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AGNES: a technique for determining the concentration of free metal ions. The case of Zn(II) in coastal Mediterranean seawater.

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Abstract

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is a recently suggested electroanalytical technique designed for the determination of the free concentration of heavy metals (such as Zn, Cd or Pb) which is here developed and applied to seawater samples. A key improvement for the implementation of AGNES with complex matrices is the development of a new blank, called the shifted blank (presented in this work for the first time), which can be applied to the same sample where the measurement is intended. The careful selection of the required parameters for the determination of the free Zn concentration (or activity) at the nanomolar level is described in detail. The methodology has been validated with a synthetic solution containing Zn and nitrilotriacetic acid (NTA) and then applied, as a first case, to two coastal seawater samples taken close to Barcelona and Tarragona (Catalonia, North Eastern Spain) finding values in the range of 1-3 nM, representing around 25% of total Zn. This technique can, in the near future, be crucial in helping to elucidate the role of the free zinc(II) concentration in natural waters.

Keywords: Chemical speciation, Zinc, Seawater, stripping analysis, heavy metals, FIAM

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1. Introduction

Knowledge of the concentration of free metal ions in natural waters is essential for understanding the role and fate of nutrient and pollutant elements [1-5]. Indeed, the Free Ion Activity Model [6] or the Biotic Ligand Model [7,8] highlight the free concentration as more relevant than the total metal concentration, which can be determined with well established techniques. In this context, the implementation and development of a variety of techniques for reliable measurements of free metal ion concentrations (or well-defined labile fractions) can be of interest [9-15].

In the particular case of Zn ion, there has been a long debate on the potential limiting role of Zn(II) concentration in the marine environment [16-20]. A coherent answer to the questions of this debate can only be done by putting together complementary information on the speciation of Zn provided by different techniques.

Some methods to measure total Zn and/or its labile fraction involve a preconcentration step, such as Anodic Stripping Voltammetry [21] or Cathodic Stripping Voltammetry [22,23]. When these kind of methods have been applied to seawater samples, they have usually supported the hypothesis of a very low availability (and limiting role) of Zn, especially in the open ocean, where a large proportion of the total Zn would be bound, mostly to organic ligands. For instance, Donat and Bruland [24,25] determined that >95% of total dissolved Zn is strongly complexed by organic ligands having concentrations of 1.6-2.2 nM and forming complexes with conditional stability constants of 10^{10}-10^{11}. A number of other reports indicate, however, a much lower proportion of organically complexed zinc [26-28].

As far as we know, there is no standard method for the determination of Zn(II) free concentration, despite intense work such as the search for an Ion Selective Electrode for Zn [29] and many other strategies [30,31]. So, the availability of techniques allowing such determination could be helpful in the solving of an important question for the scientific community.

Recently, we have designed [32] a new stripping technique aiming at the determination of free metal ion concentration, whose name, AGNES, indicates that the stripping step is applied when a special situation has been achieved. One advantage of this new electroanalytical technique is its simple interpretation, in contrast with the difficulties encountered in many current determinations of the "labile metal" concentration whose exact meaning depends on a number of assumptions and ill-unknown parameters. In a further development [33] we have shown that AGNES can determine free Zn concentrations in various synthetic solutions with labile or non-labile complexes. So, we aim here at implementing and developing AGNES methodology for the measurement of Zn in Mediterranean seawater samples, tackling the problem of reducing the limit of detection and the difficulties of a complex matrix.

2. Theoretical background

2.1 Principles of AGNES

AGNES has been presented in detail in previous works [32,33]. We summarize here the principles of this electroanalytical technique for the particular case of Zn. AGNES consists of two stages: deposition or first stage and stripping or second stage. In the simplest potential program (see Fig 1), only one constant potential E_1 is applied along the deposition time t_1, while stirring is on during a time t_1-t_w.

2.1.1 First stage

A key feature of AGNES is that by the end of the first stage a special situation is reached:

i) "Absence of Gradients": there are no concentration profiles at either side of the electrode surface. So, the free Zn concentration at the bulk is the same as in the electrode surface and we represent them here as $[\mathrm{Zn}^{2+}]$.

ii) "Nernstian Equilibrium". The couple $\mathrm{Zn}^{2+}/\mathrm{Zn}^{\mathrm{o}}$ has reached the equilibrium corresponding to the applied potential (E_1). Nernst equation allows to compute the ratio of concentrations at each side of the electrode surface (which we call the preconcentration factor or "gain" Y) as

$$Y = \frac{[\mathrm{Zn}^{\mathrm{o}}]}{\left[\mathrm{Zn}^{2+}\right]} = \exp\left[-\frac{2F}{RT}\left(E_1 - E^{\mathrm{o}\,\prime}\right)\right] \tag{1}$$

where F is the Faraday, R the gas constant, T the temperature and $E^{\mathrm{o}\prime}$ is the standard formal potential (where the activity coefficients are embedded). In the present implementation of AGNES, the preconcentration factor is determined from E_{peak}, the potential peak of a Differential Pulse Polarography (DPP) experiment [32]:

$$Y = \sqrt{\frac{D_{\mathrm{Zn}}}{D_{\mathrm{Zn}^0}}}\exp\left[-\frac{2F}{RT}\left(E_1 - E_{peak} - \frac{\Delta E_{\mathrm{DPP}}}{2}\right)\right] \tag{2}$$

where D_{Zn} and D_{Zn^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.

2.1.2 Second stage

The aim of the second stage is the determination of the concentration of Zn^{o} 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.

The response function in this implementation of AGNES is the current I at a certain time t_2.

2.1.3 The proportionality factor, *h*

Due to the linear properties of the diffusion of Zn° inside the amalgam [32], there is a direct proportionality between the faradaic current I_f and the concentration of Zn° built up inside the mercury drop. But eqn. (1) indicates another direct proportionality between this Zn°-concentration and that of free Zn. So, regardless of processes such as electrodic adsorption, complexation or hydrodynamic regimes, the following fundamental relationship between faradaic current and free metal concentration applies:

$$I_{\mathrm{f}} = h\left[\mathrm{Zn}^{2+}\right] \quad (3)$$

As the measured current, I, contains other components other than the faradaic one of the analyte (see next section), there is a need to subtract a blank from the total current.

2.2 An improved blank: the "shifted" blank

The subtracted blank in AGNES has been, up to the present date [32,33], determined in a synthetic solution with the same composition of the sample and no added metal. This "synthetic" blank exhibits some limitations: a) the presence of traces of the analysed metal (via contamination) cannot be avoided (if this trace concentration in the blank is not well determined there will be a loss of accuracy in the determination of low concentrations in the sample) and variations in the contamination concentration leads to a (relatively) high value for the Limit of Detection (LOD); b) for natural samples it is very difficult to mimic such complex matrices via synthetic solutions. So, we develop here a new blank which will be essential in the improving of the LOD of AGNES and suitable for natural samples.

In principle, in any response current I measured with AGNES (either in any blank or in a sample with a given amount of metal) at a certain time t_2, we recognise the following components:

i) the charging or capacitive current, which we denote I_c.

ii) the current due to the reduction of O_2 , I_{O2}. Even if N_2 flows through the cell, traces of oxygen are always present.

iii) the faradaic current due to non-analyte elements which have been preconcentrated along the first stage and stripped along the second stage, I_{fna}. For instance, when determining Zn, the deposition potential (E_1) will also preconcentrate Cd and if the stripping potential (E_2) was -0.1 V, then the re-oxidating preconcentrated Cd would also contribute to the measured current at t_2.

iv) the faradaic current due to the analyte (Zn in this case). We denote it as I_f.

