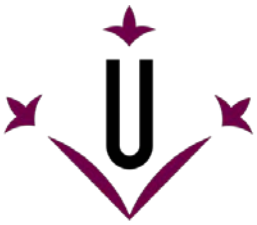

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Speciation of Zn, Fe, Ca and Mg in wine with the Donnan Membrane Technique

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Abstract

Free concentrations of Zn^{2+}, Fe^{3+}, Ca^{2+} and Mg^{2+} in a red wine (Raimat, Catalonia, Spain) have been determined, with the Donnan Membrane Technique (DMT) for the first time. The required equilibration time benefits from the acceptor solution including major cations. K^+ and Na^+, mainly unbound to any ligand in the sample, have been identified as suitable reference ions. A free Zn concentration of 1.76 μmol L^{-1} determined with DMT was in excellent agreement with the free Zn concentration independently provided by the electroanalytical technique Absence of Gradients and Nernstian Equilibrium Stripping (AGNES), 1.7 μmol L^{-1}, amounting to 14.4% of the total Zn. The free concentrations found in this wine were 1.79 μmol L^{-1} Fe^{3+}, 1.11 mmol L^{-1} Ca^{2+} and 3.4 mmol L^{-1} Mg^{2+} (8.82%, 40% and 57% of their total concentrations). Prior to the application of the techniques to the red wine, they had been cross-validated in Zn-tartrate solutions.

Keywords: Donnan Membrane Technique; Absence of Gradients and Nernstian Equilibrium Stripping; Zinc; Iron; free metal; wine

1. Introduction

Speciation usually refers to the distribution of a given element (such as a metal) over a variety of chemical species (such as the hydrated cation, different complexes with different ligands, different redox states, etc.). Great interest is devoted to speciation because it is well known that many biological effects (be toxic or nutritional) do not depend on the total concentration of the element in the medium, but on that of the free form or of a group of species. Bioavailability depends on speciation, because different species of a given element diffuse and react with different rates, and only certain species can be taken up directly.

Speciation of metal elements is also relevant for wine (Ibanez et al., 2008; Pyrzynska, 2007). For instance, Cu (and Fe) speciation is reported to be related to oxidative spoilage and stalling, so various techniques have been employed to pinpoint different forms of these elements in wine (see (Pohl and Sergiel, 2009; Rousseva et al., 2016) and references therein).

Additionally, mineral content of wine has been used to authenticate its region of origin (Coetzee et al., 2014). Free metal concentrations are expected to be more specific properties (i.e. more particular of a wine type) than the total concentration measurements, since they will depend not only on the total mineral content, but also on the concentration of ligands present. Thus, free concentrations are well suited as fingerprint markers, not only of a production region but also as ageing (due to the time evolution of compounds present in wine that act as metal ligands) and quality identifiers, another issue of great commercial relevance.

There is a need for developing and consolidating techniques that can access the free ion concentrations in all kinds of matrices (Feldmann, Salaun and Lombi., 2009; Pesavento et al., 2009). A standard technique for some metals is the use of Ion Selective Electrodes (Bakker and Pretsch, 2007). However, for some other elements, despite intense work (in Zn, (Fu et al., 2012); in Mg (Lamaka et al., 2009); in Fe, (Ali et al., 2015), etc.) there is still no commercial ISE or the commercial electrodes show limits of detection too high for being of interest in certain samples (this might be the case for Cd and Pb in many food matrices). Other techniques that can determine the free metal concentration are the Permeation Liquid Membrane (PLM) (Gramlich et al., 2012) and the Ion-Exchange Technique (Cremazy et al., 2015).

The Donnan Membrane Technique (DMT) (Temminghoff et al., 2000) is a technique designed for the simultaneous determination of various free ion concentrations relying on the selective permeation of cations through an ion exchange membrane. The sample (or donor solution) reaches equilibrium with a synthetic acceptor solution, both in separate compartments. DMT has been extensively used in soils and waters (Jones et al., 2016). A few DMT works have tackled food matrices. For instance, Gao et al. (2009) studied synthetic and reconstituted milk, but, to the best of our knowledge, the use of DMT in wine (or any hydroalcoholic medium) has not yet been reported.

AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) (Galceran et al., 2004) is another technique for determining free metal ion concentrations of amalgamating elements. Some of the systems analysed are seawater (Diaz-de-Alba et al., 2014; Galceran et al., 2007), river water (Parat and Pinheiro, 2015), dispersions of nanoparticles (Galceran et al., 2014), quantum dots (Domingos et al., 2011), clays (Rotureau, 2014), etc. AGNES has been applied to find the free Zn concentration (Companys et al., 2008) and the complexation capacity (Chito et al., 2013) of wine. A recent work (Chito et al., 2012), cross-validating and comparing advantages and drawbacks of DMT and AGNES, dealt with soil extracts and river water.

This study aims to show that DMT can be used to determine the free metal concentrations of Zn, Fe, Ca and Mg in wine. This should pave the way for future applications of DMT to other alcoholic beverages or to more detailed studies with other types of wines or musts. The results of this work first deal with the determination of Zn in synthetic (model) wine samples (essentially, with tartaric acid to simulate the principal metal-complexing ligand in wine) to validate the use of DMT with AGNES. Then, the core of this work discusses the need of reaching equilibrated concentrations and the choice of suitable reference cations, to finally compute the free concentrations of the target analytes.

2. Materials and Methods

2.1. Reagents and wine samples

Synthetic wines, either in aqueous medium or in hydroalcoholic one (13.5% of ethanol), consisted of $c_{T,Zn}$= 1.16×10^{-5} mol L^{-1} (the total concentration determined by elemental analysis) and potassium hydrogentartrate (KHTar) $c_{T,KHTar}$= 0.011 mol L^{-1} (Bradshaw et al., 2002) at pH= 3.422. DMT analysis of the synthetic wines was performed with 0.1

mol L^{-1} $NaNO_3$ as background electrolyte in both donor and acceptor solutions. To compensate for $c_{T,KHTar}$= 0.011 mol L^{-1} in the donor (i.e. to have the same initial K concentration), 0.011 mol $L^{-1}KNO_3$ was added in the acceptor. K^+ was used as the reference cation.

For DMT analysis of the red wine Raimat Clamor Tinto Roble 2012 (to act as donor solution), two homogenizations were prepared from 8 bottles of 0.75 L by putting half of each bottle of wine in homogenization 1 and the other half in homogenization 2, to finally have 3L for each homogenization.

Elemental analysis of the red wine yielded total concentrations: $c_{T,Zn}$=12±1 μmol L^{-1}, $c_{T,Fe}$=20±2 μmol L^{-1}, $c_{T,Cu}$=14±1 μmol L^{-1}, $c_{T,Na}$=0.93±0.04 mmol L^{-1}, $c_{T,K}$=33±2 mmol L^{-1}, $c_{T,Ca}$=2.8±0.2 mmol L^{-1} and $c_{T,Mg}$=6.0±0.3 mmol L^{-1} (mean ± standard deviation, with n=8). All these values are in the usual ranges in wines (Pyrzynska, 2007; Tariba, 2011). Two acceptor solutions were assayed: Acceptor 1 with only K in the acceptor that will be referred to as "just K" and Acceptor 2 with the four principal cations (K, Na, Mg and Ca) at total concentrations given at the beginning of this paragraph. This latter acceptor will be called "multimetal".

