

Measurement of Free Zinc Concentration in Wine with AGNES

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AGNES (absence of gradients and Nernstian equilibrium stripping), a voltammetric technique recently introduced to measure free metal concentration in solution and checked with different natural and synthetic aqueous media, has been applied here to determine free Zn concentration in wine. The content of ethanol in a solution increases its viscosity, and, so, the diffusion coefficient decreases. Another added effect in ethanolic solutions is the increase of the activity of the metal ions, due to the decrease of the permittivity in the alcoholic medium with respect to the aqueous one. With this taken into account, a specific methodology has been developed to apply AGNES in ethanolic media. A relevant point in this methodology has been the introduction of a new kind of blank, the EDTA blank, able to be applied in the same natural sample and with the same potential program. The free Zn concentrations of the two wines analyzed, a red and a white Raimat wine, were $4.5(2) \times 10^{-7}$ and $7.2(4) \times 10^{-7}$ M, respectively. These represent around 5% of the total Zn content. In the wine samples analyzed, it was checked that intermetallic formation of Zn–Cu does not affect the measurement of free Zn in a significant way.

KEYWORDS: Wine; free Zn concentration; ethanol; AGNES; stripping analysis; heavy metals; speciation

INTRODUCTION

Metals play an important role in wines acting as promoters of yeast formation (1). They also influence the nutritional characteristics such as the stability, color, and clarity of wines. Therefore, the role of some of these metals has been studied in depth as they affect the organoleptic characteristics of wine (2). It has been pointed out that zinc causes the persistence of the sour taste (1). An excess content of some mineral substances may cause turbidity [e.g., Cu and Fe (3) or Al and Zn (4, 5)] and may be harmful to human health (5, 6) [e.g., Pb poisoning due to homemade wine consumption (7)]. Consequently, monitoring of the content and chemical form of potentially relevant elements can be of great interest.

An assessment of the role of metals in wine or any food or beverage requires an understanding of its speciation distribution among the chemical forms (2, 8). For instance, in environmental studies there is general consensus that typically metal bioavailability and toxicity are not governed by its total concentration, but mostly by its free form.

There are a limited number of studies concerning the speciation of metals in wines. Wine is a very complex matrix, and the accurate determination of particular chemical forms of metals in wine is a formidable analytical challenge (9). Differential pulse anodic stripping voltammetry and stripping potentiometry have been used for the simultaneous determination of total and labile metal (6, 10–17). Several chro-

matographic studies also discussed ion complexation in wine samples (18, 19).

The determination of free metal concentrations has been up to now almost restricted to the use of ion selective electrodes (ISE) (20, 21), which—in their commercial form—are generally limited to situations when the total metal concentration is above 1 μ M (22). The fact that there are no commercially available ISE for some species (such as Zn) can be one reason for the lack of data on the free Zn in wine. Therefore, alternative approaches to probe free Zn concentration with conventional equipment can be useful.

Recently (23), we have designed a new voltammetric stripping technique, AGNES (absence of gradients and Nernstian equilibrium stripping), aimed at the determination of free metal ion concentration with standard voltammetric equipment. One potential advantage of the new technique relies on its simple interpretation, given that the meaning of many current determinations of the “labile metal” concentrations obtained experimentally is not completely defined (or known). In more recent work, we have shown that AGNES can determine free Zn concentrations in simple ligand solutions (oxalate and nitrilotriacetic acid) (24), in Mediterranean seawater samples (25, 26), and in samples containing humic acids (25). A comparison has been carried out to validate the technique with other electroanalytical [ISE and SSCP (27)] or non-electroanalytical [RT (28)] techniques.

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It is the aim of this work to develop a new methodology to apply the AGNES technique to determine the free Zn concentration in an ethanolic medium in the prototypical case of wine.

MATERIALS AND METHODS

Reagents and Wine Samples. Zinc stock solution was prepared from $\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Merck, analytical grade) and standardized by means of a complexometric end point titration with EDTA (29). Zn, Cu, and Cd solutions were prepared by dilution of a standard 1000 ppm solution (Merck).

Potassium nitrate was used as the inert supporting electrolyte and prepared from solid KNO_3 (Merck, Suprapur). Ethanol absolute (Panreac, 99.9% UV-IR-HPLC quality) was used to prepare ethanolic solutions for calibrations.

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 of the experiments. Purified water-saturated nitrogen $\text{N}_2(50)$ was used for deaeration and blanketing of solutions.

H_2O_2 30% (Panreac, p.a.) and HNO_3 69% (Panreac, p.a.) were both used for the digestion procedure to determine total metal concentrations.

Two commercially available Spanish wines were used, a red wine Raimat Abadia (2003) 13% and a white wine Raimat Chardonnay (2006) 13.5%. Once opened, the wines were stored at 4 °C until required for further analysis.

Instrumentation. Voltammetric measurements were carried out with Eco Chemie Autolab PGSTAT12 and PGSTAT10 potentiostats attached to Metrohm 663VA stands being controlled from 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 catalog 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 $\text{Ag}/\text{AgCl}/3 \text{ mol L}^{-1} \text{ KCl}$, encased in a jacket containing $0.05 \text{ mol L}^{-1} \text{ KNO}_3$ and 13.25% ethanol.

A glass combined electrode (Orion 9103) was attached to an Orion Research 720A ion analyzer and introduced in the cell to control the pH. A glass jacketed cell provided by Metrohm was used in all measurements. The vessel was thermostated at 25.0 °C.

