Evaluation of thin mercury film rotating disc electrode to perform Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) measurements

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*This work is dedicated to the memory of Professor Helena Maria Carapuça,
acknowledging her inspiration, her limitless support and especially her constant
friendship always demonstrated.*

Abstract

In the present work, the applicability of thin mercury film on a rotating disk electrode (TMF-RDE), to assess the free metal ion concentration by absence of gradients and Nernstian equilibrium stripping (AGNES), is evaluated. The thickness of the mercury film and several AGNES parameters has been optimized. A nominal 16 nm film is chosen due to the higher signal (faradaic current) relative to the value of the noise (capacitive current). Due to the smaller volume to area ratio, the deposition time needed to reach a certain pre-concentration factor (Y) is much shorter than in larger electrodes, like the HMDE. The limit of detection (3σ) for lead(II) is 7.4×10^{-9} M and 7.2×10^{-8} M for a Y of 5000 (deposition time of 150 s) and 1000 (deposition time of 100 s), respectively. A specific mathematical treatment is developed in order to subtract a corrected blank taking into account the degradation of the thin film (presumably, falling down of drops). The couple TMF-RDE /AGNES is successfully applied for speciation purposes in the systems Pb(II)-latex nanospheres and Pb(II)-IDA (iminodiacetic acid),

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where the stability constants calculated for both systems agree with values reported in the literature.

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1. Introduction

Absence of gradients and Nernstian equilibrium stripping (AGNES) was recently proposed for the determination of the free metal ion concentration of amalgamating elements [1]. The key idea in this technique is that during the deposition step the metal within the mercury electrode is allowed to proceed until reaching Nernstian equilibrium without concentration gradients. Therefore, the concentration of amalgamated metal and the faradaic stripping current are proportional to the free metal ion concentration in the bulk solution [1][2].

Recently, instead of using the conventional HMDE, AGNES was applied with Hg-Ir microelectrodes. Due to a reduction of the drop size in the latter case, the amount of deposited metal for a given preconcentration is much less and the deposition time can be reduced. Because of the larger ratio of the accessible area over the volume of the amalgam, shorter times will be required to attain the target conditions (Nernst equilibrium). Despite of these advantages, the preparation procedure of the microelectrode is time consuming and the mechanical resistance of this electrode still needs to be improved. Moreover, the large capacitive current only allowed a modest decrease of the limit of detection [3].

In a previous work, the thin mercury film rotating disk electrode (TMF-RDE) proved to be suitable in stripping chronopotentiometry and scanning stripping chronopotentiometry (SCP/SSCP) studies of metal cations, due to the high sensitivity

and resolution attained [4]. A relatively stable, homogeneous and reproducible *ex-situ* thin mercury film (TMF) could be easily plated, with a single electrodeposition step of high overpotential, in acidic solutions [4][5].

The aim of the present work is to evaluate the TMF-RDE to perform AGNES. For that, the evolution of the film electrode is taken into account by means of a mathematical treatment. The TMF-RDE thickness is optimized in order to obtain a stable film coating that could be used for one day, without recasting the electrode. The key parameters of the technique, such as deposition time, deposition potential and also the rotation speed applied to the rotating disk electrode (RDE) are also investigated. The main goal is to shorten the time of analysis while obtaining a low detection limit. Additionally, the features of the TMF-RDE coupled with AGNES in studies of metal ion speciation are studied, by evaluating the interaction of lead(II) cation with two ligands: i) iminodiacetic acid (IDA) and carboxylated latex nanospheres (P(S/V-COOH)) with 40.0 nm radius.

2. Theoretical background

We now summarize the principles of AGNES and its application with non-evolving electrodes (fixed area and fixed volume).

AGNES consists of two conceptual steps or stages: deposition or first stage, and stripping or second stage. The aim of the deposition step is to attain a special situation without concentration gradients (both inside the mercury film electrode and in the solution in contact with the electrode), while a constant ratio is kept between the concentrations of the electroactive couple due to the Nernstian equilibrium [1]. This

ratio is called gain or preconcentration factor Y , and depends on the applied potential, E_1 :

$$Y = c_{M^0}^* / c_M^* = \exp \left[-nF (E_1 - E^{0'}) / RT \right] \quad (1)$$

where $E^{0'}$ stands for the standard formal potential of the redox couple of the metal M and $c_{M^0}^*$ and c_M^* correspond to the final homogeneous metal concentrations inside the mercury electrode and the bulk solutions, respectively. The duration of the deposition stage is denoted as t_1 . The gain Y for a given applied potential E_1 is determined from the peak potential of a differential pulse polarography (DPP) experiment, E_{peak} [6]:

$$Y = \sqrt{\frac{D_M}{D_{M^0}}} \exp \left[-\frac{nF}{RT} \left(E_1 - E_{\text{peak}} - \frac{\Delta E_{\text{DPP}}}{2} \right) \right] \quad (2)$$

where D_{M^0} is the diffusion coefficient of the reduced metal, and ΔE_{DPP} is the modulation amplitude of the DPP experiment.