Potassium hydrogen L-tartrate (Fluka, analytical grade), ethanol absolute 99.9% (Merck, p.a.), 1000 mg L^{-1} Zn, Fe, Cu, K, Na, Ca, and Mg standards solutions (High Purity Standards), potassium nitrate (Fluka, Trace Select), sodium nitrate (Sigma-Aldrich, p.a.), calcium nitrate tetrahydrate (Fluka, p.a.), magnesium nitrate hexahydrate (Merck, p.a.), 0.1 M KOH and HNO_3 (Riedel de Haen) were used to prepare the solutions. A multielement isotopically-enriched standard (IES-WAK, ISC Science) with 2000 ng g^{-1} of Fe (2.91% ^{56}Fe and 95.15% ^{57}Fe), 2000 ng g^{-1} of Cu (0.90% ^{63}Cu and 99.10% ^{65}Cu) and 2000 ng g^{-1} of Zn (3.88% ^{66}Zn and 89.60% ^{67}Zn) was used for the quantification of Fe, Cu and Zn.

In all the experiments ultrapure water (Milli-Q, Millipore) was employed.

2.2. Instrumentation

Total Zn, Fe, Cu, K, Na and Mg concentrations were quantified using a 7700x ICP-MS (Agilent Technologies, Inc, Tokyo, Japan) with Ni sampler and skimmer cons, a MicroMist glass concentric nebulizer and a He collision cell.

DMT experiments were performed using lab DMT cells (see (Chito et al., 2012; Temminghoff et al., 2000)) and two peristaltic pumps (Gilson Minipuls 3) (one for each homogenization). A membrane (BDH Laboratory Supplies, Poole, UK) of polystyrene and divinylbenzene with sulfonic acid groups was used as cation exchange membrane in DMT experiments. The ion-exchange capacity of the membrane is 0.8 mmol g^{-1} and its thickness is 0.15–0.17 mm (Weng et al., 2005). Prior to the measurement of the sample, the membranes were allowed to equilibrate with the same electrolyte solution of the acceptor. Each pump was connected to 4 DMT cells to have 4 measurements for each homogenization, in which two cells were connected to acceptor solutions containing "just K" and two with K, Na, Mg and Ca ("multimetal") (see section 2.1.).

500 mL of wine was used as donor solution while 26 mL of " just K" or "multimetal" solution was used as initial acceptor solution. Aliquots of 1mL from the acceptor and donor solutions were taken at chosen time intervals and diluted 10 times (Gonzalvez et al., 2008) with a mixture of 1% HNO_3 and 0.5% HCl for further total elemental analysis by ICP-MS.

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=1.41×10^{-4} m). The auxiliary electrode was a glassy carbon electrode and the reference electrode was Ag/AgCl/3 mol L^{-1} KCl, encased in a 0.1 mol L^{-1} KNO_3 jacket. A glass jacketed cell thermostated at 25.0 ºC was used in all measurements. N_2 (99.999%) saturated in a hydroalcoholic medium (13% ethanol) was used for deaeration and blanketing the wine.

A glass combined electrode (Crison 5103) was attached to a Dual Star Orion ion analyser to control the pH.

2.3. Procedures

2.3.1. Determination of free metal ion concentrations using DMT

The Donnan Membrane Technique relies on the equilibration between a sample (or donor solution) and an acceptor solution, separated by a cationic exchange membrane

blocking the practical permeation of negative species. This equilibration between the transferable cations means that their electrochemical potential is eventually the same in the acceptor as in the donor solutions. After simple algebra, the activity of an analyte M in the donor solution can be computed from the activity of this analyte in the acceptor and the activities of a reference cation (R):

$$\left\{M^{z_M}\right\}_D = \left\{M^{z_M}\right\}_A \left(\frac{\left\{R^{z_R}\right\}_D}{\left\{R^{z_R}\right\}_A} \right)^{z_M / z_R} \quad (1)$$

where subscripts D and A label the Donor and Acceptor solutions and z_j stands for the charge of the cation j (Temminghoff et al., 2000).

Equation (1) indicates that equilibrium might have not been reached when the activities of a given cation have a common value in both donor and acceptor solutions. Instead, both actitivities can differ at equilibrium by the presence of a Donnan factor represented by the second factor of the right hand side due to the arising of an electric potential between both solutions. Indeed,

$$\Pi = \left(\frac{\left\{R^{z_R}\right\}_D}{\left\{R^{z_R}\right\}_A} \right)^{1 / z_R} \quad (2)$$

corresponds to the Donnan factor for a monovalent cation, so that for a probe cation of charge z_M, its Donnan factor corresponds to the last factor appearing in Eqn. (1).

If the activity coefficients are similar in the donor and acceptor solutions, the free concentration of M in the donor solution, can be computed from concentrations as:

$$\left[M^{z_M}\right]_D = \left[M^{z_M}\right]_A \left(\frac{\left[R^{z_R}\right]_D}{\left[R^{z_R}\right]_A} \right)^{z_M / z_R} \quad (3)$$

In practice, to avoid a slow step due to mass transport, the acceptor and donor solutions are recirculated (by means of a peristaltic pump) until they impinge on the cationic exchange membrane within a specially designed chamber. For details, see refs (Weng et al., 2005; Weng et al., 2011).

2.3.2. Elemental analysis by ICP-MS

The operating conditions were as follows: RF power 1550 W, carrier gas flow rate 1.01 L min^{-1}, helium collision gas flow rate 4.3 mL min^{-1}, spray chamber temperature 2.0 ºC, sample depth 10.0 mm, nebulizer pump 0.1 rps, extract lens 1 voltage 0.0 V and extract lens 2 voltage -195.0 V. For Fe and Zn, the measurement was conducted through isotope dilution analysis, using the multielemental isotopically-enriched spike solution. The monitored isotopes were ^{56}Fe and ^{57}Fe for Fe, ^{63}Cu and ^{65}Cu for Cu and ^{66}Zn and ^{67}Zn for Zn. The isotopic ratio is determined from a metal solution with a known concentration which is prepared from a standard. Once this ratio is established it is possible to know the concentration of any sample by comparing the isotopic ratios. For Na, Mg, K and Ca, ^{23}Na, ^{24}Mg, ^{39}K and ^{44}Ca were monitored, respectively, and external calibration was conducted for the quantification. Compared to the direct mass measurement, the isotope dilution analysis is less vulnerable to matrix effect in the ICP-MS analysis and more accurate (Centineo et al., 2001; Quetel et al., 2001).

The limit of detection (LOD) of DMT strongly depends on the quantifying technique used for the total element in the acceptor compartment (Pesavento et al., 2009). For the ICP-MS configuration used in this work, a LOD of 1 μg/L for Zn, 9 μg/L for Fe, 48 μg/L for Ca and 0.3 μg/L for Mg has been reported.

2.3.3. Determination of free Zn concentrations using AGNES

Absence of Gradients and Nernstian Equilibrium Stripping consists of two stages. In the first stage, the reduced form (Mº) of the analyte (M^{z+}) is accumulated in a mercury electrode until a special situation of equilibrium is reached: the concentration profiles inside and outside the electrode are flat and there is a fix relationship (gain, *Y*) between these concentrations:

$$Y = \frac{\left[\mathrm{M^o}\right]}{\left[\mathrm{M^{z+}}\right]} = \exp\left[-\frac{zF}{RT}\left(E_1 - E^{o\prime}\right)\right] \quad (4)$$

where *F* is the Faraday constant, *R* is the gas constant, *T* is the absolute temperature and $E^{0\prime}$ is the standard formal potential. In the case of wine, E_1, the deposition potential corresponding to the desired gain *Y*, can be computed for the peak potential of a Differential Pulse Polarogram obtained in the same hydroalcoholic medium via:

$$E_j = {}_{\mathrm{EtOH}}E_{\mathrm{peak}} + \frac{\Delta E}{2} + \frac{RT}{nF}\ln\left({}_{\mathrm{EtOH}}Y_j\sqrt{\frac{D_{\mathrm{M^0}}}{{}_{\mathrm{EtOH}}D_{\mathrm{M}}}}\right) \quad (5)$$

where pre-subscript EtOH indicate the 13.5% ethanolic medium. This equation is the correct form of eqn. 4 in (Chito et al., 2013) and of eqn. 10 in (Companys et al., 2008), where an error of transcription led to an undue replacement of $D_{\mathrm{M^0}}$ by ${}_{\mathrm{w}}D_{\mathrm{M}}$.