A microwave 1200 Milestone was used for sample digestion prior to the determination of total Zn concentration, performed with an ICP-OES Activa Horiba Jobin-Yvon. The flow conditions for plasma gas, auxiliary gas, and nebulizer gas were 13, 0.3, and 1 L/min, respectively. Power was set at 1200 W. The wavelength used for Zn was 213.8 nm. Standard solutions containing 2% HNO_3 were prepared with appropriate dilutions of a standard of 1000 ppm of Zn (Horiba Jobin-Yvon) to cover the concentration range.

Procedures. Electroanalytical Techniques. *AGNES.* The potential program of AGNES (23–26, 28, 30) consists of two stages: reduction of Zn^{2+} at the electrode surface and amalgamation or first stage and reoxidation or second stage. The deposition stage aims at reaching a situation of no concentration gradient while keeping a fixed ratio of the electroactive couple concentrations due to the Nernstian equilibrium (determined by the applied potential E_1)

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

where F is the Faraday, R the gas constant, T the temperature, and E^0 the standard formal potential of the redox couple of the metal M. c_{Zn^0} and c_{Zn} refer to the final homogeneous concentration value inside the mercury electrode and in the solution, respectively, and γ_{M} and γ_{M^0} refer to the activity coefficients of the free metal in solution and of the reduced metal, respectively.

The preconcentration factor Y can be determined from E_{peak} , the potential peak of a differential pulse polarography (DPP) experiment (23), which—for a planar electrode—can be computed as

$$E_{\text{peak}} = E^0 + \frac{RT}{nF} \ln\left(\frac{D_{\text{Zn}}}{D_{\text{Zn}^0}}\right)^{1/2} - \frac{\Delta E_{\text{DPP}}}{2} \quad (2)$$

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 the pulse amplitude of the DPP experiment. Combining eqs 1 and 2, we obtain

$$Y = \sqrt{\frac{D_{\text{Zn}}}{D_{\text{Zn}^0}}} \exp\left[-\frac{nF}{RT}\left(E_1 - E_{\text{peak}} - \frac{\Delta E_{\text{DPP}}}{2}\right)\right] \quad (3)$$

In the simplest implementation of AGNES only one potential step is applied along the deposition time t_1 , whereas stirring is on during a time $t_1 - t_w$ (i.e., t_w is the resting time). To reduce the deposition time, another potential program has been proposed (which we call 2P or “2 pulses”): this consists of splitting the first stage into two different substages (see **Table 1**): a potential step corresponding to diffusion limited conditions, $Y_{1,a}$, during a time $t_{1,a}$ followed by a potential step at the desired concentration gain Y , also denoted $Y_{1,b}$ during a time $t_{1,b}$. This strategy has proven to reduce the deposition time needed to reach the target situation of the first step (24).

The second stage aims at quantifying the concentration of M^0 reduced in the amalgam. For that purpose, a simple strategy consists of applying a sufficiently less negative potential (E_2) producing a stripping current under diffusion-limited conditions. The current at a certain time t_2 is the response function of AGNES (for more details see refs 23 and 24).

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

$$I = hc_{\text{M}}^* \quad (4)$$

(which can also be seen as the sensitivity of the method). As the measured current contains other components different from the faradaic one, there is a need to subtract a blank from the total current.

The current I_2 at a certain time t_2 after the application of the reoxidation potential E_2 (once the blank has been subtracted) is the output of AGNES when the residual current I_∞ (the limit at long enough t_2) is negligible. When the residual current is not negligible (as in the case of wine), we have to subtract this value from the I_2 measured at a time t_2 .

Other Voltammetric Techniques. Standard voltammetric techniques other than AGNES are also used in this work. Differential pulse polarography (DPP) is applied to the drop of the Metrohm 663 VA stand set to size 3 and with a drop time 1 s, a scan rate of 0.9 mV s^{-1} , a pulse time of 50 ms, and $\Delta E_{\text{DPP}} = 50 \text{ mV}$. Anodic stripping voltammetry (ASV) is applied to the drop of the Metrohm 663 VA stand set to size 1 and with deposition potential -1.3 V during 30 s, 15 s of equilibration time, drop time of 1 s, scan rate of 6.9 mV s^{-1} , and pulse time of 50 ms.

KNO_3 (0.05 M) was added to all synthetic solutions to keep an ionic strength similar to that computed for wine (31, 32).

Pretreatment. Sample Digestion. For total concentration determinations, an acid digestion procedure in a microwave was followed (6). Two milliliters of the wine sample was introduced in a Teflon vessel and digested with a mixture of 1 mL of concentrated HNO_3 and 0.5 mL of H_2O_2 . After 2 h at room temperature, the vessel was covered and put into a microwave according to the program specified in ref 6. After cooling, the resulting solution was transferred into a 10 mL volumetric flask and made up to the final volume with ultrapure water.

RESULTS AND DISCUSSION

Effect of Ethanol on Electroanalytical Techniques. Prior to the determination of the free metal concentration in wine, we have performed some experiments to determine the impact of the ethanolic medium in different standard electroanalytical techniques.

