

Determination of the Complexing Capacity of Wine for Zn Using the Absence of Gradients and Nernstian Equilibrium Stripping Technique

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S Supporting Information

ABSTRACT: The complexing capacity of synthetic (0.011 M tartrate in 13.5% ethanol) and real wine (*Raimat Abadia*) in titrations with added total Zn concentrations up to 0.03 M has been determined following the free Zn concentrations with AGNES (absence of gradients and Nernstian equilibrium stripping) technique. A correction to find the preconcentration factor or gain (Y_1) really applied at each one of the ionic strengths reached due to Zn additions along the titration has been applied. The standard implementation of AGNES to real wine led to the observation of two anomalous behaviors: (a) an increasingly negative current in the deposition stage (labeled as “HER” effect) and (b) a minimum in the currents of the stripping stage plot (labeled as the “dip” effect). A practical strategy to apply AGNES avoiding the dip effect has been developed to quantify properly free Zn concentrations. The van den Berg–Ružic–Lee linearization method (assuming the existence of just 1:1 complexes) has been adapted to consider the dilution effect and the ionic strength changes. Aggregated stability constants and total ligand concentrations have been calculated from synthetic and wine titration data. The found complexing capacity in the studied wine ($c_{T,L} = 0.0179 \pm 0.0007$ M) indicates the contribution of ligands other than tartrate (which is confirmed to be the main one).

KEYWORDS: AGNES, complexing capacity, Zinc, free metal, wine, tartrate

INTRODUCTION

Wine is a complex matrix consisting of a large number of organic and inorganic compounds, which are responsible for wine quality.^{1–3} Trace amounts of heavy metals are present in the inorganic fraction. The analysis of their content and speciation is of interest, since they play an important role in the fermentative process.^{4,5} Fe, Zn, Mn, and Cu are needed in low quantities by yeast (due to their catalytic activity).^{1,2,6} They influence the stability, color, and organoleptic quality of wine.^{7,8} Specifically, Zn is related with the persistence of a sour taste in wine.³ Hue color ranging from orange to purple in red wines is conferred by the presence of polyphenolic compounds, mainly, anthocyanins.⁹ Polyphenols have shown an important complexing activity on metals, and a color modification due to the distortion of the natural existing equilibrium of metal–polyphenol complexation has been reported.¹⁰ It is well-known that toxic/nutritional effects are attributed to the free form of the metals, which can actively interact with sites of biological ligands. Hence, there is a need to develop and improve analytical techniques for speciation studies in complex samples like wine.¹¹

Some authors have tackled the complexation of metal ions with organic molecules, which act as ligands in wine, for example, polyphenols and anthocyanins,^{12–14} but a detailed consideration of the complexation of Zn with tartaric acid is lacking. In addition, electroanalytical techniques have been applied to real wine.^{10,15–18} For instance, Esparza et al.¹⁰ found labile fractions of Zn and Cu by applying ASV to diluted wine in acetate buffer 1:10; Vasconcelos et al.¹⁵ determined various fractions of Pb and Cu in white and red wines using AAS (atomic absorption spectroscopy), ISE (ion selective electrode), and SWCV (square wave cathodic voltammetry) techniques. In this context, AGNES (absence of gradients

and Nernstian equilibrium stripping) appears as a promising alternative, as it is a robust technique, suitable for the determination of free metal concentrations,^{19,20} especially of Zn (for which there is no commercial ISE).^{20–27} Furthermore, no previous dilution or pretreatment (other than purging) of samples is required.

The aim of this study is to determine the complexing capacity of a red wine toward Zn by applying AGNES directly to it, with minimum disturbance of the natural equilibrium occurring in the matrix. Tartaric acid, which is described as the main complexing agent of metals in red wines,^{10,28} has been chosen as the ligand in a model solution that we will refer to as synthetic wine.

The outline of this work is as follows. We start with the titration of the synthetic wine, extending the implementation of AGNES in hydroalcoholic media reported in ref 20 by also taking into account that the variation of the ionic strength (along the titration) will modify the nominal gain (or preconcentration factor). Then, we describe two anomalous behaviors of the currents in the wine matrix (which we label as the “HER” and “dip” effects) and develop strategies to properly measure free Zn in it. Finally, we adapt the van den Berg–Ružic–Lee^{29–31} linearization method and apply it to synthetic and red wine titration data to determine the complexing capacity.

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MATERIALS AND METHODS

Principles of AGNES. The application of AGNES involves a deposition stage, designed for the attainment of a special equilibrium situation, followed by a stripping stage that allows the quantification of the accumulated metal. In the first stage (deposition or preconcentration stage), a suitable potential program (e.g., E_1 is applied for a time t_1) leads to the absence of gradients in the concentration profiles at each side of the mercury electrode surface and to Nernstian equilibrium. The preconcentration factor or gain Y_j (corresponding to a given applied potential E_j) is the relationship, at the mercury–solution interphase, between reduced metal concentration, $[M^0]_0$, and free metal ion concentration in solution, $[M^{n+}]_0$, prescribed by the Nernst equation

$$Y_j = \frac{[M^0]_0}{[M^{n+}]_0} = \frac{\gamma_{M^{n+}}}{\gamma_{M^0}} \exp\left[-\frac{nF}{RT}(E_j - E^0)\right] = \exp\left[-\frac{nF}{RT}(E_j - E^0)\right] \quad (1)$$

where $\gamma_{M^{n+}}$ and γ_{M^0} are the activity coefficients of species M^{n+} and M^0 , n is the number of exchanged electrons, F is the Faraday constant, R is the gas constant, T is the temperature, E^0 is the standard redox potential, and E^0 is the standard formal potential. For a complete list of symbols, see SI-A in the Supporting Information. By the end of the first stage, we have reached the gain Y_1 , and the bulk concentration of M^{n+} equals the one at the electrode surface.

In practice, the potential E_j associated to a given gain (e.g., Y_1 for deposition, Y_2 for stripping) is determined from the peak potential of a differential pulse polarogram (DPP), E_{peak} , by means of

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

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 is the modulation amplitude defined in the DPP experiment.

Up to date, there are two modalities for the application of this deposition stage. They are known as AGNES one pulse (AGNES 1P) and AGNES two pulses (AGNES 2P). One pulse consists of the application of a potential E_1 during a deposition time t_1 .¹⁹ In the two pulses variant, the first stage is split into two substages with gains $Y_{1,a}$ and $Y_{1,b}$ during deposition times $t_{1,a}$ and $t_{1,b}$, respectively. This first substage at $Y_{1,a}$ under diffusion-limited conditions is added to reduce the deposition time needed to reach equilibrium.^{23,32} According to the evolution of the registered currents in the second substage, two situations can be distinguished: an overshoot or an undershoot (i.e., when there is a higher or lower amount of preconcentrated metal inside the drop than that aimed at). The selection of optimal gains and deposition times to analyze an unknown sample, in the absence of complications such as those described later on, can follow the algorithm described in ref 33.

