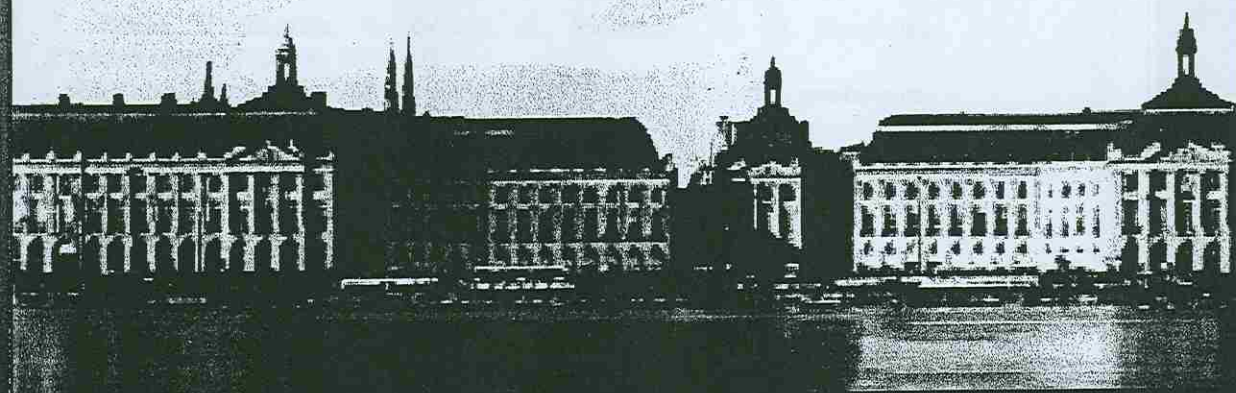**ESEAC 2006**

# 11th International Conference on Electroanalysis

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# Book of Abstracts

# Electrochemical and colorimetric assessment on the influence of target metals on wine color

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Three year old samples of *Vitis vinifera* origin-controlled red wine samples were spiked with adequate amounts of metals and subsequent colorimetric parameters evolution and complexing capacity behaviour were checked.

The used approach consisted in the study of the complexing capacity of natural occurring ligands on wine with respect to Zn and Cu by means of stripping voltammetry in acetate pH 4 buffered 1:5 diluted samples onto which increasing amounts of standard metals were added. The resulting titration curves allowed the ligand concentration to be estimated, and the Scatchard and/or Langmuir algorithm transformation permitted the elucidation of the predominant stoichiometries of existing complexes. This technique has proved very sensitive to detect changes in the composition of samples along fermentation time as a function of major ligand populations [1].

Samples treated in exactly the same way were subjected to spectrometric analysis at selected wavelengths. Stability of polyphenol-metal complexes and its possible redistribution on increasing metal concentration can be followed-up by both classical and Cielab space colorimetric parameters. We have already observed that Fe addition modified certain colorimetric parameters reflecting a change in the polyphenol-metal complexes nature [2].

1. Í. Salinas, I. Esparza, S. Gómez, C. Santamaría, J. M. Fernández. *Electroanalysis.* 17 (2005) 469 – 475
2. I. Esparza, C. Santamaría, J. M. Fernández, Anal. Chim. Acta, in press, available on-line (doi: 10.1016/j.aca.2005.09.043)

# Electrochemical and theoretical assessment of wine by means of complexation processes with Zn and Cu

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## INTRODUCTION

Fermentation of wines is a process which involves many different chemical transformations affecting the concentration of most of the components of this complex matrix. Previous studies[1] showed a dramatic evolution in polyphenol and metal concentrations during the first three or four days, remaining almost constant after that time.
Metal concentrations are generally determined both by Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP- AES)[2], but electrochemical techniques have also been applied to calculate the total and labile metal concentrations depending on the chemical treatment of the sample (digestion, acidification, dilution with water with no pH control)[3].
Dilution of a wine under no pH control conditions induces important changes in the natural equilibria of the matrix, as has been reported elsewhere[4].
In the present work, an electrochemical technique DPASV will be used to follow up the evolution of ligand-metal complexes during the first sixty days of a wine fermentation under dilution at pH control conditions. The concentration of ligand and conditional complexing constants were calculated by Scatchard and Langmuir algorithms[5].
Molecular modelling studies of the main polyphenols present in wine were undertaken with Hyperchem®. These studies were done in the presence of Cu and Zn in order to confirm the stoichiometry of the most energetically favourable ligand-metal complexes, since the application of Scatchard and Langmuir algoritms implies the formation of 1:1 metal – ligand complexes.