In the second (or quantification) step, a sufficiently less negative potential, E_2 , is applied in order to produce a stripping current under diffusion limited conditions. The measured response function is the current I at a certain time t_2 . The faradaic component of this current is linearly related with $c_{M^0}^*$, due to the linear features of the metal diffusion inside the mercury film. So, the free metal ion concentration is directly proportional to the faradaic current with a proportionality factor h (which can be obtained from the calibration), since, according to equation (1) [1], $c_{M^0}^*$ is Yc_M^* . So

$$I_f = hc_M^* \quad (3)$$

The measured current contains other components apart from the faradaic current; therefore it is necessary to subtract a blank from the total current.

3. Experimental

3.1. Reagents

All chemicals were of analytical reagent grade and all solutions were prepared with ultra-pure water (18.3 MΩ cm, Milli-Q systems, Millipore-waters). Sodium nitrate, potassium chloride, nitric acid 65% (Merck, suprapur), hydrochloric acid 37% (trace select, Fluka) and 1000 ppm Hg and Pb AA-Spectrosol metal ion standards (Merck) were also used. Ferricyanide standard solution (1.929×10^{-3} mol dm⁻³) in 1.0 mol dm⁻³ KCl was used for chronoamperometry. Nitric acid (1 M) and sodium hydroxide solutions (1 M) were used for pH adjustments. Stock solution of MES 2-(N-morpholino-ethanesulfonic acid) buffer (0.1 M) was prepared from the solid (Merck). Solutions of ammonium acetate (NH₄Ac 1.0 M/0.5 M HCl) pH buffer and ammonium thiocyanate 1.0 M were prepared monthly and used without further purification. Biohit Proline pipettes equipped with disposable tips were used for appropriate dilutions. All measurements were carried out at room temperature (20 °C).

3.2. Apparatus

An Ecochemie Autolab PGSTAT12 potentiostat (controlled by GPES 4.9 software from EcoChemie, the Netherlands) was used in conjunction with a Metrohm 663 VA stand (Metrohm, Switzerland). The three electrode configuration was used comprising a TMF plated onto a rotating glassy carbon (GC) disc (1.9 mm diameter, Metrohm) as the working electrode, a GC rod counter electrode and a double junction Hg | Hg₂Cl₂ | KCl (3 M) encased in a 0.1 M NaNO₃ solution, as the reference electrode. The electrochemically active surface area of the glassy carbon electrode, ($3.098 \pm$

0.015) mm², was measured by chronoamperometry (in 1.929×10⁻³ M ferricyanide/1.0 M KCl solution; two polishing experiments, each with four replicate determinations).

A combined glass electrode (Radiometer Analytical pH3006-9) connected to a pH meter (Thermo Electron Corporation, Model Orion 3-Start) was used for pH measurements.

The microscope examinations were carried out with an optical inverted microscope Zeiss Axiovert 40 MAT connected to a digital camera Cannon Powershot A620 and an AxioVision LE Canon Module software.

3.3. Preparation of the thin mercury film electrode

Prior to coating, the GCE was conditioned following a reported polishing/cleaning procedure [7]. The GCE was polished with an alumina slurry (grain size 0.3 μm, Metrohm) and sonicated in pure water for 60 seconds, to obtain a renewed electrode surface. Then, an electrochemical pre-treatment was carried out with a multicycle voltammetric scanning (50×) between -0.8 and +0.8 V at 0.1 V s⁻¹, in NH₄Ac-HCl solution. These polishing and electrochemical pre-treatments were repeated daily.

The thin mercury film was then ex-situ prepared in 0.12 mM mercury (II) nitrate and adjusted to pH~1.9, with a 1 M nitric acid solution, by electrodeposition at -1.3 V for different deposition times (240 s and 480 s) and a rotation rate of 1000 rpm [5, 8].