The presence of ethanol in a Zn solution results in a shift in the potentials of the DPP peak, as can be observed in **Figure**

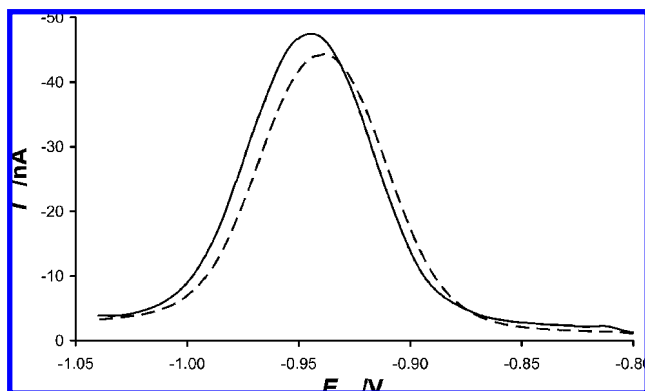


Figure 1. DPP for an aqueous solution (continuous line) and for an ethanolic solution (12% v/v) (dashed line) of 1×10^{-6} M Zn and 0.1 M KNO_3 .

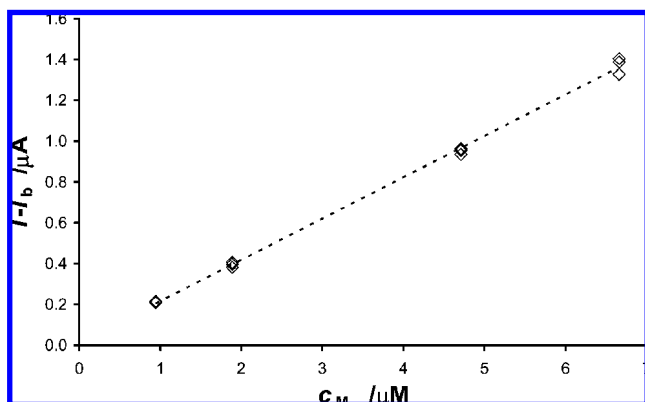


Figure 2. Faradaic current in terms of the free metal concentration for a Zn calibration experiment in an aqueous solution with 0.05 M KNO_3 . Parameters: $Y_{1,a} = 1.11 \times 10^8$, $Y_{1,b} = 111$, $Y_2 = 1.11 \times 10^{-8}$, $t_{1,a} = 70$ s, $t_{1,b} = 210$ s, $t_2 = 0.2$ s. From the slope we get a w/h value of 0.202.

1. The potential of the DPP peak has become 6.4(4) mV more positive, and a reduction of the peak current of 10(7)% has also been quantified. The observed effects had been previously reported by Arévalo et al. (33) and Matsuyama et al. (34), who stated that the addition of ethanol increases the viscosity of the solution, yielding a lower diffusion coefficient. This effect can be explained through the Stokes–Einstein equation

$$D_M = \frac{kT}{6\pi\eta r} \quad (5)$$

where D_M is the diffusion coefficient, η is the viscosity, and r is the radius of the assumed spherical particle).

Reasonably, the reduction of the diffusion coefficient implies a reduction of the current of the DPP peak. Quantitatively, the maximum peak current (in uncomplicated solutions) is proportional to the free metal concentration linearly with $\sqrt{D_M}$ (23)

$$I_{\text{peak}} = nFA\sqrt{\frac{D_M}{\pi t_p}} c_{T,M} \quad (6)$$

where I_{peak} is the peak current of a DPP peak, t_p is the pulse time, and $c_{T,M}$ is the total metal concentration when there are ligands in the system. This equation is acceptable only for fully labile complexes with the same diffusion coefficient as for the metal ion or in the case of no complexation. Following ref 35, we assume here that there is a negligible complexation between Zn and ethanol at this low ethanolic content. If such complexation was discovered and quantified,

Table 1. Outline of the Potential Program of a Simple Implementation of AGNES (1 POTENTIAL) and the Strategy of Splitting the First Stage into Two Potential Steps (2 POTENTIALS) (24)

AGNES	first stage	second stage
1 POTENTIAL	Y_1 during $t_1 - t_w$ stirring	Y_2 during t_2 no stirring
2 POTENTIALS	$Y_{1,a}$ during $t_{1,a}$ stirring	$Y_{1,b}$ during $t_{1,b}$ no stirring

Table 2. Diffusion Coefficients for Zn and Cd in Aqueous and Alcoholic Media

	this work		literature values	
	without EtOH	with EtOH (12%), 0.1 M KNO_3	without EtOH, 0.1 M KCl	with EtOH (10%), 0.1 M KCl
$D_M(\text{Cd}) / \text{m}^2\text{s}^{-1}$	7.30×10^{-10a}	5.81×10^{-10}	7.11×10^{-10b}	5.20×10^{-10b}
$D_M(\text{Zn}) / \text{m}^2\text{s}^{-1}$	7.03×10^{-10a}	6.19×10^{-10}	7.09×10^{-10c}	5.37×10^{-10c}

^a Data from ref 42. ^b Data from Arévalo et al. (43). ^c Data from Arévalo et al. (44).

our results could be reinterpreted in terms of the found stability constant.

Applying expression 6 to data from solutions with and without ethanol, we can easily obtain a new relationship that allows us to determine the diffusion coefficient of the metal in ethanolic media in terms of the peak currents with and without ethanol

$$\frac{w I_{\text{peak}}}{\text{EtOH} I_{\text{peak}}} = \sqrt{\frac{w D_M}{\text{EtOH} D_M}} \quad (7)$$

where the presubscripts w and EtOH refer to aqueous and ethanolic media, respectively. Using this expression, we were able to determine the diffusion coefficient of the metal in an ethanolic solution (see **Table 2**). We performed this measurement also in Cd solution to compare this procedure with values available in the literature. Notice the good agreement of the values of the D_M of Zn and Cd obtained with those reported previously.

We expect that the reduction of the diffusion coefficient in ethanolic media increases the time needed to reach AGNES conditions (for the same gain) with respect to an aqueous solution, as the entrance of metal to the electrode is slowed.