## EXPERIMENTAL

The studied samples were must and wine (*V. vinifera* Tempranillo variety grown in Navarra), fermented at different times ranging from 0 to 60 days, being 0 the time when grapes were introduced in the vessel where fermentation will take place. Commercial wines selected for this study were also Tempranillo variety from grapes grown in different parts of Navarra and La Rioja (Fortius (2002), Alex (2004), Orvalaiz (2003), Palacio de Azcona (2004), Ochoa (2001), Piedemonte (2004), Campo Viejo (2004) and Castillo Rioja (2003)).
All must and wine samples were diluted 10 times with a pH 4 acetic – acetate  buffer. Zn and Cu were the metals studied and titration curves for both metals were registered after spiking the diluted wine samples with 50µL of the standard solutions of Zn (2.5 ppm) or Cu (2.0 ppm).
Total concentrations of Cu and Zn were obtained from AAS measurements of the corresponding digested samples.

## RESULTS

Current – Potential curves registered for must and wine samples at different fermentation times  and for commercial wines after the addition of increasing amounts of the corresponding metal.
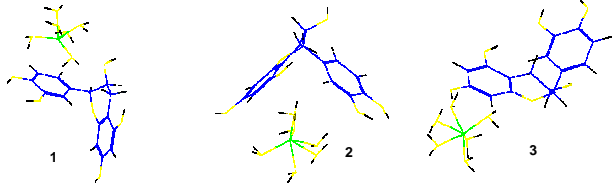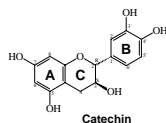
### Zn
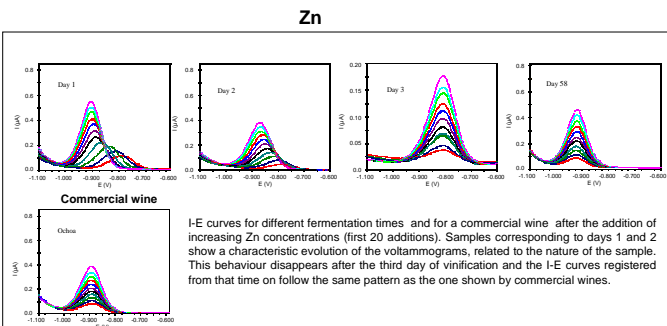


I-E curves for different fermentation times  and for a commercial wine  after the addition of increasing Zn concentrations (first 20 additions). Samples corresponding to days 1 and 2 show a characteristic evolution of the voltammograms, related to the nature of the sample. This behaviour disappears after the third day of vinification and the I-E curves registered from that time on follow the same pattern as the one shown by commercial wines.

**Table 1**: Evolution of total zinc concentration, total ligand concentration ($L_t$) and conditional stability constant ($k'_{Cu}$) for the different times of vinification and for commercial wines, calculated from Scatchard and Langmuir algorithms. The applicability of both algorithms means that the stoichiometry of metal – ligand complex is 1:1. The values show a good correlation between both algorithms.

