and Cd(II) in the inset vary from 10 to 55 nM, 2 to 12 nM and 32 to 170 nM respectively.

under the conditions used, which is expected if only a small fraction of the sites are occupied by the test metals. The average normalized calibration slopes, calculated from five replicate calibration curves of the three metals measured simultaneously, and the detection limits ( $LOD$ ) for  $t_{acc} = 1$  h, computed based on a signal to noise ratio = 2, determined from batch measurements (Amel system) and automatic on-line measurements (VIP system) are reported in Table 1. The difference in the normalized slope values obtained with both systems are due to the different slope frequency used for the SWASV measurements and to a  $10\times$  internal amplification of the signal for the Amel potentiostat. These results demonstrate the reproducibility of the CGIME chemical accumulation step and SWASV measurements of the adsorbed metal species, in particular under VIP automatic on-line control for which the relative standard deviation ( $RSD$ ) is  $< 5\%$ . The larger variability observed under batch

Table 1. Average normalized calibration slopes, calculated from replicate calibration curves ( $N=5$ ) of the target metals measured simultaneously, and lower detection limits ( $LOD$ ), computed based on a signal to noise ratio = 2, determined from batch measurements (Amel system) and automatic on-line measurements (VIP system).

Metal	Potentiostat	Normalized slopes [ $\text{nA/nM } t_{acc}$ ]	$RSD$ [%]	$LOD$ [a] [ $\mu\text{M}$ ]
Cu	Amel	45.7	3.9	23
	VIP	18.8	3.3	21
Pb	Amel	100.4	11.5	10
	VIP	44.5	4.5	9
Cd	Amel	16.6	10.8	60
	VIP	6.8	2.9	59

[a] for an accumulation time of 1 h.

conditions is mainly due to a lack of reproducibility of the time interval between the exchange of the test solution by the acidic solution and the start of the SWASV measurements, which may lead to a larger loss of the accumulated metal due to back diffusion of metal to the solution prior the electrochemical reduction and preconcentration start.

The stability of the CGIME sensor for long term measurements was also tested by performing replicate measurements over a period of five days without renewal of the Hg semidrops. The sensor was left in open circuit in the electrolyte between measurements. Results obtained for a test solution of 5 nM Cu(II), 2 nM Pb(II) and 14 nM Cd(II) in 0.1 M NaNO<sub>3</sub> and a chemical accumulation time of 1 hour are given in Figure 6. The average currents for Cu(II), Pb(II) and Cd(II) over the five day measurement period were respectively 221, 211 and 212 nA with relative standard deviations of 9%, 8% and 5% ( $N = 15$ ). These results show that, as for the GIME [16, 24], continuous CGIME measurements without sensor maintenance, can be performed over several days with a good precision.

### 3.2. Validation of Free Ion Concentration Measurements in Synthetic Complexing Solutions

The last important analytical test of the CGIME, before the application, was to validate its selectivity toward free metal ions irrespective of the presence of other metal species. In natural waters, in addition to the free ions, M may be present under various forms. In particular they can be adsorbed on particles, colloids or polymers, or complexed with small organic or inorganic ligands. Metals adsorbed on particles/colloids with radius > 30 nm are excluded from the agarose gel [13, 16, 17]. The smaller but chemically inert complexes does not react with the resin. Therefore, apart from free  $M^{n+}$ , the small (thus quickly diffusing) and labile metal complexes are the only species which might react with the resin and contribute to the CGIME signal.

Hence, measurements were performed on line with the CGIME-VIP system, in buffered pH 6.5 (MES) 0.1 M NaNO<sub>3</sub> solutions, containing constant concentrations of

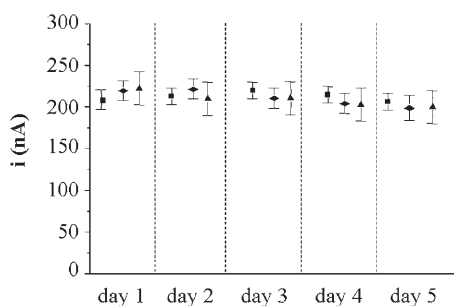


Fig. 6. Stability of the SWASV signals obtained for replicate measurements of the three target metals over extended period using the same CGIME. Test sample solution: (◆) 5 nM Cu(II), (■) 2 nM Pb(II), and (▲) 14 nM Cd(II) in 0.1 M NaNO<sub>3</sub>. Chemical accumulation time = 1 h. SWASV conditions as in Figure 2.