So, the current obtained at t_2, for any given preconcentration factor Y, can be split into

$$I = I_{\mathrm{c}} + I_{\mathrm{O_2}} + I_{\mathrm{fna}} + I_{\mathrm{f}} \tag{4}$$

Now, assume that we perform 2 experiments with two different Y-values, being one Y-value negligible in front of the other. For simplicity in the exposition in this section, we take *Y*=500 and *Y*=0.01, but many other combinations would be possible. If we subtract both measured currents,

$$\begin{aligned}I_{Y=500}-I_{Y=0.01}&=\left(I_{\mathrm{c},Y=500}-I_{\mathrm{c},Y=0.01}\right)\\&+\left(I_{\mathrm{O_2},Y=500}-I_{\mathrm{O_2},Y=0.01}\right)+\left(I_{\mathrm{fna},Y=500}-I_{\mathrm{fna},Y=0.01}\right)+\left(I_{\mathrm{f},Y=500}-I_{\mathrm{f},Y=0.01}\right)\end{aligned}\tag{5}$$

As detailed in section 4 (sub-section 4.1) below, a suitable selection of the potential program parameters allows the neglecting of all the terms in the r.h.s. of previous eqn. (5), except the last one (the one corresponding to the faradic current). Under such conditions

$$I_{Y=500}-I_{Y=0.01}=I_{f,Y=500}-I_{f,Y=0.01}=[\mathrm{Zn}^{2+}]\left(h_{Y=500}-h_{Y=0.01}\right)\tag{6}$$

where we have used the proportionality between faradaic current and free metal concentration given by the fundamental eqn. (3). If we take into account the proportionality between *h* and *Y* (see Appendix in [32]):

$$I_{Y=500}-I_{Y=0.01}=[\mathrm{Zn}^{2+}]\left(h_{Y=500}-h_{Y=0.01}\right)=[\mathrm{Zn}^{2+}]h_{Y=500}\left(1-\frac{0.01}{500}\right)\approx[\mathrm{Zn}^{2+}]h_{Y=500}\tag{7}$$

So, the measurement performed at *Y*=0.01 (i.e. for a *Y* which is negligible with respect to the *Y*-value of the main measurement) would act as the blank to be subtracted in order to experimentally access to the proportionality between faradaic current and concentration. This negligible *Y* (of the blank) corresponds to a change in the (main) deposition potential E_1 by an amount which can be called ΔE_{shift}. The essence of the new blank consists, as will be detailed in sub-section 4.1 below, in applying basically the same potential program as in the main measurement, but with the applied potentials shifted a fixed ΔE_{shift} towards less negative potentials. For this reason, we suggest to call it the "shifted blank" and to label its parameters with the subscript "sb".

3. Experimental

3.1 Instrumentation and reagents

Voltammetric measurements were carried out with an Eco Chemie Autolab PGSTAT30 and with a PGSTAT12 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, which according to the catalogue corresponds to a radius around $r_0 = 1.41\times10^{-4}$ 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} KNO_3 jacket.

A glass combined electrode (Orion 9103) was attached to an Orion Research 920A ion analyser and introduced in the cell to control the pH. A glass jacketed cell and a Teflon (PFA) cell provided by Metrohm were used in all measurements. The vessel was thermostated at 25.0 °C.

To stir the solution, the PTFE tip stirrer of the Metrohm 663VA Stand, which is screwed onto the driving axis, was used. The rotation rate is set on the 663 VA Stand and it was fixed at 1500 rpm for all experiments.

All laboratory ware and the equipment for sampling and filtering were extensively washed using the following procedure: they were stored in 3 M nitric acid ultra pure for at least 1 week, then the solution was replaced by fresh 0.1 M nitric acid and they were stored in this solution just before their use. Finally they were rinsed with ultrapure Milli-Q water and air-dried under a cleaned hood.

Zinc standard solutions were prepared by adequate dilution from the Merck 1000 mg L^{-1} stock solution. Potassium nitrate was used as the inert supporting electrolyte and prepared from solid KNO_3 (Merck, Suprapur). Nitrilotriacetic acid (NTA) (Fluka, analytical grade) in the H_3L form, was used as ligand.

KOH and HNO_3 titrisol (Merck) were added to fix the pH to the desired values.

Ultrapure water (Milli-Q plus 185 System, Millipore) was employed in all the experiments. Purified water-saturated nitrogen N_2(50) was used for deaeration and blanketing of solutions.

3.2 Seawater sample collection

Seawater samples were collected at 50 m from the shore and placed in 5 L plastic bottles. The collections took place on 7th June 2005 (Castelldefels, Barcelona) and on 13th October 2005 (La Rabassada, Tarragona) from beaches located in the western Mediterranean sea in Catalonia, Spain. All of them were surface samples (~ 0.25 m). After their collection, seawater samples were transferred within a few hours to the laboratory where they were filtered through nitric cellulose Millipore 0.45 μm filters.

The pH of the Castelldefels and Tarragona seawaters were 8.20 and 8.18, respectively, and the temperatures were 21ºC and 23ºC. The conductivities of Tarragona and Castelldefels seawaters were 48.5 mS cm^{-1} and 49.5 mS cm^{-1} respectively.

4. Results and Discussion

4.1 Selection of the conditions for the shifted blank to be used for the determination of Zn in seawater.

The use of the shifted blank procedure requires the cancelling (or neglecting) of components other than the faradaic current of Zn in eqn (5). Now, we analyse these components in order to select the best conditions for eqn. (7) to be applied.

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i) The capacitive current, I_c, for this electrode, decays faster than the faradaic component [34], so that its absolute value (for the main measurement) is expected to be low at the relatively long measuring time t_2 such as 0.25 s. However, specially for low faradaic currents, I_c can be still relevant at t_2, so we conducted a series of experiments, in synthetic solutions with just traces of Zn, to see how the different potential programs would affect the capacitive current term we want to minimise, i.e. $\left(I_{c,Y=500} - I_{c,Y=0.01}\right)$.

Fig 2 shows AGNES currents I_{sb} (at t_2=0.2 s) measured in a solution with just KNO_3 and in another solution with KNO_3 and EDTA. The presence of EDTA has not a noticeable impact on the currents, despite reducing dramatically the free Zn concentration of an already very low total metal concentration (just from the existence of traces of Zn) and forming very inert complexes. So, we conclude that the changes in the total measured currents are mostly due to the variations in the capacitive component (the oxygen component can be considered practically constant in the range of potentials scanned by E_2 in the figure and EDTA forms complexes with other existing trace metals, so that their faradaic contribution should also be totally negligible). The key conclusion from Fig 2 is that I_c depends mostly on the difference in the potentials applied during the first stage (E_1) and the second stage (E_2); when this potential jump between stages (E_2-E_1) increases, I_c increases, as expected from the usual charging model for the capacitive current [34]. When comparing experiments performed at different deposition potentials E_1 corresponding to Y_{sb}=0.01 and Y_{sb}=0.0025 (e.g. diamond and square markers for the case without added EDTA and triangles and circles in the solution with added EDTA) we see no relevant impact on the current. The capacitive current term depends, then, on the difference of E_2-E_1, rather than on the particular values of E_1 and E_2. So, we conclude that the term $\left(I_{c,Y=500} - I_{c,Y=0.01}\right)$ will be minimised when E_2-E_1 is kept fixed for both the main measurement (e.g. at Y=500) and the shifted blank measurement (e.g. at Y_{sb}=0.01).