The second stage of AGNES quantifies the amount of Mº in the mercury electrode. A simple derivation (Galceran et al., 2014) leads to a direct proportionality between the faradaic current and the free Zn concentration in the sample

$$I_{\mathrm{faradaic}} = \eta Y_1\left[\mathrm{Zn^{2+}}\right] \quad (6)$$

The proportionality factor η can be found from a calibration.

3. Results and discussion

3.1. Synthetic solutions

In the aqueous synthetic wine (see Section 2.1), two major cationic species containing Zn can be identified: free Zn^{2+}, and $HZnTar^{+}$, so that both species could have reached equilibrium across the DMT membrane. However, the retrieved free Zn^{2+} concentration from DMT analysis $[Zn^{2+}]_{DMT}$= 5.2 ± 0.5 µmol L^{-1} is consistent with the prediction of the speciation code VMinteq (Gustafsson, 2010) $[Zn^{2+}]_{VMinteq}$= 5.35 µmol L^{-1} (see Fig 1), assuming that only free Zn^{2+} species reached equilibrium. Actually, if other positive or neutral Zn species (e.g. $HZnTar^{+}$ or ZnTar) had also reached equilibrium, the total Zn concentration measured in the acceptor would have increased by more than 30% (see table SI-1, with the predicted species distribution). The preferential "filtering" of just the free divalent cations can be justified considering the membrane as an extra phase domain (which we will label with the subscript "membrane") so that 3 phases are present in the system: donor, membrane and acceptor. Applying the Donnan equilibrium conditions at the donor/membrane interphase, a Boltzmann (or partitioning) factor Π≈35 appears for a monovalent cation, following from the application of (Puy et al., 2014; Temminghoff et al., 2000)

$$\Pi = \exp\left(-\operatorname{arcsinh}\left(-\frac{\rho}{2I}\right)\right) \quad (7)$$

considering an estimated ρ=-3.5 mol L^{-1} charge density of the membrane phase and a ionic strength of the background electrolyte *I*=0.1M $NaNO_3$. This factor indicates that the equilibrium concentration of a monovalent cation at the membrane/donor interface in the membrane side is 35 times its concentration at the donor side. A rough model for the (initial) ratio of fluxes of Zn-containing species can consider the initial concentrations in donor and acceptor solutions with a steady-state profile stablished inside the membrane for each Zn species (see schematic diagram in Fig SI-1). So, for a concentration ratio of 12.3 (in solution, as predicted by VMINTEQ, see Table SI-1) between Zn^{2+} and $ZnHTar^{+}$, the same ratio of concentrations in the cation exchange membrane (at the interface with the donor solution) becomes:

$$\frac{\left[Zn^{2+}\right]_{membrane}}{\left[ZnHTar^{+}\right]_{membrane}} = \frac{\left[Zn^{2+}\right]_{solution} \times 35^{2}}{\left[ZnHTar^{+}\right]_{solution} \times 35} = 12.3 \times 35 = 432 \quad (8)$$

For the concentrations in this synthetic solution, even assuming a common diffusion coefficient for these two species, the initial flux across the membrane of $ZnHTar^{+}$ will be 432 times smaller than that of Zn^{2+} assuming zero concentration at the acceptor/membrane interface. Similarly, the ratio of Zn^{2+} to the neutral species ZnTar is 1.87 in the solution, and the flux of Zn^{2+} is 2289 times that of the neutral species. In addition, the absence of tartrate in the initial acceptor solution will lead to dissociation of $HZnTar^{+}$ or ZnTar once they enter the acceptor, further slowing down the equilibration of these species (while favouring the attainment of the equilibrium for free Zn).

Measurements in the ethanolic medium required longer equilibration times (Fig 2) than in aqueous medium. This longer equilibration time might be related to a slower permeation process in the membrane. Comparison with the expected VMINTEQ concentrations (shown in Fig 2) serves just as a guideline, given that VMINTEQ does not take into account the presence of ethanol. At equilibrium, the free Zn concentration measured with AGNES (at two gains, each with 2 different deposition times: *Y*=11.4, t_1=75s and t_1=100 s; *Y*=22.74, t_1=150s and t_1=200s) resulted in $[Zn^{2+}]_{AGNES}$= 6.50 ± 0.10 µmol L^{-1}, which agrees (within the experimental error) with $[Zn^{2+}]_{DMT}$= 7.3 ± 0.7 µmol L^{-1}.

3.2. Analysis of real wine

To compute the free concentration with eqn. (3), one needs to identify a suitable reference cation. In the following, several candidates are considered, starting with K, a typical choice.

The total concentration of K in the acceptor of the replicates with initially "just K" in the acceptor experienced a clear decrease, while no such a change in the "multimetal" acceptors was observed (Fig 3). To explain this change in the "just K" acceptor, one starts by noticing that the concentrations of the rest of principal cations (Ca, Mg and Na) can only increase in the acceptor due to their initial absence in the acceptor and their trend towards equilibration close to the concentrations of those cations in the donor (see Fig SI-2). Thus, the required transference of Ca, Mg and Na from donor to acceptor has to be compensated with transference of K from the acceptor to the donor in order to keep electroneutrality. We conclude that K can be used as reference cation in the "multimetal" acceptor configuration, but the "just K" configuration might be unreliable (due to the requirement of longer time to reach true equilibrium of all species). Indeed, in these experiments of “just K”, the concentrations of Mg, Ca and Na in the acceptor at 200 h are still increasing (see Fig SI-2). Notice that, in general (see Figs 3-5), the found equilibration times are even slightly longer than those needed for synthetic alcoholic wine.

Na concentration remains stable in the "multimetal" acceptor (see Fig 4). This indicates that its free concentration in the acceptor is very close to the free concentration in the donor solution, and both solutions have similar ionic strength. So, Na can also be used as reference cation in this case.

At 216 hours $\frac{[K^+]_D}{[K^+]_A} \approx \frac{[Na^+]_D}{[Na^+]_A}$ in "multimetal", but not in "just K" acceptors. This equality confirms the attainment of equilibrium for K^+ and Na^+ in "multimetal” conditions.

Ca and Mg concentrations decrease with elapsing time in the "multimetal" acceptor (Fig 4). This could be due to the fact that these divalent cations are relevantly complexed in the wine (and not just free), so the free concentration in the acceptor is initially higher than in the donor, so that some transference of Ca and Mg to the donor is needed for

296 equilibration. Because of the much larger volume of the donor, the change in the
297 composition of the wine is negligible. Due to the significant difference between the total
298 and free concentration of Ca or Mg in the donor (of the order of the drop from the initial
299 to equilibrium concentrations seen in Fig 4), none of them can be safely used as
300 reference cation.

301 The evolution of the trace analytes in the acceptor can be considered to have reached
302 equilibrium also around 200 h (see Figs 5 and SI-3). The application of equation (3)
303 directly with the average values of the ICP-MS concentrations for times longer than 150
304 h leads to results in Table 1. According to what has been said up to now, the safer [Zn^{2+}]
305 is the one obtained in "multimetal" acceptors when taking K (or Na) as the reference
306 cations. The average of these eight data (considering the 2 replicates at each
307 homogenization) is 1.76 ± 0.07 μmol L^{-1} which is in excellent agreement with the
308 totally independent AGNES technique that yielded 1.7 ± 0.2 μmol L^{-1}. Reported free Zn
309 concentrations in similar red wines (with AGNES) were within the same order of
310 magnitude: 0.45 μmol L^{-1} (Companys et al., 2008) and 1.49 μmol L^{-1} (Chito et al.,
311 2013). Computations of [Zn^{2+}] with equation (3) when using either Ca or Mg as
312 reference ion (see table 1) are clear overestimations, though by a factor not larger than
313 2.5 (i.e. the order of magnitude is correct). The use of "just K" acceptors (with Ca, Mg
314 and Na away from equilibrium) produced either around 20% underestimation (acceptors
315 homogenization 1), or around 8% overestimation (acceptors homogenization 2).