The shift of the DPP peak (see **Figure 1**) toward more positive potentials, apart from the decrease in the diffusion coefficient, can also be attributed to the increase of the activity of the metal ions in solution, which can be explained in terms of the decrease of the permittivity in the alcoholic medium with respect to the aqueous one (36–38).

We can derive an expression for the calculation of the activity coefficients in ethanolic media from the shift of the DPP peak (39) by combining eq 2 applied to both solutions

$$w E_{\text{peak}} - \text{EtOH} E_{\text{peak}} = \frac{RT}{nF} \ln \frac{w \gamma_M}{\text{EtOH} \gamma_M} + \frac{RT}{nF} \ln \left(\frac{w D_M}{\text{EtOH} D_M} \right)^{1/2} \quad (8)$$

where E_{peak} stands for the peak potential of the DPP and γ_M for the activity coefficient of M.

By using expression 8, we computed the ratio $\text{EtOH} \gamma_M / w \gamma_M$, which resulted in 1.40(7) obtained through four replicates of DPP experiments performed with a Zn solution with and without ethanol. The activity coefficient in ethanol is higher than in

aqueous media, and this means a larger gain for a fixed potential applied, E_1 (see eq 1).

The same effects observed in DPP have also been observed in ASV (data not shown).

Procedure To Determine Free Metal Concentration in an Ethanolic Solution with AGNES. For the determination of the free metal concentration with AGNES in a given medium (ethanolic in this work), we need to know the proportionality factor h defined in eq 4 in this particular medium. We have developed the following specific methodology:

1. Obtain the proportionality factor w_h at a known gain wY through a calibration in an aquatic medium, as usual in previous works (23–26, 28) (see **Figure 2**).

2. Compute the gain in the ethanolic medium, $E_{\text{EtOH}}Y$. We would need the standard formal potential E^{of} in this medium, given that eq 1 for an ethanolic medium can be written as

$$E_{\text{EtOH}}Y = \frac{c_{\text{M}^0}^*}{c_{\text{M}}^*} = \exp\left[-\frac{nF}{RT}(E_1 - E_{\text{EtOH}}E^{\text{of}})\right] \quad (9)$$

We can avoid the explicit determination of $E_{\text{EtOH}}E^{\text{of}}$ by relating the gain with the peak potential in the ethanolic solution (as surrogate of the wine matrix for the activity coefficient contribution). By combining equations for an aqueous solution, eqs 8 and Y, we have the following expression for $E_{\text{EtOH}}Y$

$$E_{\text{EtOH}}Y = \sqrt{\frac{E_{\text{EtOH}}D_{\text{M}}}{wD_{\text{M}}}} \exp\left[\frac{nF}{RT}\left(E_1 - E_{\text{EtOH}}E_{\text{peak}} - \frac{\Delta E}{2}\right)\right] \quad (10)$$

3. Obtain the proportionality factor $E_{\text{EtOH}}h$ to be used in the measurement. Due to the fact that the presence of an aqueous solution or an ethanolic solution is irrelevant for the diffusion inside the amalgam, we know that the factor h/Y is constant (23). Thus, we can compute the proportionality in ethanolic solution $E_{\text{EtOH}}h$ from the information found in previous steps 1 and 2:

$$E_{\text{EtOH}}h = E_{\text{EtOH}}Y \left(\frac{w_h}{wY}\right) \quad (11)$$

Free Zn Determination in Wine. Selection of the Parameters. The application of AGNES in a specific medium requires an adequate selection of times and potentials (e.g., the gain Y has to be moderate) to set up the potential program. To determine a combination of safe parameters to measure c_{Zn} in wine, we performed preliminary tests: (i) applying AGNES with only 1 potential in the first step with $Y_1 = 50$ and $Y_2 = 10^{-8}$ and at different t_1 ; (ii) applying AGNES with two potentials in the first step, $Y_{1,\text{a}} = 10^{10}$, $Y_{1,\text{b}} = 50$, $Y_2 = 10^{-8}$, and $t_{1,\text{a}} = 5$ s and at different $t_{1,\text{b}}$; and (iii) applying AGNES with two potentials in the first step, $Y_{1,\text{a}} = 10^{10}$, $Y_{1,\text{b}} = 50$, $Y_2 = 10^{-8}$, and $t_{1,\text{a}} = 2.5$ s and at different $t_{1,\text{b}}$.

In **Figure 3** we plot the current measured in terms of the deposition time for our red wine. Notice that the experiments with only one potential in the first step (see marker +) needed a very long time to reach AGNES conditions (for a moderate Y such as 50). Indeed, the times needed to reach the target of AGNES with $Y = 50$ to determine Zn in wine (around 1000 s) appear to be much longer than the ones to determine Zn in hydroalcoholic solutions (around 600 s). Probably, this behavior can be ascribed to the irreversibility of Zn induced by some components of wine, as has also been previously reported (40). To check this irreversibility phenomenon, we performed two AGNES experiments with and without stirring in the deposition