**Table 1**

| | Total Cu (mg/L) | LANGMUIR | | | SCATCHARD | | |
|---|---|---|---|---|---|---|---|
| | | $L_t$ (µM) | $K'_{Cu}$ x $10^5$ (M⁻¹) | log K'Cu | $L_t$ (µM) | $K'_{Cu}$ x $10^5$ (M⁻¹) | log K'Cu |
| **FERMENTATION PROCESS** | | | | | | | |
| 1 | 0.446 | 0.330 | 2.594 | 6.414 | | | |
| 2 | 0.295 | 0.571 | 1.114 | 6.047 | 0.599 | 0.954 | 5.980 |
| 3 | 0.256 | 2.633 | 0.228 | 5.358 | 2.754 | 0.214 | 5.331 |
| 4 | 0.230 | 0.713 | 0.753 | 5.877 | 0.729 | 0.734 | 5.866 |
| 5 | 0.201 | 1.021 | 0.805 | 5.906 | 1.052 | 0.773 | 5.888 |
| 6 | 0.531 | 1.515 | 0.713 | 5.853 | 1.505 | 0.726 | 5.861 |
| 7 | 0.339 | 3.478 | 0.492 | 5.692 | 3.455 | 0.498 | 5.697 |
| 8 | 0.270 | 1.439 | 0.586 | 5.768 | 1.488 | 0.548 | 5.739 |
| 9 | 0.225 | 1.535 | 0.970 | 5.987 | 1.569 | 0.923 | 5.965 |
| 32 | 0.151 | 3.404 | 0.209 | 5.319 | 3.524 | 0.204 | 5.309 |
| **COMMERCIAL WINES** | | | | | | | |
| Fortius | 0.752 | 1.107 | 3.376 | 6.528 | 1.539 | 1.018 | 6.008 |
| Alex | 0.495 | 2.320 | 0.589 | 5.770 | 2.509 | 0.510 | 5.707 |
| Orvalaiz | 1.055 | 2.434 | 0.683 | 5.835 | 2.492 | 0.647 | 5.811 |
| Palacio de Azcona | 0.875 | 2.089 | 0.759 | 5.880 | 2.067 | 0.773 | 5.888 |
| Ochoa | 0.768 | 2.615 | 0.649 | 5.812 | 2.626 | 0.644 | 5.809 |
| Campo Viejo | 0.727 | 1.453 | 2.291 | 6.360 | 1.554 | 1.704 | 6.231 |
| Piedemonte | 0.393 | 1.156 | 0.679 | 5.832 | 1.255 | 0.592 | 5.772 |
| Castillo Rioja | 0.415 | 3.918 | 0.315 | 5.499 | 4.049 | 0.302 | 5.480 |

### Cu



**A**: I-E curves for day 1 must. Red line shows the response of the diluted sample; the rest of the lines correspond to the first 20 additions of copper. Evolution of the curves with increasing amounts of copper: both peaks increase  in intensity with copper concentration.
**B**: I-E curves for day 1 must for the last 20 additions. Peak 1 keeps increasing with copper concentration but peak 2 stops increasing.
**C**: Titration curve for peak 1: Typical shape for ligand species in solution. **D**: Titration curve for peak 2: unusual shape, ligand species adsorbed on the electrode surface.  Both titration curves (C and D) show the presence of three different slopes which match with the same copper concentration ranges. The first slope in plot **C** corresponds to Cu(Hg)⁰/Cu(L)ₐds⁺ reaction. The second slope in the same plot corresponds mainly to Cu(Hg)⁰/Cu(L)²⁺ [and to a lesser extent to the Cu(Hg)⁰/Cu(L)ₐds⁺ reaction], and the third slope to Cu⁰/Cu²⁺(free metal in solution). Titration curve  **D** corresponds to Cu(L)ₐds⁺/Cu²⁺ reaction. The three consecutive slopes decrease continuously with added copper concentration indicating the progressive saturation of the complexing capacity of the adsorbed ligand. Similar results for natural waters have been published elsewhere[6].
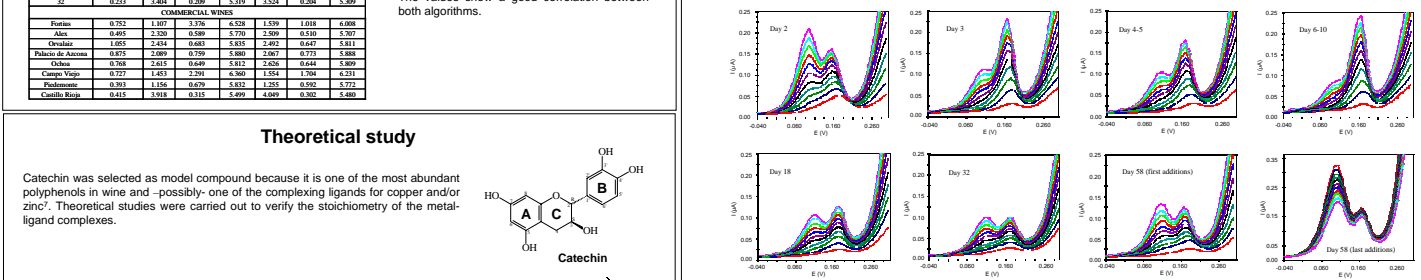
I-E curves shown below correspond to longer fermentation times and display a similar pattern to the one explained above, although the profiles of both peaks are not exactly the same: they evolve with the vinification time and for long times and high concentrations of added copper, they behave similarly to commercial wines, e.g. Ochoa. Peak 2 decreases in intensity from day 1 (plot B) to day 58 (last additions) and tends to disappear for commercial wines, as can be seen in the plot corresponding to Ochoa. Following this behaviour, titration curves corresponding to peak 1 display a shortening in the range of copper concentration where the first slope is measured. Even for some commercial wines, peak 2 was not observed at all as in the case of Palacio de Azcona, for which peak 1 titration curve shows just two sections instead of the three sections displayed above because the corresponding ligand is not present in solution anymore.