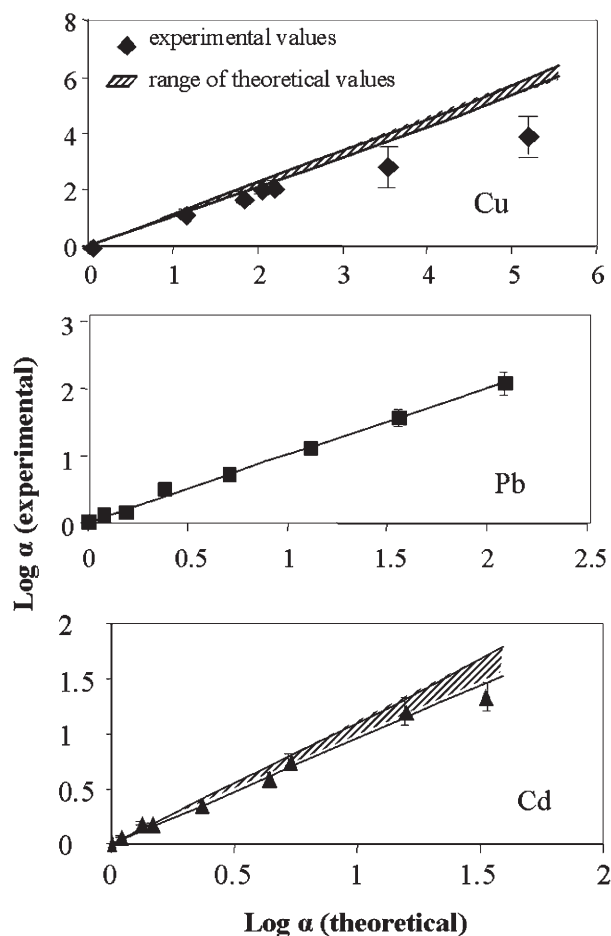
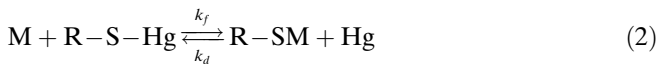


Fig. 7. Experimental vs. theoretical values of degrees of complexation,  $\alpha$ , of the three test metals;  $\alpha_{\text{experimental}} = [M]_{\text{tot}}/[M^{n+}]_{\text{CGIME}}$ ,  $\alpha_{\text{theoretical}} = [M]_{\text{tot}}/[M^{n+}]$  with  $[M^{n+}]$  computed using the minimum and maximum values of stability constants  $K$  reported in the literature. Total metal concentrations:  $2 \times 10^{-7}$  M Cu(II) and  $10^{-7}$  M Pb(II) and Cd(II) in 0.1 M NaNO<sub>3</sub> solutions buffered at pH 6.5 with  $10^{-2}$  M MES. The solutions contained variable concentrations of malonate. Accumulation time: 1 h; SWASV conditions as in Figure 2.

Cu(II), Pb(II) and Cd(II) in presence of various concentrations of malonate. Malonate was chosen as it forms labile complexes and its stability constants with the target metals are well-known [25]. The experimental values of  $[M^{n+}]_{\text{CGIME}}$  and degrees of complexation  $\alpha_{\text{exp}} = [Me]_{\text{tot}}/[Me^{n+}]_{\text{CGIME}}$  were determined from the  $i_p$  values of the three metals, and the  $\alpha_{\text{exp}}$  values were compared with the theoretical ones computed by using the minimum and maximum values of stability constants reported in the literature [18] (Fig. 7). For all metals, an excellent agreement was obtained between the experimental and theoretical data for  $[M^{n+}]$  larger than the LOD reported in Table 1. These results confirm the selectivity of the CGIME for the free metal ions.