The suitability of this strategy is shown in Fig 3 where currents are obtained with a fixed E_2-E_1=0.1982 V (this value was chosen given the relatively flat region in 2). The “shifted blank” currents obtained for Y_{sb}=0.01, $Y_{sb}=5\times10^{-3}$ and $Y_{sb}=2.5\times10^{-3}$ are essentially constant, regardless the presence or absence of EDTA. The main measurements at Y=50 and Y=100 are much higher when there is no EDTA (we are measuring the current of the existing traces of Zn), but they fall down to practically the same values of the shifted blanks (around 1 nA) when the added EDTA dramatically reduces the free metal concentrations. So, we observe that the shifted blank yields the current of the main measurement when there is no faradaic (of the analye) component in it.

ii) I_{O2} could depend on E_1 and E_2 and on the achievement of some steady state regime. We can assume that the concentration profile of O_2 is essentially the steady-state profile for diffusion limited conditions in spherical semi-infinite diffusion after some 20 s from the stopping the stirring (in the waiting period while we apply E_1) have elapsed. It is also reasonable to assume that this concentration profile is not essentially altered by switching off the potential to E_2, because O_2 is totally reduced on the electrode surface at both potentials. So, the measured current will depend on the reduction process at E_2. The well-known O_2 reduction wave exhibits a plateau between -0.8 V and -0.2 V. So, the term $\left(I_{O_2,Y=500}-I_{O_2,Y=0.01}\right)$ will be negligible as long as the E_2-values, for both the main measurement and the corresponding shifted blank, fall within this plateau.

iii) I_{fna} can be minimised if $E_{2,\mathrm{sb}}$ is still negative enough for the non-analytes (Cd and Pb, mostly) as to not be stripped back to the solution. This stripping back will be satisfactorily avoided if the time t_1 is insufficient for them to be preconcentrated up to the resulting $Y_{2,\mathrm{sb}}$ (for these non-analytes) corresponding to $E_{2,\mathrm{sb}}$. As an illustration of these issues, Figure 4 shows an anodic striping voltamogram of a sample containing Zn and also some traces of Cd and Pb (non-analytes) together with the potentials applied both in the AGNES experiment and the shift blank (depicted as vertical lines). The relative position of each AGNES potential with respect to a given peak provides a first indication whether the corresponding species is entering or leaving the mercury drop. Regarding this issue iii), notice how $E_{2,\mathrm{sb}}$ is still quite more positive than the peak potential of Cd.

Obviously, a very low concentration of Cd or Pb (as existing in seawater) also renders this condition less important. We checked (data not shown) that the currents of shifted blanks in a solution of KNO_3 0.1 M did not change when varying t_1, this indicating that traces of Cd were not discharged under our conditions and parameters.

Taking into account the previous points, the parameters selected for the shifted blank were a potential program of just one potential step, $E_{1,\mathrm{sb}}$ corresponding to Y_{sb}=0.01, such as -0.9070 V, for the deposition stage (t_1-t_{w}=350 s; t_{w}=50 s) and a stripping potential $E_{2,\mathrm{sb}}$ so that the potential jump was the same as in the main measurement, i.e. (E_2-E_1)= 0.3165 V leading to $E_{2,\mathrm{sb}}$ such as -0.5905 V.

4.2 Optimising the deposition time

A key issue in the implementation of AGNES is the use of a suitable deposition time (and potential program) for the fulfillment of conditions i) and ii) stated in section 2.1.1

by the end of the first stage. This deposition time has to be long enough so that all dynamic processes (diffusion, electron transfer kinetics, complexation kinetics, adsorption kinetics, etc.) have reached the equilibrium state. Previous work [33] showed that the application of two potential steps ($E_{1,a}$ during a time $t_{1,a}$ and $E_{1,b}$ during a time $t_{1,b}+t_{w}$ as shown in Fig 5) along the first stage could reduce the overall deposition time (t_1) needed with respect to that of the simplest implementation depicted in Fig 1. The first potential ($E_{1,a}$) corresponds to diffusion limited conditions and the second one ($E_{1,b}$) corresponds to the desired Y of the experiment.

Preliminary tests in seawater indicated that the AGNES Zn-reoxidation current obtained with Y=500 was sufficiently different from the shifted blank current; so this Y was taken as the target preconcentration factor.

The "recipe" developed in previous work [33] prescribed a ratio of times given by $t_{1,b}=3\times t_{1,a}$. Taking into account that the goal of this work is the measurement of one concentration (and not very different from one sampling site to the other) in each solution (i.e. there is no change of ligand concentrations), we decided to optimize the required times of the potential program for the particular conditions of our Mediterranean seawater. The key idea is to apply the first potential step $E_{1,a}$ (in diffusion limited conditions) for a convenient time $t_{1,a}$ so that the desired number of moles of Zn have practically entered the mercury drop. Thus, a time $t_{1,b}$ (say $t_{1,b} = t_{1,a}$) much lesser than $3\times t_{1,a}$ can be used for the fine-tuning and stabilization of [Zn^{o}] to the desired value $Y\times[\mathrm{Zn}^{2+}]$.

Fig 6 shows the last phase of the search for a convenient $t_{1,a}$. When we conducted the experiment with $t_{1,b} = t_{1,a}$ =800 s (see open square) we obtained (within the experimental accuracy) the same current as when applying the "recipe" $t_{1,a}$ =800 s; $t_{1,b}$ =2400 s (see open diamond). In order to decide whether to increase or to reduce $t_{1,a}$, we analysed whether at $t_{1,a}$ =800 s the actual concentration of Zn^{o} built up inside the drop was higher or lower than the desired value $Y\times[Zn^{2+}]$. We call "overshoot" [33] to the existence (inside the drop) at $t = t_{1,a}$ of an amount of Zn^{o} larger than desired, i.e. $[Zn^{o}]_{actual} > Y\times[Zn^{2+}]$; this leads to a decaying current for $t_{1,a} < t < t_1$ because the excess of accumulated Zn^{o} has to be re-oxidized to reach the prescribed Nernstian equilibrium by $t=t_1$. For samples with high metal concentrations, this overshoot can be easily seen in the evolution of the current along the first stage [33]. We can call "undershoot" the opposite situation where, at $t=t_{1,a}$, we have $[Zn^{o}]_{actual} < Y\times[Zn^{2+}]$, so that Zn continues to enter the mercury drop for $t > t_{1,a}$. Due to the low Zn concentration in the samples here analysed, the currents in case of "overshoot" or "undershoot" are difficult to be distinguished in the evolution of the currents. So, we decided to probe the "overshoot" or "undershoot" situation by measuring the currents at the second stage (i.e. at t_2=0.25 s within the second stage) with a very short $t_{1,b}$ =50 s (this usually leaves not enough time for the reaching of conditions i and ii of section 2.1.1 by the end of the first stage). As seen on the right of Fig 6 (open circle), the use of $t_{1,a}$ =800 s with $t_{1,b}$ =50 s produces a current higher than with a $t_{1,b}$ (say 800 s or 2400 s) allowing AGNES conditions to be attained: we conclude that with the combination $t_{1,a} = t_{1,b}$ = 800 s there has been overshoot, so we can look for a $t_{1,a}$ shorter than 800 s. On the left of Fig 6, we see that using $t_{1,a}$ =650 s with $t_{1,b}$ =50 s produces a current less than the one obtained with $t_{1,b}$=2400 s, this indicating that there is undershoot when using $t_{1,a}$ =650 s and that we could try a better $t_{1,a}$ in between 650 s and 800 s. We finally found that $t_{1,a} = t_{1,b}$=700 s was a safe combination exhibiting neither overshoot nor undershoot and fully agreeing with the results from the recipe $t_{1,b}=3\times t_{1,a}$.