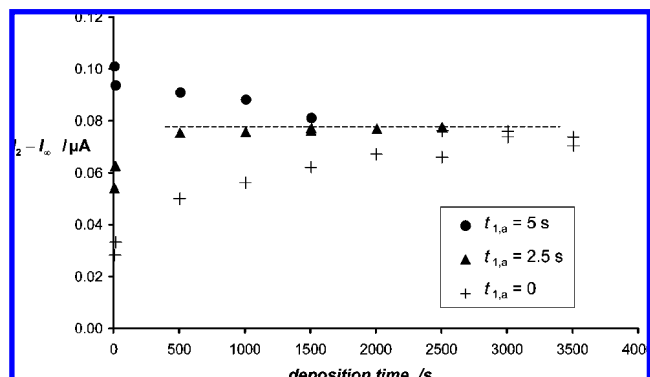


Figure 3. Reoxidation current I_2 (measured with respect to the residual current I_{∞}) versus the deposition time ($t_{1,\text{a}} + t_{1,\text{b}} + t_w/10$) for the red wine Raimat Abadia using different $t_{1,\text{a}}$ values. The continuous dashed line has been drawn to approximately show the current obtained when AGNES conditions are attained.

step (see **Figure 4**). The fact that the currents I_2 obtained from both experiments were almost the same, 3.94×10^{-7} and 3.90×10^{-7} A, respectively, is consistent with our hypothesis of irreversibility, because the limiting step for deposition is the electron transfer at the electrode surface and not diffusion (which is the usual limiting step). To avoid the need of waiting so a long time, we performed experiments with two potentials in the first stage. The added short time under diffusion-limited conditions greatly enhanced the metal supply to the drop. The decrease of the current with $t_{1,\text{b}}$ observed in **Figure 3**, for $t_{1,\text{a}} = 5$ s (bullets), indicates that there has been “overshoot”. Overshooting appears when the potential step corresponding to $Y_{1,\text{a}}$ has been applied for too long of a time $t_{1,\text{a}}$ and an excess of metal enters the mercury electrode; therefore, we need also a sufficiently long ($t_{1,\text{b}}$) second potential step at the desired gain Y to avoid a too large c_{Zn^0} by the end of the deposition stage. To avoid this excess of metal in the drop that afterward has to be reoxidated, a better choice is to apply a $t_{1,\text{a}}$ of 2.5 s. In this case a much shorter time $t_{1,\text{b}}$ seems to be enough to attain the constant value (depicted with a continuous dashed line in **Figure 3**). Therefore, we have taken $t_{1,\text{a}} = 2.5$ s and $t_{1,\text{b}} = 1000$ s with $Y_{1,\text{a}} = 10^{10}$ and $Y_{1,\text{b}} = 50$ as safe parameters to determine free Zn in wine.

As an additional check of the validity of the parameters optimized to apply AGNES to the wine with $Y = 50$, we performed also the experiment at $Y = 100$ (data not shown). With a sufficiently long deposition time, we obtained for $Y = 100$ a faradaic current twice the one obtained at $Y = 50$. The proportionality of the current with the gain Y lends support to the validity of our measurement with $Y = 50$.

Blank. A crucial step in the application of AGNES is the subtraction of the blank to the measured current to obtain the faradaic current. The classical or synthetic blank, which consists of the application of the same potential program, but to a solution containing the same matrix without metal, is not realistic in a complex matrix such as wine.

To avoid the matrix problems with the synthetic blank, we developed another blank (25), which can be applied to the same sample, but with a shift in the potentials, in a potential region where all but the analyte is sensed (for details see ref 25). The main problem that can limit the use of this blank is the presence of other electroactive species in the region of the shifted potentials that were not present in the potentials region of the analyte.

To check the validity of the shifted blank used in this work we have developed a third kind of blank, which we call the

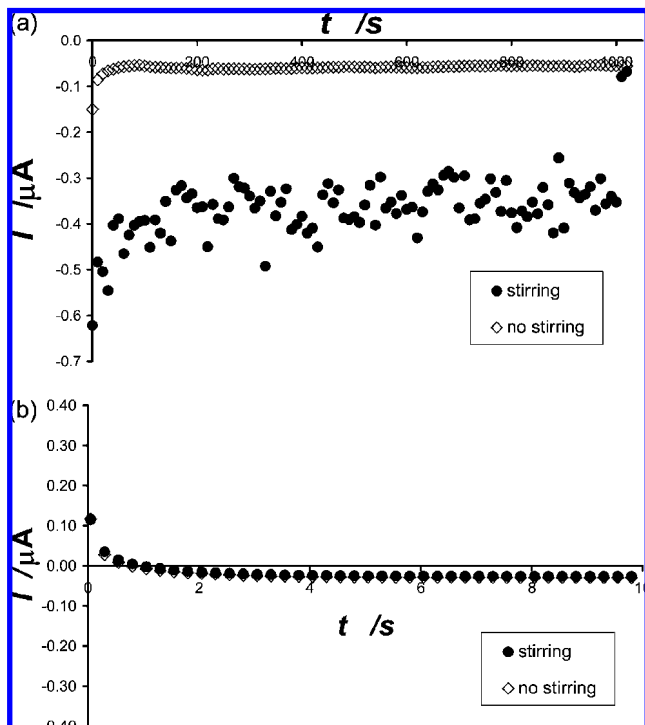


Figure 4. Current versus time for the first (a) and second stages (b) of AGNES for the red wine Raimat Abadia with and without stirring during the deposition time, with $t_1 = 1000$ s and $Y = 100$. Only one of each five experimental points is depicted for the sake of clarity.