316 For Fe, we assume that the relevant oxidation form is its trivalent state given that the
317 acceptor solution and the wine are in contact with the atmosphere. Similar reasonings
318 (on the various acceptor solutions) to those expound for Zn apply also to Fe(III). The
319 average of the most reliable data (those with K and Na as reference ion in equilibrated
320 "multimetal" acceptors) is 1.79±0.08 μmol L^{-1}, which represents an 8.82% of free
321 Fe(III) with respect to the total Fe content in the wine. Comparison with previous works
322 (Ajlec and Stupar, 1989; Costa and Araujo, 2001; Pyrzynska, 2007) is hindered by the
323 fact that there the speciation is considered between total Fe(II) and Fe(III) without
324 distinguishing free and complexed fractions of each oxidation state. Some Fe(II)
325 (Danilewicz, 2016) might be still present in the wine at the equilibration time. However,
326 it will be probably mostly preserved in the form of strong complexes rather than free
327 Fe(II), so that [Fe^{2+}] in the acceptor can be assumed to be negligible in comparison with
328 [Fe^{3+}] given that the acceptor solution has no compounds able to keep a redox cycle

329 while the solution is open to the air. In any case, the current results about Fe (III) have
330 to be taken as a first rough approximation.

331

332 The free concentrations of Ca and Mg in the wine can also be estimated taking K or Na
333 as reference ion (see Table 1) in the configuration with "multimetal" acceptor. Their
334 averages are 1.11± 0.09 mmol L^{-1} and 3.4± 0.3 mmol L^{-1}, respectively. They represent a
335 40% of free Ca and 57% of free Mg in wine. The measured percentage of free Ca in this
336 wine is in the range from 33 to 64% of ionised Ca found by Cardwell et al. (1991)
337 using an ion selective electrode in different kind of wines.

338 In this work, the Cu free concentrations could not be accurately determined. The data
339 indicate a level around 0.10 μmol L^{-1}. This concentration represents a 0.71% of the total
340 Cu, lower than the free copper range of 3.3–31% reported in Wiese and Schwedt
341 (1997). However, the lack of a clear stabilization of the acceptor Cu concentration leads
342 us to point it just as a very rough estimation. Future work could be devoted to tackle Cu
343 speciation in wine using DMT technique.

344 4. Conclusions

345 DMT measurement of cations in complex matrices, such as wine, would benefit from an
346 iterative procedure where, in successive experiments, the acceptor composition of the
347 non-analyte ions (or even of the analytes) approached the composition of the free ions in
348 the sample. However, for practical reasons it might be simpler to endure longer
349 equilibration times (rather than prepare and equilibrate the acceptors with closer
350 compositions) once certain proximity to the ideal initial configuration is obtained.

351 Faster designs of DMT are needed to tackle some complex media (as wine and other
352 food matrices). Even if, currently, there are relatively long equilibration times, DMT
353 gives access to the key information of the free ion concentration, which is a rigorously
354 defined physicochemical quantity (in contrast to many operationally defined ones). This
355 method is different and complementary to those currently prescribed in international
356 wine legislation (e.g. the International Organisation of Vine (OIV) or European Union),
357 which relies just on total metal concentrations.

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Supplementary data

Electronic supplementary information related to this article can be found at http://dx.doi.org/XXXXX.

Literature cited

Ajlec R. and Stupar J. (1989) Determination of Iron Species in Wine by Ion-Exchange Chromatography Flame Atomic-Absorption Spectrometry. *Analyst.* **114**, 137-142.

Ali T. A., Mohamed G. G., and Farag A. H. (2015) Electroanalytical Studies on Fe(III) Ion-Selective Sensors Based on 2-methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one Ionophore. *Int. J. Electrochem. Sci.* **10**, 564-578.

Bakker E. and Pretsch E. (2007) Modern Potentiometry. *Angew. Chem. , Int. Ed.* **46**, 5660-5668.

Bradshaw M. P., Prenzler P. D., and Scollary G. R. (2002) Square-wave voltammetric determination of hydrogen peroxide generated from the oxidation of ascorbic acid in a model wine base. *Electroanal.* **14**, 546-550.

Cardwell T. J., Cattrall R. W., Mrzljak R. I., Sweeney T., Robins L. M., and Scollary G. R. (1991) Determination of Ionized and Total Calcium in White Wine Using A Calcium Ion-Selective Electrode. *Electroanal.* **3**, 573-576.

Centineo G., Rodriguez J. A., and Munoz E. (2001) On-line isotope dilution analysis with the 7700 Series ICP-MS: Analysis of trace elements in high matrix samples. Agilent Publication 5990-9171EN.

Chito D., Galceran J., Companys E., and Puy J. (2013) Determination of the Complexing Capacity of Wine for Zn Using the Absence of Gradients and Nernstian Equilibrium Stripping Technique. *J. Agric. Food Chem.* **61**, 1051-1059.

Chito D., Weng L., Galceran J., Companys E., Puy J., van Riemsdijk W. H., and van Leeuwen H. P. (2012) Determination of free Zn^{2+} concentration in synthetic and natural samples with AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique). *Sci. Total Envir.* **421-422**, 238-244.

Coetzee P. P., van Jaarsveld F. P., and van Haecke F. (2014) Intraregional classification of wine via ICP-MS elemental fingerprinting. *Food Chem.* **164**, 485-492.

Companys E., Naval-Sanchez M., Martinez-Micaelo N., Puy J., and Galceran J. (2008) Measurement of free zinc concentration in wine with AGNES. *J. Agric. Food Chem.* **56**, 8296-8302.

Costa R. C. D. and Araujo A. N. (2001) Determination of Fe(III) and total Fe in wines by sequential injection analysis and flame atomic absorption spectrometry. *Anal. Chim. Acta* **438**, 227-233.

Cremazy A., Leclair S., Mueller K., Vigneault B., Campbell P., and Fortin C. (2015) Development of an In Situ Ion-Exchange Technique for the Determination of Free Cd, Co, Ni, and Zn Concentrations in Freshwaters. *Aquat. Geochem.* **21**, 259-279.

Danilewicz J. C. (2016) Fe(II):Fe(III) Ratio and Redox Status of White Wines. *Am. J. Enol. Vitic.* **67**, 146-152.

405 Diaz-de-Alba M., Galindo-Riano M. D., and Pinheiro J. P. (2014) Lead electrochemical
406 speciation analysis in seawater media by using AGNES and SSCP techniques. *Environ.*
407 *Chem.* **11**, 137-149.

408 Domingos R. F., Simon D. F., Hauser C., and Wilkinson K. J. (2011) Bioaccumulation
409 and Effects of CdTe/CdS Quantum Dots on Chlamydomonas reinhardtii - Nanoparticles
410 or the Free Ions? *Environ. Sci. Technol.* **45**, 7664-7669.

411 Feldmann J., Salaun P., and Lombi E. (2009) Critical review perspective: elemental
412 speciation analysis methods in environmental chemistry - moving towards
413 methodological integration. *Environ. Chem.* **6**, 275-289.

414 Fu Q., Qian S., Li N., Xia Q., and Ji Y. (2012) Characterization of a New Zn2+-
415 Selective Electrode Based on Schiff-base as Ionophore. *Int. J. Electrochem. Sci.* **7**,
416 6799-6806.

417 Galceran J., Companys E., Puy J., Cecília J., and Garcés J. L. (2004) AGNES: a new
418 electroanalytical technique for measuring free metal ion concentration. *J. Electroanal.*
419 *Chem.* **566**, 95-109.

