

A comparison between the determination of free Pb(II) by two techniques: Absence of Gradients and Nernstian Equilibrium Stripping and Resin Titration

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Abstract

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is an emerging electroanalytical technique designed to measure free metal ion concentration. The practical implementation of AGNES requires a critical selection of the deposition time, which can be drastically reduced if the contribution of the complexes is properly taken into account.

The Resin Titration (RT) is a competition method based on the sorption of metal ions on a complexing resin. The competitor here considered is the resin Chelex 100 whose sorbing properties towards Pb(II) are well known. The RT is a consolidated technique especially suitable to perform an intercomparison with AGNES, due to its independent physicochemical nature.

Two different ligands for Pb(II) complexation have been analyzed here: Nitrilotriacetic acid (NTA) and pyridinedicarboxylic acid (PDCA). The complex PbNTA is practically inert in the diffusion layer, so, for ordinary deposition potentials, its contribution is

almost negligible; however, at potentials more negative than -0.8 V vs. Ag/AgCl the complex dissociates on the electrodic surface giving rise to a second wave in techniques such as Normal Pulse Polarography. The complex Pb-PDCA is partially labile, so that its contribution can be estimated from an expression of the lability degree of the complex. These new strategies allow us to reduce the deposition time. The free Pb(II) concentrations obtained by AGNES and by RT are in full agreement for both systems here considered. The main advantage of the use of AGNES in these systems lies in the reduction of the time of the experiment, while RT can be applied to non-amalgamating elements and offers the possibility of simultaneous determinations.

Keywords: Free lead(II) concentration; stripping analysis; lability degree; Chelex 100; speciation; Absence of Gradients and Nernstian Equilibrium Stripping (AGNES)

1. Introduction

The knowledge of the free metal ion concentration in an aqueous solution is a key issue in a number of areas, ranging from the determination of stability constants to speciation studies for various purposes [1]. For instance, there is a general consensus in the environmental scientific community that the nutritional or toxic effects of many elements are typically dependent on the free ion concentration, as asserted by theories such as the Free Ion Activity Model (FIAM) [2] or the Biotic Ligand Model (BLM) [3]. Even if these theories were incomplete (and needed some corrections due to the dynamic contribution of complexes [4]), the information on the free metal ion concentration will still be needed as a key datum for the required speciation.

The most typical instrument to measure free ion concentration is the Ion Selective Electrode (ISE) [5]. Some advantages of the ISE's are the simplicity of interpretation,

their low cost, fast response, ease of use, etc. Among the drawbacks -focussing specifically on commercial devices- are the high limit of detection (unless buffered by a high total metal concentration [6]), the lack of availability of ISE for some metals such as Zn [7], stability problems, etc.

In this perspective, the proposal and testing of alternative techniques to complement existing ones deserves attention [8,9]. Validations of one technique against the others can be seen as one important step in their development, especially if the comparison between techniques permits to elucidate advantages and limitations of the analyzed techniques [10] and the comparison is also particularly important in cases where certified materials do not exist [4]. Here, we present an interlaboratory work to compare two techniques designed by our research teams: the Resin Titration (RT) technique [11] and AGNES [12] (Absence of Gradients and Nernstian Equilibrium Stripping). The interest of such comparison is supported by the very different physicochemical nature of both techniques: RT being based on an ionic exchange resin while AGNES being a voltammetric technique. As systems, we have selected two ligands: NTA (Nitrilotriacetic acid) and PDCA (2,6-pyridinedicarboxylic acid) which form complexes with lead. These ligands have been selected to represent different complex behaviour in the pH range analyzed. For RT, based on the consideration of thermodynamic equilibria, NTA is weaker -in the resin complexation competition- than PDCA; while for AGNES, NTA is much more inert (a non-equilibrium characteristic) than PDCA, and -in principle- should require much longer deposition times. Moreover, PDCA exhibits some extra interesting characteristics: it is present in nature as a component of fulvic acid and as a degradation product of vitamins, coenzymes and alkaloids and was suggested as a model compound of natural pedogenic fulvic acids [13]; only the fully

protonated form [14,15] and the complex PbPDCA adsorb onto the electrode [16,17]; PDCA+transition element ions could be helpful in understanding diabetes [18], etc.

The layout of this paper is as follows. We summarize the principles and practical implementation of RT and AGNES in a theoretical section followed by an experimental section. In section 4 we analyze the strategies and results of each technique. In the conclusions we compile the main differences between both techniques.

2. Theory

2.1 The basis of the AGNES method

AGNES principles have been described mainly in [12,19], but further details can be found in previous applied work such as the measurement of free Zn^{2+} in Mediterranean seawater [20], the study of the complexation of Cd^{2+} and Zn^{2+} to humic acids [21], the use of microelectrodes [22], etc. We summarize here the two conceptual steps or stages: deposition or first stage and stripping or second stage of AGNES.

2.1.1 First stage

The aim of the deposition stage is to reach *–by the end of it–* a situation of no concentration gradient (neither inside the mercury electrode nor in the solution in contact with the electrode), while keeping a fixed ratio of the electroactive couple concentrations due the Nernstian equilibrium (determined by the applied potential E_1)

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

where F is the Faraday, R the gas constant, T the temperature and $E^{\circ'}$ stands for the standard formal potential of the redox couple of the metal M, and $c_{\text{M}^0}^*$ and c_{M}^* refer to

the final homogeneous concentration value inside the mercury electrode and in the solution, respectively. We highlight that the attainment of this special condition of Nernstian equilibrium with no gradients in the concentration profiles of the metal species on both sides of the electrode surface along the deposition step (and before any stripping), is the key point of AGNES: so the deposition time and potential have to be judiciously selected.

The preconcentration factor Y can be determined from E_{peak} , the potential peak of a Differential Pulse Polarography (DPP) experiment [12], through:

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

where D_M and D_{M^0} are the diffusion coefficients for the free metal ion and the reduced metal (inside the amalgam) and ΔE_{DPP} is a characteristic parameter of the DPP experiment.

In the simplest implementation of AGNES only one potential step is applied along the deposition time t_1 , while stirring is on during a time $t_1 - t_w$ (i.e. t_w is the resting time). In order to reduce the deposition time, another potential program has been proposed (which we call 2P or "2 pulses"): this consists in splitting the first stage into two different sub-stages (see Figure 1): a potential step corresponding to diffusion limited conditions, $Y_{1,a}$, followed by a potential step at the desired concentration gain Y , also denoted $Y_{1,b}$. This strategy has proven to reduce the deposition time needed to reach the target situation of the first step [19].

If the potential step corresponding to $Y_{1,a}$ is applied for too long time $t_{1,a}$ then an excess of metal might enter the mercury electrode (i.e. we have a higher metal concentration inside the amalgam than wished), and then we need a sufficiently long ($t_{1,b}$) second potential step at the desired gain Y to avoid an overshooting of the final current (see Figure 2a). It has been demonstrated [19] that, when working with a practically constant concentration of free ligand, a ratio of $t_{1,b}=3\times t_{1,a}$ is enough to compensate for any possible overshooting and reach AGNES conditions.

2.1.2 Second stage

The aim of the second stage is the determination of the concentration of M^0 reduced in the amalgam. For that purpose, a simple strategy consists in applying a sufficiently less negative potential (E_2) producing a stripping current under diffusion limited conditions (see Fig 1). The measured response function of AGNES is the current at a certain time t_2 (see Fig 2b).

As the faradaic current I is linearly related with $c_{M^0}^*$ (due to the linear properties of the diffusion of M^0 inside the electrode), and $c_{M^0}^*$ is just Yc_M^* according to eqn. (1), it follows that the free metal ion concentration is directly proportional to the faradaic current, with a proportionality factor h :

$$I = h c_M^* \quad (3)$$

As the measured current, contains other components different than the faradaic one, there is a need of subtracting a blank from the total current. Under diffusion limited conditions no phenomenon in the solution (such as complexation, convection or adsorption) can affect the faradaic current.