Different processes may be considered to explain the proportionality between the accumulated metals and their free ion concentrations in solution as well as the slow kinetics of the metal accumulation in the resin observed (Secs. 3.1.1 and 3.1.2). A slow formation of the metal-resin

site complex  $RS-M$  between free  $M^{n+}$  and free resin sites. This, however, is unlikely since one would expect this process to be orders of magnitude faster than the observed kinetics, even if steric effects are operative at the reactive site. Another explanation might be a slow diffusion of  $M^{n+}$  in the resin due to its strong complexing ability, combined with a polyelectrolyte effect due to a significant charge density of the resin. However, in such case, the influence of the nature of the metal would be expected to play a much more significant role on the signal than observed in Figure 4. Alternatively, preliminary tests suggest that a significant proportion of the resin sites may be occupied by Hg after the preparation of the sensor, even though this is performed at pH 1. This could be explained by the large concentration of Hg(II) used during the plating step, and a stability constant for the Hg(II)-microchelex which was estimated, based on the data of the Me-IDA complexes, to be ca. 15 times larger than the one for Cu(II) given in Section 2.2, i.e.,  $\log K \approx 10^{12.5}$ . Under these conditions, any test metal M should react with the resin according to:



In this reaction, the formation rate of the complex  $R-SM$  is controlled by the dissociation rate of  $R-SHg$  which is expected to be very slow, due to its large stability constant, and independent of the nature of M. Based on Reaction 2, the accumulation rate of M on the resin, before the equilibrium is reached, is given by:

$$d\{R-SM\}/dt = k_f \{R-SHg\}[M^{n+}] \quad (3)$$

where  $\{R-SM\}$  and  $\{R-SHg\}$  are the concentration of M and Hg in the resin respectively. Since  $R-SHg$  is in very large excess compared to M,  $k_f \{R-SHg\} = k_a$ , the accumulation rate constant, and since the consumption of M by the resin is negligible compared to the amount of M in solution, integration of Equation (3) gives:

$$\{R-SM\} = k_a [M^{n+}]t \quad (4)$$

This rate law is similar to the observed one (Eq. 1) under conditions where the measured current is proportional to  $\{R-SM\}$ . Equation 4 also shows that only  $M^{n+}$  reacts with  $R-SHg$ , even when metal complexes exist in solution.

A more detailed mechanistic study, of in particular the slow step process governing the measurement step 1, is required to verify the above hypothesis and to allow sound theoretical description of the CGIME measurements. This is out of the scope of this work and is a goal of a future study.

### 3.3. Environmental Application

The first applications of the CGIME sensor for direct measurements in natural samples were performed at the Kristineberg Marine station (Sweden, August 18–26 2002).

The free ion concentrations of Cu(II) and Pb(II) in sea water are very low (see Fig. 8) and there are very few methods (and no unambiguous one) to compare with CGIME. Hollow fiber based permeation liquid membrane (HF-PLM) coupled to ICP-MS detection was chosen because it is a very sensitive technique which has been shown to measure the free ion concentration with a large number of ligands [7, 8]. Measurements with both techniques were performed in the fjord-shore laboratory, on filtered and/or raw, nonacidified samples collected with Go-Flo bottles, at different stations and depths in Gulljmar fjord. The samples were stored at 4°C directly after sampling and the measurements performed as soon as possible (i.e., max. 24 h later). Typical results obtained for Cu and Pb are reported in Figs. 8a and b respectively. Cadmium concentrations were found to be below detection limit for both the HF-PLM and CGIME in most of the samples. Considering the very low concentration levels, copper and lead concentrations measured with the CGIME can be considered as similar to those obtained with HF-PLM (Fig. 8a et b). Interestingly, the HFPLM copper results are systematically slightly higher than CGIME, even though the difference is close to experimental variability (note that each point corresponds to a different sampling site). Nevertheless, this slight difference does not exist for lead and might be due to the presence of lipophilic copper