The charge associated to the deposited mercury (Q_{Hg}) was calculated by electronic integration of the linear sweep stripping peak of mercury, for $\nu = 0.005 \text{ V s}^{-1}$. The electrolyte solution was ammonium thiocyanate 5 mM (pH 3.4). The stripping step began at -0.15 V and ended at +0.6 V [8]. All charges quoted are mean values of 6 replicate measurements (RDS<7%). The thickness of the TMF-RDE was estimated

using this mean value of Q_{Hg} and assuming a uniform spread of the mercury on top of the entire disk surface [5, 8]. Assuming a thin-film condition, the volume of the mercury electrode, $V_{\text{Hg,dep}}$, can be estimated from the amount of deposited mercury measured as the charge, Q_{Hg} , using the Faraday law and assuming the Hg atomic radius (r_{Hg}) as 1.44×10^{-10} m [7]. For the thin mercury film used in this work, the Q_{Hg} was 1.28×10^{-3} C (mean value of 6 replicate measurements with an RDS<7%), leading to a $V_{\text{Hg,dep}}$ 5.01×10^{-14} m³.

When not in use, the GCE was stored dry in a clean atmosphere [5].

3.4. AGNES procedures

AGNES experiments for optimisation of parameters and for calibration were carried out in 20 mL 0.01 M NaNO₃ solutions spiked with lead(II) at pH of *ca.* 4. The potential program used consists in applying one potential step along the first stage (“1 pulse” or “1P” strategy) following the scheme in Fig.1 [6].

The measured response is the current I at a certain time t_2 . To this current we have to subtract a blank to remove the contributions other than the faradaic one. The synthetic blank [1, 2, 9] (obtained applying the same potential program in a solution with the same composition of the sample but no metal) is not suitable in case of presence of traces of contaminants or when dealing with natural samples with complex matrices. So, we developed a new kind of blank, the “shifted blank” [3, 6, 10, 11, 12]. The “shifted blank” is applied to the solution containing the metal but with a potential program shifted with respect to the main measurement; the deposition potential $E_{1,\text{sb}}$ corresponds to a very low gain ($Y_{\text{sb}}=0.01$) and a $E_{2,\text{sb}}$ is applied keeping the same potential jump ($\Delta E = E_{1,\text{sb}} - E_{2,\text{sb}}$) of the main measurement. To avoid the effects caused

by the presence of residual oxygen in the test solution, all of the current values (of the main and the shifted blank measurements) are corrected with the residual current (I_{∞}), which is obtained at very large stripping time (usually 20 s) [3].

All solutions were purged for 60 minutes at the beginning of each series of AGNES experiments and 60s (assisted by mechanical stirring of the rotating GCE for the experiments performed using the rotation of the electrode) after each measurement.

Prior to the addition of the ligand (latex nanospheres or IDA) for the speciation assays, a calibration plot was performed using the optimized experimental parameters (t_1 , t_2 , Y and rotation speed of the rotating disk electrode).

4. Results and discussion

4.1. AGNES with evolving electrodes

4.1.1. Mechanical stability of the TMF-RDE

One of the problems reported with the use of the TMFE, especially when applied in the rotating mode of the electrode, is its mechanical degradation due to the coalescence or falling of the small drops of Hg. The stability of the mercury coating was assessed by evaluating the repeatability of the current measured at t_2 of 2 ms and for a Y of 2000. A set of 29 consecutive AGNES measurements, each of them immediately followed by a measurement of the shifted blank (a total of 58 measurements in 29 pairs of main measurement and shifted blank), is shown in Fig 2. A “synthetic blank” with just background electrolyte (NaNO_3 0.01 M solution, pH c.a. 4) in the solution was run prior to the addition of the metal to the electrolyte solution.

Despite of this slight drift, we accept that this electrode can be used for a maximum of 58 AGNES experiments. A strategy (not essayed here) could be to discard the first few AGNES measurements, since the highest current deviations occur along these initial values.

4.1.2. Mathematical treatment

One interpretation of the observed decrease of the main measurements and blank currents is the falling down of some of the small drops that make up the film. Up to date, AGNES technique has only been used with electrodes whose area and volume remained fixed during the experimental course. Given that the shifted blanks could not be used because of their anomalously high values, we develop next a new mathematical treatment for this electrode with non-fixed area and volume.

We assume that AGNES principles apply to each individual drop, so that the faradaic current of a drop ($I_{f,j}^{\text{drop}}$) is proportional to the free metal concentration c_j at that moment or addition. j denotes a given configuration in the life of the evolving film – e.g. the electrode situation for a pair of main measurement and shifted blank in Fig 2. So,

$$I_{f,j}^{\text{drop}} = h^{\text{drop}} c_j \quad (4)$$

where h^{drop} depends on the area of the drop. For the sake of simplicity, we assume that all drops are identical.