2.2 The basis of the Resin Titration method

In the resin titration (RT) technique, the resin is the titrant of the metal ion, and it is used in excess with respect to the metal ion. The procedure is based on the sorption of the metal ion on the resin at different values of V/w , i.e., the ratio of the volume of the solution phase (V , in mL) to the amount of resin (w , in g). The resin titration curve is obtained by plotting the concentration of the metal ion adsorbed on the resin (c , in nM) as a function of $1/w$. Figures 3 and 4 are examples of these curves for the synthetic systems here considered.

The concentration of sorbed metal ions depends on the species stability in solution phase and on the sorbing properties of the complexing resin. The stability of the species in solution is measured by the side reaction coefficient of the metal, $\alpha_{M(L)}$, the ratio of total to free metal ion in solution [23]. The sorbing properties of the resins are measured by the partition coefficient K^* [11,24], which is the ratio of the metal ion concentration in the resin phase to free metal ion concentration in solution. K^* can be calculated at each particular set of experimental conditions as described in previous papers [11,24]. The concentration of metal ions sorbed at each point of the titration is modelled by the following relationship:

$$c = \frac{c_{\text{tot},\text{res}}}{1 + \frac{\alpha_{M(L)} \cdot V}{K^* \cdot w}} \quad (4)$$

$c_{\text{tot},\text{res}}$ is the total concentration of the metal ion that is sorbed on the resin when $\alpha_{M(L)}V/K^*w$ is negligible with respect to 1, i.e. at low V/w .

The reaction coefficient $\alpha_{M(L)}$ can be evaluated only if it is in a well defined range of values, (“detection window”), which strongly depends on the nature of the resin and on the conditions. The range is defined by [11,25]:

$$\frac{0.1 K^* w}{V} < \alpha_{M(L)} < \frac{10 K^* w}{V} \quad (5)$$

If the reaction coefficient $\alpha_{M(L)}$ is lower than $0.1 K^* w/V$, the metal is quantitatively sorbed by the resin (because M was either free or in complexes with very weak ligands completely dissociated by the resin). If $\alpha_{M(L)}$ is higher than $10 K^* w/V$, the metal ion is so strongly bound to the complexes that it is not sorbed on the resin. The presence of such strongly complexing species cannot be excluded in real samples and, for their determination, one must select a resin sufficiently strong. $c_{tot,res}$ and $\alpha_{M(L)}/K^*$ can be evaluated by a non-linear regression procedure.

3. Experimental

3.1 Reagents

All chemicals were analytical reagent grade. Solutions were prepared with ultrapure water (Milli-Q). Chelex 100 [CAS 68954-42-7] (Bio-Rad Laboratories), with a particle size of 100-200 mesh was delivered in the sodium form. It was washed and converted into the H^+ form with ultrapure HNO_3 as previously described [26].

The capacity of the resin (mmol of active groups per g of dry resin) was found to be 2.0 mmol per g of dry resin Chelex 100 in H^+ form, and the amount of water sorbed by the resin was determined as described elsewhere [26].

For the RT experiments, a lead atomic spectroscopy standard solution (Fluka) of 1000 mg L⁻¹ [CAS 10099-74-8] and Pb(NO₃)₂ [CAS 10099-74-8] (Riedel De Haen) were used to have the proper Pb(II) concentration in the solution phase. For the AGNES experiments, lead standard solutions were prepared by adequate dilution from a 1000 mg L⁻¹ stock solution (Merck).

The considered complexing agents were nitrilotriacetic acid, C₆H₉NO₆ [CAS 139-13-9] (Fluka); pyridinedicarboxylic acid, C₇H₅NO₄ [CAS 499-83-2] (Merck-Schuchardt). Solutions were prepared from a proper dissolution of solids.

3.2 Apparatus

3.2.1 Apparatus for the AGNES Procedure

Voltammetric measurements were carried out with an Eco Chemie Autolab PGSTAT30 potentiostat attached to a Metrohm 663VA Stand and to a computer by means of the GPES (Eco Chemie) software package. The working electrode was a Metrohm multimode mercury drop electrode. The smallest drop in our stand was chosen for AGNES experiments, which according to the catalogue corresponds to a radius around $r_0 = 1.41 \times 10^{-4}$ m. The auxiliary electrode was a glassy carbon electrode and the reference electrode was Ag/AgCl/3 mol L⁻¹ KCl, encased in a 0.1 mol L⁻¹ KNO₃ jacket.

A glass combined electrode (Orion 9103) was attached to an Ioncheck45 Radiometer analytical ion analyzer and introduced in the cell to control the pH. A glass jacketed cell thermostated at 25.0 °C was used in all measurements, which were performed under a purified nitrogen atmosphere.

To stir the solution, the PTFE tip stirrer of the Metrohm 663VA Stand was used at a rotation rate of 1500 rpm in all experiments.

3.2.2 Apparatus for the Resin Titration Procedure

A PHM 84 Research pH meter, Radiometer Copenhagen, with a combined Orion glass electrode was used for pH measures. The potentiometric cell was standardised in H^+ concentration as previously reported [27]. A small overpressure of nitrogen was applied in the vessel that was thermostated at 25 °C.

An ICP (PerkinElmer SCIEX ELAN 6000 ICP-MS Instrument) was used to determine lead(II) concentrations in the stripping solutions. The detection limit (LOD) in the stripping solution (HNO_3 1.0 M) was 0.1 nM.

3.2.3 Procedure for the Resin Titration

The titration is carried out following a batch procedure, with the same volume of sample solution and varying the amount of resin (w). The contact time is 24 h on a shaking plate. A long contact time is useful when complexes with unknown dissociation rate are present in the sample. If metal species do not dissociate within 24 hours, they are considered to be either kinetically inert or thermodynamically stronger than the active groups of the resin. After equilibration, the final pH is measured, the solution phase is separated by suction and the metal ion is eluted from the resin with 10 mL 1 M nitric acid, placed directly into the RT bottles. The concentrations of Pb(II), are determined in each eluate by ICP-MS.

4. Results and discussion

4.1 System Pb-NTA (nitrilotriacetic acid)

4.1.1 AGNES determinations: developing the use of the second wave.

Diamonds in figure 5 stand for the NPP wave of Pb^{2+} (no added ligand). If we add a large amount of nitrilotriacetic acid (NTA), we can see (squares) how this wave practically disappears in the region -0.3 to -0.8 V due to the dramatic reduction in concentration of free Pb(II) and the inert characteristics of PbNTA, but one can also see a new wave developing at a much more negative potential (around -0.8 V). This *second wave* is due to the direct reduction of the complex PbNTA, without previous dissociation in the solution. This effect had already been described by many authors [28-32] and sometimes called "lability dependence on potential".

We aim now at using this "second" wave of PbNTA to reduce the deposition time. In the modality of AGNES with 2 pulses, we can apply a potential $E_{1,a}$ sufficiently negative to take advantage of this second wave. Indeed, when diffusion limited conditions hold, not only for the free Pb^{2+} but also for the complex PbNTA, the supply to the electrode will be much larger, i.e. the direct reduction of PbNTA at the electrode surface will favour the mass transport of Pb^{2+} from the solution to the drop. Afterwards, we apply the deposition potential $E_{1,b}$ necessary for the desired gain Y during a certain time $t_{1,b}$ to be sure that any missing Pb^0 is supplied or any exceeding Pb^0 is eliminated and we can attain AGNES conditions.

Suitable values of $t_{1,a}$ depend on the desired Y . In principle, we prefer to fix the value of the current I_2 that we want to obtain: this total current should be high enough so that the

value of the blank could have an almost negligible (or low) importance. With this I_2 -value fixed, we will change the value of preconcentration factor or “gain” (Y) every time that the relationship of free and total concentration of Pb^{2+} varies. Indeed, equation (6) comes from equating the amount of metal arrived (either free or in the form of a complex with the same diffusion coefficient D_M as that of the free metal, because NTA is a small ligand) during $t_{1,a}$ with the metal accumulated inside the drop at the desired Y

$$(4\pi r_0^2) \frac{D_M c_{TM}^*}{\delta} t_{1,a} = \left(\frac{4}{3}\pi r_0^3\right) Y c_M^* \quad (6)$$

where δ stands for the diffusion layer thickness (in planar diffusion, i.e. when $r_0 \gg \delta$).