For the quantification of the amalgamated metal in the second stage (or stripping), the simplest variant consists in the application of a reoxidation constant potential E_2 , under diffusion-limited conditions for a certain time (usually 50 s). From the faradaic intensity current at a fixed time (typically $t_2 = 0.2$ s), the free metal concentration can be computed using

$$I_{\text{faradaic}} = \eta Y_1 [Zn^{2+}] \quad (3)$$

where η is the proportionality factor that only depends on the diffusion process inside the drop and is experimentally determined in a calibration (with known free concentrations in a medium similar to the sample). Extra components of the measured current different than the faradaic one (capacitive current, due to other oxidants and other analytes, etc.) are subtracted with a blank.^{20,24}

Reagents and Wine Samples. Zinc stock solutions were prepared by proper dilution from the Merck 1000 ppm standard solution or from solid $Zn(NO_3)_2 \cdot 4H_2O$ (Merck, analytical grade). Potassium hydrogen L-tartrate (Fluka, analytical grade) was used as a

ligand in hydroalcoholic medium. Ethanol 13.5% was prepared by dilution from ethanol absolute 99.9% (Merck, p.a.). NaOH (1 M, Merck) was added to potassium hydrogen L-tartrate (KHTar) to be dissolved. Suitable volumes of KOH and HNO_3 (Riedel de Haen) 0.1 M were added to adjust the pH to that of the original wine (3.42).

Potassium nitrate (Fluka, Trace Select) was used as the supporting electrolyte. In all of the experiments, ultrapure water (Milli-Q, Millipore) was employed. Nitric acid (69%, Fluka, Trace Select) and hydrogen peroxide (30%, Panreac, p.a.) were used to digest the samples to determine the total Zn concentration. A K-TART kit (Megazyme) was used to determine tartaric acid concentration in red wine.

Commercial bottles of red wine, Raimat Abadia 2007, made from grapes variety Tempranillo, Origin Denomination Costers del Segre (Lleida, Catalonia, Spain), were used in this study. Its alcohol content was 13.5%, and the pH was 3.42.

Instrumentation. Voltammetric measurements were carried out with an Eco Chemie Autolab PGSTAT12 or a μ -Autolab type III potentiostat attached to a Metrohm 663VA Stand and to a computer by means of the NOVA 1.7 (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 ($r_0 = 1.41 \times 10^{-4}$ m), and the largest drop was chosen for differential pulse polarographic (DPP) experiments ($r_0 = 2.03 \times 10^{-4}$ m). The auxiliary electrode was a glassy carbon electrode, and the reference electrodes were Ag/AgCl/3 M KCl and Hg/Hg₂Cl₂/3 M KCl, encased in a 0.1 M KNO_3 jacket, but mentioned potentials here are given versus Ag/AgCl. A glass combined electrode (Orion 9103) was attached to an IONcheck 45 Radiometer analytical ion analyzer and introduced into the cell to control the pH. The pH electrode was calibrated in aqueous medium, and no further correction was applied as the essential point was to have a fixed pH in all hydroalcoholic solutions (including wine) regardless of the Zn addition. A glass-jacketed cell thermostatted at 25.0 °C was used in all measurements. Purified water-saturated nitrogen N_2 (99.999%) was used for deaeration, and a blanketing system of N_2 saturated with hydroalcohol was used to avoid evaporation (see more details of special setting in section SI-B in the Supporting Information) of the solutions along AGNES experiments.

A microwave oven (1200 Milestone) was used to digest wine samples. Inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Analytical Instruments and Activa-S, Horiba Scientific) was used to determine total Zn and Cu contents in digested wine samples. A spectrophotometer (Perkin-Elmer Lambda XLS) and plastic cubets were used to measure the absorbance in tests using K-TART kit to determine the tartaric acid concentration in wine. The VMINTEQ database has been used to predict the speciation in aqueous media.³⁴

Procedures. Determination of Total Metal Concentration. A routine procedure for the analysis of commercial wines,^{20,35} which includes the acid digestion in a microwave oven as pretreatment before ICP-OES analysis, was used to determine total Zn and Cu concentrations.

AGNES Calibration. A calibration in aqueous 0.0560 M KNO_3 (close to the mean ionic strength of the samples along the titration; see Figure 1) was obtained by application of AGNES 1P¹⁹ after different additions of the total Zn concentrations in the range of 1×10^{-6} to 8×10^{-6} M. The applied AGNES parameters are gathered in Table 1. The potentials E_j (corresponding to desired gains Y_j) applied in the different stages were computed (see eq 2) from the DPP peak potential in an aqueous medium with ionic strength $\mu = 0.0560$ M. The normalized proportionality factor η (see eq 3) was retrieved from the slope of the calibration and the applied gain $Y_1 = 20$. Because η parameter only depends on the diffusion process inside the amalgam,¹⁹ it is expected to be a constant value (for a given electrode and fixed t_2) regardless of the medium or the composition of the solution.²⁰ Typical values of η for HMDE are around $2.00 \times 10^{-3} \text{ A M}^{-1}$.^{22,25,36}

In the cell containing the last addition of Zn, pure ethanol was added until reaching the alcohol content in the real red wine (13.5%), and a new DPP was run ($\mu = 0.0491$ M). From its peak potential, the

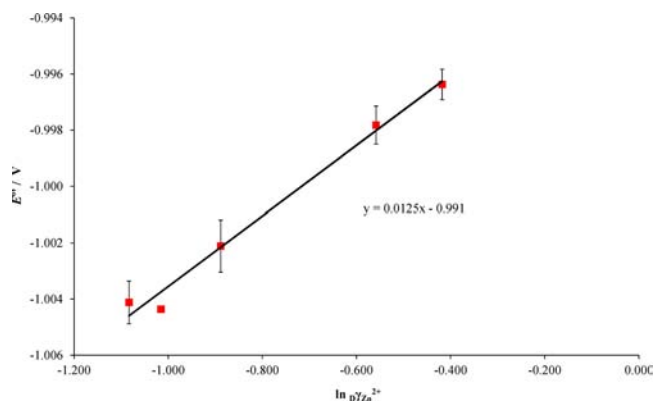


Figure 1. Dependence of $E^{\circ'}$ with $\ln n_D \gamma_{Zn^{2+}}$ (computed from the composition of the sample as if it was an aqueous solution; see eq 8) in 13.5% ethanolic solution ($c_{T,KNO_3} = 0.0100$ M in 13.5% ethanol). Each point corresponds to an average of the standard redox potentials computed with eq 5 from the AGNES charge measured at least in duplicate at each ionic strength in between 0.0102 and 0.139 M reached by the addition of Zn. Vertical lines show the standard deviation. The thick line represents the linear regression.

Table 1. AGNES Settings Used in the Calibration^a

AGNES	Y_1	t_1 (s)	t_w (s)	Y_2	t_2 (s)
shifted blank	0.04	50	50	2×10^{-11}	50
measurement	20	200	50	10^{-8}	50

^aParameters: $D_{Zn^{2+}} = 1.81 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ and $wD_{Zn^{2+}} = 7.03 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$.

potentials E_j (corresponding to the various gains) to be applied in hydroalcoholic medium were computed using (see eq 4 in ref 20):

$$E_j = E_{\text{EtOH}} E_{\text{peak}} + \frac{\Delta E}{2} + \frac{RT}{nF} \ln \left(E_{\text{EtOH}} Y_j \sqrt{\frac{w D_M}{E_{\text{EtOH}} D_M}} \right) \quad (4)$$

where presubscripts EtOH and w indicate the medium: 13.5% ethanolic and aqueous, respectively.