420 Galceran J., Huidobro C., Companys E., and Alberti G. (2007) AGNES: a technique for
421 determining the concentration of free metal ions. The case of Zn(II) in coastal
422 Mediterranean seawater. *Talanta* **71**, 1795-1803.

423 Galceran J., Lao M., David C., Companys E., Rey-Castro C., Salvador J., and Puy J.
424 (2014) The impact of electrodic adsorption on Zn, Cd or Pb speciation measurements
425 with AGNES. *J. Electroanal. Chem.* **722-723**, 110-118.

426 Gao R., Temminghoff E. J. M., van Leeuwen H. P., van Valenberg H. J. F., Eisner M.
427 D., and van Boekel M. A. J. S. (2009) Simultaneous determination of free calcium,
428 magnesium, sodium and potassium ion concentrations in simulated milk ultrafiltrate and
429 reconstituted skim milk using the Donnan Membrane Technique. *Int. Dairy J.* **19**, 431-
430 436.

431 Gonzalvez A., Armenta S., Pastor A., and de la Guardia M. (2008) Searching the most
432 appropriate sample pretreatment for the elemental analysis of wines by inductively
433 coupled plasma-based techniques. *J. Agric. Food Chem.* **56**, 4943-4954.

434 Gramlich A., Tandy S., Slaveykova V. I., Duffner A., and Schulin R. (2012) The use of
435 permeation liquid membranes for free zinc measurements in aqueous solution. *Environ.*
436 *Chem.* **9**, 429-437.

437 Gustafsson J. P. (2010) Visual MINTEQ version 3.0. Available at
438 <www.lwr.kth.se/English/ Oursoftware/vminteq/index.htm>.

439 Ibanez J. G., Carreon-Alvarez A., Barcena-Soto M., and Casillas N. (2008) Metals in
440 alcoholic beverages: A review of sources, effects, concentrations, removal, speciation,
441 and analysis. *J. Food Compos. Anal.* **21**, 672-683.

442 Jones A. M., Xue Y., Kinsela A. S., Wilcken K. M., and Collins R. N. (2016) Donnan
443 membrane speciation of Al, Fe, trace metals and REEs in coastal lowland acid sulfate
444 soil-impacted drainage waters. *Sci. Total Envir.* **547**, 104-113.

445 Lamaka S. V., Taryba M. G., Zheludkevich M. L., and Ferreira M. G. (2009) Novel
446 Solid-Contact Ion-Selective Microelectrodes for Localized Potentiometric
447 Measurements. *Electroanal.* **21**, 2447-2453.

448 Parat C. and Pinheiro J. P. (2015) ISIDORE, a probe for in situ trace metal speciation
449 based on Donnan membrane technique with related electrochemical detection part 1:
450 Equilibrium measurements. *Anal. Chim. Acta* **896**, 1-10.

451 Pesavento M., Alberti G., and Biesuz R. (2009) Analytical methods for determination of
452 free metal ion concentration, labile species fraction and metal complexation capacity of
453 environmental waters: A review. *Anal. Chim. Acta* **631**, 129-141.

454 Pohl P. and Sergiel I. (2009) Evaluation of the Total Content and the Operationally
455 Defined Species of Copper in Beers and Wines. *J. Agric. Food Chem.* **57**, 9378-9384.

456 Puy J., Galceran J., Cruz-Gonzalez S., David C. A., Uribe R., Lin C., Zhang H., and
457 Davison W. (2014) Metal accumulation in DGT: Impact of ionic strength and kinetics
458 of dissociation of complexes in the resin domain. *Anal. Chem.* **86**, 7740-7748.

459 Pyrzynska K. (2007) Chemical speciation and fractionation of metals in wine. *Chem.*
460 *Speciation Bioavail.* **19**, 1-8.

461 Quetel C. R., Nelms S. M., Van Nevel L., Papadakis I., and Taylor P. D. P. (2001)
462 Certification of the lead mass fraction in wine for comparison 16 of the International
463 Measurement Evaluation Programme. *J. Anal. At. Spectrom.* **16**, 1091-1100.

464 Rotureau E. (2014) Analysis of metal speciation dynamics in clay minerals dispersion
465 by stripping chronopotentiometry techniques. *Colloids Surf. A* **441**, 291-297.

466 Rousseva M., Kontoudakis N., Schmidtke L. M., Scollary G. R., and Clark A. C. (2016)
467 Impact of wine production on the fractionation of copper and iron in Chardonnay wine:
468 Implications for oxygen consumption. *Food Chem.* **203**, 440-447.

469 Tariba B. (2011) Metals in Wine-Impact on Wine Quality and Health Outcomes. *Biol.*
470 *Trace Elem. Res.* **144**, 143-156.

471 Temminghoff E. J. M., Plette A. C. C., van Eck R., and van Riemsdijk W. H. (2000)
472 Determination of the chemical speciation of trace metals in aqueous systems by the
473 Wageningen Donnan Membrane Technique. *Anal. Chim. Acta* **417**, 149-157.

474 Weng L. P., van Riemsdijk W. H., and Temminghoff E. J. M. (2005) Kinetic aspects of
475 donnan membrane technique for measuring free trace cation concentration. *Anal. Chem.*
476 **77**, 2852-2861.

477 Weng L. P., Vega F. A., and van Riemsdijk W. H. (2011) Strategies in the application
478 of the Donnan membrane technique. *Environ. Chem.* **8**, 466-474.

479 Wiese C. and Schwedt G. (1997) Strategy for copper speciation in white wine by
480 differential pulse anodic stripping voltammetry, potentiometry with an ion-selective
481 electrode and kinetic photometric determination. *Fresenius J. Anal. Chem.* **358**, 718-
482 722.
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Figures

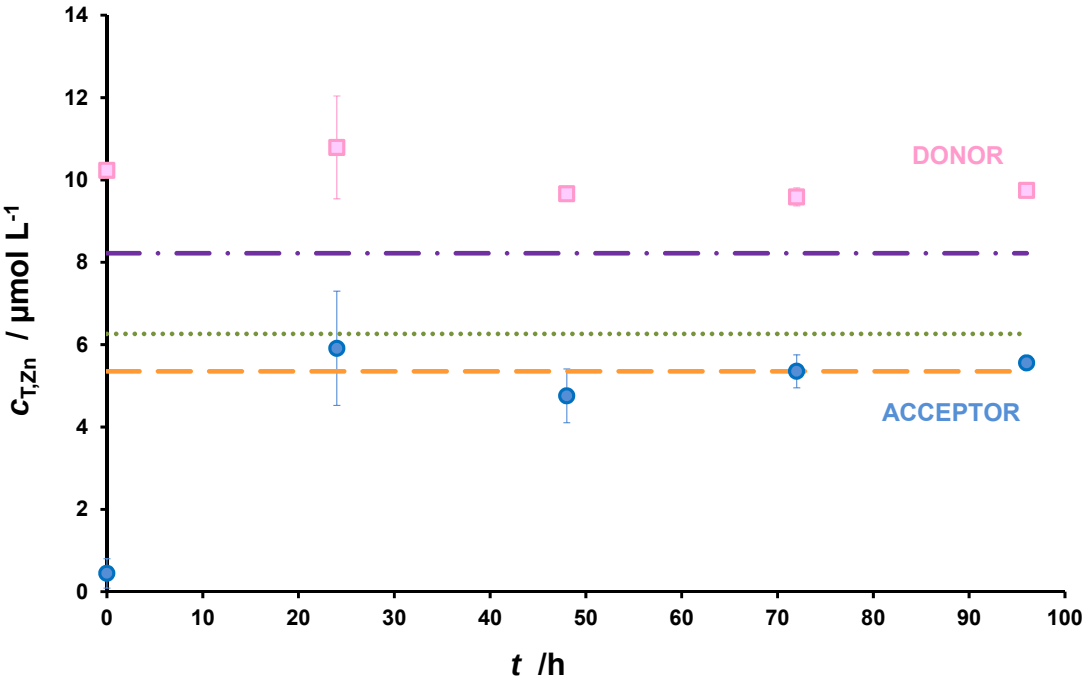


Figure 1. Evolution of total Zn concentrations with time in the donor (pink squares) and the acceptor solutions (blue circles) for the synthetic solution in aqueous medium. The orange dashed line stands for the free zinc concentration in the donor solution predicted by VMinteq considering the measured total Zn concentration at equilibrium ($c_{T,Zn}$= 9.64 µmol L^{-1}). The green dotted line stands for the sum of free zinc and the positive complexes and the purple dotted-dashed line for the sum of free zinc and the neutral complexes. Other conditions for donor: $c_{T,KHTar}$= 0.011 mol L^{-1} pH=3.422 . Initial conditions for acceptor: $c_{T,NaNO3}$ = 0.1 mol L^{-1}, $c_{T,KNO3}$= 0.011 mol L^{-1}, pH=3.422.The error bars represent the standard deviation (n=3).