This equation leads to

$$t_{1,a} = \left(\frac{\delta r_0}{3D_M}\right) Y \frac{c_M^*}{c_{TM}^*} \quad (7)$$

which, given the usual parameters for our condition with HMDE, boils down to the approximate relationship:

$$t_{1,a} \approx Y \frac{c_M^*}{c_{TM}^*} \quad (8)$$

If we use a value of $Y_{1,b}=250$ and the relationship between total and free concentrations of Pb(II) is taken approximately 10, then the theoretical value of $t_{1,a}$ is 25 s.

Preliminary results (data not shown) with $t_{1,b}$ fixed to 200 s, $Y_{1,b}=250$ and a relationship between total and free concentrations=10, indicated that, experimentally, around $t_{1,a}=11$ s was sufficient for AGNES conditions to be achieved. Then, with $t_{1,a}$ fixed in 11 s, we seek a refined $t_{1,b}$ (other than the initial 200 s), long enough to eliminate any possible excess of Pb^0 preconcentrated during the first substage. We see in figure 6 that the

measured current decreases with increasing $t_{1,b}$ (indicating that even $t_{1,a}=11$ s produces a slight overshoot) and allowed us to accept $t_{1,b}=300$ s as a correct (and safe) time for these conditions. We see in figure 6 that, using $t_{1,b} = 300$ s, the I -values with the second wave strategy and the standard (i.e. without taking advantage of the second wave) 2P strategy are practically the same. On the other hand, following ref. [19], when we use the standard 2P and $Y=250$, the values of $t_{1,a}$ and $t_{1,b}$ are 175 s and 525 s, respectively.

From these results, we can conclude that working with the system Pb-NTA, the deposition time of the experiments ($11+300+50=361$ s) can be reduced, for $Y=250$, to less than one half of the time needed with the standard 2 pulses strategy ($175+525+50=750$ s).

We performed experiments in which Pb-NTA complexation was varied via a change in the value of pH. The total concentration of Pb(II) was 2×10^{-6} M and the concentration of NTA added was 5×10^{-6} M.

For these experiments we applied the parameters previously optimized ($t_{1,a}=11$ s, $t_{1,b}=300$ s, $Y_{1,a}=10^{20}$, $Y_{1,b}=250$), which were obtained at pH=5.07 and at a ratio between total and free concentration of Pb(II) of 10.

To check the validity of the combination for pH other than 5.07, we have analyzed the currents along the first stage. Figure 7 shows these currents for three different values of pH. The difference in the observed behaviours, working with the second wave strategy, is due to the dramatic change in the relationship between total and free concentration of Pb(II).

- At pH= 4.09 (square markers in Figure 7), the relationship between total and free metal ion concentration is around 5. This relationship value is smaller than the optimized one (which was 10) and, therefore, $t_{1,a}$ is not enough to reach AGNES conditions during the first substage. In the second substage a slight “undershoot” can be appreciated, but the applied $t_{1,b}$ (300 s) is enough to eventually attain AGNES conditions (i.e. we see in Figure 7 that the residual current is reached by the end of the stirring time).
- At pH=5.07, the pH-value previously optimized (see triangle markers in Figure 7), a small or negligible “overshoot” can be appreciated in the second substage.
- At pH=6.01 (cross markers in Figure 7), the relationship between total and free Pb(II) concentration is around 100. Notice that in this case the “overshoot” is higher than in the previous ones, but, even in this case, the $t_{1,b}$ applied is enough to eliminate the excess of Pb⁰ deposited during the first substage.

4.1.2 RT determinations and comparison of AGNES and ISE techniques

Independent synthetic solutions of KNO₃ 0.1 M all containing Pb(II) 2×10^{-6} M and NTA 5×10^{-6} M, but at different acidity, were analyzed by RT. In the range of pH here considered (from 3 to 6), the complexation between Pb(II) and NTA is weaker than that between the metal and the active groups of the resin. Thus, the useful points of the titration, to obtain a first estimation of $\alpha_{M(L)}$, are those with the less quantities of Chelex 100. In fact, only in these cases there is a competition between the resin and the ligand NTA in solution for the complexation of Pb(II). This observation is immediately evident

from the shape of the RT curves which show a small decay rate (see some examples in Fig. 3).

The RT experimental conditions and the results obtained are reported in Table 1. In all the considered samples, the concentration of total lead(II) found by RT, $c_{\text{tot, res}}$, is in good agreement with the total metal content (c_{tot}). As previously demonstrated with certified samples [33], the choice of strong sorbents -like the iminodiacetic Chelex 100 used here, able to compete with strong ligands for metal complexation- allows the determination of the total metal concentration directly from the RT and without the need of any other independent measurements. A good reproducibility of total metal concentration indicates that there is no loss or contamination of the metal during the RT procedure.

Moreover, from the values of the reaction coefficient $\alpha_{\text{M(L)}}$ experimentally obtained, we can calculate the concentration of the free metal ion in solution, as the total Pb(II) content in each system investigated is known.

The results obtained are compared (see figure 8) with those found with AGNES (second wave and standard 2P) and ISE techniques and also with the theoretical calculations. We can see that for pH=2.99 (where the concentration of free Pb is high because the value of pH is acidic and most NTA³⁻ is protonated) we only performed the experiments with AGNES standard 2P technique because the contribution of the complex PbNTA is negligible and we cannot take advantage of the second wave.

To compare our experimental results with theoretical values, we have first to solve the problem about the choice of a suitable set of conditional constants among the huge literature data. We used the SC_Query database by IUPAC (SC-Query Vn 5.18) to collect the protonation and the complexation constants for the systems here considered. Given the variety of the reported stability constants, we averaged (as done in [34]) the values at the same ionic strength of our solutions (0.1 M) and in the temperature range between 20 and 25 °C. For NTA³⁻ we obtained: $\beta^H_1=(5\pm2)\times10^9\text{ M}^{-1}$, $\beta^H_2=(17\pm7)\times10^{11}\text{ M}^{-2}$ and $\beta^H_3=(11\pm5)\times10^{13}\text{ M}^{-3}$ for the protonation equilibria and $\beta^{Pb}_1=(3\pm1)\times10^{11}\text{ M}^{-1}$ for Pb²⁺ complexation. These average values were used to compute the free metal concentration, in the whole range of the pH of our systems, by SC_Query speciation code SolEq (SC-Query Vn 5.18) [35], see continuous lines in figure 8. The error bars (of each calculated value) are derived from the propagation of errors on the input values employed in the free metal ion concentration.

We can see that the results of AGNES, ISE, and the theoretical data are practically the same and just a little bit lower than those of RT for very acid pH. It is worthwhile to note the importance of the formation of PbNO₃⁺, whose concentration reaches almost one half of that of free Pb according to the speciation codes used in this work. Indeed at lower pH, the formation of PbNO₃⁺ (a complex too weak to be distinguished from free Pb with this resin) could be the reason for the small discrepancies between AGNES and RT.

For other pH-values, the results with all techniques are in excellent agreement with each other. From a methodological point of view, as we have explained before, we can

conclude the usefulness of the second wave strategy in AGNES to reduce the deposition time while retaining the accuracy of the determination of the free ion concentration.

4.2 System Pb(II)-PDCA (2,6-pyridinedicarboxylic acid)

4.2.1 AGNES determinations.

We performed experiments in which Pb–PDCA complexation was varied by changing the pH. The total concentration of Pb was 2×10^{-6} M and the total concentration of PDCA added was 4.5×10^{-6} M. The range of pH, with AGNES, was from 2.7 to 7.0.

When non-inert complexes contribute to the flux (as expected in the case of PbPDCA), one can reduce the deposition time of AGNES [19]). This contribution can be used, for example, in standard 2P to reduce the total deposition time for a given Y . Alternatively, we have chosen here to fix a sufficiently high current (with respect to the blank) and, so, increase Y without the need of extremely large deposition times.

To obtain the values of Y (also called $Y_{1,b}$) we fixed the value of the current (I_2) in a value high enough (8×10^{-8} A) so that the intensity of the blank could be negligible. As we can estimate the free concentration of Pb via numerical codes (e.g. MEDUSA [36]), we can calculate the required value of h to obtain the desired I with equation (3).