#### Commercial wines



**Table 2**: Evolution of total copper concentration, total ligand concentration ($L_t$) and conditional stability constant ($k'_{Cu}$) for the different times of vinification and for commercial wines, calculated from Scatchard and Langmuir algorithms. The applicability of both algorithms means that the stoichiometry of metal – ligand complex is 1:1. The values show a good correlation between both algorithms.

**Table 2**

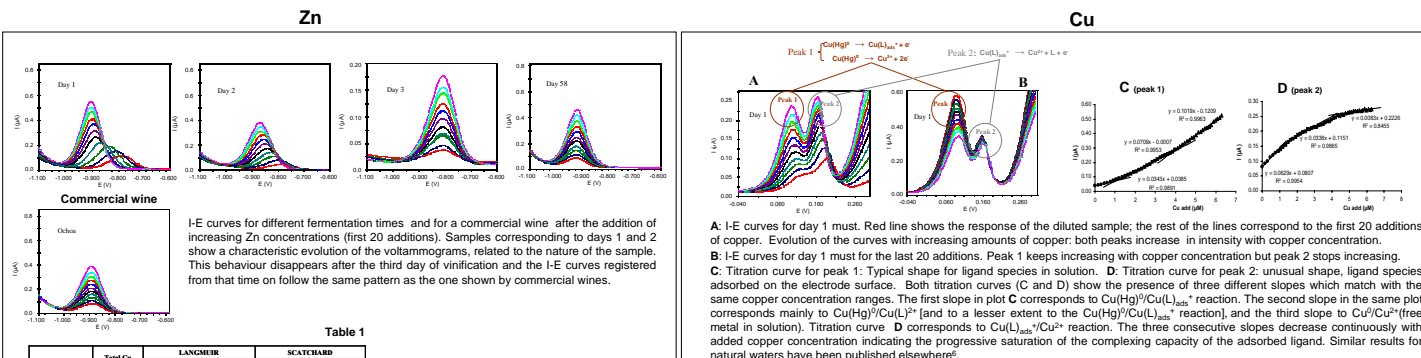| | Total Cu (mg/L) | LANGMUIR | | | SCATCHARD | | |
|---|---|---|---|---|---|---|---|
| | | $L_t$ (µM) | $K'_{Cu}$ x $10^6$ (M⁻¹) | log K'Cu | $L_t$ (µM) | $K'_{Cu}$ x $10^6$ (M⁻¹) | log K'Cu |
| **FERMENTATION PROCESS** | | | | | | | |
| 1 day | 0.958 | 3.18 | 1.61 | 6.21 | 3.16 | 1.60 | 6.20 |
| 2 days | 0.433 | 2.63 | 1.32 | 6.12 | 2.55 | 1.50 | 6.18 |
| 3 days | 0.290 | 4.15 | 1.18 | 6.07 | 4.07 | 1.26 | 6.10 |
| 4 days | 0.207 | 1.98 | 3.53 | 6.55 | 1.98 | 3.47 | 6.54 |
| 6 days | 0.170 | 2.30 | 2.60 | 6.42 | 2.28 | 2.75 | 6.44 |
| 7 days | 0.180 | 3.59 | 1.80 | 6.26 | 3.55 | 1.89 | 6.28 |
| 8 days | 0.167 | 2.69 | 1.55 | 6.19 | 2.66 | 1.63 | 6.21 |
| 9 days | 0.178 | 2.36 | 1.69 | 6.23 | 2.36 | 1.73 | 6.24 |
| 10 days | 0.202 | 2.29 | 1.83 | 6.26 | 2.30 | 1.79 | 6.25 |
| 18 days | 0.190 | 1.80 | 2.14 | 6.33 | 1.81 | 2.10 | 6.32 |
| 32 days | 0.131 | 1.39 | 2.04 | 6.31 | 1.39 | 2.00 | 6.30 |
| 58 days | 0.158 | 1.65 | 1.63 | 6.21 | 1.66 | 1.60 | 6.21 |
| | 0.063 | 1.30 | 1.60 | 6.20 | 1.31 | 1.66 | 6.22 |
| **COMMERCIAL WINES** | | | | | | | |
| Fortius | 0.049 | 0.636 | 1.27 | 6.10 | 0.627 | 1.35 | 6.13 |
| Alex | 0.037 | 1.89 | 0.478 | 5.68 | 1.90 | 0.482 | 5.68 |
| Orvalaiz | 0.157 | 0.587 | 1.81 | 6.26 | 0.589 | 1.80 | 6.26 |
| Palacio de Azcona | 0.117 | 0.795 | 3.91 | 6.59 | 0.773 | 4.63 | 6.67 |