Determination of Free Zn Concentrations Using AGNES. Total Zn concentrations in ranges from 5.03×10^{-7} to 3.66×10^{-2} M and from 1.13×10^{-3} to 4.24×10^{-2} M were attained by the addition of Zn solutions to 50 mL of solution containing 0.011 M KHTar in ethanolic (13.5%) medium and real wine, respectively. After each Zn addition, the pH was adjusted to 3.42. The free metal concentration was determined by application of AGNES 1P¹⁹ and AGNES 2P,²³ as detailed in the Results and Discussion.

In all cases, the current at $t_2 = 0.2$ s was chosen as a response function, and AGNES parameters always sought to yield currents around 5.00×10^{-8} A,²² and at least two replicates were performed. The nominal gains (Y_1) (i.e., those intended with eq 2 and the $E_{\text{EtOH}} E_{\text{peak}}$ in ionic strength $\mu = 0.0491$ M) are detailed in Tables 2 and 3.

RESULTS AND DISCUSSION

Impact of the Ionic Strength on the Really Applied Gain. A titration up to high concentrations of Zn (e.g., more than double the tartrate concentration) involves a substantial change in the ionic strength of this system, where the ionic strength is essentially given by the ligand. A change in ionic strength can have a large impact on the computation of the free metal concentration, because the change in the activity coefficients implies a change in the applied gain when keeping the deposition potential fixed. This can also be seen as the ionic strength affecting each $E^{\circ'}$ (see eq 1). To take into account this

Table 2. AGNES Parameters Applied in the Zn-Tartrate Titration in 13.5% Hydroalcoholic Solution^a

$c_{T,Zn}$ (M)	$Y_{1,a}$	$t_{1,a}$ (s)	$Y_{1,b}$	$t_{1,b}$ (s)	Y_2	t_2 (s)
5.04×10^{-7}						
5.14×10^{-7}	10^{10}	35	50	105		
2.28×10^{-6}						
2.33×10^{-6}	—	—	5	200		
5.23×10^{-6}		35	50	105		
5.34×10^{-6}	10^{10}	13	25	50		
9.03×10^{-6}		35	50	105		
9.22×10^{-6}						
2.04×10^{-5}			5	200		
6.01×10^{-5}						
1.00×10^{-4}			3	100		
2.99×10^{-4}			1	100		
7.04×10^{-4}			0.5	50	10^{-8}	50
1.13×10^{-3}						
1.15×10^{-3}			0.3	50		
5.02×10^{-3}						
5.03×10^{-3}	—	—	0.1	50		
8.00×10^{-3}			0.01	50		30
1.10×10^{-2}			0.01	50		
1.11×10^{-2}						
1.75×10^{-2}			0.01	50/30		
2.50×10^{-2}						
2.99×10^{-2}						
3.01×10^{-2}			0.01	50		

^aGains computed before ionic strength correction (i.e., the nominal gains). Parameters: $E_{\text{EtOH}} D_{Zn^{2+}} = 6.19 \times 10^{-10} \text{ m}^2 \text{ s}^{-120}$ and $wD_{Zn^{2+}} = 7.03 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$.

influence, we determine an experimental $E^{\circ'}$ value for a set of synthetic solutions of known free concentration at various ionic strengths with dedicated AGNES experiments. The $E^{\circ'}$ for each ionic strength can be computed from the combination of Nernst and Faraday laws (see eq 9 in ref 37),

$$E^{\circ'} = E_1 + \frac{RT}{nF} \ln \left(\frac{Q}{n F V_{\text{Hg}} [Zn^{2+}]} \right) \quad (5)$$

where Q is the experimental accumulated faradaic charge up to reach Nernstian equilibrium, V_{Hg} is the electrode mercury volume, and $[Zn^{2+}]$ is the free Zn concentration computed with Visual Minteq.

To interpolate the values of $E^{\circ'}$ (to be used in the samples at the different ionic strengths) from a reduced set of dedicated experiments (in solutions of known free Zn concentration), we look for a simple correlation. The correlation expected in any medium is (see eq 2.1.45 in ref 38)

Table 3. AGNES Parameters Applied in the Zn Titration in Red Wine^a

$c_{T,Zn}$ (M)	$Y_{1,a}$	$t_{1,a}$ (s)	$Y_{1,b}$	$t_{1,b}$ (s)	Y_2	t_2 (s)
1.16×10^{-5} no addition	10^{10}	18	50	50/500/1000		
		9	25			
		1.8	5			
1.13×10^{-3}			5	50/400/800		
			2			
			0.5			
5.03×10^{-3}			1	50/400/800		
			0.5			
			0.01			
1.10×10^{-2}	-	-	0.1	50/400/800	10^{-8}	50
			0.01			
			0.001			
1.80×10^{-2}			0.01	50/400/800		
			0.05			
			0.001			
2.30×10^{-2}	100	1.0	0.1	30/120/500		
		0.1	0.05			
			0.01			
3.41×10^{-2}	100	0.2	0.1	30/120/500		
		0.05	0.05			
		0.03	0.01			

^aGains computed before ionic strength correction. The first total Zn concentration corresponds to that contained in wine (see Table 4). The rest of $c_{T,Zn}$ includes the initial amount of Zn. Same parameters as in Table 2.

$$E^{0'} = E^0 + \frac{RT}{nF} \ln \gamma_{M^{2+}} \quad (6)$$

Unfortunately, the determination of the activity coefficients in hydroalcoholic media would be too time-consuming, so we assay (as a rough approximation) to compute the coefficients in these media as if the medium was water.

$$\ln {}_D\gamma_{Zn^{2+}} = -0.51 \times 2^2 \left(\frac{\sqrt{\mu}}{1 + \sqrt{\mu}} - 0.3\mu \right) \quad (7)$$

That is, the coefficients computed using Davies equation are labeled with subscript D. These coefficients are then used in eq 6. The resulting plot is approximately linear, see Figure 1, so that the following empirical relationship for $E^{0'}$ in terms of $\ln {}_D\gamma_{M^{2+}}$ is suggested:

$$E^{0'} = C_1 + C_2 \ln {}_D\gamma_{M^{2+}} \quad (8)$$

We have found a slope C_2 of around 0.013 V, which is the expected value RT/nF (at 25 °C) in the rigorous eq 6, which suggests a direct proportionality between aqueous and hydroalcoholic activity coefficients in the range of explored conditions.

Possible potential drifts of the reference electrode (in the elapsed time between the dedicated experiments and the calibration) just impact C_1 , which can be re-evaluated from the $E^{0'}$ associated to the E_{peak} obtained in the calibration:

$$E^{0'} = E_{peak} - \frac{RT}{nF} \ln \sqrt{\frac{D_{M^0}}{D_{M^{2+}}}} \quad (9)$$

Once we know C_2 (from eq 8 in dedicated experiments) and C_1 (from eqs 8 and 9), we can interpolate $E^{0'}$ at any μ with eq 8 to be used in the samples with unknown free concentrations.

With the $E^{0'}$ at the μ of the addition, we can compute the corrected gain of the first stage, $Y_{corrected}$, using eq 1. Finally, we compute the free Zn concentration by applying eq 3 using $Y_{corrected}$ in place of Y_1 .