Assuming that the capacitive current (e.g. via the area) is proportional to the number of drops:

$$I_{\text{sb},j} = n_j I_{\text{sb},j}^{\text{drop}} \quad (5)$$

we can divide the previous eqn. applied to a general j with respect to a fixed (selected) “reference” moment or addition:

$$\frac{I_{\text{sb},j}}{I_{\text{sb,ref}}} = \frac{n_j I_{\text{sb},j}^{\text{drop}}}{n_{\text{ref}} I_{\text{sb,ref}}^{\text{drop}}} = \frac{n_j}{n_{\text{ref}}} \quad (6)$$

We assume that, for each j , we can use a certain synthetic blank, which denoted $I_{\text{NaNO}_3,j}$. This value cannot be experimentally measured for each moment j , but rather is estimated from the initial measurement $I_{\text{NaNO}_3,0}$ (synthetic blank with just background electrolyte):

$$\frac{I_{\text{NaNO}_3,j}}{I_{\text{NaNO}_3,0}} = \frac{n_j}{n_0} \Rightarrow I_{\text{NaNO}_3,j} = \frac{n_j}{n_0} I_{\text{NaNO}_3,0} = \frac{I_{\text{sb},j}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0} \quad (7)$$

Thus, the faradaic component of one main measurement is:

$$I_{\text{f},j} = I_j - I_{\text{NaNO}_3,j} = I_j - \frac{I_{\text{sb},j}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0} = n_j I_j^{\text{drop}} = n_j h^{\text{drop}} c_j \quad (8)$$

where I_j is the total current of the main measurement.

Let us divide the previous eqn. applied to a general moment or addition j with respect to a fixed (selected) “reference” moment or addition:

$$\frac{I_j - \frac{I_{\text{sb},j}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0}}{I_{\text{ref}} - \frac{I_{\text{sb,ref}}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0}} = \frac{n_j h^{\text{drop}} c_j}{n_{\text{ref}} h^{\text{drop}} c_{\text{ref}}} = \frac{n_j c_j}{n_{\text{ref}} c_{\text{ref}}} \quad (9)$$

We can isolate the faradaic component as

$$I_{\text{f},j} = I_j - \frac{I_{\text{sb},j}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0} = \left(I_{\text{ref}} - \frac{I_{\text{sb,ref}}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0} \right) \frac{I_{\text{sb},j}}{I_{\text{sb,ref}}} \frac{c_j}{c_{\text{ref}}} \quad (10)$$

which, by defining a dimensionless or normalized current as

$$I_{\text{norm},j} \equiv \frac{I_j - \frac{I_{\text{sb},j}}{I_{\text{sb},0}} I_{\text{NaNO}_3,0}}{I_{\text{sb},j}} = \frac{I_j}{I_{\text{sb},j}} - \frac{I_{\text{NaNO}_3,0}}{I_{\text{sb},0}} \quad (11)$$

the relationship can be simply written as

$$I_{\text{norm},j} = h_{\text{eff}} c_j \quad (12)$$

with h_{eff} being the “effective” proportionality between concentration and the ratio between the faradaic and the shift blank currents:

$$h_{\text{eff}} \equiv \frac{\frac{I_j}{I_{\text{sb},j}} - \frac{I_{\text{NaNO}_3,0}}{I_{\text{sb},0}}}{c_j} \quad (13)$$

From the calibrations, we compute h_{eff} as the slope of $\frac{I_j}{I_{\text{sb},j}} - \frac{I_{\text{NaNO}_3,0}}{I_{\text{sb},0}}$ vs c_j and

then we use this h_{eff} in eqn. (12) to determine the free metal concentration in a speciation experiment.

As a simple verification of this proposal, we have computed h_{eff} with eqn. (13) for the experiments shown in Fig. 2 and plot the results in Fig 3. The variation of h_{eff} is around 20% from $j=1$ to $j=29$, but is around 10% if we go from $j=1$ to $j=15$ (30 experiments). This variation in h_{eff} is reasonably acceptable for the small number of experiments performed by us in one day (e.g. in the speciation experiments). We speculate (with the support of a simple model based on the proportionality between capacitive current with area and the functional dependence for h given in eqn A9 of ref. [Galceran20004AGNES1]) that the increase with time of h_{eff} could be due to the falling down of the smallest droplets, while the largest ones would remain attached.