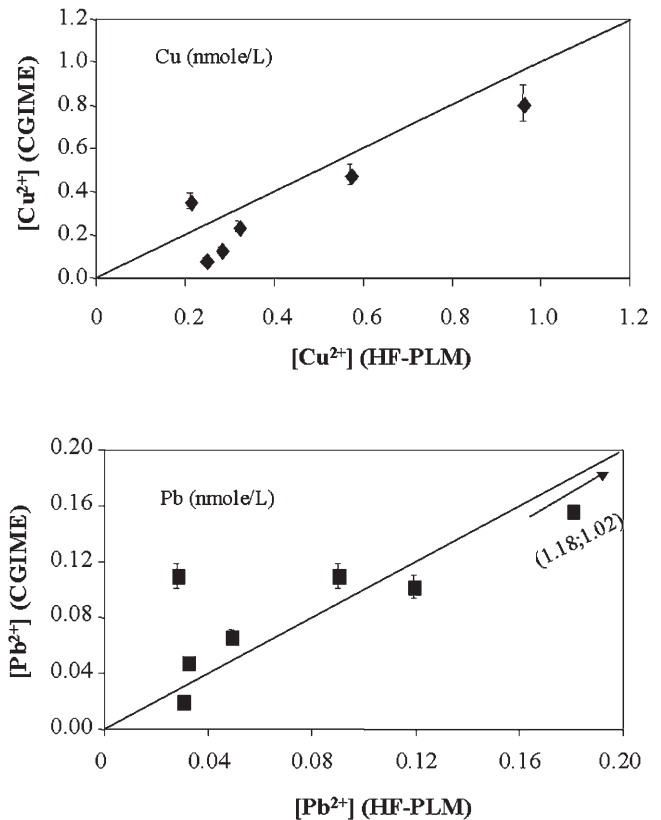


Fig. 8. Comparison of a) Cu and b) Pb concentrations measured in Gulljmar fjord samples using CGIME-SWASV and HF-PLM coupled to ICP-MS techniques.

complexes which may also be measured by HF-PLM [12] whereas they would not be measured by CGIME. These results confirm the selectivity of the CGIME towards free metal ions and its sensitivity and reliability for their direct measurements in natural waters.

The CGIME sensor was later installed in a multiphysical-chemical profiler (MPCP) and successfully applied for in-situ continuous monitoring of Cu and Pb free ion concentrations in estuaries and coastal sea water [26]. The results obtained have demonstrated the great interest of the in-situ CGIME measurements for the assessment of the toxicological impact of the trace metal in natural aquatic systems.

#### 4. Conclusions

The development of a sensor for the direct measurements of free metal ion concentrations in complex media has been reported. This has been achieved by coupling various analytical techniques into a micro-sized device to allow efficient exclusion of fouling material, virtually nonperturbing chemical accumulation of metal on a microbead size chelating resin and sensitive voltammetric detection. Systematic laboratory tests in synthetic media were performed to optimise, characterize and calibrate this Microchelex-based CGIME sensor. The results have shown that the thin complexing resin layer inserted between the surface of the microelectrode array and the agarose gel of a GIME sensor does not impair the Hg layers deposition nor the reproducibility and reliability of trace metal SWASV measurements. Selectivity of the CGIME to the free metal ions in presence of small labile metal complexes was validated by tests performed in model complexing media. Measurements in real sea water, and comparison with the results obtained with HFPLM-ICPMS techniques have demonstrated the potential of the CGIME sensor developed for direct, simultaneous detection of free ion concentrations of several metals, with a sensitivity at the ppt level, in aquatic media. The great interest of this sensor for environmental and pollution control monitoring has been confirmed by the results of the CGIME-MPCP in-situ measurements reported elsewhere [26].

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