Zn Titration of Synthetic Wine. Prior to the study of the complexation of tartrate with Zn in synthetic and real wine, a total tartaric acid concentration of 0.01128 ± 0.00006 M ($n = 2$) was experimentally determined in Raimat Abadia wine 2007. Hence, the model wine was prepared as close as possible to the real one: a solution 0.011 M in potassium hydrogen tartrate (KHTar), 0.00153 M in sodium hydroxide, and 13.5% (v/v) in ethanol and adjusted to pH 3.42, by addition of acid or base, resulting an ionic strength equal to 0.012 M. The use of NaOH (instead of KOH) is a here-suggested modification to the preparation described in refs 39 and 40 to avoid KHTar precipitation due to its decreased solubility in hydroalcoholic solutions.⁴¹ A simple device (see Figure SI-1 in Supporting Information) was designed to avoid evaporation of the hydroalcoholic solutions.

Pink circle markers in Figure 2 show the Zn titration curve obtained in synthetic wine. Empty and filled markers represent the free Zn concentrations before and after the ionic strength correction, respectively. Two regions, regardless of the application of the correction, can be distinguished: low $c_{T,Zn}$ (where there is an excess of ligand) and high $c_{T,Zn}$ (where there is an excess of free Zn). A break (i.e., sudden change of slope) in the initial linearity of the curve occurs at a total Zn

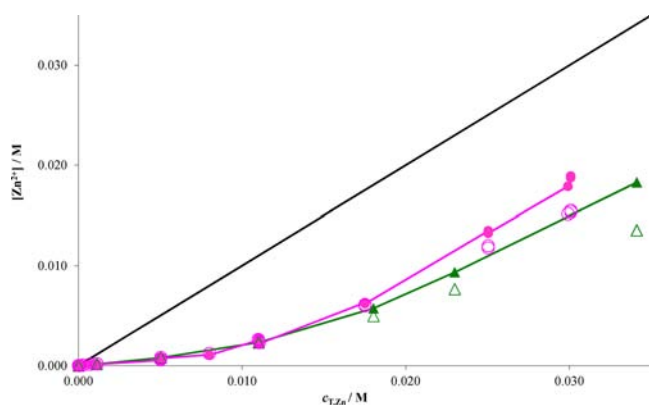


Figure 2. Titration curves of 0.011 M tartrate with Zn obtained with AGNES in (pink filled circle, pink open circle) synthetic (13.5% ethanolic) and (green filled triangle, green open triangle) real wine at pH 3.42. Empty markers represent AGNES free Zn concentrations computed with the nominal gains Y_1 (original data before applying the correction due to the effect of ionic strength change). Full markers represent free Zn concentrations using $Y_{\text{corrected}}$ (see eq 8). (—) Line for free Zn equal to total Zn. The composition of synthetic wine is given by $[\text{KHTar}] = 0.011 \text{ M}$, $[\text{NaOH}] = 0.00153 \text{ M}$, pH 3.42. AGNES parameters are detailed in Tables 2 and 3.

concentration ca. 0.011 M (which is the KHTar concentration). This suggests that Zn reacts with tartrate in 13.5% ethanol (and in the range of used concentrations) mainly following a stoichiometry 1:1, as happens in water solution (where the main complex species are Zn-Tar and Zn-HTar). As expected, the corrected free Zn concentrations (see filled pink circles) align practically parallel (for $c_{\text{T,Zn}} > 0.011 \text{ M}$) to the $[\text{Zn}^{2+}] = c_{\text{T,Zn}}$ line (thick black line), which indicates that each new addition of total Zn becomes free Zn, because all of the ligand is saturated. Additionally, by comparing empty and filled markers, we can see that the correction is larger for higher $c_{\text{T,Zn}}$ because of the larger ionic strength increase at higher additions of Zn (where Zn^{2+} is the ion with the greatest contribution to the ionic strength).

These free Zn^{2+} concentrations in ethanolic medium are lower than those computed with VMINTEQ in aqueous medium with standard constants.⁴² This can be understood from a lower permittivity in alcohol than in water^{43–45} yielding a lower screening of the attractions between Zn and tartrate. See refs 20 and 46 for more details.

Analysis of Real Wine. Implementation of AGNES Technique in Wine. The total concentrations of Zn and Cu (measured with ICP-OES) and of free Zn (measured with AGNES) in different samples from one bottle of untreated wine (i.e., before any Zn addition) are shown in Table 4.

Table 4. Total Zn and Cu Concentrations and Free Zn Concentration Determined with ICP-OES and AGNES, Respectively, for Red Wine Raimat Abadia 2007^a

$c_{\text{T,M}} (\mu\text{M})$		$[\text{Zn}^{2+}] (\mu\text{M})$	% free Zn
Cu	Zn		
9.24 ± 0.10 ($n = 3$)	11.6 ± 0.2 ($n = 3$)	1.49 ± 0.04 ($n = 2$)	12.9 ± 0.4

^aThe used AGNES parameters are detailed in the first row of Table 3. Free Zn corresponds to the concentration obtained after ionic strength correction (eq 8). The number of replicates (different samples on the same bottle of wine) is in between brackets.

The application of AGNES to the analysis of wine with added Zn exhibited deposition and stripping currents with marked differences (see Figure 3) with respect to the typical currents found, for instance, in refs 19, 23, 25, 27, 33, 47, and 48.

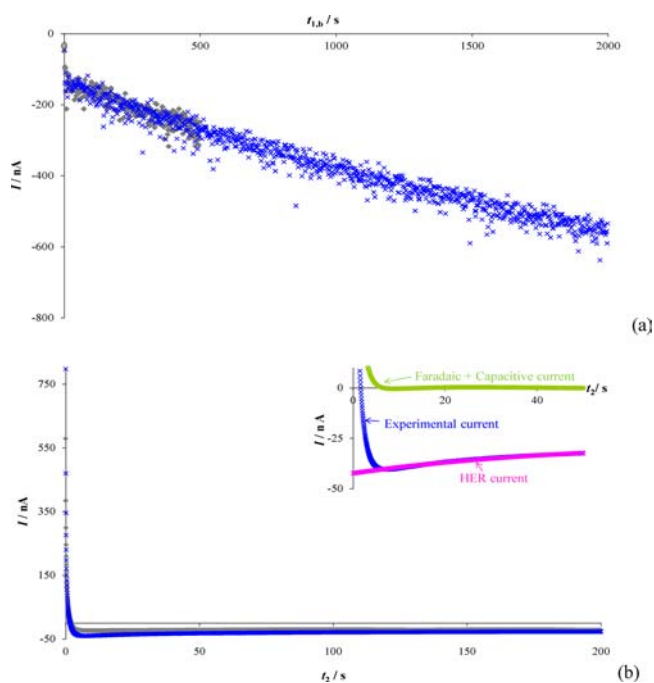


Figure 3. Currents recorded in the (a) first and (b) second stages of the application of AGNES in wine showing HER and dip effects. Two experiments with different deposition times $t_{1,b}$ are represented showing that the dip effect is larger with $t_{1,b} = 2000 \text{ s}$ (blue times sign) than with $t_{1,b} = 500 \text{ s}$ (gray diamond). The inset in b allows one clearly to see the dip effect for $t_{1,b} = 500 \text{ s}$. The inset also depicts the components of the resulting current as a combination of HER (pink times sign) (computed with eq SI-2 in the Supporting Information with $\alpha = 0.0218 \text{ s}^{-1}$ and $d = 15.22 \text{ nA}$) and the faradaic + capacitive current (green open circle), computed with eq SI-4 in the Supporting Information for the experimental currents obtained with $t_{1,b} = 2000 \text{ s}$ (blue crosses) by extrapolation of HER current. Parameters: $c_{\text{T,Zn}} = 3.76 \times 10^{-2} \text{ M}$, $Y_{1,a} = 100$, $t_{1,a} = 0.1 \text{ s}$, $Y_{1,b} = 0.01$, and $t_2 = 200 \text{ s}$.