So, the potential program for the main measurements (i.e. not blanks) applied in this work is:

- $E_{1,\mathrm{a}}$ under reduction diffusion limited conditions (with stirring) corresponding to $Y=10^{10}$ for $t_{1,\mathrm{a}}=700$ s.
- $E_{1,\mathrm{b}}$ corresponding to $Y=500$ for $t_{1,\mathrm{b}}=700$ s (with stirring) and waiting time $t_\mathrm{w}=50$ s (without stirring).
- E_2 corresponding to re-oxidation diffusion limited conditions corresponding to $Y=10^{-8}$ for 50 s, with the response current being read at $t_2=0.25$ s.

4.3. Validation of the determination of free Zn^{2+} in a synthetic solution

Prior to the application of AGNES to real samples, a validation procedure was designed based on the determination of a known low concentration of free Zn (due to known NTA and Zn total concentrations with known pH) in a medium of higher ionic strength than those used with AGNES up to now [33].

The value of h for the medium KNO_3 0.5 M was obtained from AGNES values using a concentration range of Zn between 0.1 to 1 μM. One calibration plot is shown in Fig 7: in this particular instance an $h=1.04$ A M^{-1} was found from the linear regression of I vs. [Zn^{2+}] data.

The total concentrations of the prepared mixture were 10^{-5} M in NTA and 1.2×10^{-7} M in Zn. The pH of the solution was varied around the interval 5.2-6.1. The difference between the pH values of the validation procedure and that of the seawater sample was considered immaterial, given experimental evidence of h being the same for a large set of pH values. According to the speciation codes VMINTEQ [35] and MEDUSA [36], in this range of pH, with the combination of total concentrations of Zn and NTA here considered, the concentration of free Zn moves around 2-20 nM. The logarithm of the concentrations of free Zn obtained with AGNES (in two different days) at each pH is shown in Fig. 8 with markers. The slight disagreement (around 0.2 log units) between the replicates of different days can be mainly ascribed to small differences in the prepared sample and uncertainities in pH measurements. The continuous line represents the computed values obtained considering the complexation equilibria Zn/NTA and the hydrolysis of Zn at the ionic strength used with the code MEDUSA. The dashed line corresponds to the theoretical values predicted by MINTEQ for the same mixture at the same pH-range. Differences between MEDUSA and MINTEQ can be traced to be mainly due to differences in: i) the computation of the activity coefficients (MINTEQ uses Davies' equation while MEDUSA uses equations by Helgeson, Kirkham and Flowers [37] and ii) the value of the stability constant for the complex ZnNTA (MINTEQ takes log K_{ZnNTA}= 12.01 while MEDUSA takes 11.84). Considering the difficulties in theoretically ascertaining the free metal concentration [38], the experimental results can be accepted as satisfactory.

4.4 Determination of free Zn^{2+} in seawater samples

From the experience gained in the previous results, the parameters used for AGNES aiming at probing the seawater samples were: i) for measurement $t_{1,\mathrm{a}}=t_{1,\mathrm{b}}=700$ s; $Y=500$; $t_2=0.25$ s; $Y_2=10^{-8}$ ii) for the shifted blank $t_{1,\mathrm{sb}}=400$ s; $Y_{\mathrm{sb}}=0.01$; $t_2=0.25$ s; $Y_{2,\mathrm{sb}}=2\times10^{-13}$. For each speciation determination the required *h*-value was determined in a calibration, conducted within a few days proximity, in a medium with a similar ionic strength to that corresponding to the seawater. For this purpose, a concentration of background electrolyte KNO_3 0.7 M was used.

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An estimation of the limit of detection (LOD) and the limit of quantification (LOQ) of this implementation of AGNES (basically defined by Y=500 and the shifted blank) was obtained carrying out 10 repetitions of shifted blanks in a solution of KNO_3 0.7 M without any added Zn (notice that, from the 0.005 ppm maximum impurities given by the manufacturer, one estimates up to 5 nM in Zn for this solution which could have been taken for the "classical blank"). The same kind of experiment was performed also in seawater, in order to compare the results. Table 1 gathers the average value of the current, the standard deviation (SD) and the values of LOD and LOQ (computed respectively from the ratios 3 × SD/slope of the calibration curve and 10 x SD/slope of the calibration curve) [39]. These results show the high sensitivity and the good precision of the AGNES measurement even in a natural complex matrix as seawater, provided a low blank, such as the shifted one developed here, can be accepted. It is also important to highlight that the capability to determine free Zn concentration at so low concentration, renders AGNES a very promising technique for speciation analysis, especially if one takes into account that better limits would be obtained with larger Y-values.

Results of the application of AGNES to the sample from Castelldefels are gathered in Table 2. Measurement currents around 5.6 nA and blank currents around 0.8 nA lead to difference currents around 4.8 nA. Applying eqn. (3), with h=1.53 A M^{-1} from its corresponding calibration at I=0.7 M, one finally reaches the average of this determination of $[Zn^{2+}]$ as 3.1(4) nM.

In order to check whether an intermetallic [21,22,40,41] amalgam complex between Cu^{o} and Zn^{o} could affect the response from AGNES current at t_2=0.25 s, we performed an experiment with Castelldefels seawater as usual and obtained a current *I*=4.89 nA. After the additions of Cu up to a total concentration of 5 nM, the new currents in the two replicates were 4.94 nA and 5.30 nA. If there was an interference due to the formation of an intermetallic complex, there would be a substantial decrease of the current of Zn^o-reoxidation, which did not appear in our experiment. So, we concluded that for the level of Cu and Zn concentrations present in our seawater, the used preconcentration factor and deposition time, there is not evidence of the formation of intermetallic Cu-Zn. Results for the sample from La Rabassada are gathered in Table 3. One can see a very similar structure to the results of Castelldefels. Measurement currents span from 2.0 nA to 2.8 nA yielding concentrations from 1.0 nM to 1.4 nM via the corresponding *h*-value of 1.40 A M^{-1}. The resulting average is 1.2 nM with a standard deviation 0.2 nM.

Total Zn concentrations were determined by Anodic Stripping Voltammetry and Square Wave Stripping Voltammetry in samples acidified to pH 1.5. The average value for Castelldefels sample was 10.6(6) nM. This means that the free fraction in Castelldefels is about 29% of the total. The average value for La Rabassada sample was 9(1) nM. So, the free fraction in La Rabassada seawater was around 21% of the total Zn.

The high values of total concentrations are consistent with the general trend of higher concentration in coastal waters than in open ocean ones [16,18,42,43]. Regarding the fraction of free Zn over total, our results differ from several authors who have found that Zn in seawater is more than 95% complexed by organic matter [18,24,25,27,44]. However, there exist some other reports finding -as we have done- a much lower organic fraction. For instance, Lewis *et al.* [27] found ca 28% of total Zn was free or weakly bound according to their pseudopolarographic method; [28] found 30% of total

Zn as organically bound in seawater; [26] reported 26% of organically bound in South Atlantic surface waters. Caution must, thus, be exercised because of the particularity of the speciation of any sample from a given location, depth, season, etc. [18,43,45], which hinders a proper comparison between different experimental techniques with just literature data. In any case, one should keep in mind that different experimental techniques are -in fact- measuring different fractions of any metal in a given complex matrix [15].