Due to the proportionality between h and Y , we can compute the Y required for a given h with the data from a calibration. For instance, for Pb, with $Y_{1,b}=100$ we usually have an h around 0.26, so

$$Y_{1,b} = \frac{100h}{0.26} \quad (9)$$

Using this equation, we have obtained a convenient value of $Y_{1,b}$ at each pH (see Table 3).

The lability degree (ξ) is a measure of the capacity of the complex to dissociate and contribute to the flux. The higher the lability degree, the more favoured the global mass transport from the solution to the drop will be. For planar finite diffusion, under excess of ligand conditions or approximately constant free ligand concentration (e.g. by buffering of a much larger total ligand concentration), the lability degree -when $\varepsilon K' \gg 1$ and $\delta \gg \mu^\infty$ - can be approximated [37] via

$$\xi \approx \frac{\delta}{\delta + (\varepsilon K' \mu^\infty)} \quad (10)$$

where δ is the diffusion layer thickness, ε is the ratio between the diffusion coefficients of the complex ML and the metal M (in this work ε is taken as unity), K' is the relationship between $[\text{PbPDCA}]$ and $[\text{Pb}^{2+}]$ and μ^∞ is the reaction layer thickness whose value is calculated with

$$\mu^\infty = \sqrt{\frac{D_M}{k'_a}} \quad (11)$$

where D_M is the diffusion coefficient and k'_a is

$$k'_a = k_a c_L^* \quad (12)$$

and k_a is the association kinetic constant of the complexation complex and c_L^* is the concentration value of PDCA^{2-} at the bulk of the solution. The degree of lability is not

constant across all our experiments because the change in the value of the pH implies a different concentration of PDCA^{2-} in each of them.

Equating the number of moles of Pb^0 to be accumulated with the supply along the first substage ($t_{1,a}$), we obtain

$$4\pi r_0^2 \frac{D_M (1 + \varepsilon K' \xi) c_M^*}{\delta} t_{1,a} = \frac{4}{3} \pi r_0^3 Y c_M^* \quad (13)$$

The plot of the computed factor $(1 + \varepsilon K' \xi) c_M^*$ of the contribution to the flux, for these systems with parameters $k_a = 9.44 \times 10^7 \text{ m}^3 \text{mol}^{-1} \text{s}^{-1}$; $D_M = 9.45 \times 10^{-10} \text{ m}^2 \text{s}^{-1}$; $r_0 = 1.41 \times 10^{-4} \text{ m}$; $\delta = 2 \times 10^{-5} \text{ m}$; $\varepsilon = 1$ can be seen in figure 9.

From eqn. (13), one can isolate

$$t_{1,a} = \frac{\delta r_0 Y}{3 D_M (1 + \varepsilon K' \xi)} \quad (14)$$

The values of the computed parameters, which can serve as a guideline for the experiments with this system, are shown in table 3. Notice that we rounded the Y to powers of ten or five times a power of ten. With this rounded Y , we computed $t_{1,a}$ which was again rounded by excess to 50 s or 80 s. In all cases we observed overshoot along the first stage (data not shown), but as we took $t_{1,b} = 3 \times t_{1,a}$ [19] and we assume that free ligand concentration is approximately constant, the system achieved the desired AGNES conditions.

4.2.2 RT determinations and comparison with AGNES and ISE techniques

The Pb(II)-PDCA complexation is studied also by the RT method, with the aim to compare the results with those obtained with AGNES and ISE techniques. The considered solutions have the same composition (KNO_3 0.1 M, Pb(II) 2×10^{-6} M and PDCA 4.5×10^{-6} M), but different acidity. The range of the pH is between 2.8 and 7.0: in this interval of acidity, and particularly for the lowest values of pH, the soluble ligand PDCA is able to give a stronger complexation with respect to the previously considered NTA, so PDCA competes with the active groups of the resin also for the RT points with a high content of Chelex.

In the range of pH here considered, from the value of K^* it is possible to verify that a quantitative sorption is expected within the hypothesis that the metal is free in solution. In the presence of a ligand strong enough to compete with the resin, only a fraction of the metal is sorbed and, obviously, this fraction decreases as the reaction coefficient of the ligand in solution increase. Also the shapes of the RT curves are different, depending on the ratio M:L and on the strength of the complex between the metal and the added ligand.

In Figure 4, examples of RT curve for the system Pb(II)-PDCA here considered are shown. As highlighted before, the RT curve falls steeply at low pH where a strongest competition between the soluble ligand PDCA and the iminodiacetic group of the resin appears.

Table 2 reports the RT experimental conditions and the results obtained for all the Pb-PDCA solutions here examined.

As for the previously investigated Pb(II)-NTA systems, from the values of the experimental reaction coefficients (Table 2, line 8) the free metal concentration for each solutions were calculated to perform a direct comparison with the results obtained by AGNES and ISE techniques.

As done with Pb-NTA in section 4.1.2, we compute the theoretical free lead concentration from an average of previously published stability constants. Using SC-Query Vn 5.18, for PDCA²⁻ we obtained: $\beta^H_1=(5\pm1)\times10^4\text{ M}^{-1}$ and $\beta^H_2=(7\pm1)\times10^6\text{ M}^{-2}$ for protonation. We also computed $\beta^{Pb}_1=(5\pm1)\times10^8\text{ M}^{-1}$ and $\beta^{Pb}_2=(40\pm9)\times10^{10}\text{ M}^{-2}$.

In figure 10, the comparison between AGNES, RT and ISE techniques is shown. The concentration values by AGNES were experimentally obtained with the parameters calculated before and shown in table 3. We see that these results are very similar compared with RT, ISE and theoretical SolEq (continuous lines with error bars). The results of the three techniques seem to suggest that Pb+PDCA constants in the literature might be slightly underestimated.

5. Conclusions

AGNES is a recent electroanalytical technique designed to quantify the free metal activity (or concentration) and RT is a consolidated technique especially suitable to perform an intercomparison with AGNES, due to its independent nature (i.e. non-electroanalytical).

AGNES has the advantage of being relatively cheap if compared to spectroscopic methods (i.e. ICP-MS, ETAAS) and is clearly cheaper than RT. AGNES results can be obtained faster (even in the case of low concentrations) than RT (which require overnight equilibration) and the procedure is usually less laborious than RT. However, work is in progress in order to optimize this issue in RT.

One important restriction of AGNES, in comparison with RT, is the necessity of amalgam formation, which, although can be seen as an increase of the selectivity, reduces the range of studied elements essentially to a few metals. This is a restriction common to many stripping techniques, such as, for example, pseudo-polarography [38] or Scanned Stripping Chronopotentiometry (SSCP) [39], to which AGNES is closed related given that AGNES conditions can be fulfilled in the foot of the wave (i.e. potentials close to the standard potential) of such techniques. Interferences of other electroactive species in the region of used potentials and the formation of intermetallic compounds in the amalgam have to be carefully considered in AGNES.

On the other hand, a key interesting feature of the RT method is that many metal ions can be investigated simultaneously (provided that their binding constants with the ligands fall within the analytical window). Besides, using a strong chelating resin as the iminodiacetic Chelex 100, the total metal ion content is directly determined from the titration curves without any other independent measurement and the free metal ion is determined from the value of the reaction coefficient $\alpha_{M(L)}$ experimentally obtained.

An essential point in the application of AGNES is the suitable selection of the deposition program. The optimal deposition program depends on several issues ranging

from complex kinetics to hydrodynamics characteristics. Thus, this selection implies a certain difficulty if one wishes to use minimal values for the deposition times. It has been demonstrated that the contribution of the complexes can reduce the time needed to reach AGNES conditions. In this work, two new strategies for the proper selection of the deposition time have been suggested.

A first strategy consists in the use of the second wave when inert complexes are present. It has been experimentally checked with PbNTA which is practically inert in the diffusion layer, so, for ordinary deposition potentials, its contribution is almost negligible; however, at very negative potentials the complex dissociates on the electrodic surface giving rise to a second wave. This direct dissociation results in a significant reduction of the deposition time.

The second strategy consists in the qualitative assessment of the kinetic contribution of the complexes. In this case, we have worked with the complex PbPDCA because is partially labile. This contribution has been estimated here, for the first time, from an expression of the lability degree of the complex, and thus, the deposition time has been conveniently reduced.