In the first stage, see Figure 3a, the current decreases steadily (practically with a linear dependence with the deposition time), instead of the typical behavior of an increasingly more positive current tending asymptotically to a stable constant. We attribute this phenomenon to the hydrogen evolution reaction (HER);^{49,50} see SI-C-1 in the Supporting Information for details. When HER has previously appeared in systems other than wine, we did not observe any impact on the analytical signal.

In the second stage, see Figure 3b, the plot I_2 vs t_2 goes through a minimum at relatively long t_2 (around 3–10 s). We label the formation of this minimum as “dip effect”. We also observe that the current due to remaining oxidants in the sample at very long t_2 , that is, residual current $I_{2,\text{exp},\infty}$, is more negative than usual (of the order of -30 to -50 nA in front of the usual -0.2 to -0.6 nA in aqueous medium). In consequence, the dip effect could impact the proper reading of $I_{2,\text{exp},\infty}$ and the response function (i.e., I taken at $t_2 = 200 \text{ ms}$).

Details on the facts affecting the dip effect can be found in SI-C-1 in the Supporting Information, as well as its possible causes. The HER current in the first moments of the second stage can be estimated by extrapolating the behavior in the last section to an assumed exponentially decaying model (see eq SI-2 in SI-C-2 in the Supporting Information). The splitting of the experimental current into the faradaic + capacitive current and a HER interference is schematically depicted in the inset of Figure 3b.

Practical Strategy for the Quantification of Free Zn Concentration with AGNES in Wine. We aim at finding a methodology to obtain a correct determination of free Zn in wine at each total Zn concentration attained in the titration. A possible strategy consists of minimizing the deposition time. So, by reducing the time along which HER develops, we obtain a smaller dip effect. In general, the 2 Pulses modality allows us to reduce the deposition time (even if the more negative potential, i.e., $E_{1,a} < E_{1,b}$, could initially induce a somewhat larger HER).

We distinguish *low* (up to $c_{T,M} = 1.10 \times 10^{-2}$ M) and *high* ($c_{T,M} \geq 1.80 \times 10^{-2}$ M) total Zn concentrations. Low $c_{T,Zn}$ requires higher gains Y_1 and longer deposition times, while high $c_{T,Zn}$ requires smaller Y_1 and shorter deposition times. So, we use 500 s as the maximum deposition time $t_{1,b}$ (applying AGNES 2P) or t_1 (applying AGNES 1P) for high concentrations and 1000 s for low concentrations; in the 2 Pulses experiments, $t_{1,a}$ is negligible in front of 500 s. These strategies, labeled as *low Zn* and *high Zn*, led to very low dip effects. As a further checking, we applied two extra deposition times shorter than 500 or 1000 s for each case (see Table 5)

Table 5. Deposition Times ($t_{1,b}$) Applied in the Strategies of Quantification of Free Zn Concentration in Wine

$t_{1,b}$ (s)	strategy	
	<i>low Zn</i>	<i>high Zn</i>
$t_{1,b,1}$	50	30
$t_{1,b,2}$	400 or 500	120
$t_{1,b,3}$	800 or 1000	500

and computed the average of currents when applying different deposition times. As the currents at the two longer different times did not differ more than 10%, we can consider that AGNES equilibrium has been reached.

For the small dip currents obtained in this work (due to the short deposition times), the difference in the retrieved concentrations using the standard computation of $I_{2,f+c}$:

$$I_{2,f+c} = I_{2,exp}(t_2 = 0.2 \text{ s}) - I_{2,exp,\infty} \quad (10)$$

or a refined one taking into account the HER extrapolation (eq SI-5 in the Supporting Information) is below 1.8%. So, we have considered the correction of HER as negligible in the experimental conditions of these titrations and kept the standard procedure.

Additionally, the global methodology can increase its reliability by comparing two concentrations determined at two different gains $Y_{1,b}$. For that, we applied three $Y_{1,b}$: $Y_{1,b,1} > Y_{1,b,2} > Y_{1,b,3}$ with $Y_{1,b,2} = Y_{1,b,1}/2$ and $Y_{1,b,3} = Y_{1,b,1}/10$. The resulting currents $I_{2,f+c}(t)$ (denoted now $I_{Y_{1,b,j}}$ with $j = 1, 2, \text{ or } 3$, to distinguish the currents obtained by application of the different gains) contain a faradaic component (proportional to the free concentration as prescribed by eq 3) and a capacitive component. The splitting at $t_2 = 0.2$ s yields:

$$I_{Y_{1,b,j}}(t_2 = 0.2 \text{ s}) = I_C^{Y_{1,b,j} \rightarrow Y_2} + Y_{1,b,j} \eta [Zn^{2+}] \quad (11)$$

where $I_C^{Y_{1,b,j} \rightarrow Y_2}$ corresponds to the capacitive current due to the potential jump from the deposition gain ($Y_{1,b,j}$) to the stripping gain (Y_2). By subtracting eq 11 for two deposition gains and assuming the canceling of the capacitive current

$$[Zn^{2+}]_1 = \frac{I_{Y_{1,b,1}}(t_2 = 0.2 \text{ s}) - I_{Y_{1,b,3}}(t_2 = 0.2 \text{ s})}{(Y_{1,b,1} - Y_{1,b,3})\eta} \quad (12)$$

and

$$[Zn^{2+}]_2 = \frac{I_{Y_{1,b,2}}(t_2 = 0.2 \text{ s}) - I_{Y_{1,b,3}}(t_2 = 0.2 \text{ s})}{(Y_{1,b,2} - Y_{1,b,3})\eta} \quad (13)$$

This method of subtracting two currents at relatively close gains (instead of subtracting a blank^{20,24,27}) aims at canceling out the dip effect as much as possible, although we neglect the difference in capacitive current between the signals from these two close gains, for example, the potential from $Y_{1,b} = 50$ ($E_{1,b} = -1.0502$ V) to $Y_2 = 1 \times 10^{-8}$ ($E_2 = -0.7723$ V) jumps 287 mV, while from $Y_{1,b} = 5$ ($E_{1,b} = -1.0207$ V) to $Y_2 = 1 \times 10^{-8}$ ($E_2 = -0.7723$ V) jumps 257 mV. The percentage of difference between free Zn concentrations calculated by means of eqs 12 and 13 is required to be less than 10%. Finally, the resulting free Zn concentration is taken as the average between $[Zn^{2+}]_1$ and $[Zn^{2+}]_2$. The algorithm in Figure SI-8 in the Supporting Information details the two strategies (*high Zn* and *low Zn*) followed to determine Zn in wine. Parameters used in each addition are gathered in Table 3, being the nominal gains computed from DPP peak potential at $\mu = 0.0491$ M (i.e., before the correction for ionic strength change).