5. Summary and perspective

AGNES is a recent electroanalytical technique designed to quantify the free metal activity (or concentration) [32]. The first stage consists in the preconcentration of the reduced metal inside the amalgam up to a level prescribed by the preconcentration factor (Y) which is controlled by the applied potential via Nernst equation (1). The second stage consists in the measurement of the preconcentrated metal via stripping. In the current implementation there is a proportionality factor (h) between the faradaic intensity current (I) and the concentration of the free metal ion in the solution (3).

The development of the shifted blank strategy, introduced for the first time in this work, allows a crucial lowering of the limit of detection of AGNES with respect to the "synthetic" blanks, where it was hard to obtain a synthetic reproduction of a complex matrix (such as seawater) free from trace amounts of the analyte. In essence, the shifted blank consists in the application of an analogous potential program to that of the main measurement, but with the potential values shifted towards a region where the analyte is not appreciably preconcentrated along the first stage. Thus, the shift blank fundamentally evaluates the capacitive current for the same potential jump between the two stages of AGNES in the same sample where the measurement is intended.

The two-pulse strategy (i.e. the application of two potential steps along the first stage, see Fig 5), described in a previous work [33], has been specifically optimised for our particular setup with Zn^{2+} and Y=500, so that an important reduction of the deposition time has been achieved ($t_{1,a}=t_{1,b}$).

The developed methodology has been applied to the system NTA+ Zn (with changing pH, see Fig 8) in a validation phase. The results obtained by AGNES are in good agreement with theoretical predicted concentrations by two independent speciation codes.

Two Mediterranean seawater samples have been analysed with the described strategies. The sample from Castelldefels yielded a free Zn(II) concentration of 3.1 nM (see Table 2) from a determined total of 10.6 nM, while the sample from La Rabassada (Tarragona) yielded $[Zn^{2+}]$=1.2 nM (see Table 3) from a total of 9.2 nM. The results of this first implementation of AGNES to measure seawater indicate that Zn in Mediterranean coastal waters is much more available than in reported estimations corresponding to open oceans [24,25], but similar to a few other measurements [26-28,46].

AGNES has been shown to determine free Zn in these seawater samples. We have begun by tackling Zn due to the lack of a standard procedure to measure its free concentration and its intrinsic interest, but AGNES can also be a useful tool in the determination of free metal concentrations in seawater for a series of elements (such as Cu, Cd or Pb). Further work, with Zn and other metals, should address other aquatic systems of environmental interest where an even lower limit of detection is required.

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Tables

Table 1: Limit of detection (LOD) and limit of quantification (LOQ) for AGNES procedure with Y=500 in KNO_3 0.7 mol L^{-1} and in a seawater sample (Castelldefels sample).

	I_{sb} **average** /nA	I_{sb} **SD** /nA	**LOD** nM	**LOQ** nM
KNO_3 0.7 M	2.0	0.1	0.20	0.65
Seawater	1.61	0.09	0.18	0.59

Table 2: Results obtained applying AGNES to the seawater sample collected in Castelldefels. Free Zn^{2+} concentrations, $[Zn^{2+}]$, calculated with eq (3) with an h value of 1.53 A M^{-1} obtained in a calibration experiment with KNO_3 = 0.7 M. Number between brackets indicates the standard deviation and refers to the last significant digit. Parameters used: i) for measurement $t_{1,a}=t_{1,b}$=700 s; Y=500; t_2=0.25 s; $Y_2=10^{-8}$ ii) for the shifted blank $t_{1,sb}$=400 s; Y_{sb}=0.01; t_2=0.25 s; $Y_{2,sb}=2\times10^{-13}$.

experiment (n replicates)	I /nA	I-I_{sb} /nA	$[Zn^{2+}]$ /nM	pH
1 (n = 3)	6.0(6)	5.2(5)	3.4(3)	8.7
2 (n = 5)	5.2(3)	4.4(4)	2.8(2)	8.9

Table 3: Results obtained applying AGNES to the sea water sample collected in Tarragona (La Rabassada). Free Zn^{2+} concentrations, $[Zn^{2+}]$, calculated with eq (3) with an h value of 1.40 A M^{-1} obtained in a calibration experiment with KNO_3 = 0.7 M. Parameters used: i) for measurement $t_{1,a}=t_{1,b}=700$ s; $Y=500$; $t_2=0.25$ s; $Y_2=10^{-8}$ ii) for the shifted blank $t_1=400$ s; $Y_{sb}=0.01$; $t_2=0.25$ s; $Y_{2,\,sb}=2\times10^{-13}$.

experiment (*n* replicates)	I /nA	I-I_{sb} /nA	$[Zn^{2+}]$ /nM	pH
1 (*n* = 6)	2.3(2)	2.0(2)	1.4(2)	8.9
2 (*n* = 5)	2.0(2)	1.64(8)	1.17(5)	8.9
3 (*n* = 6)	2.8(3)	1.44(9)	1.0(7)	8.9

Figures

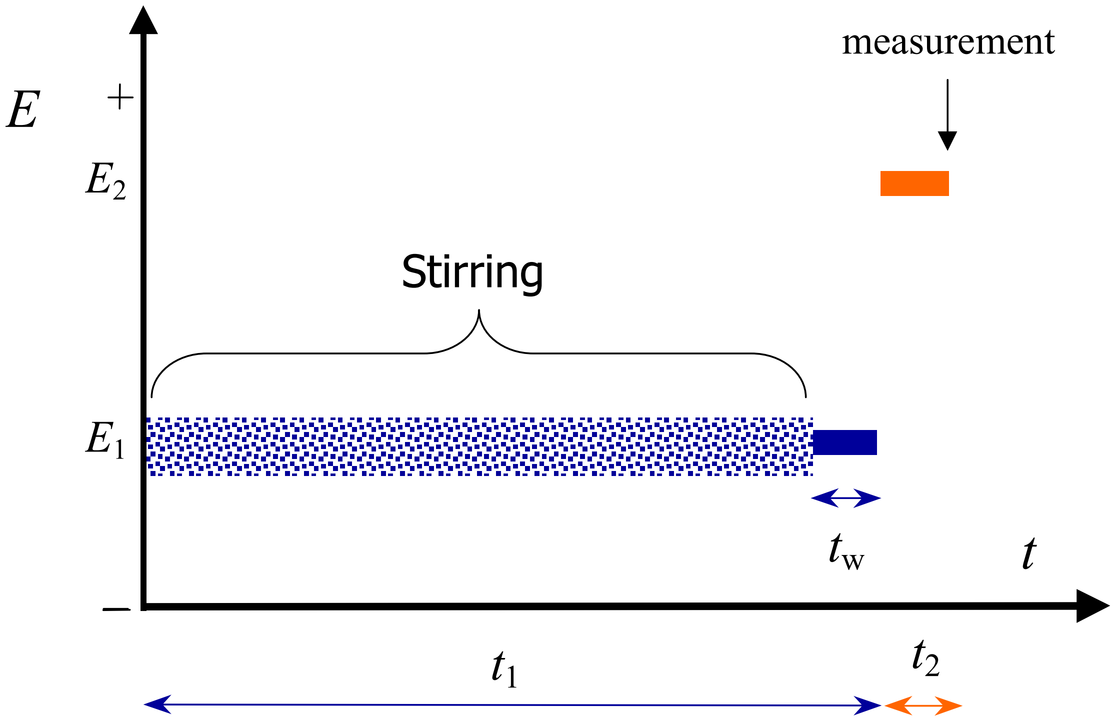

Fig 1: Schematic potential program for the simplest implementation of AGNES (one potential step along the first stage). The thicker line indicates that stirring is on along most of the deposition time (t_1). One key point of AGNES is that t_1 must be sufficiently long and the required preconcentration factor Y sufficiently small so that equilibrium is reached by the end of the first stage. A second important aspect is that E_2 must correspond to diffusion limited conditions for the reoxidation.