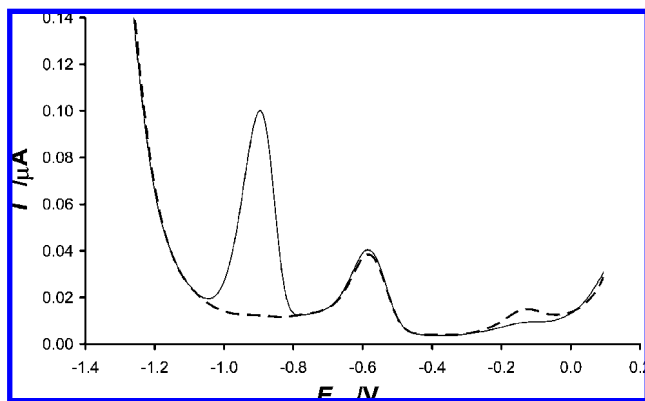


Figure 5. Anodic stripping voltammogram corresponding to the red wine before (continuous line) and after (dashed line) the addition of EDTA in order to complex the free Zn.

EDTA blank. It can also be applied in the same sample, but after the addition of a certain amount of a strong complexant, enough to drastically cut down the free metal in solution. The absence of free metal when EDTA is added can be followed with the performance of an ASV to the sample (see **Figure 5**). In this way, when we apply (to the sample with added EDTA) the AGNES potential program (i.e., the same that we applied to the untreated sample), the expected faradaic component due to the free analyte is practically null.

For comparison purposes, in this work we have performed the shifted blank and the EDTA blank to all of the samples. The good agreement between both kinds of blanks leads us to conclude that both can be applied to our wine samples (see **Figure 6**).

Free and Total Zn Concentration in Red and White Wines. To determine the free Zn in wine by AGNES, we used the previously selected parameters $t_{1,a} = 2.5$ s and $t_{1,b} = 1000$ s

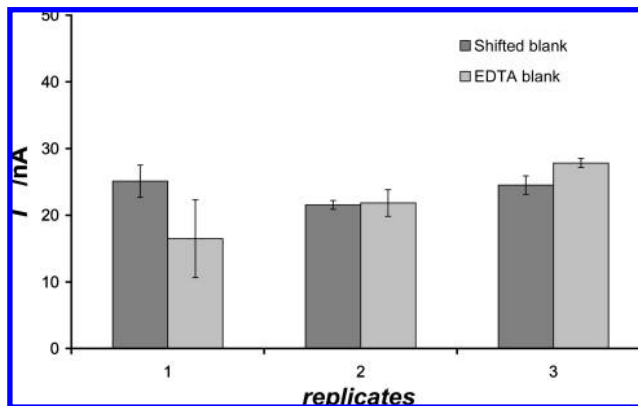


Figure 6. I_2 currents obtained for the shifted blank (dark gray) and the EDTA blank (light gray) corresponding to the three replicate experiments performed with red wine.

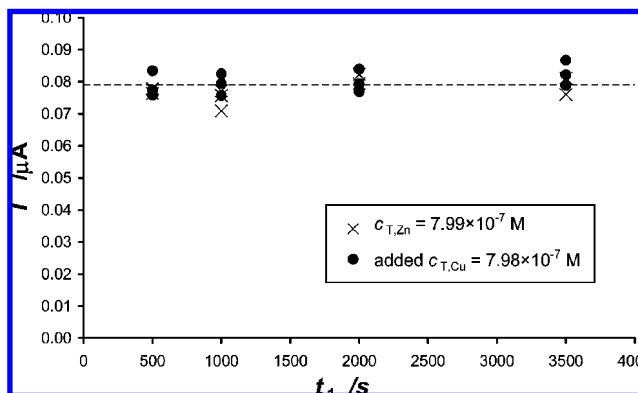


Figure 7. Reoxidation current I_2 versus the deposition time for an aqueous solution containing only 7.99×10^{-7} M Zn and for the same solution after an addition of 7.98×10^{-7} M Cu, for $Y_1 = 50$ and $Y_2 = 10^{-8}$. The ionic strength was given by 0.05 M KNO_3 . The dashed line has been drawn to show the average current obtained when AGNES conditions are attained and shows no significant effect of the Cu addition.

Table 3. Free Zn in Red and White Wine Measured with AGNES and the Total Concentration Determined by ICP-OES^a

	red wine Raimat Abadia	white wine Raimat Chardonnay
c_{Zn}/M (AGNES, $n = 3$)	$4.5(2) \times 10^{-7}$	$7.2(4) \times 10^{-7}$
$c_{\text{T,Zn}}/\text{M}$ (ICP-OES, $n = 3$)	$1.06(2) \times 10^{-5}$	$9.4(5) \times 10^{-6}$
% free Zn	4.3(2)	7.6(6)

^a Numbers in parentheses indicate the standard deviation and refer to the last significant digit. n stands for the number of samples of wine analyzed. AGNES measurements were repeated four to six times on each sample.

with $Y_{1,a} = 10^{10}$ and $Y_{1,b} = 50$. For each set of replicates of each sample of red or white wine, we performed a quick check of the suitability of the settings by applying also an AGNES experiment with a shorter and longer time ($t_{1,b} = 50$ s and $t_{1,b} = 2000$ s). A constant value for both $t_{1,b} = 1000$ s and $t_{1,b} = 2000$ s indicated that the selected parameters were correct. **Table 3** gathers the values obtained.

The total Zn concentration was determined with an ICP-OES after digestion of the wine samples in a microwave, as commented in a previous section (see **Table 3**). This leads to percentages of free Zn of 4.3(2) and 7.6(6)% in red and white wines, respectively. The lack of literature values on free Zn in wine does not allow us to perform a comparison.

These found percentages can be seen as consistent with the optimized $t_{1,a}$ of 2.5 s (see **Figure 3**). Indeed, a rough estimation

(see eq 7 in ref 28) of the supply assuming a fully labile mixture with the same diffusion coefficient as the free metal in ethanol (e.g., due to a predominant complexation of Zn with tartrate), a thickness of the diffusion layer $\delta = 10^{-5}$ m, and a ratio of free to total metal of 0.05 yields $t_{1,a} = 1.9$ s, which is of the same order of magnitude as the optimized 2.5 s.