4.2. Selection of the mercury film thickness in AGNES

The TMF-RDE is subject to mechanical deterioration of the electrode surface during the experimental course. To improve the electrode stability, the electrodeposition of mercury was performed in very acidic solutions (pH *ca.* 1.9) as in [5]. The result was the formation of highly reproducible thin mercury films, consisting of a collection of very small radius mercury droplets, as can be seen in the optical microphotographs (Fig. 4). The performance, i.e. reproducibility, stability/durability, of the TMF-RDE prepared by this procedure is similar to that achieved for the mercury films, *ex-situ* prepared in thiocyanate media [7], as reported in a previous work [4].

The capacitive currents exhibited by microelectrodes [3] and by the TMF-RDE (probed in this work) are relatively large (i.e. of the same order of magnitude as the main measurement, so that the faradaic component is less well-defined). Here, the thickness of the mercury coating has revealed as key to reduce the charging currents [3]. Indeed, for the TMF-RDE of 9.1 nm the current obtained in the main experiment was practically the same as the capacitive current (provided by the shifted blank and for a concentration of lead(II) of 2.0×10^{-7} M) and so, a thicker, of *ca.* 16 nm, TMF-RDE was used in this work.

4.3. Optimization of AGNES parameters

Now we evaluate the impact of the different AGNES experimentally controlled parameters, such as the interval time used to measure the current (t_2) during the stripping step, the deposition time (t_1) and the rotation speed applied to the disk electrode, on the performance of the TMFE.

4.3.1. Selection of t_2

The new mathematical treatment for evolving electrodes relies on the application of eqn. (12). The faradaic current I_{fj} (see eqn (10)) is obtained by subtraction of the corrected synthetic blank (7)) from the main measurement of the current, being all currents measured at the same fixed t_2 .

The selection of the measuring time t_2 stems from the idea of minimizing the relative importance of the capacitive current (estimated with the corrected synthetic blank) in the total current, which can be reformulated as obtaining larger values for the normalised current (11)). In order to look for the ideal t_2 , the ratio of the measured current over the shifted blank was plotted (Fig. 5) within the time interval of [1-10] ms, for a Y of 2000 and for two concentrations of lead(II).

The results point out that the suitable t_2 values are within the interval of [1-3] ms, where the I/I_{sb} ratio goes through a maximum around 2 ms. Therefore, 2 ms was the time chosen as the standard t_2 for the present work, representing a time not too short (where large initial capacitive current arise) nor too long (where the gradual extinction of the faradaic current proceeds). Of course that, depending on the experimental set-ups, the optimum t_2 might suffer slight variations, but they can be easily followed given that the stripping current is recorded for a large number of t_2 -values.

4.3.2. Effect of the deposition time, t_1

The deposition time is a key parameter in AGNES experiments. Higher t_1 values will favour the approach to the Nernst equilibrium, although too long deposition times would render the technique less useful. Therefore, using a one pulse strategy (i.e. the simplest potential program, that consists in applying only one deposition potential), the effect of the deposition time t_1 on the reaching of the sought equilibrium was evaluated.

The deposition gain (Y) used in this experiment was 5000 and the total lead(II) concentration selected was 1.49×10^{-7} M. In a first stage (see open markers in Fig 6), no rotation was applied to the rotating disk electrode during the deposition step. The results reveal that for a gain (or preconcentration factor) of 5000, deposition times (t_1) longer than *ca.* 1600 s at the AGNES first stage, are required to reach the equilibration with flat profile.

Previous works of AGNES with the HMDE have systematically used stirring as a means of improving the efficiency of the mass transport, this resulting in a reduction of the required deposition time (t_1) [1]. This feature is particularly relevant to measure trace levels of free metal ions, where high preconcentration factors are frequently required. Therefore, the effect of the rotation speed in the experimental time of AGNES first stage under the same experimental conditions, i.e. using a Y of 5000, was evaluated (full markers in Fig. 6).

As expected, Nernst equilibrium is attained much faster with the rotation of the electrode, during the application of the potential deposition E_1 . For the Y of 5000 used in this experiment, the plateau is reached for a deposition time of 100 s with a rotation speed of 500 rpm, which is sixteen times less the time needed without the rotation of the RDE (1600 s). In both cases, a stabilized faradaic current of *ca.* 2×10^{-5} A is achieved. The small t_1 values needed to reach the equilibrium at the TMF-RDE allow the use of higher preconcentration factors, thus lowering the limit of detection (LOD) of the technique.