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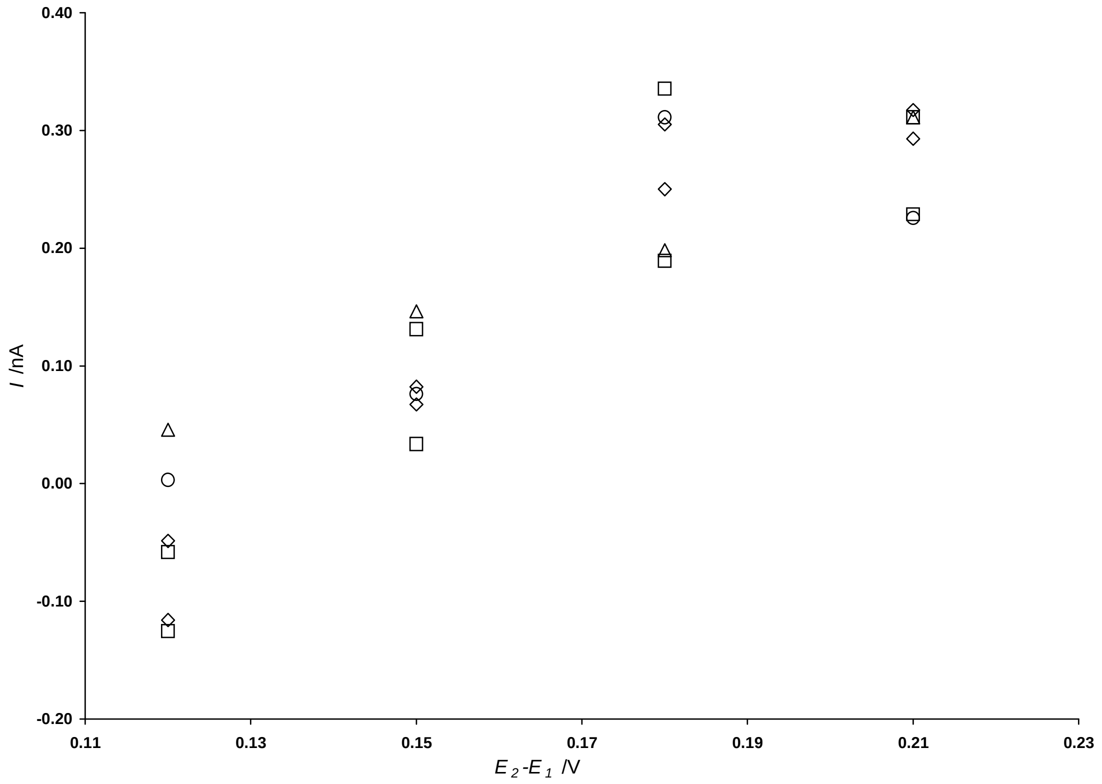

Fig 2: Currents measured at t_2=0.2 s for different potential differences (E_2-E_1) between the first potential step and the second potential step of the implementation of AGNES with only one potential step in the deposition stage (see Fig 1). Diamonds: Y=0.01; squares: Y=0.0025; triangles: Y=0.01 with added EDTA; circles: Y=0.0025 with added EDTA. The presence or absence of the ligand is not relevant in the measured shifted blank, while the value of E_2-E_1 is a crucial parameter in the shifted blank current.

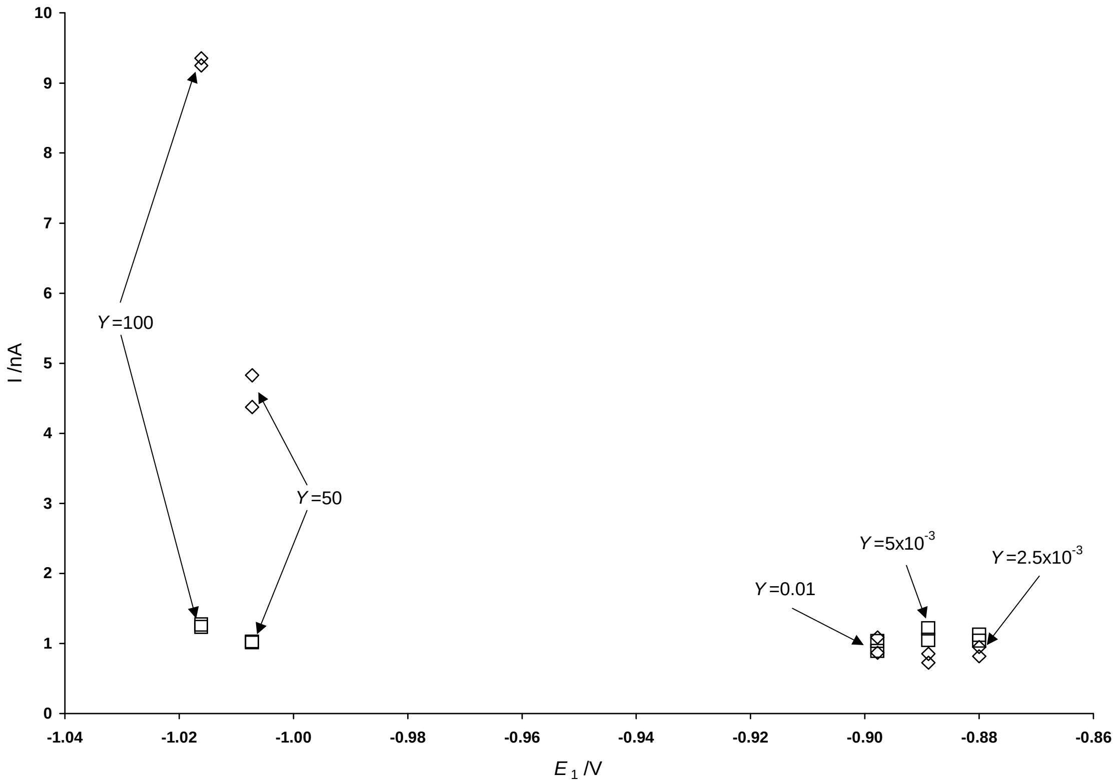

Fig 3: Current measured at t_2=0.2 s for different deposition potentials while keeping a fixed E_2-E_1=0.1982 V. Diamonds: KNO_3 0.1 M (without any EDTA); squares: same solution after adding EDTA 2×10^{-5} M.

