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AGNES at vibrated gold microwire electrode for the direct quantification of free copper concentrations

Rute F. Domingos[1,2,*], Sara Carreira[1], Josep Galceran[3], Pascal Salaün[4], José P. Pinheiro[5]

[1] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Torre Sul lab 11-6.3, Av. Rovisco Pais # 1, 1049-001 Lisbon, Portugal

[2] Institut de Physique du Globe de Paris, Sorbonne Paris Cité, Université Paris Diderot, UMR CNRS 7154, 75205 Paris Cedex 05, France (Present address)

[3] Department of Chemistry, University of Lleida and Agrotecnio, Rovira Roure 191, 25198 Lleida, Spain

[4] School of Environmental Sciences, University of Liverpool, 4 Brownlow Street, Liverpool L693 GP, United Kingdom

[5] LIEC/ENSG, UMR 7360 CNRS – Université de Lorraine, 15 Avenue du Charmois, 54500 Vandoeuvre-les-Nancy, France

*Corresponding author

email: rdomingos@ipgp.fr

phone number: (+33) 01 83 95 74 43

Abstract

The free metal ion concentration and the dynamic features of the metal species are recognized as key to predict metal bioavailability and toxicity to aquatic organisms. Quantification of the former is, however, still challenging. In this paper, it is shown for the first time that the concentration of free copper (Cu^{2+}) can be quantified by applying AGNES (Absence of Gradients and Nernstian equilibrium stripping) at a solid gold electrode. It was found that: i) the amount of deposited Cu follows a Nernstian relationship with the applied deposition potential, and ii) the stripping signal is linearly related with the free metal ion concentration. The performance of AGNES at the vibrating gold microwire electrode (VGME) was assessed for two labile systems: Cu-malonic acid and Cu-iminodiacetic acid at ionic strength 0.01 M and a range of pH values from 4.0 to 6.0. The free Cu concentrations and conditional stability constants obtained by AGNES were in good agreement with stripping scanned voltammetry and thermodynamic theoretical predictions obtained by Visual MinteQ. This work highlights the suitability of gold electrodes for the quantification of free metal ion concentrations by AGNES. It also strongly suggests that other solid electrodes may be well appropriate for such task. This new application of AGNES is a first step towards a range of applications for a number of metals in speciation, toxicological and environmental studies for the direct determination of the key parameter that is the free metal ion concentration.

Keywords: gold electrode, VGME, copper, speciation, AGNES, stripping scanned voltammetry

1. Introduction

When entering in a natural aquatic environment, trace metals are rapidly complexed by biotic and abiotic ligands, thus modifying their speciation and their bioavailability. The understanding and prediction of their ecotoxicological impact is dependent on our ability to measure the most relevant fractions of the trace metals in terms of bioavailability, i.e., the free and labile metal ion concentrations.

The critical importance ascribed to the free ion in models, such as the free-ion activity model (FIAM) and the biotic ligand model (BLM) [1], has led to an increasing interest in quantifying these species in environmentally relevant media. However, there is only a limited number of techniques able to quantify the free ion concentration with the required selectivity and detection limit. These include potentiometry with ion-selective electrodes (ISEs) [2], the Donnan membrane technique (DMT) [3], the permeable liquid membrane (PLM) [4, 5], the ion-exchange technique (IET) [6], and the absence of gradients and Nernstian equilibrium stripping technique (AGNES) [7]. Reviews of these (and other) techniques are available [8-10]. Although all these techniques have the ability to measure the free metal ion concentration, each of them has its own limitations. For instance, the main drawback of commercial ISE is its restricted use to the quantification of proton and those elements present in total concentrations above 10^{-6} M due to the solubilization and contamination/adsorption of the electrode membrane. In the last decades potentiometric sensors using polymeric membranes and special inner solutions were developed increasing the ISE's detection limit (10^{-10}-10^{-13} M), however, their operation is still difficult and most are not commercially available [11, 12]. DMT performs simultaneous measurements of several elements at very low detection limit (dependent on the analytical technique used to quantify the metal content; e.g., 10^{-11}-10^{-12} M by using inductively coupled plasma mass spectrometry), but the equilibration time is very long, often 24 or

48 h. PLM is a dynamic technique where the speciation answer depends on the rate-limiting step; the free metal ion can be measured when the diffusion across the membrane is governing the flux. However, despite the multielement quantification at the 10^{-11}-10^{-12} M level (also dependent on the analytical technique used for the elemental analysis) when using PLM, there is a high risk of measurement of lipophilic metal complexes together with the free ions. The detection limits in IET are directly related with the quantity (and binding capacity) of the resin (10^{-9}-10^{-6} M) and in some systems the equilibration times might be long. Moreover, positive interferences do occur in the presence of complex amino acid ligands.

AGNES, which has been increasingly used in the past decade, has been suggested as a free metal ion quantification technique overcoming most of the limitations shown by the above described techniques [13-16]. This electroanalytical technique was specifically designed at a Hg electrode to quantify low free metal ion concentrations (as low as 10^{-10} M), via two stages: i) deposition, where a potential E_1 is applied to preconcentrate the metal inside the working electrode until the redox couple reaches Nernstian equilibrium (i.e., until there are no concentration gradients of reduced or oxidized metal); ii) stripping, where the concentration of the reduced metal is quantified. The analytical signal is the response taken from the electrochemical technique used in the stripping stage. One clear advantage of AGNES is its predictable Nernstian response, similar to an ISE, since a suitable analytical signal is linearly related with the free metal ion concentration with a proportionality factor obtained from the calibration. However, two main disadvantages need to be pointed out: i) AGNES typical implementation can only quantify free ion concentrations of metal ions that can amalgamate reversibly on a Hg electrode, and ii) the time involved in the preconcentration stage to reach equilibrium can vary from minutes to hours, although much shorter than when using techniques such as DMT or even IET. AGNES had been originally applied only at mercury electrodes, with these arising

concerns due to Hg toxicity and not ideal for Cu quantifications due to the proximity of their reduction potentials (see [17] and references therein). Recently, AGNES was successfully implemented at bismuth film electrodes, highlighting the possibility of applying AGNES at other solid electrode materials [18]. Unfortunately, copper speciation is not possible at Bi electrodes due to the similarity of their reduction potentials. Contrarily, gold electrodes are well suited for Cu speciation [19-22], without the need for oxygen removal, since the metal reduction wave is at more positive potentials than the O_2 wave [19]. Evidently, this is a significant advantage in analysis of environmental samples, since both CO_2 and pH are maintained in the sample, avoiding changes in metal speciation [23]. Another advantage is that the gold electrodes can be used as such, without an electrodeposited film (like for thin film Hg [24], Bi [18] and Sb [25] electrodes), which could become an issue over long measuring time periods.