The analytical window for RT is well defined, as detailed in Section 2.2, and it is strongly dependent on the nature of the resin and on the nature of all the complexes present in the samples. In the case of AGNES, the number and/or nature of the complexes (weak or strong, labile or inert) is relatively irrelevant for the free metal ion determination, but they have an impact (e.g. if they are labile or not) on the time needed to reach a given gain. In AGNES, the analytical window depends on the parameters

used, i.e. size of the electrode, applied gain(s) and characteristic times, etc. Estimations of the detection limit with AGNES have been reported previously for different particular conditions [12,20,22]. In practice, the upper limit of the analytical window in AGNES is not restricted because solubility problems in the amalgam can be overcome by decreasing the prescribed gain.

In summary, AGNES and RT can correctly describe the metal speciation in the two considered systems (NTA and PDCA complexation with lead) as shown by the successful agreement of the results from both techniques and from ISE and theoretical calculations. Although they cannot be applied for in-situ speciation analysis (at least in their current implementations), they have already proved effective to assess the free metal concentration in synthetic and natural samples.

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Tables

Table 1 – Results of RT of Pb(II)-NTA systems by Chelex 100. (Number in parenthesis is the standard deviation).

Pb(II)-NTA systems						
pH ^(a)	3.15	3.23	3.58	3.86	4.55	5.88
V (mL)	100	50	100	50	100	50
w (g)	0.010 – 1.905	0.007 - 0.952	0.010 – 1.218	0.004 - 0.936	0.009 - 1.862	0.007 - 0.934
K* ^(b)	8.03×10 ³	1.04×10 ⁴	2.99×10 ⁴	6.87×10 ⁴	4.21×10 ⁵	8.48×10 ⁶
c _{tot} (M)	2.02(1) ×10 ⁻⁶	2.05(2) ×10 ⁻⁶	2.04(3) ×10 ⁻⁶	2.04(1) ×10 ⁻⁶	2.03(1) ×10 ⁻⁶	2.03(2) ×10 ⁻⁶
<i>Estimated parameters obtained from Eq. 1</i>						
c _{tot,res} (M)	1.96(2) ×10 ⁻⁶	1.96(2) ×10 ⁻⁶	1.95(2) ×10 ⁻⁶	2.02(1) ×10 ⁻⁶	2.04(3) ×10 ⁻⁶	1.99(2) ×10 ⁻⁶
α _{M(L)}	1.00(3)	1.00(5)	1.51(6)	2.8(1)	10.1(9)	95(20)
<i>Statistics</i>						
r ^(c)	0.991	0.993	0.995	1.000	0.992	0.849
R.E. ^(d)	0.016	0.011	0.012	0.007	0.011	0.342

(a) After equilibration with the resin.

(b) K* evaluated at the pH of the resin titration, and for the ionic composition of the sample.
Values obtained from the intrinsic complexation constants of lead(II) in resin phase [40]

(c) Correlation coefficient.

$$(d) \text{ R.E.} = \sqrt{\sum (c - c_{calc})^2 / \sum c_{calc}^2} .$$

Table 2 – Results of RT of Pb(II)-PDCA systems by Chelex 100. (Number in parenthesis is the standard deviation).

Pb(II)-PDCA systems						
pH ^(a)	2.79	2.74	3.49	4.05	5.00	6.24
V (mL)	100	50	100	50	100	50
w (g)	0.015 – 1.844	0.061 – 1.385	0.051 – 1.844	0.049 – 1.387	0.045 - 0.825	0.017 - 0.362
K* ^(b)	2.69×10 ³	2.31×10 ³	2.48×10 ⁴	1.38×10 ⁵	1.87×10 ⁶	3.76×10 ⁷
c _{tot} (M)	2.03(1) ×10 ⁻⁶	2.01(2) ×10 ⁻⁶	2.03(1) ×10 ⁻⁶	2.01(1) ×10 ⁻⁶	2.03(1) ×10 ⁻⁶	2.03(2) ×10 ⁻⁶
<i>Estimated parameters obtained from Eq. 1</i>						
c _{tot,res} (M)	2.1(8) ×10 ⁻⁶	1.99(4) ×10 ⁻⁶	2.1(6) ×10 ⁻⁶	2.0(8) ×10 ⁻⁶	2.2(2) ×10 ⁻⁶	2.04(2) ×10 ⁻⁶
α _{M(L)}	15(8)	14.2(3)	94(4)	3.1(4) ×10 ²	1.4(2) ×10 ³	2.70(2) ×10 ³
<i>Statistics</i>						
r ^(c)	0.994	0.999	0.985	0.996	0.971	0.997
R.E. ^(d)	0.214	0.028	0.261	0.033	0.069	0.008

(a) After equilibration with the resin.

(b) K* evaluated at the pH of the resin titration, and for the ionic composition of the sample.
Values obtained from the intrinsic complexation constants of lead(II) in resin phase [40]

(c) Correlation coefficient.

$$(d) \text{ R.E.} = \sqrt{\sum (c - c_{calc})^2 / \sum c_{calc}^2} .$$

Table 3– Values of the necessary parameters to perform the different AGNES experiments for the system Pb(II)-PDCA in the range of pH 2.8-7.0.

pH	expected C_M^* / M	$h^{(a)}$ / $A\ M^{-1}$	calculated $Y^{(b)}$	Applied Y	calculated $t_{1,a}^{(c)}$ /s	applied $t_{1,a}$ /s
2.79	1.45×10^{-7}	0.553	2.13×10^2	5×10^2	51	50
3.07	8.49×10^{-8}	0.942	3.62×10^2	5×10^2	37	50
3.51	3.12×10^{-8}	2.56	9.86×10^2	10^3	30	50
4.00	1.12×10^{-8}	7.14	2.75×10^3	5×10^3	69	80
4.46	4.68×10^{-9}	17.1	6.58×10^3	5×10^3	38	50
5.03	2.45×10^{-9}	32.6	1.26×10^4	10^4	52	50
6.18	1.62×10^{-9}	49.3	1.90×10^4	10^4	40	50
6.97	1.58×10^{-9}	50.4	1.94×10^4	10^4	39	50

^(a) h has been computed with eqn. (3)

^(b) $Y=Y_{1,b}$ has been computed with eqn. (9)

^(c) with eqn. (14) using (rounded) applied Y values.

Figures

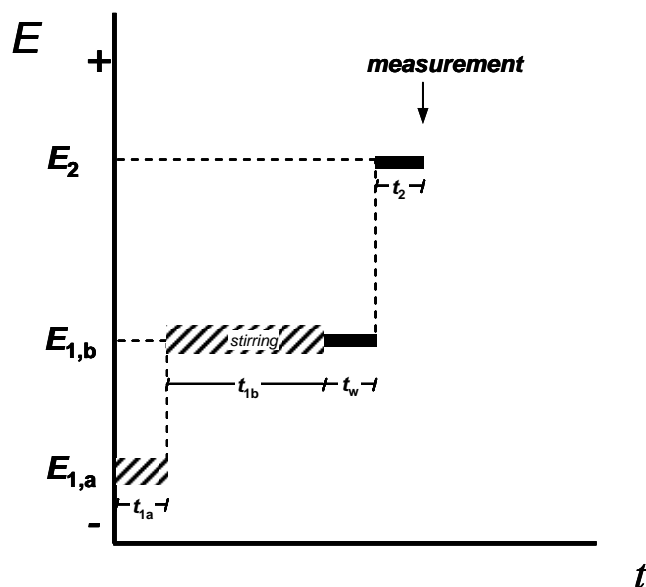


Fig 1 : Outline of the potential program of AGNES with the strategy of splitting the first stage into two potential steps or pulses (2P). Thick discontinuous lines indicate the longer part of the first stage (when stirring is applied), whereas thin continuous lines indicate that the solution remains without stirring.