Comparing the performance of the TMF-RDE with that of the Hg-Ir microelectrode [3], apart from the differences in the composition of the test solution used in the set-ups of experiments (the measurements using the microelectrode were made in 0.1 M KNO_3 media, at a pH 6.1), the results reveal that shorter t_1 values are

required for the film electrode to reach AGNES conditions. For a gain of 5000, the microelectrode requires a t_1 of 400 s and reaches a LOD of $3.43 \times 10^{-8} \text{M}$, while the TMF-RDE for the same Y , reaches a lower LOD of $7.20 \times 10^{-9} \text{M}$ with a shorter t_1 (150s) for a rotation speed of XXXX.

The thin mercury film electrode is considered to be morphologically characterized not as a true film, but as a collection of small mercury droplets (*cf.* Fig. 4). The increased efficiency of the TMF-RDE with respect to the Hg-Ir microelectrode is a combination of a favourable ratio accessible area over volume and to the possibility of enhancing the mass transport via rotation [1, 3, 13].

In this work, other experiments were performed using a rotation speed of the RDE of 1000 rpm. The results were quite similar to the ones obtained with a rotation of 500 rpm, i.e. the time needed to obtain the plateau was basically the same. Since higher rotation speeds promote the faster mechanical degradation of the TMFE, the rotation speed of 500 rpm was selected as the more adequate, being applied in the subsequent studies.

4.4. Determination of the working concentration range for AGNES at the TMF-RDE

The proportionality between I_{norm} and c_{pb}^* (given by eqn. (12)) on the TMF-RDE of 16 nm of thickness was evaluated. The calibration curves for three Y values (1000, 2000 and 5000) are displayed in Fig. 7. Before the additions of the metal to the electrolyte solution (NaNO_3 0.01 M solution, pH c.a. 4), a “synthetic blank” was made.

The interval of linearity between the faradaic current and the concentration of the free lead(II) ion clearly depends on the gain applied in the experiment. A higher gain produces higher faradaic current and as a result, the deviation to the linearity occurs at

lower metal ion concentrations. Fig.8 shows the chronoamperograms obtained in the second stage of AGNES for several concentrations of the total lead(II) and for a Y of 5000. The distortion in the AGNES signals for a concentration of the total lead(II) above 5×10^{-7} M is clearly seen. The anomalous stripping behaviour of Zn and Cd in AGNES experiments has been recently described in [14] and mostly attributed to a low ratio of the electroactive to background electrolyte concentrations [15]. One strategy, to avoid this distortions in the current values consists in increasing the ionic strength (provided this change or selection is allowed in the specific study undertaken, e.g. in many titrations of Humic Acids with metals). Alternatively, one can use the stripped charge (by integration of the stripping current all along the second stage) as response function, thus, increasing the range of linearity of the response function with the reduced metal concentration quite close to the saturation conditions.

Table 1 presents the interval of operational concentration values (expressed by the total lead(II) concentration) at this ionic strength, the limit of detection (LOD) and the calibration data, i.e. the slope (the proportionality factor, h_{eff}) and the correlation coefficient (r).

By applying a Y of 1000 it is possible to work with higher concentrations, but impossible to go below 7.4×10^{-8} M of lead(II), since the metal current is mainly capacitive current. The analytical window can be lowered applying a Y of 5000. Though, at this preconcentration factor it is not possible to go above a total lead(II) concentration of 2.0×10^{-7} M, due to the anomalous behaviour of the stripping currents at high reduced metal concentrations (for this ionic strength), but further work could take advantage of the wider analytical window available with the charge as response function [14]. The limit of detection (LOD) of this technique at the TMFE was 7.4×10^{-8} M and 7.2×10^{-9} M for a Y of 1000 and 5000, respectively. Lower LOD can –in principle– be

achieved increasing the preconcentration factor applied in the experiment, though –in practice- the degradation with the required increased deposition times might pose restrictions. AGNES with the TMF-RDE and the essayed selection of the preconcentration gains, has measured the free metal concentration within a wide range of metal concentrations, i.e. from 7.2×10^{-9} M to 1×10^{-6} M, depending on the chosen conditions.

The factor h_{eff} , that represents the direct proportionality between the normalised current measured and the free metal concentration (Eq. 3), was plotted as a function of the preconcentration factor Y (Fig 9). The linearity attained confirms the direct proportionality between these two parameters.

4.5. AGNES for metal speciation studies

4.5.1. Labile system

Monodisperse carboxylated latex nanospheres (P(S/VCOOH)) with 40.0 nm radius constitute a good model for macromolecular and particulate binding. The interaction of lead(II) with the latex nanospheres was studied with AGNES and the values obtained were compared with the experimental values obtained at the HMDE [11]. The experiments were performed for a total concentration of lead(II) of 6.0×10^{-7} M, at ionic strength 0.01 M in NaNO_3 , and a MES 2-(N-morpholino-ethanesulfonic acid) buffer concentration of 2.0×10^{-3} M to fix the value of the pH to 5.5 and 6.1. The experiment at the TMF-RDE was carried out using a Y of 5000 and 2000, a deposition time of 150 s and a rotation speed of 500 rpm. Prior to the addition of the ligand to the test solution, the calibration plots for both preconcentration factors were obtained (pH *ca.* 4), see Table 2.