Traditionally, gold electrodes are not widely employed in environmental samples since they can be easily contaminated and their detection limit is not sufficiently low. Recently, a vibrating gold microwire electrode (VGME) was proposed [20], and appears to be an optimal candidate for the Cu quantification in natural samples due to its high sensitivity. These gold electrodes have already been shown to successfully quantify i) total metal concentrations of various metals, including Cu [19], and ii) the conditional complex stability of inert metal complexes by scanned stripping voltammetry (SSV) [21]. The electrode vibrations offer additional advantages over other common electrode setups: i) they eliminate the need for external stirring of the solution, thus also simplifying the *in situ* detection in the environment [20], ii) they produce much more stable hydrodynamic conditions at the surface of the electrode leading to better reproducibility and, thus, lower detection limits [26, 27], iii) they produce a significantly higher flux of species towards the electrode due to a smaller diffusion layer, again decreasing detection limits [26], and

iv) the stripping can start immediately after stopping the vibration, without any equilibrium time.

AGNES theoretical framework was recently developed for solid electrodes [18]. The key requirement for AGNES is the attainment -by the end of the deposition stage- of Nernstian equilibrium involving the activity of the free metal ion in solution and that of the reduced metal. The AGNES principle offers the possibility to choose the activities ratio (which, in an amalgam, is proportional to the concentrations ratio or gain) just by changing the deposition potential. When M^o deposits on a solid electrode as a bulk solid phase (i.e., when depositing a metal on its own substrate), its activity is always unity regardless of the metal amount deposited and in that case, AGNES cannot be applied. Fortunately, the activity of the reduced metal can be changed by varying the coverage of adsorbed atoms on the foreign solid surface, leading to the possibility of performing AGNES also at solid electrodes in conditions of sub-monolayer coverage [18].

The aim of this work is to develop and test an optimal electrochemical methodology based on AGNES principles for the direct determination of the free metal ion concentration at the solid gold electrode. The system was tested in the presence of ligands forming labile Cu complexes and results (free metal ion concentrations and conditional stability constants) were compared with speciation modeling predictions (Visual MinteQ). Additional validation was obtained by comparison between the performances of AGNES and scanned stripping voltammetry (SSV).

2. Experimental setting

2.1. Reagents and solutions

All solutions were prepared in ultrapure water from a MilliQ Integral 3 (resistivity > 18 MΩ cm). The Cu stock solutions were prepared by dilution of its standard solution (1000 mg L^{-1} Fluka), and the $NaNO_3$ used to adjust the ionic strength solution was prepared

from the solid (Merck, suprapur). HCl (Merck, suprapur) and H_2SO_4 (Panreac), NaCl (Merck, 99.6 %), potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$; Merck, p.a. 99 %), and KCl (Merck, p.a., 99.5%) were used for the preparation and characterization of the VGME. Malonic acid (MAL) and iminodiacetic acid (IDA) were purchased from Sigma-Aldrich (ReagentPlus 99 and 98%, respectively). HNO_3 (Merck, suprapur) and NaOH (Merck, p.a.) solutions were used to adjust the pH, which was measured using a Denver Instrument (model 15) and a Radiometer analytical combined pH electrode.

2.2 Instrumentation, electrode preparation and maintenance

Voltammetric experiments were performed using an Eco Chemie μAutolab III potentiostat in conjunction with a Metrohm 663VA stand and a personal computer using the GPES 4.9 software (Eco Chemie). Electrodes included a calomel reference electrode with a 0.1 M $NaNO_3$ salt bridge, a platinum counter electrode, and the VGME as the working electrode. The measurements were carried out at room temperature (20-23 °C). The gold microwire (99.99%, hard, Goodfellow, diameter (d) = 25 μm) was heat-sealed in a 200 μL polypropylene pipette tip together with a copper wire (to make contact with the working electrode cable) that was previously dipped in a conductive silver solution (Leitsiber L100; that is used as a conductive adhesive), and this tip was then fitted onto a 1 mL polypropylene pipet tip, which had a vibrator incorporated [20, 28]. The vibrator was driven by a 1.5 V power supply (home-built converter of 5 to 1.5 V), which was interfaced to, and powered by, the IME (Autolab, Metrohm, Switzerland), and controlled by the stirrer on/off trigger in the software (GPES, Autolab). The surface of the VGME was cleaned electrochemically by hydrogen generation at -2 V for 60 s in 0.5 M H_2SO_4 and its behavior was checked by running 5 cyclic voltammetry scans between 0 and 1.5 V (0.1 V s^{-1}) until the response was stable, which was usually from the second scan onwards [19]. The thickness of the diffusion layer (δ) of the microwire electrode was