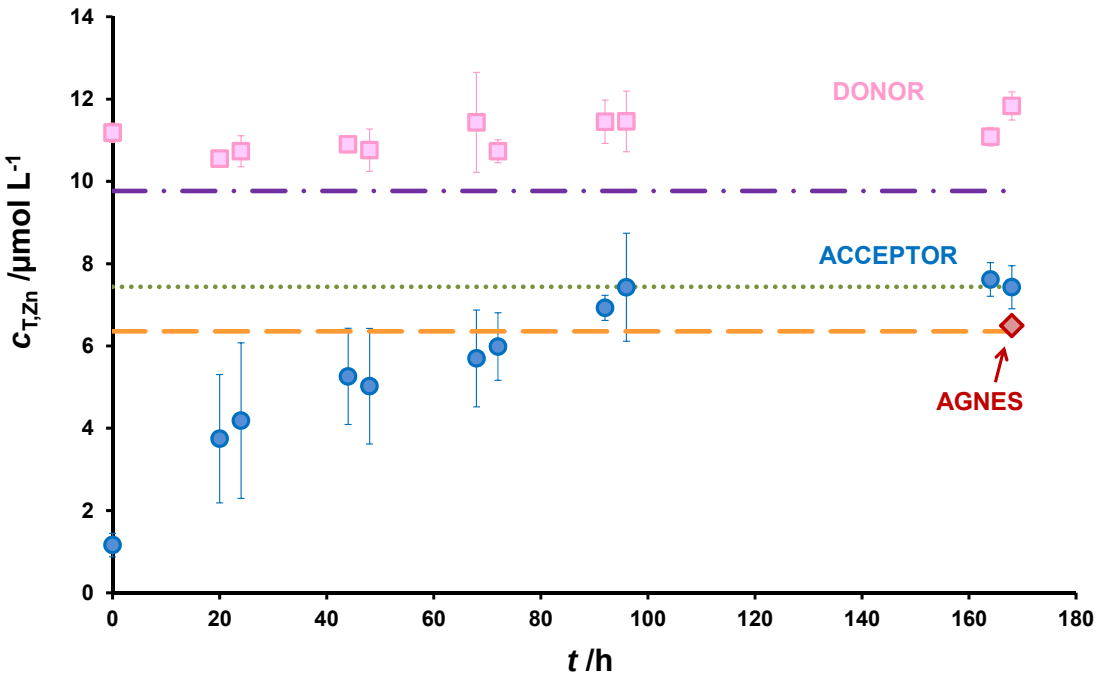


Figure 2. Evolution of total Zn concentrations with time in the donor (pink squares) and the acceptor solutions (blue circles)of the synthetic solution in ethanolic medium. The orange dashed line stands for the free zinc concentration predicted in the Donor by Vminteq –if the solution was aqueous- considering the measured total Zn concentration ($c_{T,Zn}$= 11.5µmol L^{-1}). The green dotted line for the sum of free zinc and the positive complexes and the purple dotted-dashed line for the sum of free zinc and the neutral complexes. The red diamond marker corresponds to AGNES data. Other conditions for donor: $c_{T,KHTar}$= 0.011 mol L^{-1}, 13.5% ethanol, pH=3.422. Initial conditions for acceptor: $c_{T,NaNO3}$ = 0.1 mol L^{-1}, $c_{T,KNO3}$= 0.011 mol L^{-1}, pH=3.422 The error bars represent the standard deviation (n=4).

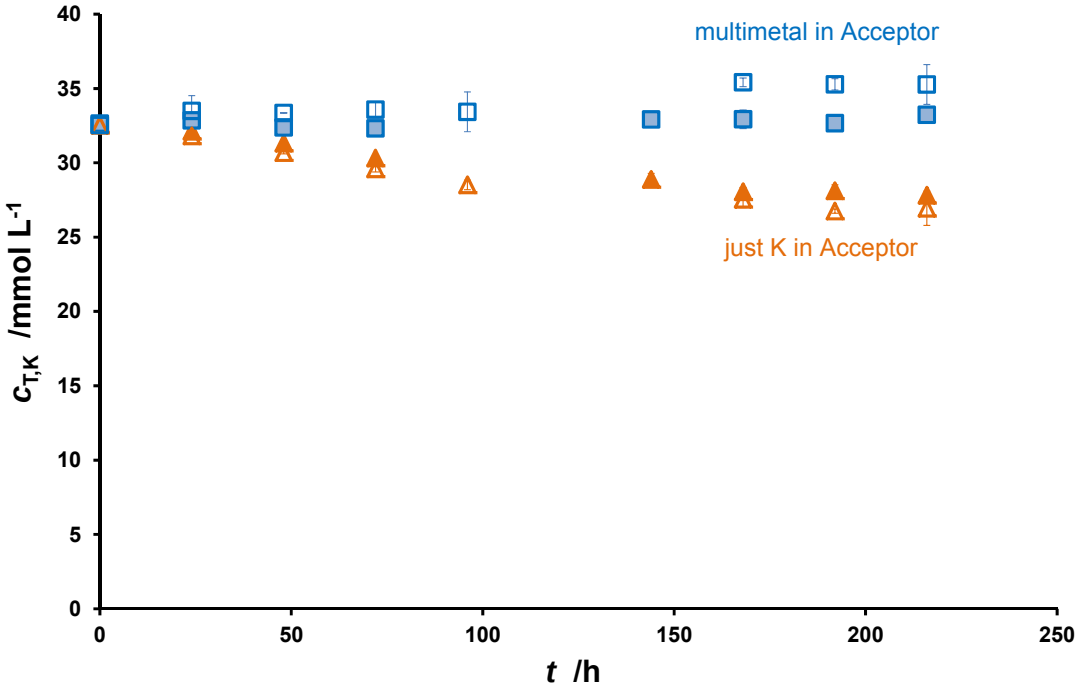
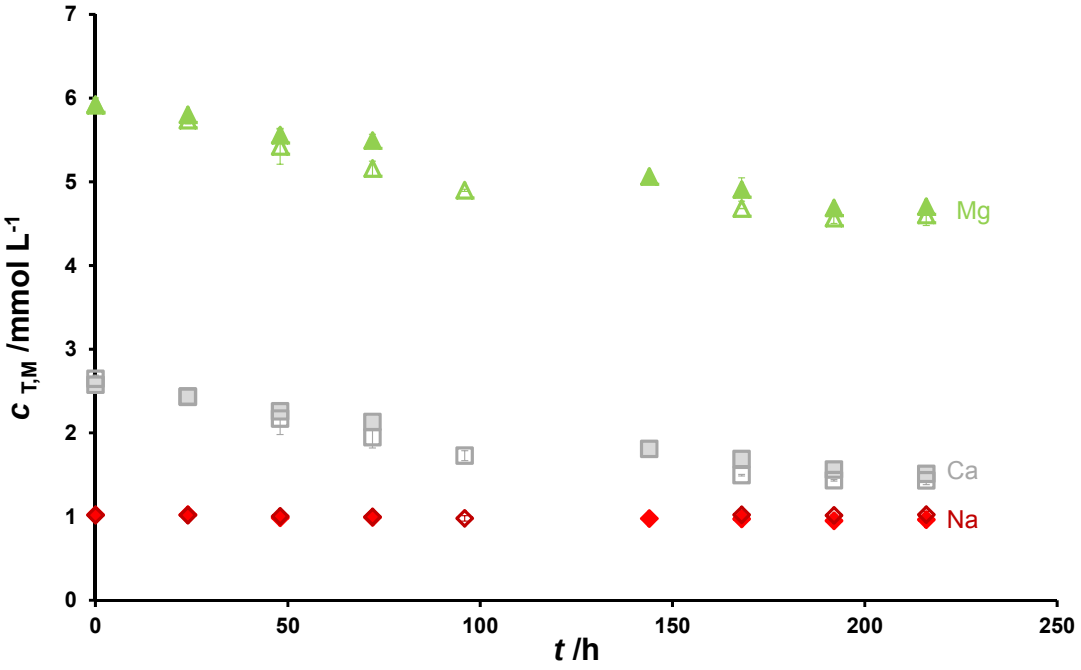