To compare with existing literature, we have computed the conditional stability constant as

$$K = \frac{c_{ML}}{c_M c_L} = \frac{c_{M,t} - c_M}{c_M (c_{L,t} - c_{M,t} + c_M)} \quad (14)$$

(i.e. by assuming that M only forms the complex ML with L, and neglecting other forms of species M and L, such as PbNO_3^+). We include these retrieved values of K in the last column of Table 2.

The calculated values perfectly agree with the values of [4.9-5.0] and [5.3-5.6] for pH 5.6 and 6.1, respectively, obtained with the HMDE by Domingos *et al.*[11].

4.5.2. Quasi-labile system

The interaction of lead(II) with iminodiacetic acid (IDA) was studied with AGNES at the TMFE. In order to assess this, a titration was performed by adding different concentrations of IDA to a test solution, containing a total concentration of lead(II) of 2.49×10^{-7} M and a MES 2-(N-morpholino-ethanesulfonic acid) buffer concentration of 2.0×10^{-3} M to fix the value of the pH to 5.5. The experiment was performed using a Y of 5000 and the other AGNES parameters (deposition time and rotation speed of the RDE) were the same as in 4.5.1. The results are presented in Table 3.

For the Pb-IDA system, the values obtained for $\log K$, mean value of 8.61 ± 0.06 for 0.01 mol dm^{-3} ionic strength, are in reasonable agreement with the $\log K$ value of $8.48 \text{ dm}^3 \text{ mol}^{-1}$ computed with Davies correction from the reported value in reference [16, 17]. However, one obtains an average $\log K$ value 8.1 from works reported in MiniSCD where Davies correction [J Felcman, J da Silva; Talanta, 30, 565 (1983); J

Ambrose, A Covington, H Thirsk; Trans. Faraday Soc., 65, 1897 (1969); J Ambrose, A Covington, H Thirsk; Trans. Faraday Soc., 65, 1897 (1969); G Atkinson, J Bauman; Inorg. Chem., 2, 64 (1963) **Luciana, please compile**] is applicable.

5. Conclusions

The free metal determination with AGNES using a TMF-RDE has been implemented. The thickness of the film (16 nm) and the rotation of the electrode (500 rpm) has been optimised, as well as the parameters of AGNES technique (deposition time, gain and reoxidation measuring time). A specific mathematical treatment (*cf.* section 4.1.2 and eqn. (12)) has also been developed to take into account the degradation suffered with time and stirring with this kind of film electrodes.

Lower LOD, in comparison with the conventional dropping mercury electrode or the Ir-Hg microelectrode, can be attained with this electrode for comparable times. Special care has to be taken to avoid anomalous stripping currents for higher metal ion concentrations (relative to the background electrolyte) which lead to a loss of linearity in the calibration plot. The measurement of the charge is expected, in further work, to allow access to higher concentrations.

Good results have also been obtained with TMF-RDE when performing speciation determinations. The free metal concentration, and, thus, the conditional stability constants, determined with AGNES for the two systems considered (the complexation of Pb(II) with monodisperse carboxylated latex nanospheres and the complexation of Pb(II) with iminodiacetic acid (IDA), are in reasonable agreement with values reported previously.

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Figures

Fig. 1. Schematic representation of the potential and stirring program of AGNES applied in the strategy of one potential step along the first stage. E_1 corresponds to the deposition potential controlling the concentration gain Y ; E_2 is the potential for the reoxidation under diffusion-limited conditions; t_w is the time without stirring at the end of the deposition stage.

Fig. 2. Evolution of the measured current I (grey bars) and the current of the shifted blank I_{sb} (black bars) over 29 consecutive measurements. Parameters $t_2 = 2$ ms, $Y=2000$ and $t_l = 150$ s. The experiments were performed in 0.01 M NaNO_3 media, at a pH c.a. 4 and for a total concentration of lead(II) of 3.96×10^{-7} M. The RDE was rotated at 500 rpm.