182 determined as described previously [19]. Briefly, an oxide monolayer was first formed
183 by running (at 100 mV s^{-1}) a cyclic voltammogram between 0 and 1.5 V in 0.5 M H_2SO_4
184 and its reduction charge used to calculate the total area, assuming a 450 μC cm^{-2} charge
185 density for an oxide monolayer. The length was calculated from the chronoamperometric
186 current obtained in a solution of 0.01 M $K_3[Fe(CN)_6]$ (prepared in 0.5 M KCl) at -0.3 V
187 during 15 s (at a sampling frequency of 0.01 s^{-1}) assuming a constant diameter of 25 μm
188 (commercial specification). The δ was calculated from the diffusion-limited current
189 obtained under vibrated conditions.
190
191 *2.3 Measuring procedure*
192 Contrary to the Hg drop electrode where a new surface is produced for each single
193 measurement, an electrochemical procedure must be used to ensure a reproducible surface
194 of the solid electrode (in terms of adsorbed species) before each measurement. This was
195 done here by applying at least 5 times (until the analytical signal of interest is undistorted
196 and shoulderless) a desorption step including two consecutive potentials in the same
197 solution as that being measured: E = -2 V during 15 s and E = 0.550 V during 5 s. A
198 similar cleaning procedure of the electrode between measurements was also required for
199 optimum stability in seawater [29]. However, in presence of ligands, a more efficient
200 cleaning procedure was necessary; after each measurement 5 cyclic voltammetry scans
201 between 0 and 1.5 V (100 mV s^{-1}) in a solution of HNO_3 0.02 M were performed.
202 Each AGNES measurement consisted in the application of a deposition potential, E_1, for
203 a specific time (t_1) followed by a desorption pulse (E = - 2 V, t = 1 s), limiting the
204 adsorption of species that can interfere with the stripping peak, and, thus, contributing for
205 a reproducible gold surface. This desorption pulse must be applied after each 30 s of
206 deposition, to avoid build-up of adsorbed species leading to a continuously changing of
207 the gold surface, and thus irreproducible replicates. For depositing times larger than 30 s,

sequential procedures of 30 s deposition at E_1 followed by the desorption pulse were applied while keeping a standby potential ($E_{standby} = E_1$) between them (i.e., the total number of sequential deposition procedures times 30 s equals the total deposition time). Such sequential procedure was previously found to significantly increase the linear range of Sb(III) at the gold electrode in similar electrolyte conditions, presumably by limiting the building-up of adsorbed acetate anions at the gold surface [30]. Because only mobile and labile complexes are tackled here, differential pulse with anodic stripping voltammetry (DPASV) was used in this work for the stripping stage. A background scan was made after each analytical scan consisting of the desorption pulse (-2 V for 1 s) followed by the same stripping as for the analytical scan, and was subtracted from the analytical scan allowing the removal of this pulse (indeed negligible compared with the main signal) from the analytical signal. The background scan was kept the same, irrespective of the deposition time and thus, irrespective of the number of desorption pulses that were applied during the deposition step. The metal reoxidation was performed by using a scan rate of 0.040 V s^{-1} and modulation amplitude of 0.025 V, modulation time of 0.008 s, and interval time 0.1 s. The derivative (i.e., the sum of the two highest derivative values corresponding to the inflection points of the peaks) of the background-corrected peak signal was used for quantification.

2.4 Cu deposition on gold

The nature of Cu deposition on gold was evaluated by applying a stepwise DPASV, i.e., a DP stripping scanned voltammetry (SSV). A forward and backward SSV were performed by doing scans from 0.000 to 0.300 V and from 0.300 to 0.000 V, respectively, with a deposition time of 30 s. The SSV waves were performed with 1.0×10^{-7} M of Cu at pH 4 and I = 0.01 M.

234 *2.5 AGNES at the VGME*

235 2.5.1 Effect of deposition potential and deposition time

236 The possibility of applying AGNES by using a VGME was first evaluated by performing
237 the so-called "trajectory" studies where the peak intensity is measured as a function of
238 deposition time and this is repeated at various deposition potentials. These studies
239 together with linearity evaluation were obtained in 0.01 M $NaNO_3$ solutions adjusted at
240 pH 4 with HNO_3.

241 Trajectories (i.e., time profiles) were determined by applying a set of deposition potentials
242 (0.185, 0.200, 0.205, 0.215, 0.230 and 0.250 V) for sufficiently long deposition times
243 (450 to 1200 s) until the attainment of the equilibrium. A set of E_1 and t_1 that were found
244 sufficient to attain AGNES conditions (E_1 = 0.230 V and t_1 = 240 s) were chosen for the
245 evaluation of the linearity between the stripping signal and the free metal ion
246 concentration ($c_{Cu,free}$ = 4.9×10^{-9} to 4.9×10^{-6} M).

247

248 2.5.2 Complexation studies of Cu-MAL and Cu-IDA

249 AGNES measurements were carried out by applying an E_1 = 0.230 V and t_1 = 240 s in 20
250 mL of 0.01 M $NaNO_3$ solutions. The calibration plot included Cu concentrations from
251 5.0×10^{-9} to 1.0×10^{-7} M measured at pH 4 designed to avoid Cu losses by adsorption onto
252 the polystyrene container walls. Solutions of the MAL and IDA were prepared by dilution
253 of the stock solution (prepared from the solids) into the prepared electrolyte solution
254 (Milli-Q water with adjusted pH and ionic strength) to give the desired final
255 concentrations of 1.0×10^{-5} and 1.0×10^{-4} M for the MAL and 1.0×10^{-6} and 1.0×10^{-5} M for
256 IDA. Samples were prepared using a range of solution conditions: pH 4.0-6.0 for
257 solutions containing MAL and pH 4.0-5.5 for IDA, ionic strength (I) 0.01 M, and Cu
258 concentration of 1×10^{-7} M added as metal salt.

The results are the mean and standard deviation of at least three replicates, performed on different days using freshly prepared samples.