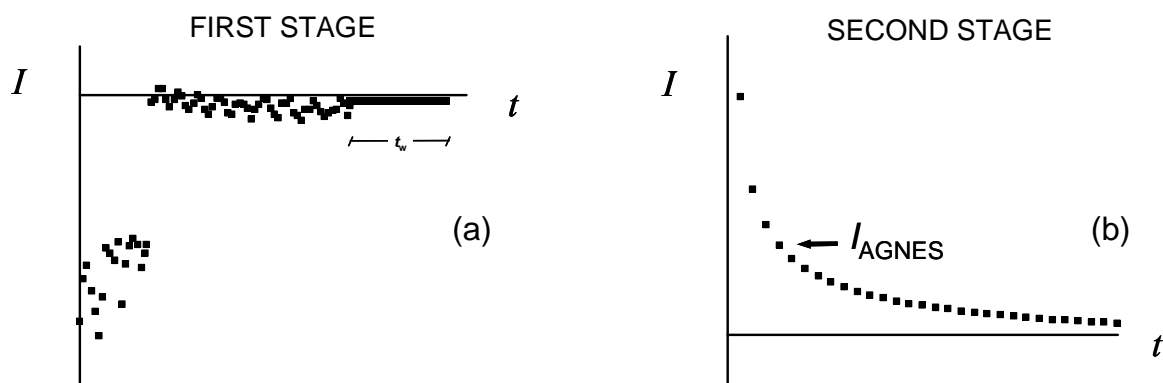


Fig 2 : Examples of currents along the first stage (panel a, in a case of overshoot) and second stage (panel b) of an AGNES experiment of two potentials (2P) in the first stage. The arrow indicates the current measured after t_2 seconds of application of the reoxidation potential. This I -value leads to the determination of the free metal concentration.

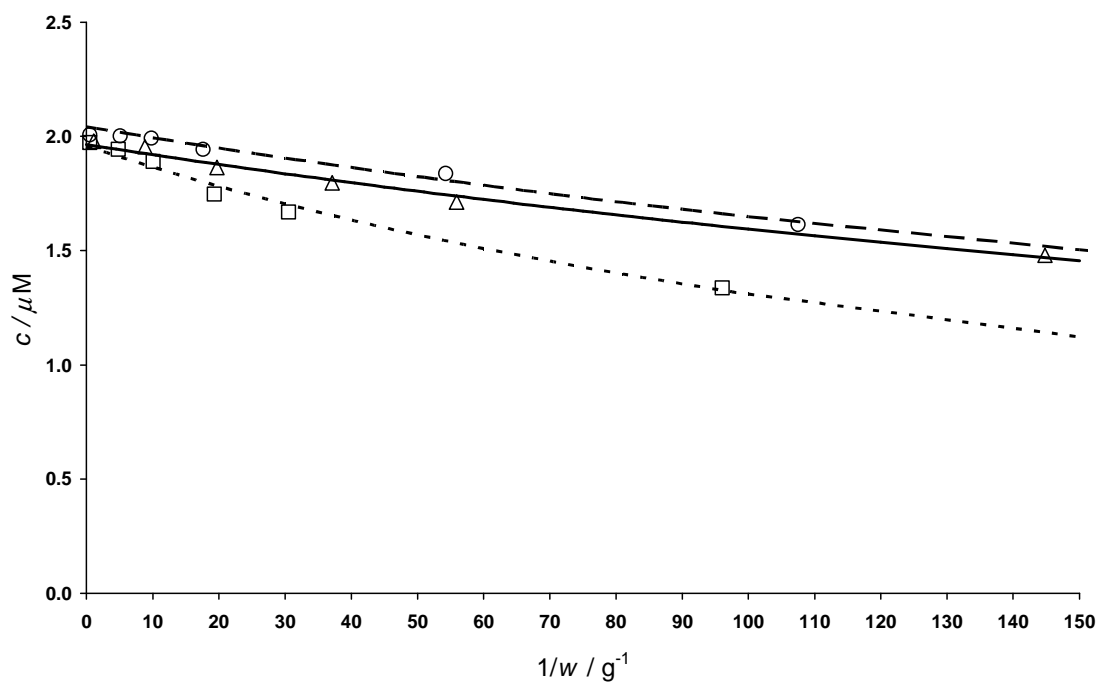


Fig 3: RT curves obtained at different acidities for Pb(II)-NTA systems in KNO_3 solution with Pb(II) 2×10^{-6} M and NTA 5×10^{-6} M. Markers: □ corresponds to pH = 3.15, $V = 100$ mL, $w = 0.010 \div 1.905$ g; Δ corresponds to pH = 3.23, $V = 50$ mL, $w = 0.007 \div 0.952$ g; and ○ corresponds to pH = 4.55, $V = 100$ mL, $w = 0.009 \div 1.862$ g. The curves are calculated from the parameters of equation 4 experimentally determined as described in the text.

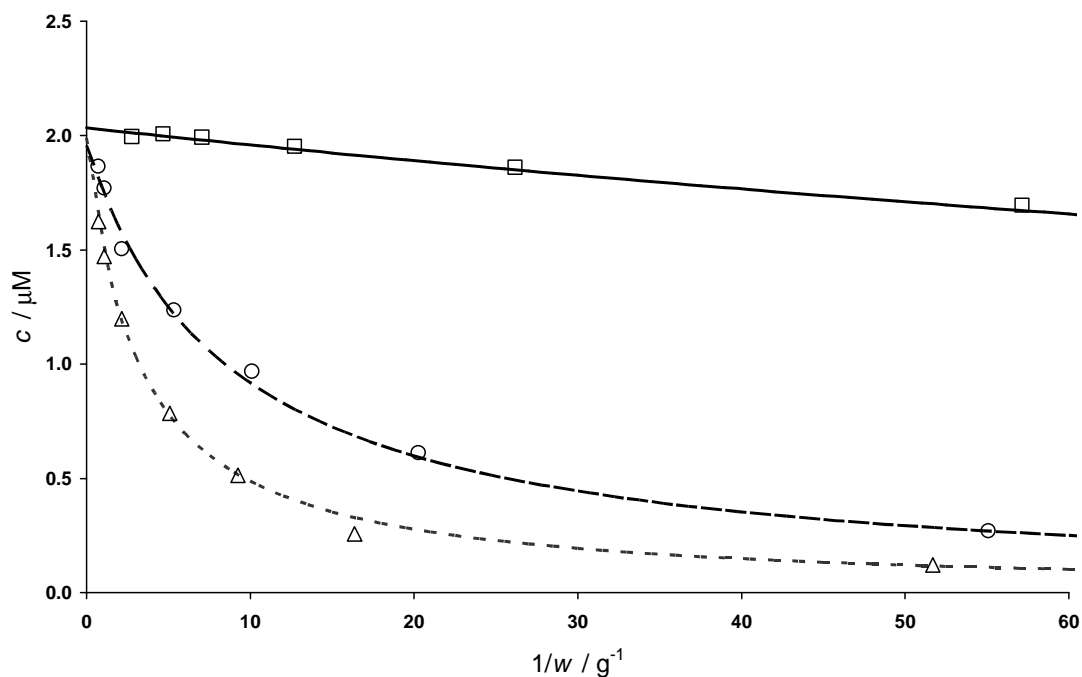


Fig 4: RT curves obtained at different acidities for Pb(II)-PDCA systems in KNO_3 solution with Pb(II) 2×10^{-6} M and PDCA 4.5×10^{-6} M. Markers: Δ corresponds to pH = 2.74, $V = 50$ mL, $w = 0.061 \div 1.385$ g; \circ corresponds to pH = 4.05, $V = 50$ mL, $w = 0.049 \div 1.387$ g; and \square corresponds to pH = 6.24, $V = 100$ mL, $w = 0.017 \div 0.362$ g. The curves are calculated from the parameters of equation 4 experimentally determined as described in the text.