Titration of Wine with Zn. Green open triangles in Figure 2 show the free Zn concentrations obtained considering the nominal gain (i.e., before applying the correction due to the variation of ionic strength in the medium), and green full triangles markers represent the concentrations obtained with the correction of ionic strength change (i.e., using $Y_{corrected}$ from eq 8). Notice a larger correction at higher Zn concentrations. Two distinct linear portions are observed with the change of slope in the region 1.1×10^{-2} to 1.8×10^{-2} M. At higher $c_{T,Zn}$, corrected free concentrations tend to align parallel to the line corresponding to absence of ligand, that is, $[Zn^{2+}] = c_{T,Zn}$.

On the other hand, we check here the possible formation of intermetallic compounds Cu_xZn during the application of AGNES, given that they can become interferences. The condition $[Cu^{2+}] < 45 \times [Zn^{2+}]^{22}$ was derived for uncomplicated systems (where the rule $t_1 = 7Y$ applies), while wine is a complex matrix. So, we must estimate the reaction quotient for possible intermetallic compounds to be formed: $CuZn$, Cu_6Zn , and Cu_3Zn in the most favorable case for intermetallic formation (i.e., longest time and highest free Zn concentration).

$[Zn^\circ]$ can be estimated from the measured charge at the higher total Zn concentration in the titration:

$$[Zn^\circ] = \frac{Q}{nFV_{Hg}} \approx \frac{9.63 \times 10^{-7} \text{ C}}{2.27 \times 10^{-3} \text{ CM}^{-1}} = 4.25 \times 10^{-4} \text{ M} \quad (14)$$

$[Cu^\circ]$ can be overestimated assuming all of its complexes are labile²² and highly mobile:

$$[\text{Cu}^\circ] = \frac{(AD_{\text{Cu}}c_{\text{T,Cu}}/\delta)(t_{1,\text{a}} + t_{1,\text{b}})}{V_{\text{Hg}}} \quad (15)$$

where A is the electrode surface area, D_{Cu} is the diffusion coefficient of Cu^{2+} (and all of its complexes) in water, $c_{\text{T,Cu}}$ is the total Cu concentration in wine (see Table 4), δ is the effective diffusion layer thickness, and $t_{1,\text{a}}$ and $t_{1,\text{b}}$ are the deposition times used in AGNES with a $c_{\text{T,Zn}} = 3.41 \times 10^{-2}$ M in the titration of wine (see Table 3). Replacing with the appropriate parameters, we have

$$[\text{Cu}^\circ] = 3.38 \times 10^{-3} \text{ M} \quad (16)$$

Reaction quotients computed using $[\text{Zn}^\circ]$ (eq 14) and $[\text{Cu}^\circ]$ (eq 16) for each stoichiometry are gathered in Table 6. We

Table 6. Solubility Product Constants of $\text{Cu}_x\text{Zn}^{22}$ and Reaction Quotient Computed from $[\text{Cu}^\circ]$ and $[\text{Zn}^\circ]$ Estimated in Wine Assuming Fully Labile and Mobile Complexes and $c_{\text{T,Zn}} = 3.41 \times 10^{-2}$ M (the Highest $c_{\text{T,Zn}}$ Considered in the Titration) and $c_{\text{T,Cu}} = 9.24 \times 10^{-6}$ M (Determined in Wine; See Table 4)

intermetallic Cu_xZn compound	reaction quotient	$K_{\text{Cu}_x\text{Zn}}$
CuZn	$1.44 \times 10^{-6} \text{ M}^2$	$3.30 \times 10^{-6} \text{ M}^2$
Cu_3Zn	$1.65 \times 10^{-11} \text{ M}^4$	$3.10 \times 10^{-11} \text{ M}^4$
Cu_6Zn	$6.41 \times 10^{-19} \text{ M}^7$	$6.20 \times 10^{-18} \text{ M}^7$

compare these values with those of the solubility product constants reported in ref 51, and we see that for the deposition times experimentally used and the total Zn concentrations added to wine along the titration, the formation of intermetallics Cu_xZn is not expected. In addition, we must consider that the reaction quotients are overestimations; for example, in the computation of $[\text{Cu}^\circ]$, we took the diffusion coefficient in water, which is greater than in hydroalcoholic medium.²⁰ The expected lack of intermetallic formation is consistent with the experimental currents ($t_2 = 200$ ms) measured at shorter and longer deposition times differing less than 10%.

Determination of the Complexing Capacity of Wine by Applying the van den Berg–Ružić–Lee Linearization Method. To the synthetic and real wine titration results, we applied a linearization treatment, which will provide (a) an estimation of the total amount of complexing ligand(s), also known as complexing capacity (CC or $c_{\text{T,L}}$) and (b) the binding strength measured through the aggregated conditional stability constant (K_{eff}).

van den Berg,³⁰ Ružić,²⁹ and Lee⁵² proposed a linearizing model for the interpretation curves for 1:1 complex formation mechanism:



where L represents the ligand (which in macromolecules corresponds to the free active sites) and ML represents the complex.

Some modifications have been introduced to the model to adapt it to our real system including aspects derived from the experimental process such as (i) dilution effect due to different additions of Zn and acid or base to adjust the pH, (ii) ionic strength change, and (iii) the formation of two main complexes: ZnTar and ZnHTar (as known for the aqueous speciation):



The adapted expression (see the details in SI-E in the Supporting Information) reads

$$\frac{[\text{Zn}^{2+}]f_{\text{V}}}{c_{\text{T,Zn}} - [\text{Zn}^{2+}](1 + K_{\text{ZnNO}_3^\circ}^\circ \gamma_{\text{Zn}^{2+}}[\text{NO}_3^-])} = \frac{1}{K_{\text{eff}}c_{\text{T,L}}} + \frac{[\text{Zn}^{2+}]}{c_{\text{T,L}}} \quad (20)$$

where f_{V} is the dilution factor, $\gamma_{\text{Zn}^{2+}}$ is the activity coefficient of species Zn^{2+} , $K_{\text{ZnNO}_3^\circ}^\circ$ is the thermodynamic formation constant for ZnNO_3° complex and K_{eff} is the effective constant.

Experimental data obtained from Zn titrations in 13.5% ethanolic media and in wine were treated with the extended model, eq 20. The left-hand term of the equation is taken as ordinate, and $[\text{Zn}^{2+}]$ is taken as the independent variable in Figures 4 and 5, for the two systems studied here: Zn-KHTar in

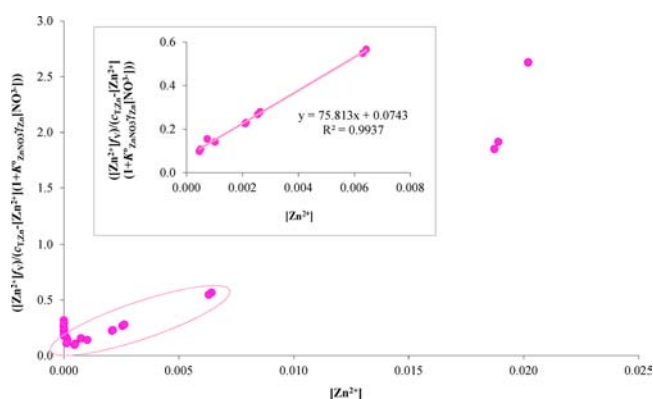


Figure 4. Extended van den Berg–Ružić–Lee plot for a model considering two 1:1 complexes (see eq 20) in the titration of KHTar in 13.5% hydroalcoholic medium (processing the corrected data shown as pink circles in Figure 2). Selected points of this ethanolic titration for the linear regression are enclosed in an oval. The final regression is shown in the inset.