Fig. 3. Variation of the h_{eff} throughout 29 consecutive measurements. The current was measured at t_2 of 2 ms and the other experimental parameters used were: $Y=2000$ and $t_l=150$ s. The experiments were performed in 0.01 M NaNO_3 media, at a pH c.a. 4 and for a total concentration of lead(II) of 3.96×10^{-7} M. The RDE was rotated at 500 rpm.

Fig. 4. Optical micrographs: $\times 50$ (A.), $\times 100$ (B.) and $\times 500$ (C.) of a TMFE (16 nm of nominal thickness), plated on a glassy carbon electrode.

Fig. 5. Ratio between the measured current and the current of the shifted blank along the second stage of AGNES, within the t_2 interval [1-12] ms, in a given moment of the life of the evolving electrode (i.e. same j). The experiments were performed in 0.01 M NaNO_3 medium at a pH c.a. 4 and for a total concentration of lead(II) of: (□) 1.99×10^{-7} M and (■) 5.99×10^{-7} M. Other experimental conditions: $Y=2000$ and $t_1=150$ s; no rotation speed of the RDE was used .

Fig. 6. Variation of the normalised current (I/I_{sb}) as a function of the deposition time (t_1), measured at t_2 of 2 ms and for: Y of 5000 for a rotation speed of the RDE of 0 rpm (white triangles) and 500 rpm (black triangles), respectively. The experiments were performed in 0.01 M NaNO_3 media, at a pH c.a. 4 and for a total concentration of lead(II) of 1.49×10^{-7} M .

Fig. 7. Plot of I_{norm} (see eqn. (11)) vs the free metal concentration of lead(II) (c_{pb}^*), for different Y values: 1000 ($\blacksquare$ and $\square$), 2000 ($\blacktriangle$ and $\triangle$) and 5000 ($\blacklozenge$ and $\lozenge$). Full markers are related to the linear interval of concentrations and the open markers to the concentration range where the TMF-RDE stripping current deviates from the linear behaviour. The experiments were performed in 0.01 M NaNO_3 media, at a pH *ca.* 4. The current was measured at a t_2 of 2 ms and the deposition times used were: $t_1=150$ s for a Y of 5000 and $t_1=100$ s for a Y of 1000 and 2000. The RDE was rotated at 500 rpm.

Fig. 8. Stripping signals along the first 40 ms of the AGNES second stage after reaching a Y of 5000. The concentrations of the total lead(II) used are (from lower curves to upper curves): 1.0×10^{-7} , 5.0×10^{-7} , 9.9×10^{-7} and 20×10^{-7} M .

Fig. 9. Plot of the proportionality factor h_{eff} versus the preconcentration factor Y based on the calibration data presented on table 1. The calibration data was: slope= 9.13×10^2 and correlation factor: 0.9999.

Table. 1. Maximum workable concentration ($c_{\text{Pb max}}$) values and the corresponding LOD and calibration data (h_{eff} and r) for three gains at the 16 nm thick TMFE (plots presented in Fig. 6) for a rotation speed of XXXX.

Y	$c_{\text{Pb max}} / \text{M}$	Calibration data		LOD /M
		$h_{\text{eff}} / \text{M}^{-1}$	r	
1000	1.0×10^{-6}	1.32×10^6	$0.995_{(N=5)}$	7.4×10^{-8}
2000	6.0×10^{-7}	2.28×10^6	$0.999_{(N=6)}$	2.1×10^{-8}
5000	2.0×10^{-7}	4.97×10^6	$0.998_{(N=4)}$	7.2×10^{-9}

Table 2- Values of the logarithm of the free metal ion concentration and bulk stability constant value obtained for two measurements for the lead(II)- latex nanospheres (40 nm radius latex and 0.08 and 0.1 % (w/w)) at the TMF-RDE. Total concentration of lead(II) 6.0×10^{-7} M.

pH	Y	c_L / M	$\log c_M^*$	$\log K$
5.51	5000	1.6×10^{-5}	-6.56	4.77
6.12	5000	1.6×10^{-5}	-7.06	5.46
5.50	2000	1.8×10^{-5}	-6.59	4.87
6.12	5000	1.8×10^{-5}	-7.00	5.37

Table 3- Values of the logarithm of the free metal ion concentration and bulk stability constant value obtained for the lead(II)-IDA system at the TMF-RDE. Total concentration of lead(II) 2.49×10^{-7} M.

c_L / M	$\log c_M^*$	$\log K$
2.5×10^{-5}	-6.75	8.63 _(N=2)
4.9×10^{-5}	-6.86	8.62 _(N=2)
9.8×10^{-5}	-7.00	8.58 _(N=2)
2.5×10^{-4}	-7.54	8.63

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