2.6 Scanned Stripping Voltammetry (SSV)

The conditional stability constants of the formed metal complexes was evaluated by performing DP SSV [21, 27] from 0.000 to 0.300 V with a t_d = 30 s (scan rate of 0.080 V s^{-1} and modulation amplitude of 0.05 V, modulation time of 0.004 s, and interval time 0.1 s). The same desorption step and pulse as explained in section 2.3 was applied as well as the background scan subtraction. The calibration plot was performed with 1.0×10^{-7} M of Cu at pH 4 and I = 0.01 M ($NaNO_3$). Samples were prepared using a range of solution conditions: $c_{Cu,T} = 1.0\times10^{-7}$ M, $c_{MAL,T} = 1.0\times10^{-5}$ M, $c_{IDA,T} = 1.0\times10^{-5}$ M, pH 4.5-6.0, I = 0.01 M.

The results are the mean, minimum and maximum of at least three replicates, performed on different days using freshly prepared samples. The indicated error arises from that of the analytical signal (5 %) together with that propagated from the +/- 2 mV uncertainty in the half-wave deposition potential (estimated error of the reference electrode on different days).

3. Results

3.1 The nature of Cu deposition on gold

The electrodeposition of metals at solid electrodes proceeds via (i) the formation of a monolayer of reduced metal onto the electrode, the so-called underpotential deposition (UPD), followed by (ii) the formation of new layers of metal atoms on top of previously deposited metal, which is called the bulk metal deposition that occurs at a less positive potential [31]. Thus, at a solid electrode, two (or more) reduction and stripping processes can occur, which may result in a complex interpretation of the analytical signal. Under

the conditions used here (0.01 M $NaNO_3$ and up to 1.0×10^{-7} M of total Cu), only UPD occurs with the stripping potential of the copper monolayer from the gold surface occurring at *ca.* 0.275 V *vs.* SCE (Figure 1). This value is in accordance with previous studies where the stripping potential of the UPD peak was found at 0.255-0.305 V *vs.* SCE, while the stripping peak of the bulk deposition occurred at much less positive potentials, 0.005-0.105 V *vs.* SCE [31-33].

The influence of the deposition potential on the UPD signal in a non-complexing medium was evaluated by sequential SSV performed from 0.000 to 0.300 V (forward wave) and from 0.300 to 0.000 V (backward wave). Half-wave deposition potentials ($E_{d,1/2}$) values were 0.218 and 0.219 V for the forward and backward scans, respectively (Figure 2). A reasonable fit between the sequential waves was obtained indicating that the cleaning procedure that is applied between each measurement is efficient.

3.2 Implementation of AGNES using the VGME

There are two stringent requirements for AGNES to be successfully implemented: i) at any selected E_1, the equilibrium must be attained for sufficiently long deposition times t_1, and ii) there must be a fixed (preferably linear) relationship between the stripping signal and the free metal ion concentration (for the chosen set of E_1 and t_1).

A third check performed here, though not a strict requirement for the application of AGNES, is the Nernstian behavior of the measured signal with the applied potential (i.e., the linearity between obtained signal and applied gain, see next section).

3.2.1 Trajectories

The possibility of performing AGNES with the VGME is strictly related with the possibility to reach equilibrium at a certain E_1 for sufficiently long deposition times (t_1).

311 Evidently, while larger t_1 values will favor the attainment of Nernst equilibrium, too long
312 deposition times will render the technique less useful or even unworkable. The
313 performance of the AGNES signal as a function of t_1 at various E_1 (ranging from 0.185
314 to 0.250 V) was evaluated (Figure 3), and the equilibrium (recognized by a plateau) was
315 attained after 2 to 15 minutes, depending on E_1; whereas for an E_1 between 0.250 to 0.230
316 V the equilibrium was reached within 200 s, it is necessary to wait around 900 s when
317 using a less positive E_1 of 0.185 V. In the following AGNES experiments, a compromise
318 between the intensity of the signal and the time needed to reach the equilibrium led to the
319 choice of using the set E_1 = 0.230 V and t_1 = 240 s.
320 A typical parameter in AGNES is the gain (Y), the preconcentration or accumulation
321 factor, which -following Nernst equation- relates the concentration ratio of reduced to
322 oxidized species in the case of Hg electrodes. At solid electrodes, the gain can relate the
323 obtained surface coverage of reduced species with the bulk concentration of oxidized
324 species [18]. Given the difficulties in finding the standard formal potential, $E^{0'}$, at this
325 type of gold polycrystalline wire electrode, we work here with the operational definition:

326 $$Y = \exp\left[-\frac{nF}{RT}\left(E_1 - E_{\mathrm{d},1/2}\right)\right] \qquad \text{(Equation 1)}$$

327 where, R is the gas constant, T the temperature, n the number of exchanged electrons, F
328 the Faraday constant and $E_{\mathrm{d},1/2}$ is the half wave potential of the SSV waves (0.218 V;
329 section 3.1). The real gain will differ from the operational one by a multiplying factor,
330 but this factor cancels out between calibration and measurement, so it does not affect the
331 determined free metal concentration.
332 The equilibrium signal intensities (that were obtained on the plateau of the various E_1
333 curves displayed in Figure 3) show a linear behavior (slope = 2.6×10^{-6} with a R^2 = 0.988;
334 Figure 4) with the gain Y, confirming that the AGNES conditions were attained.
335
336 3.2.2 <u>Variation of the signal with free metal concentration</u>

The linearity between the AGNES signal (peak derivative) and the concentration of Cu^{2+} was evaluated for the E_1 and t_1 set chosen above (E_1 = 0.230 V and t_1 = 240 s). Moreover, the range of Cu concentrations that can be used with this potential-time set was defined. For the chosen experimental setting, a linear behavior was obtained between the DP signal and the range of free Cu concentrations of 7.5×10^{-9} to 9.8×10^{-8} M ((y = 19.2 (± 0.3) x + 7.5 (± 1.6)$\times10^{-8}$, R^2 = 0.999; Figure 5a). In fact, a linear behavior is still obtained up to a free copper concentration of 9.8×10^{-7} M (y = 15.97 (± 0.09) x + 2.1 (± 0.3)$\times10^{-7}$, R^2 = 0.998), with a deviation from the linearity observed at higher concentrations (Figure 5b). Obviously, the linearity range can be modified by using different experimental settings. The limit of detection (LOD = $(3.3\,SEy)/m$; where *SEy* is the standard error for the y estimate, and *m* is the slope) for the E_1 and t_1 set chosen (E_1 = 0.230 V and t_1 = 240 s) is 1.1×10^{-8} M. It was not the focus of this study to reach lower LOD. However, this can easily be achieved by applying higher gains, although longer deposition times would be required to attain AGNES conditions (Figures 3 and 4), leading to analysis time between 15 and 30 min per measurement. Another option is the use of smaller diameter electrodes and/or increasing vibrations to increase the diffusion flux, allowing the achievement of higher gains with similar deposition times.