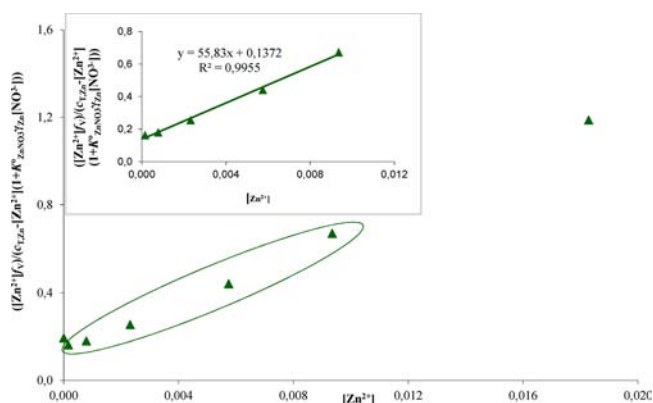


Figure 5. Extended van den Berg–Ružić–Lee plot for a model considering two 1:1 complexes in the titration of wine (processing the corrected data indicated by green triangles in Figure 2). Points of this wine titration selected for the linear regression are enclosed in an oval. The final regression is shown in the inset.

13.5% ethanol solution and Zn in wine. Activity coefficients of Zn^{2+} in water are computed with Davies expression, while activity coefficients in the hydroalcoholic media are computed with eq 8 of ref 20 together with an analogous interpolation procedure as the one used here with eq 8.

From the linear regression, that is, $y = mx + b$, K_{eff} and $c_{\text{T,L}}$ can be computed as:

$$K_{\text{eff}} = \frac{m}{b} (\text{M}^{-1}) \quad (21)$$

$$c_{\text{T,L}} = \frac{1}{m} (\text{M}) \quad (22)$$

From the visual inspection of the obtained van den Berg–Ružic–Lee plots in both media, synthetic and natural (Figures 4 and 5), we notice that some points of the data, at very high and at very low $c_{\text{T,Zn}}$ concentrations ($c_{\text{T,Zn}}$ below 10^{-3} M), lie outside the expected linear region (probably due to the amplification of errors or the presence of other complexes not included in our adaptation), and so, we discard them. The points included in each linear regression are enclosed in an oval, and the final regression is shown in the corresponding inset of each plot.

For the synthetic medium, the computed total concentration of binding sites using this linearization was $c_{\text{T,L}} = 0.0132 \pm 0.0003$ M and the equilibrium constant $\log K_{\text{eff}} = 3.01 \pm 0.03$. For wine, $c_{\text{T,L}} = 0.0179 \pm 0.0007$ and $\log K_{\text{eff}} = 2.61 \pm 0.04$.

We can see that the retrieved $c_{\text{T,L}}$ value in synthetic medium (0.0132 ± 0.0003 M) differs around 20% from the expected one (0.011 M). For the case of real wine, the extended van den Berg–Ružic–Lee model estimates a $c_{\text{T,L}} = 0.0179 \pm 0.0007$ M (while the determined tartrate concentration was also 0.011 M), suggesting that other ligands (probably anthocyanins and polyphenols¹⁴) also contribute to the resulting complexing capacity of this wine.

Regarding the aggregated conditional stability constants, differences less than 15% between synthetic medium ($\log K_{\text{eff}} = 3.01$) and real wine ($\log K_{\text{eff}} = 2.61$) suggest that the complexation strength is mainly due to tartrate, at least for sufficiently high Zn concentrations. At lower total Zn concentrations (as in the nonspiked wine), complexation to ligands other than tartrate might be relevant.

■ ASSOCIATED CONTENT

Supporting Information

Symbols, blanketing system used during AGNES experiments, HER and dip effects: observations and interpretations, estimation of HER current in the second stage and rigorous computation of the faradaic + capacitive current, quantification algorithm, and adapted van den Berg–Ružic–Lee linearization. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

- (1) Karadjova, I.; Izgi, B.; Gucer, S. Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry. *Spectrochim. Acta B* **2002**, *57* (3), 581–590.
- (2) Nicolini, G.; Larcher, R.; Pangrazzi, P.; Bontempo, L. Changes in the contents of micro- and trace-elements in wine due to winemaking treatments. *Vitis* **2004**, *43* (1), 41–45.
- (3) Pereira, C. F. The importance of metallic elements in wine—A literature survey. *Z. Lebensm. Unters. Forsh.* **1988**, *186* (4), 295–300.
- (4) Ibanez, J. G.; Carreon-Alvarez, A.; Barcena-Soto, M.; Casillas, N. Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis. *J. Food Compos. Anal.* **2008**, *21* (8), 672–683.
- (5) Grindlay, G.; Mora, J.; Gras, L.; de Loos-Vollebregt, M. T. C. Atomic spectrometry methods for wine analysis: A critical evaluation and discussion of recent applications. *Anal. Chim. Acta* **2011**, *691* (1–2), 18–32.
- (6) Salinas, I.; Esparza, I.; Gomez, S.; Santamaria, C.; Fernandez, J. M. A study of heavy metal complexation in grape juice. *Electroanalysis* **2005**, *17* (5–6), 469–475.
- (7) Pohl, P. What do metals tell us about wine? *TrAC, Trends Anal. Chem.* **2007**, *26* (9), 941–949.
- (8) Alberti, G.; Guiso, M. G.; Biesuz, R. Usage of Empore (TM) membrane in alcoholic media for copper(II) distribution studies. *Talanta* **2009**, *79* (3), 603–612.
- (9) Marquez, A.; Serratos, M. P.; Lopez-Toledano, A.; Merida, J. Colour and phenolic compounds in sweet red wines from Merlot and Tempranillo grapes chamber-dried under controlled conditions. *Food Chem.* **2012**, *130* (1), 111–120.
- (10) 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* (1–2), 267–274.
- (11) Gonzalez, A.; Armenta, S.; Cervera, M. L.; de la Guardia, M. Non-chromatographic speciation. *TrAC, Trends Anal. Chem.* **2010**, *29* (3), 260–268.
- (12) Bodini, M. E.; del Valle, M. A.; Tapia, R.; Leighton, F.; Berrios, P. Zinc catechin complexes in aprotic medium. Redox chemistry and interaction with superoxide radical anion. *Polyhedron* **2001**, *20* (9–10), 1005–1009.
- (13) Le Nest, G.; Caille, O.; Woudstra, M.; Roche, S.; Guerlesquin, F.; Lexa, D. Zn-polyphenol chelation: Complexes with quercetin, (+)-catechin, and derivatives: I optical and NMR studies. *Inorg. Chim. Acta* **2004**, *357* (3), 775–784.
- (14) Le Nest, G.; Caille, O.; Woudstra, M.; Roche, S.; Burlat, B.; Belle, V.; Guigliarelli, B.; Lexa, D. Zn-polyphenol chelation: Complexes with quercetin, (+)-catechin, and derivatives: II Electrochemical and EPR studies. *Inorg. Chim. Acta* **2004**, *357* (7), 2027–2037.
- (15) Vasconcelos, M. T.; Azenha, M.; de Freitas, V. Electrochemical studies of complexation of Pb in red wines. *Analyst* **2000**, *125*, 743–748.
- (16) Vasconcelos, M. T.; Azenha, M.; de Freitas, V. Role of polyphenols in copper complexation in red wines. *J. Agric. Food Chem.* **1999**, *47* (7), 2791–2796.
- (17) 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* (12), 2333–2339.
- (18) Azenha, M. A. G. O.; Vasconcelos, M. T. S. D. Pb and Cu speciation and bioavailability in port wine. *J. Agric. Food Chem.* **2000**, *48* (11), 5740–5749.
- (19) 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.