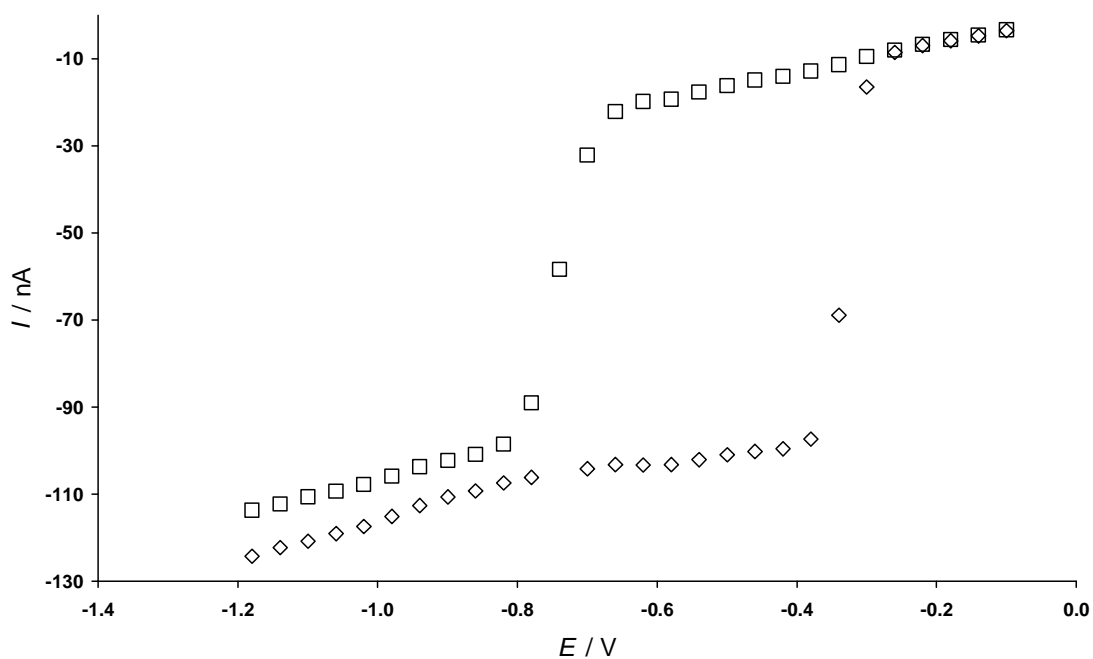


Fig 5: NPP wave for Pb and PbNTA. Solution: $[\text{Pb}^{2+}]_{\text{total}}=10^{-5}$ M; $[\text{NTA}^{3-}]_{\text{total}}=2.4\times 10^{-5}$ M. \diamond NPP wave for Pb^{2+} . \square NPP wave for the system PbNTA. Initial potential = -0.1 V; end potential = -1.2 V; interval time = 1.0 s; pulse time = 0.040 s; step potential = 0.040 V; drop size: $r_0 = 2.03\times 10^{-4}$ m.

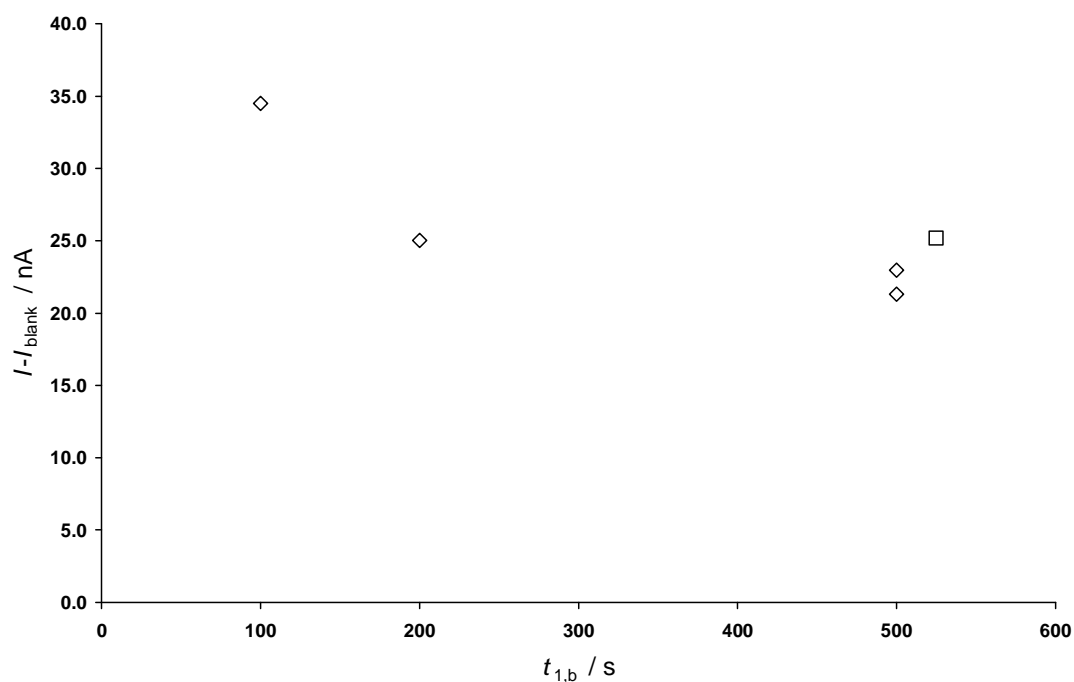


Fig 6: Representation of the faradaic current versus $t_{1,b}$. Solution: $[\text{Pb}^{2+}]_{\text{total}}=2\times 10^{-6}$ M. $[\text{NTA}^{3-}]=5\times 10^{-6}$ M. \diamond Second wave strategy: $t_{1,a}=11$ s; $Y_{1,a}=10^{20}$; $Y_{1,b}=250$. \square Standard 2P strategy: $Y_{1,a}=10^{10}$; $Y_{1,b}=250$; $t_{1,a}=175$ s and $t_{1,b}=525$ s.

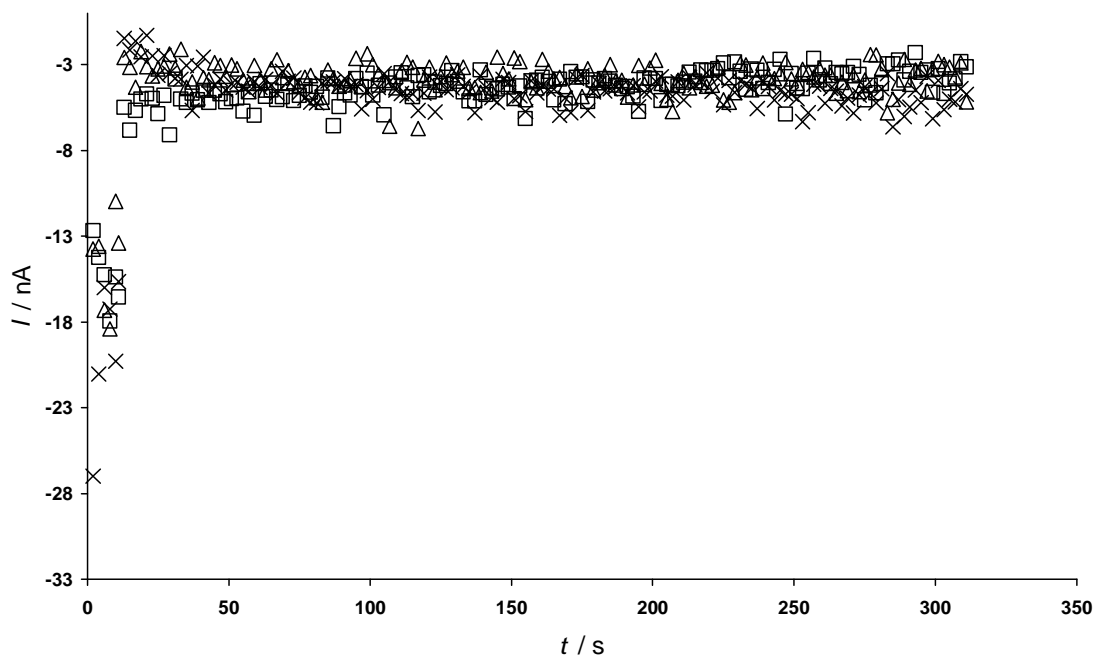


Fig 7: Representation of the currents along the first stage for different pH-values. The achievement of a horizontal final value of the current indicates AGNES conditions have been reached before the stripping stage (in this case with the second wave strategy).
 Solution: $[\text{Pb}^{2+}]_{\text{total}}=2 \times 10^{-6}$ M; $[\text{NTA}^{3-}]=5 \times 10^{-6}$ M. $t_{1,a}=11$ s. $t_{1,b}=300$ s. $Y_{1,b}=250$. \square pH=4.09. \triangle pH= 5.07. \times pH=6.01.