3.3. Equilibrium Speciation

The possibility to perform AGNES on the VGME was tested by quantifying the equilibrium speciation of Cu in presence of the two ligands forming labile complexes, malonic acid (MAL) and iminodiacetic acid (IDA) (Tables 1 and 2, respectively; obtained by using the set E_1 = 0.230 V and t_1 = 240 s; for the lability calculation see section 3.4). The free copper concentration at various concentrations of ligands and pH were directly determined from the calibration curve (Figure 5a) [7]. This concentration can be then used to calculate the conditional stability constant (*K*) by using the following equation:

$$K = \left(\frac{C_{M,T}}{C_{M,free}} - 1\right)/c_{L,dep} \quad \text{(Equation 2)}$$

where, $c_{M,T}$ and $c_{M,free}$ are the total and free metal concentrations, respectively, and $c_{L,dep}$ being the fully-deprotonated ligand concentration available for Cu complexation at each specific pH value and ionic strength (computed using Visual MinteQ [34]).

Both the quantified free Cu concentrations and the calculated log *K* values were compared with those obtained by Visual MinteQ (Tables 1 and 2). As expected, for both systems, the free Cu concentrations were found to decrease with an increase of pH, and at fixed pH, an increase of the ligand concentration resulted in lower free Cu concentrations. For both ligands, the free Cu concentrations (and, consequently, the conditional stability constants) determined by AGNES in all conditions of ligand concentrations and pH were found identical, within the experimental error, to the values obtained by the equilibrium-based computer code.

In fact, these results clearly show that AGNES at the VGME can successfully quantify free metal concentrations in simple systems at acidic pH and relatively low ionic strengths. Despite the quite laborious electrochemical set-up (optimization is underway), the data obtained are reproducible (shown by the errors associated with measurements).

3.4. Comparison between AGNES and SSV

The results obtained by AGNES were also compared to another analytical technique, the SSV analysis. The possibility of performing SSV of Cu using the VGME had already been shown by Gibbon-Walsh *et al.* [21]. SSV determinations allow the direct determination of the conditional stability constant (*K*) of labile systems from the shift in the half-wave deposition potentials considering the formation of a reversible complex [38]:

$$K = \left(\exp\left(-\left(\frac{nF}{RT}\right)\Delta E_{d,1/2} - ln\left(\frac{s^*_{ML}}{s^*_M}\right)\right) - 1\right)\Big/ c_{L,dep} \quad \text{(Equation 3)}$$

where, $\Delta E_{\mathrm{d},1/2}$ is the shift in the half-wave deposition potential between M in absence and in presence of the ligand, and s^*_{M} and s^*_{ML} the diffusion limiting values of the stripping signal in absence and in presence of the ligand.

Therefore, it is possible to directly compare the speciation data of AGNES with that of SSV, although the former measures directly the free metal concentration which allows calculation of the conditional stability constant *K* (equation 2), while for SSV it is the other way round (it measures directly *K* allowing retrieval of free metal concentration by using the same equation 2).

An extra information that can be obtained when using SSV (not possible with AGNES) is the lability coefficient of complexes, which can be calculated from the ratio between the limiting kinetic flux, J_{kin}, and the limiting diffusive flux, J_{dif} [35]:

$$\frac{J_{\mathrm{kin}}}{J_{\mathrm{dif}}} = c_{\mathrm{ML}} k_{\mathrm{d}} \mu \frac{\delta}{D_{\mathrm{M}} c_{\mathrm{M,T}}} \qquad \text{(Equation 4)}$$

where, c_{ML} is the complex concentration, μ is the reaction layer thickness, and k_{d} the dissociation rate constant. The thickness of the diffusion layer (δ) of the VGME used in this experimental set up was determined to be 5 μm, whereas the reaction layer thickness is given by:

$$\mu = \left(\frac{D_{\mathrm{M}}}{k'_{\mathrm{a}}}\right)^{1/2} \qquad \text{(Equation 5)}$$

where, $k'_{\mathrm{a}} = k_{\mathrm{a}} c_{\mathrm{L,dep}}$ and k_{a} is the association rate constant which is generally consistent with a mechanism in which the formation of an outersphere complex between the metal and the ligand, with an electrostatically determined stability constant (K^{os}), is followed by a rate-limiting removal of water from the inner coordination sphere of the metal ($k_{\mathrm{-w}}$). This is commonly known as the Eigen mechanism [36]:

$$k_{\mathrm{a}} = k_{-\mathrm{w}} K^{\mathrm{os}} \qquad \text{(Equation 6)}$$

where, $k_{\mathrm{-w}}$, which is intrinsic for each metal ion, is 1.0×10^9 s^{-1} for Cu [37], and K^{os} is given by:

$$K^{os} = \frac{4\pi N_{Av} a^3}{3} \exp(-U^{os}) \quad \text{(Equation 7)}$$

where, N_{Av} is the Avogadro number, a is the charge center-to-center distance of closest approach between the reagents, and U^{os} is the electrostatic energy which is determined by a combination of the primary Coulombic energy between the metal and the ligand, with the energy for the screening effect due to the electrolyte.