From both samples analyzed we observe a larger Zn complexation in red wine than in white wine, which can be due to the major content of polyphenols, which have been reported to be crucial for Zn complexation in wines (41).

Intermetallic Formation of Zn–Cu. It has been reported that problems may arise in several voltammetric techniques in the determination of Zn when Cu is present in the solution due to the intermetallic formation of Zn–Cu (17). This interference could lead to a lower c_{Zn} measured.

To check if the results obtained with AGNES in our conditions were affected by this problem, we performed an experiment at the highest Zn (free) concentration measured in our two wine samples, that is, 8×10^{-7} M, at different deposition times (also the long times used in our determinations with wine) and the same experiments, but with the addition of Cu at the (total) level of Cu^{2+} found in wine by ICP-OES, that is, 8×10^{-7} M. From the results obtained and plotted in Figure 7, we conclude that no difference has been observed due to intermetallic formation of Zn–Cu, when Cu is added to the Zn solution. Notice that the reoxidation current measured is practically the same at the different deposition times tested and also when Cu is added.

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LITERATURE CITED

- Pereira, C. F. The importance of metallic elements in wine—a literature survey. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 295–300.
- Pohl, P. What do metals tell us about wine? *Trends Anal. Chem.* **2007**, *26*, 941–949.
- Karadjova, I.; Izgi, B.; Gucer, S. Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry. *Spectrochim. Acta B—At. Spectrosc.* **2002**, *57*, 581–590.
- Galani-Nikolaki, S.; Kallithrakas-Kontos, N.; Katsanos, A. A. Trace element analysis of Cretan wines and wine products. *Sci. Total Environ.* **2002**, *285*, 155–163.
- Galani-Nikolaki, S.; Kallithrakas-Kontos, N. Elemental content of wines. In *Mineral Components in Foods*; Szefer, P.; Nriagu, J. O., Eds.; CRC Press, Taylor and Francis: Boca Raton, FL, 2007.
- Brainina, K. Z.; Stozhko, N. Y.; Belysheva, G. M.; Inzhevato, O. V.; Kolyadina, L. I.; Cremisini, C.; Galletti, M. Determination of heavy metals in wines by anodic stripping voltammetry with thick-film modified electrode. *Anal. Chim. Acta* **2004**, *514*, 227–234.
- Mangas, S.; Visvanathan, R.; van Alphen, M. Lead poisoning from homemade wine: a case study. *Environ. Health Perspect.* **2001**, *109*, 433–435.
- Pyrzynska, K. Analytical methods for the determination of trace metals in wine. *Crit. Rev. Anal. Chem.* **2004**, *34*, 69–83.
- Karadjova, K.; Izgi, B.; Gucer, S. Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry. *Spectrochim. Acta B* **2002**, *57*, 581–590.
- Henze, G.; Bauer, K. H. Voltammetric analysis of wine. *Chem. Unserer Zeit* **2004**, *38*, 46–54.
- Misiego, A. S.; Carra, R. M. G. M.; Carracedo, M. P. A.; Sánchez-Simón, M. T. G. Electroanalytical determination and fractionation of copper in wine. *J. Agric. Food Chem.* **2004**, *52*, 5316–5321.
- Buldini, P. L.; Cavalli, S.; Sharma, J. L. Determination of transition metals in wine by IC, DPASV-DPCSV, and ZGFAAS coupled with UV photolysis. *J. Agric. Food Chem.* **1999**, *47*, 1993–1998.
- Garrido, J.; Ayestarán, B.; Fraile, P.; Ancín, C. Influence of prefermentation clarification on heavy metal lability in Garnacha must and Rosé wine using differential pulse anodic stripping voltammetry. *J. Agric. Food Chem.* **1997**, *45*, 2843–2848.
- Green, A. M.; Clark, A. C.; Scollary, G. R. Determination of free and total copper and lead in wine by stripping potentiometry. *Fresenius' J. Anal. Chem.* **1997**, *358*, 711–717.
- Wiese, C.; Schwedt, G. Strategy for copper speciation in white wine by differential pulse anodic stripping voltammetry, potentiometry with an ion-selective electrode and kinetic photometric determination. *Fresenius' J. Anal. Chem.* **1997**, *358*, 718–722.
- Arcos, M. T.; Ancin, M. C.; Echeverria, J. C.; Gonzalez, A.; Garrido, J. J. Study of lability of heavy-metals in wines with different degrees of aging through differential-pulse anodic-stripping voltammetry. *J. Agric. Food Chem.* **1993**, *41*, 2333–2339.
- Daniele, S.; Baldo, M. A.; Ugo, P.; Mazzocchin, G. A. Determination of heavy-metals in real samples by anodic-stripping voltammetry with mercury microelectrodes. 1. Application to wine. *Anal. Chim. Acta* **1989**, *219*, 9–18.
- Szpunar, J.; Pellerin, P.; Makarov, A.; Doco, T.; Williams, P.; Medina, B.; Lobinski, R. Speciation analysis for biomolecular complexes of lead in wine by size-exclusion high-performance liquid chromatography inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectrom.* **1998**, *13*, 749–754.
- Weber, G. Speciation of iron using HPLC with electrochemical and flame-AAS detection. *Fresenius' J. Anal. Chem.* **1991**, *340*, 161–165.
- Buhlmann, P.; Pretsch, E.; Bakker, E. Carrier-based ion-selective electrodes and bulk optodes. 2. Ionophores for potentiometric and optical sensors. *Chem. Rev.* **1998**, *98*, 1593–1687.
- Bakker, E.; Buhlmann, P.; Pretsch, E. Carrier-based ion-selective electrodes and bulk optodes. 1. General characteristics. *Chem. Rev.* **1997**, *97*, 3083–3132.
- Serrano, N.; Díaz-Cruz, J. M.; Arino, C.; Esteban, M.; Puy, J.; Companys, E.; Galceran, J.; Cecilia, J. Full-wave analysis of stripping chronopotentiograms at scanned deposition potential (SSCP) as a tool for heavy metal speciation: theoretical development and application to Cd(II)-phthalate and Cd(II)-iodide systems. *J. Electroanal. Chem.* **2007**, *600*, 275–284.
- Galceran, J.; Companys, E.; Puy, J.; Cecilia, J.; Garcés, J. L. AGNES: a new electroanalytical technique for measuring free metal ion concentration. *J. Electroanal. Chem.* **2004**, *566*, 95–109.
- Companys, E.; Cecilia, J.; Codina, G.; Puy, J.; Galceran, J. Determination of the concentration of free Zn^{2+} with AGNES using different strategies to reduce the deposition time. *J. Electroanal. Chem.* **2005**, *576*, 21–32.
- Galceran, J.; Huidobro, C.; Companys, E.; Alberti, G. AGNES: a technique for determining the concentration of free metal ions. The case of Zn(II) in coastal Mediterranean seawater. *Talanta* **2007**, *71*, 1795–1803.
- Companys, E.; Puy, J.; Galceran, J. Humic acid complexation to Zn and Cd determined with the new electroanalytical technique AGNES. *Environ. Chem.* **2007**, *4*, 347–354.
- Domingos, R. F.; Huidobro, C.; Companys, E.; Galceran, J.; Puy, J.; Pinheiro, J. P. Comparison of AGNES (absence of gradients and Nernstian equilibrium stripping) and SSCP (scanned stripping chronopotentiometry) for trace metal speciation analysis. *J. Electroanal. Chem.* **2008**, *617*, 141–148.
- Alberti, G.; Biesuz, R.; Huidobro, C.; Companys, E.; Puy, J.; Galceran, J. A comparison between the determination of free Pb(II) by two techniques: absence of gradients and Nernstian equilibrium stripping and resin titration. *Anal. Chim. Acta* **2007**, *599*, 41–50.
- Vogel, A. I. *Textbook of Quantitative Chemical Analysis*, 6th ed.; Pearson Education: Harlow, U.K., 2000.