Figure 3. Plot of total concentration of K *versus* time in the acceptor for the four replicates of homogenization 1 (full markers) and homogenization 2 (empty markers) of wine. Orange triangle markers correspond to the acceptor solutions with just K and blue squares with K, Na, Mg and Ca ("multimetal"). The error bars represent the standard deviation (n=2).



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525 Figure 4. Plot of the total concentration of a metal M (such as K, Na, Mg and Ca) *versus* time
526 in the acceptors for the four replicates of homogenization 1 (full markers) and
527 homogenization 2 (empty markers) of wine. The red diamond markers correspond to Na,
528 green triangle markers to Mg and grey square markers to Ca. The error bars represent the
529 standard deviation (*n*=2).

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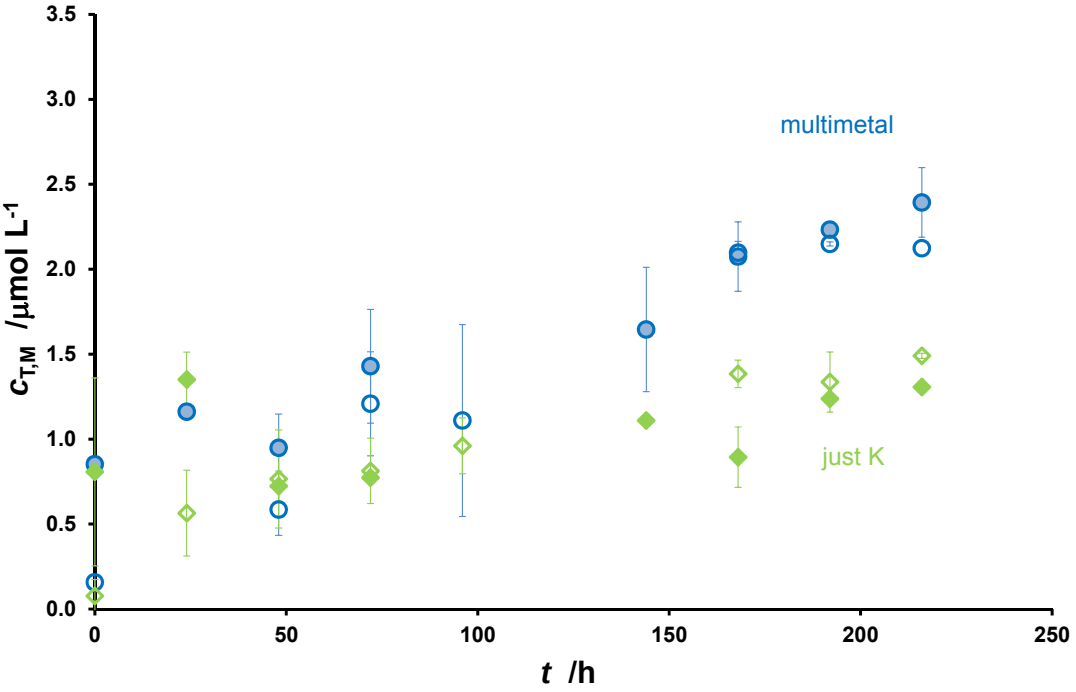

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535 Figure 5. Plot of the total concentration of Zn *versus* time in the acceptors of
536 homogenization 1 (full markers) and homogenization 2 (empty markers) of wine. The blue
537 circle markers correspond to "multimetal" acceptors and the green triangle markers to
538 "just K" ones. Initial conditions in the acceptor: pH=3.422. The error bars represent the
539 standard deviation (*n*=2).

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Tables

Table 1. Free Zn^{2+}, Fe^{3+}, Ca^{2+} and Mg^{2+} concentrations determined in the Raimat wine after the application of the correction equation(3). n=2 replicates. Bold figures indicate reliable determinations.

Acceptor/Sample	**Reference ion**	**$[Zn^{2+}]$ /µmol L^{-1}**	**$[Fe^{3+}]$ /µmol L^{-1}**	**$[Ca^{2+}]$ /mmol L^{-1}**	**$[Mg^{2+}]$ /mmol L^{-1}**
Acceptor "just K" homogenization 1	K	1.35±0.06	2.8±0.2	0.49±0.05	1.65±0.08
Acceptor "multimetal" homogenization 1	K	**1.78±0.05**	**1.81±0.09**	**1.2±0.2**	**3.7±0.2**
	Na	**1.70±0.03**	**1.69±0.06**	**1.11±0.04**	**3.2±0.1**
	Mg	2.54±0.02	3.09±0.06		
	Ca	3.39±0.07	4.8±0.2		
Acceptor "just K" homogenization 2	K	2.1±0.1	3.4±0.2	0.80±0.04	2.57±0.09
Acceptor "multimetal" homogenization 2	K	**1.81±0.09**	**1.86±0.05**	**1.04±0.07**	**3.3±0.2**
	Na	**1.8±0.1**	**1.81±0.06**	**1.08±0.04**	**3.4±0.1**
	Mg	2.9±0.3	3.7±0.3		
	Ca	4.1±0.4	6.4±0.5		