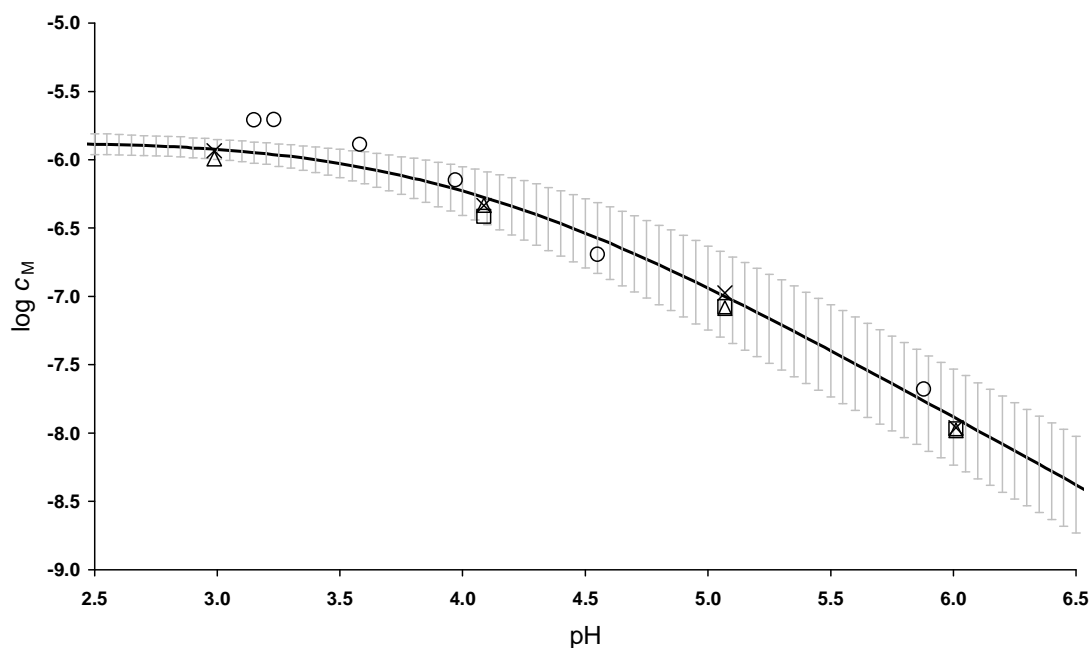


Fig 8: Representation of logarithm of [Pb] versus pH. Solution: $[\text{Pb}^{2+}]_{\text{total}}=2 \times 10^{-6}$ M. $[\text{NTA}^{3-}]=5 \times 10^{-6}$ M. $Y_{1,b}=250$. Continuous line: SolEq calculations The mean values of each constant are used to obtain the calculated solid curve. The error bars are derived from the propagation of errors from the input values employed in the free metal ion concentration; \times ISE results; \circ RT results; \square Second wave strategy; \triangle Standard 2P strategy.

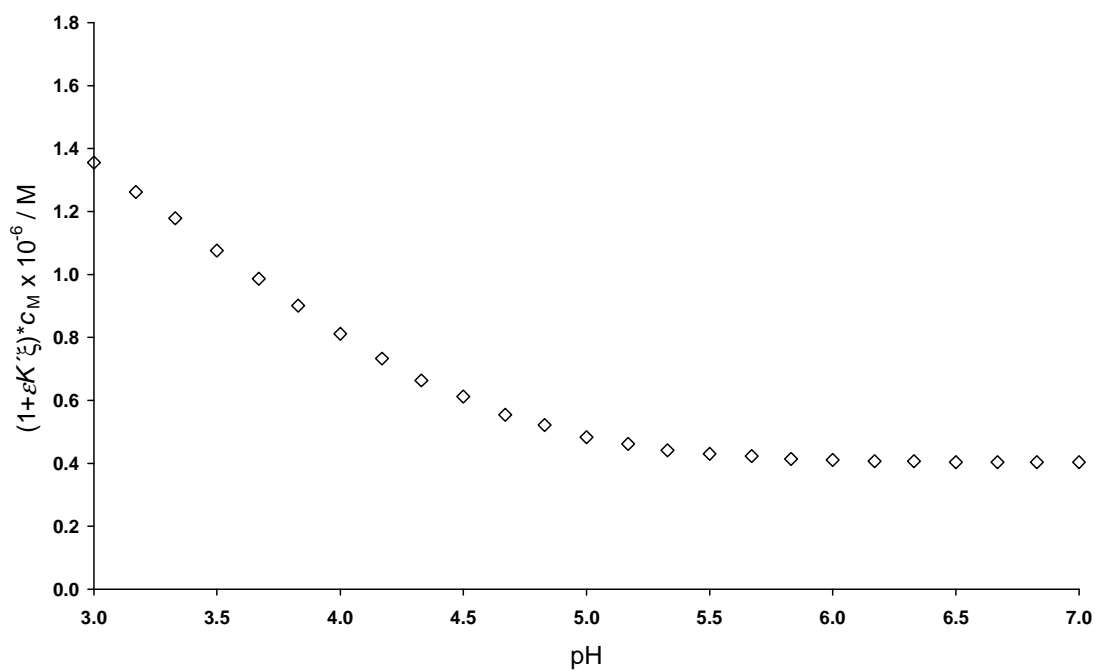


Fig 9: Plot of the value of a factor of the flux $(1 + \varepsilon K' \xi) c_M^*$ versus pH (see eqn. (13)).

Solution: $[Pb^{2+}]_{total} = 2 \times 10^{-6} M$; $[PDCA]_{total} = 4.5 \times 10^{-6} M$.

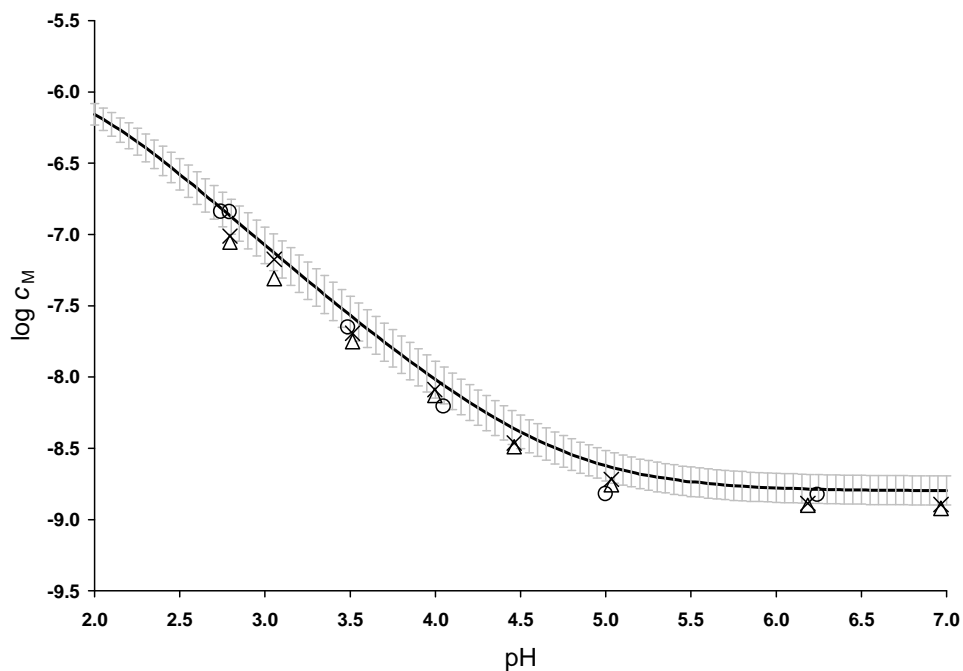


Fig 10: Representation of the logarithm of $[Pb]$ versus pH in a solution with $[Pb^{2+}]_{total}=2\times 10^{-6}$ M and $[PDCA]_{total} = 4.5\times 10^{-6}$ M. Continuous line: SolEq calculations with average constants. The mean values of each constant are considered and used to obtain the calculated curve. The error bars are derived from the propagation of errors from the input values employed in the free metal ion concentration; \times ISE results; \circ RT results; \triangle AGNES results.