- (30) Huidobro, C.; Companys, E.; Puy, J.; Galceran, J.; Pinheiro, J. P. The use of microelectrodes with AGNES. *J. Electroanal. Chem.* **2007**, *606*, 134–140.
- (31) Bradshaw, M. P.; Prenzler, P. D.; Scollary, G. R. Square-wave voltammetric determination of hydrogen peroxide generated from the oxidation of ascorbic acid in a model wine base. *Electroanalysis* **2002**, *14*, 546–550.
- (32) Vasconcelos, M. T.; Azenha, M.; de Freitas, V. Electrochemical studies of complexation of Pb in red wines. *Analyst* **2000**, *125*, 743–748.
- (33) Arevalo, A.; Acosta, J. Polarographic study of Zn(Hg)-Zn(2+). I. Limiting current in presence of KOH and NaClO₄. *An. Quim.* **1974**, *70*, 478–483.
- (34) Matsuyama, G. Ph.D. Thesis, University of Minnesota, 1948.
- (35) Fedorov, V. A.; Belevantsev, V. I. About complex formation in mixed water–organic solvents. *J. Mol. Liq.* **2003**, *103*, 83–98.
- (36) Motonaka, J.; Konishi, H.; Ikeda, S.; Tanaka, N. Behavior of the cadmium ion-selective electrode in alcohol water mixtures. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 737–740.
- (37) Koryta, J.; Dvorak, J.; Kavan, L. *Principles of Electrochemistry*, 2nd ed.; Wiley: Chichester, U.K., 1993.
- (38) Kalidas, C.; Hefter, G.; Marcus, Y. Gibbs energies of transfer of cations from water to mixed aqueous organic solvents. *Chem. Rev.* **2000**, *100*, 819–852.
- (39) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*; Wiley: New York, 1980.
- (40) Esparza, I.; Salinas, I.; Santamaria, C.; Garcia-Mina, J. M.; Fernandez, J. M. Electrochemical and theoretical complexation studies for Zn and Cu with individual polyphenols. *Anal. Chim. Acta* **2005**, *543*, 267–274.
- (41) Esparza, I.; Santamaria, C.; García-Mina, J. M.; Fernández, J. M. Complexing capacity profiles of naturally occurring ligands in Tempranillo wines for Cu and Zn. An electroanalytical approach for cupric casse. *Anal. Chim. Acta* **2007**, *599*, 67–75.
- (42) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 77th ed.; CRC Press: Boca Raton, FL, 1996.
- (43) Arevalo, A.; Tejera, E.; Vivo, A. Polarographic studies in water-ethanol media. I. Diffusion properties of Cd(II) and Pb(II) ions. *An. Quim.* **1974**, *70*, 7–14.
- (44) Arevalo, A.; Gonzalez, S.; Dominguez, B. Polarographic studies in water-ethanol media. 3. Diffusion properties of Zn(II). *An. Quim.* **1975**, *71*, 655–661.

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