The characteristic regimes of both metal complex systems, Cu-MAL and Cu-IDA, were calculated (Table 3) considering the conditions where more complexation occurs, i.e., higher ligand concentration and higher pH (stability constants obtained by Visual MinteQ were used; Tables 1 and 2). The results show that both systems are dynamic since the rates for the volume reactions are fast on the experimental time scale; $k_d t, k'_a t \gg 1$ (Table 3). The slightly larger than 1 values for the ratio of the kinetic to the diffusive fluxes indicate that both systems are still labile under these physicochemical conditions when using the VGME.

Table 1 shows the obtained values by SSV (log *K*, and the free metal concentrations calculated from the stability constants) for 1.0×10^{-7} M of total Cu in presence of 1.0×10^{-5} M of MAL at pH 4.5, 5.0 and 6.0 and I = 0.01 M. They are in very good agreement with AGNES, despite at pH 5.0 a slight difference is obtained, although still within the experimental error. The same good agreement was also obtained for the Cu-IDA system at pH 4.5 and 5.0 (Table 2). The good correlation between the data supports the SSV calculation methodology for VGME used to calculate the free metal ion concentration, however, with the AGNES procedure leading to less error than the corresponding shift in potential in SSV.

4. Conclusions

AGNES was successfully implemented at the VGME allowing for the first time the direct quantification of free Cu concentrations using a solid electrode, where a linear calibration

plot was obtained from 4.9×10^{-9} to 9.8×10^{-7} M of free Cu. The validity of AGNES at the gold electrode was verified by the trajectories reaching equilibrium values and by the linear dependency of the applied gain *Y*.

There are not many techniques that can perform the measurement of free metal ion concentration at low levels, although this is a key parameter to any speciation study and/or interpretation of the dynamic speciation data obtained with other electroanalytical techniques [16]. The AGNES methodology at the VGME could thus provide (after further developments) a powerful alternative technique for Cu speciation at the low concentration levels existing in natural waters especially due to: i) the non-toxicity of the working electrode, and ii) the ability to perform measurements without oxygen removal. Obviously, further optimization of the electrochemical set-up, which is already under way, is needed to decrease the detection limit that is reported here (11 nM). Such optimization consists in, for example, the decrease of the electrode diameter and/or increase of the vibration frequency to lower lower detection limits, which is required for applications in natural waters. Further testing of the method (e.g., in the presence of humic substances, and at wider pH and ionic strength ranges) is under way at solid gold electrodes, as well as at other material substrates (e.g., Ag and C).

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596 **Table 1 –** Free copper concentrations and log *K* values obtained from AGNES, SSV and Visual MinteQ for a total copper concentration of 1.0×10^{-7} M
597 and different total malonic acid (MAL) concentrations at *I* 0.01 M and various pH values. Deprotonated MAL concentrations ($c_{MAL,dep}$) were computed
598 by using Visual MinteQ. Diffusion coefficients: $D_{Cu} = 7.14\times10^{-10}$ m^2 s^{-1}; $D_{MAL} = 8.45\times10^{-10}$ m^2 s^{-1}. AGNES results are the mean and standard deviation,
599 whereas for SSV mean values are presented together with minimum and maximum values obtained from the SSV waves (values within square brackets).

		AGNES		SSV			VisualMinteQ	
pH	$c_{MAL,dep}$ /M	$c_{Cu,free}$ /M	log *K*	$c_{Cu,free}$ /M	log *K*	$\Delta E_{d,1/2}$ /V	$c_{Cu,free}$ /M	log *K*
4.0	9.99×10^{-6}	$(9.0 \pm 0.5)\times10^{-8}$	4.1 ± 0.1	-	-	-	9.2×10^{-8}	4.0
4.5	9.98×10^{-6}	$(7.6 \pm 0.7)\times10^{-8}$	4.5 ± 0.2	7.1×10^{-8} [$(4.4\text{-}8.7)\times10^{-8}$]	4.8 [4.2-5.1]	-0.002	8.1×10^{-8}	4.4
5.0	9.96×10^{-6}	$(6.12 \pm 0.06)\times10^{-8}$	4.80 ± 0.01	7.5×10^{-8} [$(5.4\text{-}9.9)\times10^{-8}$]	5.2 [3.4-5.6]	-0.014	6.2×10^{-8}	4.8
5.5	9.94×10^{-6}	$(4.24 \pm 0.08)\times10^{-8}$	5.14 ± 0.01	-	-	-	4.4×10^{-8}	5.1
6.0	9.93×10^{-6}	$(3.2 \pm 0.2)\times10^{-8}$	5.34 ± 0.04	3.3×10^{-8} [$(2.6\text{-}4.3)\times10^{-8}$]	5.4 [5.3-5.6]	-0.020	3.3×10^{-8}	5.3
4.0	1.00×10^{-4}	$(5.5 \pm 0.3)\times10^{-8}$	3.91 ± 0.05		-	-	5.8×10^{-8}	3.9

4.5	9.99×10^{-5}	$(3.5 \pm 0.4)\times10^{-8}$	4.27 ± 0.08		-	-	3.1×10^{-8}	4.3
5.0	9.99×10^{-5}	$(1.5 \pm 0.2)\times10^{-8}$	4.74 ± 0.08		-	-	1.4×10^{-8}	4.8

Table 2 – Free copper concentrations and log K values obtained by AGNES, SSV and Visual MinteQ for a total copper concentration of 1.0×10^{-7} M and different total iminodiacetic acid (IDA) concentrations at I 0.01 M and various pH values. Deprotonated IDA concentrations ($c_{IDA,dep}$) were computed by using VisualMinteQ; IDA constants [39, 40] were added to the database (protonation constants: 2.98 and 9.89; formation constant of Cu-IDA: 11.21). Diffusion coefficients: $D_{Cu} = 7.14\times10^{-10}$ m^2 s^{-1}; $D_{IDA} = 7.19\times10^{-10}$ m^2 s^{-1}. AGNES results are the mean and standard deviation, whereas for SSV mean values are presented together with minimum and maximum values obtained from the SSV waves (values within square brackets).