- (20) Companys, E.; Naval-Sanchez, M.; Martinez-Micaelo, N.; Puy, J.; Galceran, J. Measurement of free zinc concentration in wine with AGNES. *J. Agric. Food Chem.* **2008**, *56* (18), 8296–8302.
- (21) Chito, D.; Weng, L.; Galceran, J.; Companys, E.; Puy, J.; van Riemsdijk, W. H.; van Leeuwen, H. P. Determination of free Zn²⁺ concentration in synthetic and natural samples with AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique). *Sci. Total Environ.* **2012**, *421–422*, 238–244.
- (22) Chito, D.; Galceran, J.; Companys, E. The Impact of Intermetallic Compounds Cu₂Zn in the Determination of Free Zn²⁺ Concentration with AGNES. *Electroanalysis* **2010**, *22* (17–18), 2024–2033.
- (23) Companys, E.; Cecilia, J.; Codina, G.; Puy, J.; Galceran, J. Determination of the concentration of free Zn²⁺ with AGNES using different strategies to reduce the deposition time. *J. Electroanal. Chem.* **2005**, *576* (1), 21–32.
- (24) 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.
- (25) Galceran, J.; Chito, D.; Martinez-Micaelo, N.; Companys, E.; David, C.; Puy, J. The impact of high Zn⁰ concentrations on the application of AGNES to determine free Zn(II) concentration. *J. Electroanal. Chem.* **2010**, *638*, 131–142.
- (26) Puy, J.; Galceran, J.; Huidobro, C.; Companys, E.; Samper, N.; Garcés, J. L.; Mas, F. Conditional Affinity Spectra of Pb²⁺-Humic Acid Complexation from Data Obtained with AGNES. *Environ. Sci. Technol.* **2008**, *42* (24), 9289–9295.
- (27) Zavarise, F.; Companys, E.; Galceran, J.; Alberti, G.; Profumo, A. Application of the new electroanalytical technique AGNES for the determination of free Zn concentration in river water. *Anal. Bioanal. Chem.* **2010**, *397* (1), 389–394.
- (28) Zoecklein, B.; Fugelsang, K.; Gump, B.; Nury, F. Tartratos e Inestabilidades. In *Análisis y Producción de Vino*; Acribia, S. A., Ed.; Acribia: Zaragoza, España, 2001; pp 237–250.
- (29) Ruzic, I. Theoretical Aspects of the Direct Titration of Natural-Waters and Its Information Yield for Trace-Metal Speciation. *Anal. Chim. Acta* **1982**, *140* (1), 99–113.
- (30) van den Berg, C. M. G. Determination of Copper Complexation with Natural Organic-Ligands in Sea-Water by Equilibration with MnO₂. 1. Theory. *Mar. Chem.* **1982**, *11* (4), 307–322.
- (31) Lee, J. Complexation Analysis of Fresh Waters by Equilibrium Diafiltration. *Water Res.* **1983**, *17* (5), 501–510.
- (32) 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.
- (33) Pernet-Coudrier, B.; Companys, E.; Galceran, J.; Morey, M.; Mouchel, J. M.; Puy, J.; Ruiz, N.; Varrault, G. Pb-binding to various dissolved organic matter in urban aquatic systems: Key role of the most hydrophilic fraction. *Geochim. Cosmochim. Acta* **2011**, *75* (14), 4005–4019.
- (34) *Visual MINTEQ version 3.0*, 2010; www.lwr.kth.se/English/Oursoftware/vminteq/index.htm.
- (35) 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* (2), 227–234.
- (36) Parat, C.; Authier, L.; Aguilar, D.; Companys, E.; Puy, J.; Galceran, J.; Potin-Gautier, M. Direct determination of free metal concentration by implementing stripping chronopotentiometry as second stage of AGNES. *Analyst* **2011**, *136*, 4337–4343.
- (37) Aguilar, D.; Parat, C.; Galceran, J.; Companys, E.; Puy, J.; Authier, L.; Potin-Gautier, M. On the determination of free metal ion concentrations with AGNES in low ionic strength media. *J. Electroanal. Chem.* **2013**, *689*, 276–283.
- (38) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods. Fundamentals and Applications*, 2nd ed.; John Wiley & Sons, Inc.: New York, 2001.
- (39) 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* (7–8), 546–550.
- (40) 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* (6), 711–717.
- (41) Hu, Y.; Chen, X.; Yang, W.; Guan, J. Measurement and correlation of the solubility of urea L-tartaric acid in aqueous ethanol solution. *Thermochim. Acta* **2009**, *484* (1–2), 38–40.
- (42) Torrent-Burgues, J.; Gaus, E. Effect of tartaric acid in the electrodeposition of zinc. *J. Appl. Electrochem.* **2007**, *37* (5), 643–651.
- (43) 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* (3), 737–740.
- (44) Koryta, J.; Dvorak, J.; Kavan, L. *Principles of Electrochemistry*, 2nd ed.; John Wiley: Chichester, United Kingdom, 1993.
- (45) Kalidas, C.; Hefter, G.; Marcus, Y. Gibbs energies of transfer of cations from water to mixed aqueous organic solvents. *Chem. Rev.* **2000**, *100* (3), 819–852.
- (46) Chito, D. Methodological advancements of AGNES and its implementation for the determination of free metal ion concentrations in synthetic and natural samples. Ph.D. thesis; Universitat de Lleida, April, 2012.
- (47) Huidobro, C.; Companys, E.; Puy, J.; Galceran, J.; Pinheiro, J. P. The use of microelectrodes with AGNES. *J. Electroanal. Chem.* **2007**, *606*, 134–140.
- (48) Parat, C.; Aguilar, D.; Authier, L.; Potin-Gautier, M.; Companys, E.; Puy, J.; Galceran, J. Determination of Free Metal Ion Concentrations Using Screen-Printed Electrodes and AGNES with the Charge as Response Function. *Electroanalysis* **2011**, *23* (3), 619–627.
- (49) Doneux, T.; Ostatna, V.; Palecek, E. On the mechanism of hydrogen evolution catalysis by proteins: A case study with bovine serum albumin. *Electrochim. Acta* **2011**, *56* (25), 9337–9343.
- (50) Zivanovic, M.; Aleksic, M.; Ostatna, V.; Doneux, T.; Palecek, E. Polylysine-Catalyzed Hydrogen Evolution at Mercury Electrodes. *Electroanalysis* **2010**, *22* (17–18), 2064–2070.
- (51) Guminski, C.; Galus, Z. *Intermetallic Compounds in Mercury*; Pergamon Press: Oxford, United Kingdom, 1992; Solubility Data Series (IUPAC) Vol. 51.
- (52) Ruzic, I. Trace metal complexation at heterogeneous binding sites in aquatic systems. *Mar. Chem.* **1996**, *53* (1–2), 1–15.