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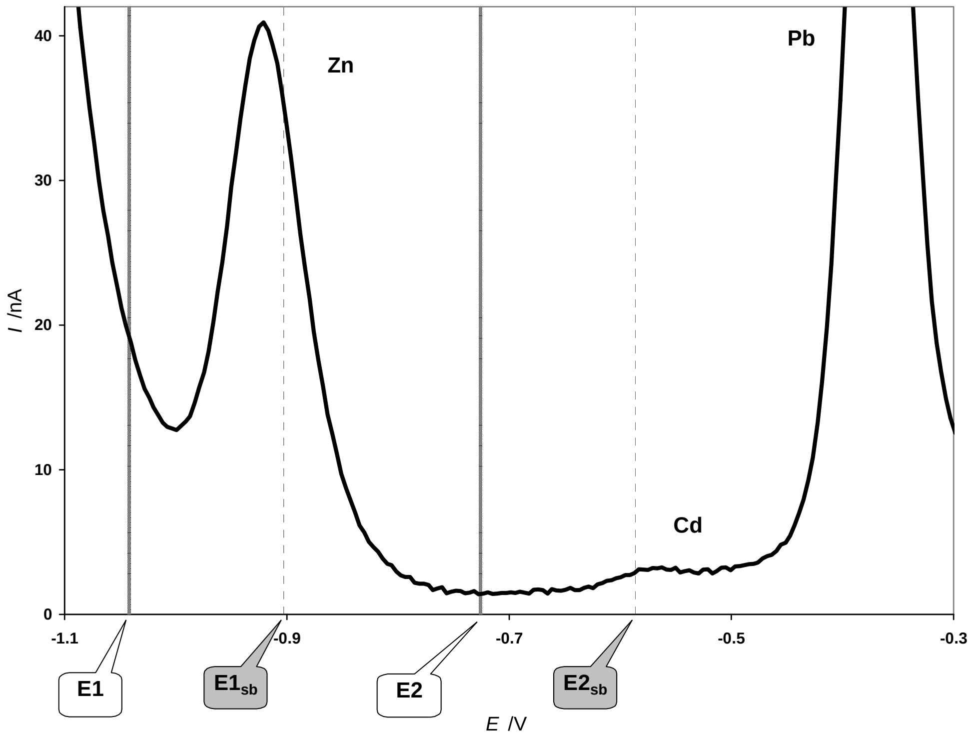

Fig 4: Anodic stripping voltammogram corresponding to a sample containing Zn, but also traces of Cd and Pb (non-analytes). The difference between potentials in the measurement (E_1 and E_2) must be the same as in the shifted blank ($E_{1,\mathrm{sb}}$ and $E_{2,\mathrm{sb}}$) and $E_{2,\mathrm{sb}}$ should not produce the reoxidation of other non-analytes (Cd and Pb in this work).

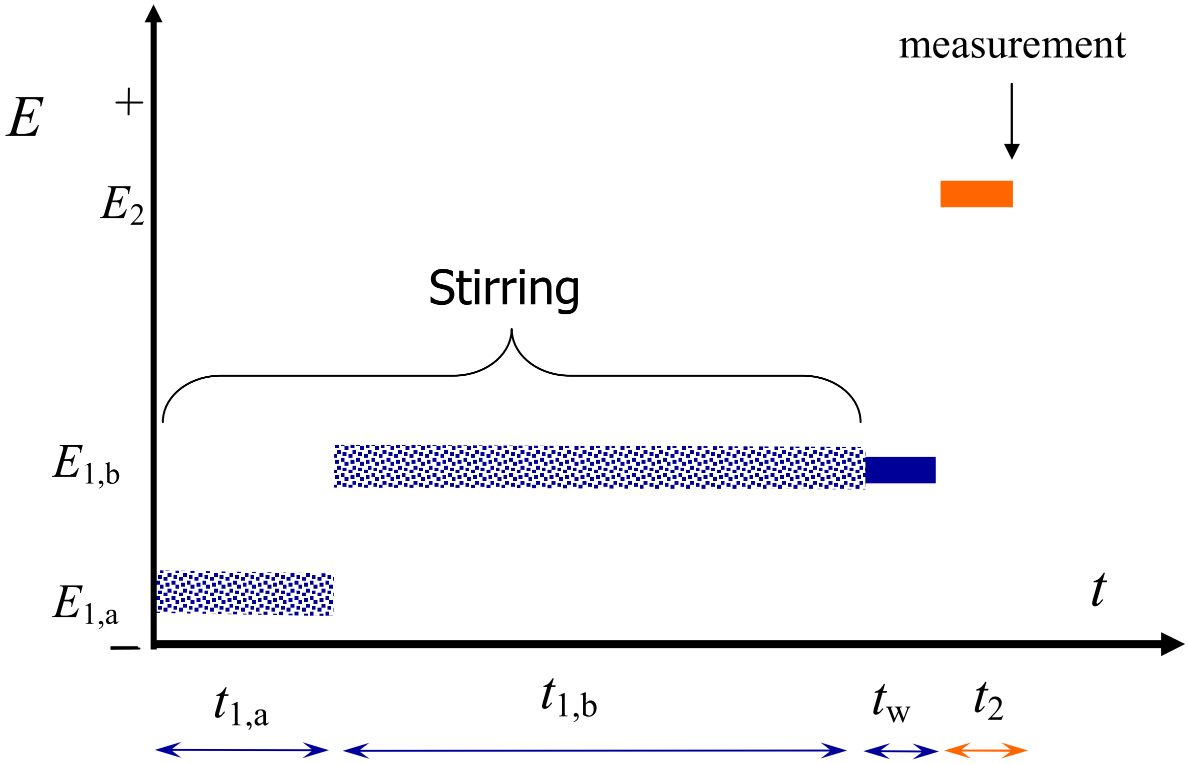

Fig 5: Potential program for AGNES with 2 potential steps. The total time of the first stage (t_1) comprises a short period under diffusion limited conditions ($t_{1,a}$), a "fine tunning" pre-concentration period ($t_{1,b}$) with the desired gain Y and a "waiting" period (t_w) without stirring. The aim of this program is to allow for shorter deposition times.

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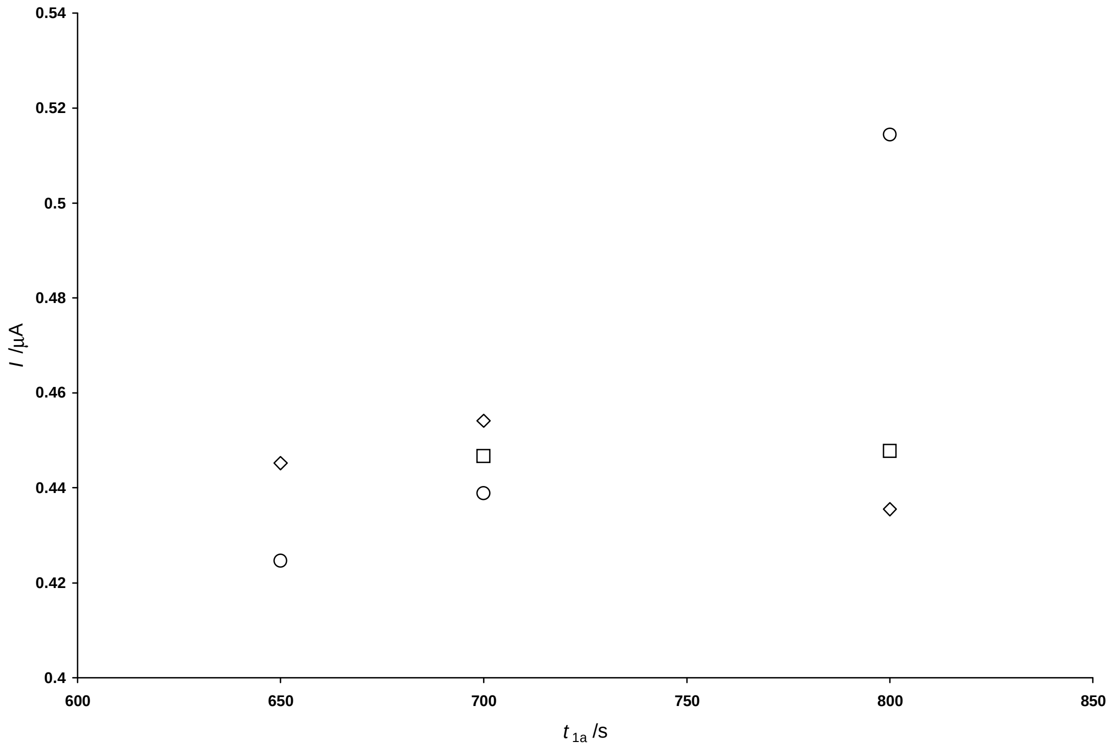