		AGNES		SSV			VisualMinteQ	
pH	$c_{IDA,dep}$ /M	$c_{Cu,free}$ /M	log K	$c_{Cu,free}$ /M	log K	$\Delta E_{d,1/2}$ /V	$c_{Cu,free}$ /M	log K
4.0	9.98×10^{-7}	$(9.27 \pm 0.03)\times10^{-8}$	4.90 ± 0.02	-	-	-	9.6×10^{-8}	4.7
4.5	9.92×10^{-7}	$(8.70 \pm 0.09)\times10^{-8}$	5.18 ± 0.04	-	-	-	9.0×10^{-8}	5.0
5.0	9.79×10^{-7}	$(7.56 \pm 0.08)\times10^{-8}$	5.52 ± 0.02	-	-	-	7.7×10^{-8}	5.5
5.5	9.55×10^{-7}	$(5.19 \pm 0.04)\times10^{-8}$	5.987 ± 0.006	-	-	-	5.4×10^{-8}	6.0
4.0	9.98×10^{-6}	$(7.4 \pm 0.3)\times10^{-8}$	4.55 ± 0.07	-	-	-	7.8×10^{-8}	4.4
4.5	9.95×10^{-6}	$(5.2 \pm 0.2)\times10^{-8}$	4.97 ± 0.04	5.3×10^{-8} [$(3.4\text{-}9.4)\times10^{-8}$]	4.9 [3.8-5.3]	-0.0037	5.3×10^{-8}	4.9
5.0	9.93×10^{-6}	$(2.6 \pm 0.1)\times10^{-8}$	5.46 ± 0.02	2.8×10^{-8}	5.4 [5.2-5.6]	-0.0059	2.7×10^{-8}	5.4

				[(2.1-3.9) $\times 10^{-8}$]				

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629 **Table 3 –** Characteristic regimes of the metal complex systems. Cu-MAL: total copper
630 concentration of 1.0×10^{-7} M in presence of 1.0×10^{-4} M of malonic acid at *I* 0.01 M and
631 pH 5.0. Cu-IDA: total copper concentration of 1.0×10^{-7} M in presence of 1.0×10^{-5} M of
632 iminodiacetic acid at *I* 0.01 M and pH 5.0. The experimental time scale is given by $t = \delta^2/D_{\mathrm{M}} = 0.035$ s. Other parameters: $k_{\text{-w}} = 1.0\times10^{9}$ s^{-1}, $K^{\mathrm{os}} = 4.4\times10^{1}$ M^{-1}, $U^{\mathrm{os}} = -4.9$, D_{M}
634 $= 7.14\times10^{-10}$ m^2 s^{-1}, $K_{\text{Cu-MAL,VisualMinteQ}} = 4.8$, $K_{\text{Cu-IDA,VisualMinteQ}} = 5.4$.

System	$k'_{\mathrm{a}}t$	$k_{\mathrm{d}}t$	$J_{\mathrm{kin}}/J_{\mathrm{dif}}$
Cu-MAL	1.5×10^{5}	2.6×10^{4}	56
Cu-IDA	1.5×10^{4}	5.6×10^{3}	33

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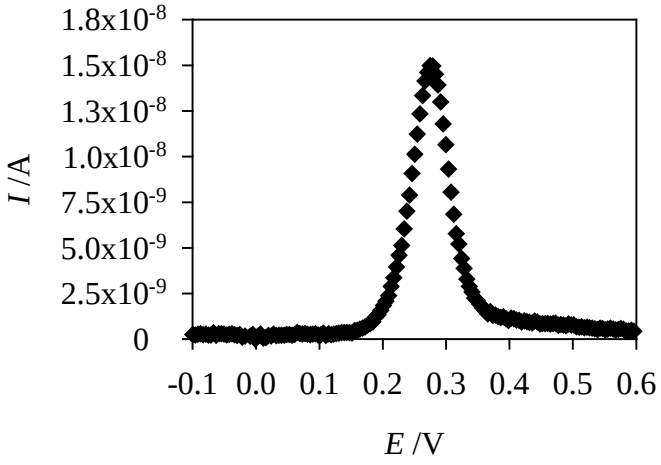
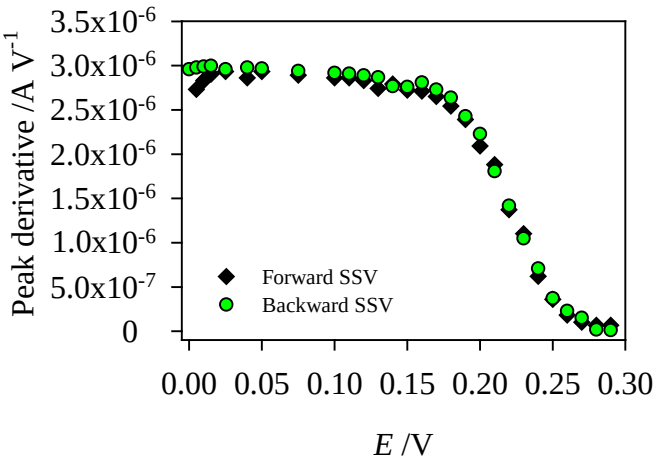


Figure 1 – Current as a function of potential for a total copper concentration of 1.0×10^{-7} M at pH 4 and *I* 0.01 M (adjusted with $NaNO_3$) obtained by DPASV after subtraction of the background scan (E_d = 0.230 V, t_d = 240 s).



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655 **Figure 2 –** Forward (◆) and backward (●) SSV of 1.0×10^{-7} M of total Cu at pH 4.0 and

656 *I* 0.01 M. Other parameters: E_d = 0.000 to 0.300 V and t_d = 30 s.