Speciation of Zn, Fe, Ca and Mg in wine with Donnan Membrane Technique

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Supporting Information

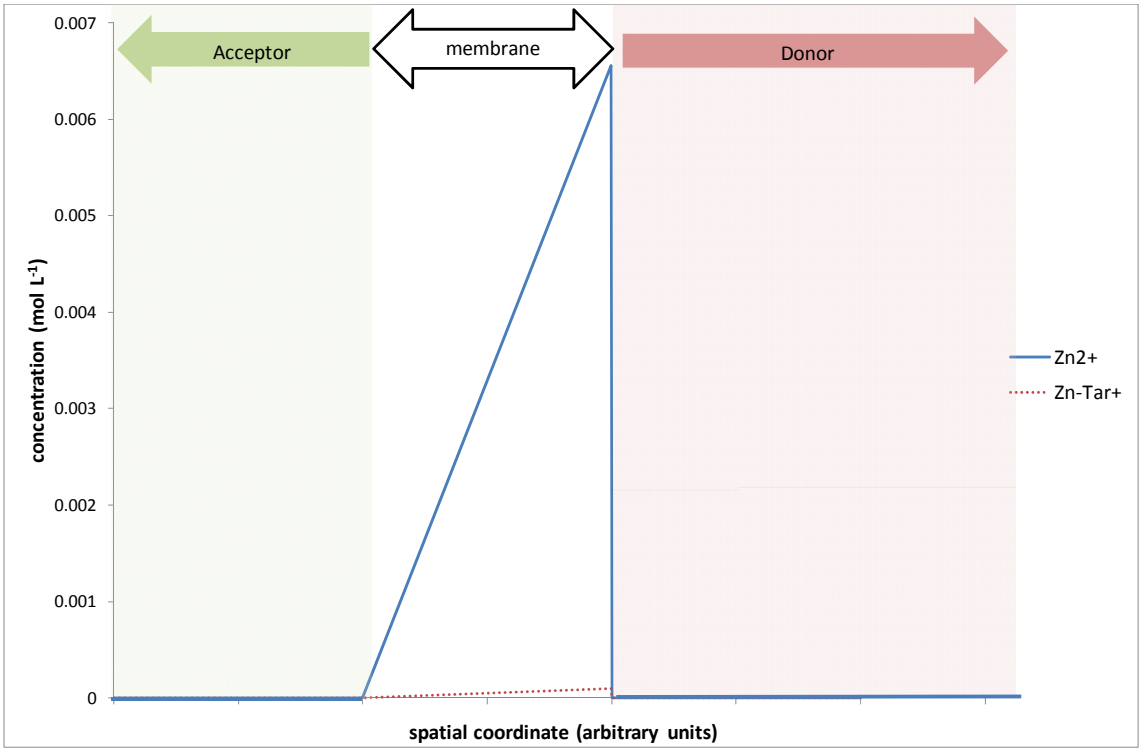

Figure SI-1. Schematic representation of the concentration profiles of Zn^{2+} and $Zn\text{-}Tar^{+}$(aq) for a very rough model to justify the large difference between fluxes across the membrane according to charge of the transported species.

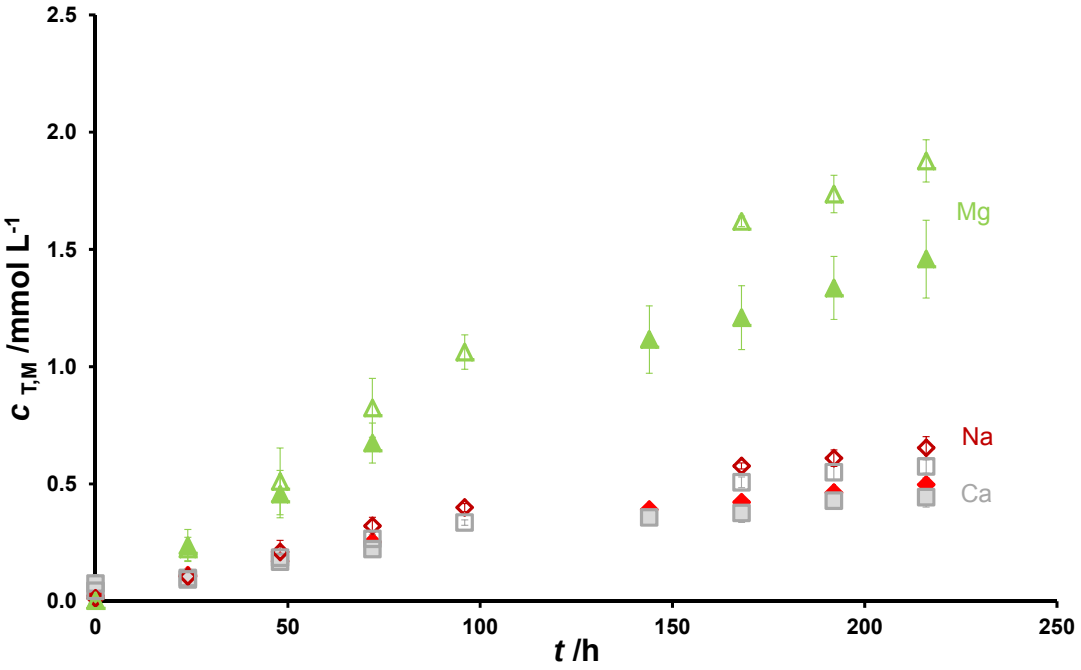

Figure SI-2. Plot of $c_{T,M}$ vs t in the acceptor (with "just K") for the four replicates of homogenization 1 (full markers) and homogenization 2 (empty markers) of wine. The red diamond markers correspond to Na, green triangle markers to Mg and grey square markers to Ca. Initial conditions in the acceptor: $c_{T,K}$=33.1 mmol L^{-1}, pH=3.422

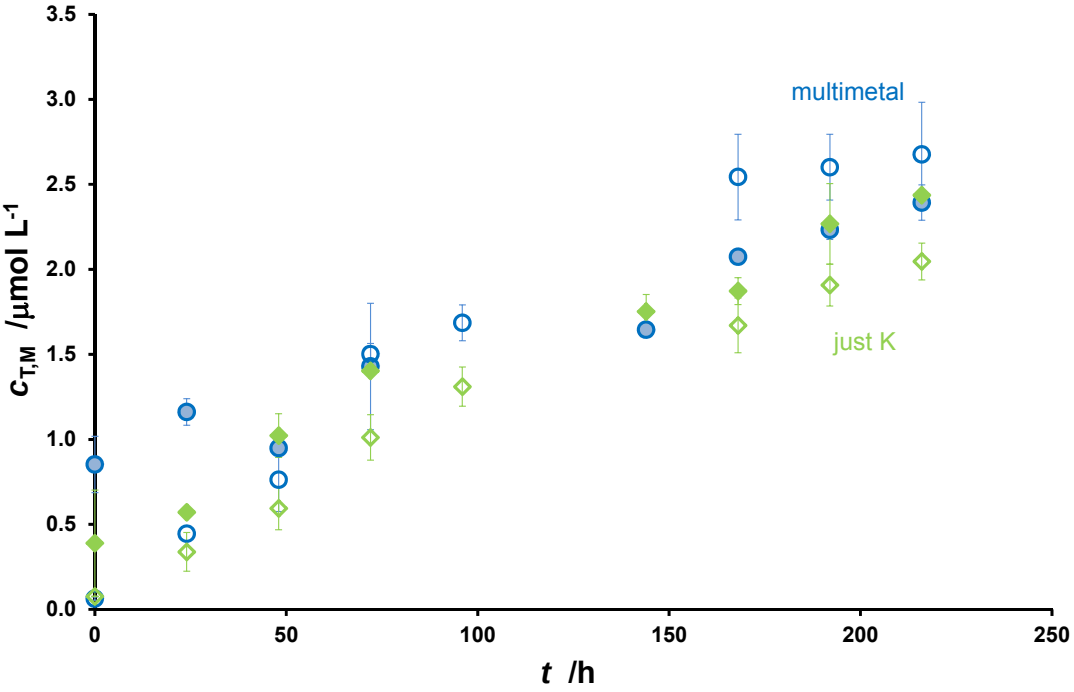

Figure SI-3. Plot of the total concentration of Fe vs *t* in the acceptor of homogenization 1 (full markers) and homogenization 2 (empty markers) of wine. The blue triangle markers correspond to "multimetal" acceptors and green square markers to "just K" ones. Initial conditions in the acceptor: pH=3.422.

Table SI-1. *Concentration and % Zn of selected species (free Zn and main neutral and positive species) predicted by VMinteq for the aqueous synthetic wine*

Specie	**Concentration /mol L^{-1}**	**% Zn**
Zn^{2+}	5.35×10^{-6}	55.5
Zn-Tartrate (aq)	2.86×10^{-6}	29.7
Zn-$(Tartrate)_2^{2-}$	5.13×10^{-7}	5.3
$ZnNO_3^+$	4.77×10^{-7}	4.9
ZnH-$Tartrate^+$	4.33×10^{-7}	4.5