Fig 6: Currents measured at t_2=0.25 s for different combinations of $t_{1,a}$ and $t_{1,b}$ in a solution with $[Zn^{2+}]=3.71\times10^{-7}$ M in KNO_3 0.5 M . Markers: open circle $t_{1,b}$=50 s; open square $t_{1,b}=t_{1,a}$; open diamond $t_{1,b}= 3\times t_{1,a}$

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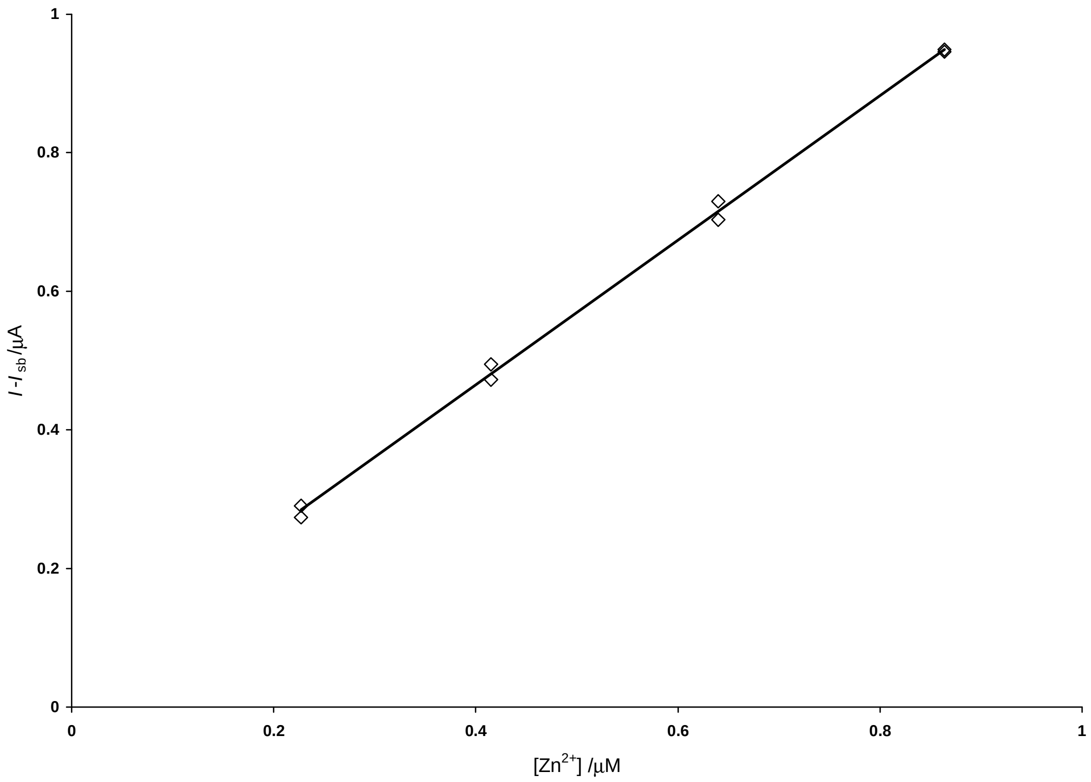

Fig 7: Calibration plot for Zn^{2+} in KNO_3 0.5 M. Parameters: $t_{1,a}$ = $t_{1,b}$ =700 s; $Y_{1,a}$=10^{10}; $Y_{1,b}$=500; Y_2=10^{-8}. From the slope we derive h= 1.04 A M^{-1} to be used in a speciation test on Zn+NTA (see Fig 8).

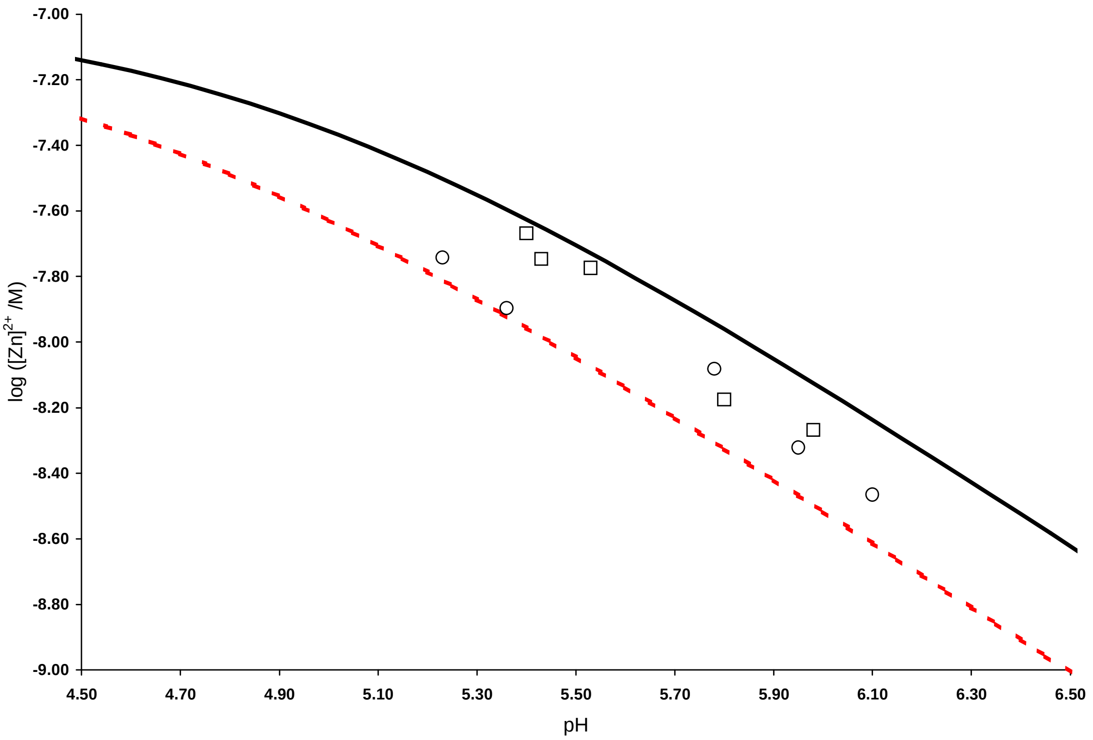

Fig 8 : Theoretical and experimental free Zn concentrations in a synthetic solution of Zn+NTA at different pH-values. Continuous line: MEDUSA calculations; dashed line: MINTEQ calculations; circle and square markers are experimental results obtained with AGNES in two different experiments carried out different days.

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