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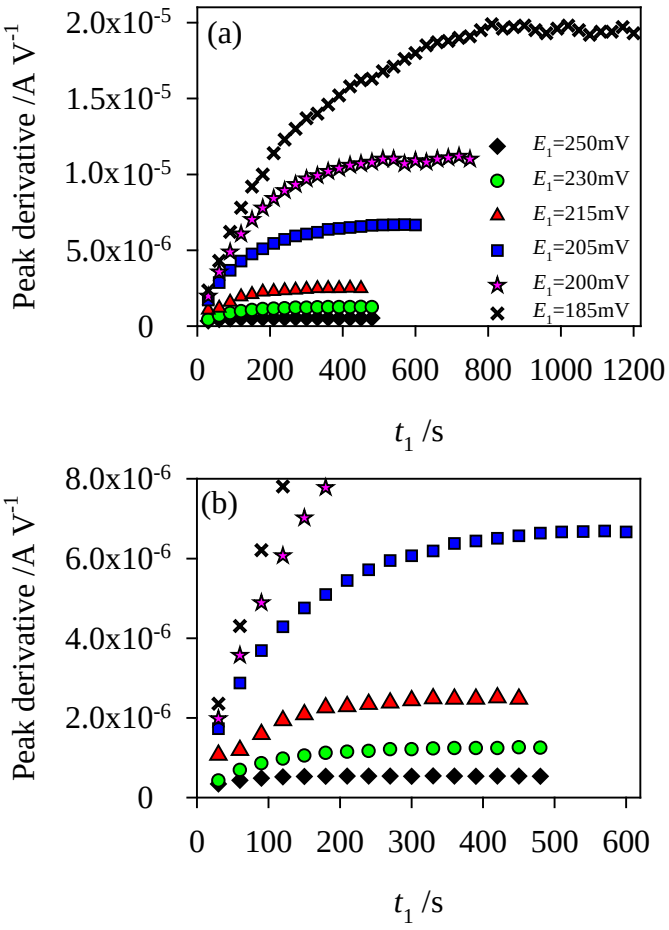

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674 **Figure 3 –** Peak derivative as a function of deposition time (t_1) for a total copper
675 concentration of 1.0×10^{-7} M at pH 4.0 and I 0.01 M, and for a range of deposition
676 potentials (E_1 = 0.185 to 0.250 V). Panel (b) is a zoom of graph in panel (a). The range of
677 deposition potentials used was: 250 (◆), 230 (●), 215 (▲), 205 (■), 200 (★), and 185
678 (✖) mV.

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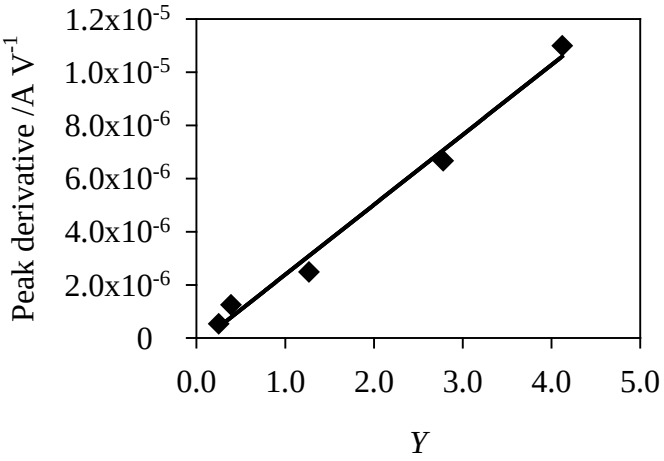

Figure 4 – DP analytical signal after attainment of equilibrium as a function of the gain for a total copper concentration of 1.0×10^{-7} M at pH 4.0 and *I* 0.01 M, and for a range of deposition potentials (0.200 to 0.250 V) and sufficiently long deposition times (240 to 750 s).

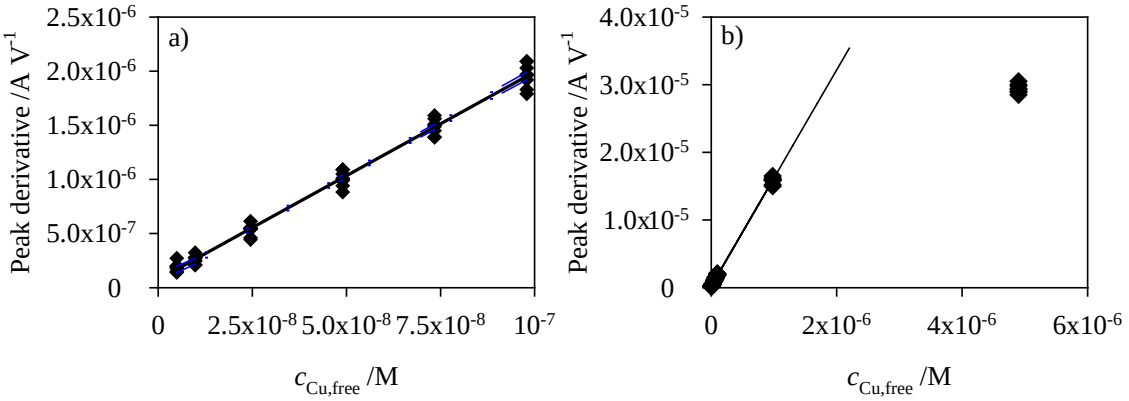

Figure 5 – Peak derivative as a function of free copper concentration at pH 4.0 and *I* 0.01 M; $c_{Cu,free}$: a) 4.9×10^{-9} to 9.8×10^{-8} M, and b) 4.9×10^{-9} to 4.9×10^{-6} M. The data points are representative of 8 replicates performed in different days, the solid line represents the average and the blue long dash lines represent a 95 % confidence level of the root mean square error. Other parameters: E_1 = 0.230 V, t_1 = 240 s.