| Ochoa | 0.113 | 1.340 | 0.649 | 5.81 | 1.25 | 0.653 | 5.81 |
| Campo Viejo | 0.084 | 1.15 | 0.468 | 5.67 | 1.30 | 0.421 | 5.62 |
| Piedemonte | 0.053 | 3.208 | 0.373 | 5.57 | 3.11 | 0.396 | 5.60 |
| Castillo Rioja | 0.166 | 0.650 | 1.37 | 6.14 | 0.683 | 1.34 | 6.13 |

### Theoretical study

Catechin was selected as model compound because it is one of the most abundant polyphenols in wine and –possibly- one of the complexing ligands for copper and/or zinc[7]. Theoretical studies were carried out to verify the stoichiometry of the metal-ligand complexes.

**Catechin**



Study of the inter- molecular approaching interaction for the system Cu-Catechin. The optimised molecular systems were calculated using molecular mechanics. In the case of Catechin, three stable interactions were obtained. Two of them with similar stability: one involving the aromatic π electrons on the rings A and C, and the phenols in 5 (ring A) and in 5' (ring C) (Structure **1**); and another involving the aromatic π electrons located on the structural group formed by rings A and B, and the oxygen of the phenol in position 5 (Structure **2**). And finally the most stable interaction involving the aromatic π electrons located on ring C and the oxygen of the phenol in position 3 (Structure **3**). It was noteworthy that those interactions involving aromatic π electrons were associated with a decrease in the aromatic character of the related rings. This fact was reflected in the conformational changes undergone by those aromatic rings.

## REFERENCES

1.- I. Esparza, I. Salinas, I. Caballero, C. Santamaría, I. Calvo, J.M. García-Mina, J.M. Fernández. Anal. Chim. Acta, 524 (2004) 215
2a. - M. Aceto, O. Abollino, M.C. Bruzzoniti, E.Mentasti, C. Sarzanini, M. Malandrino. Food Additives and Contaminants, 19 (2) (2002) 126.
2b. - R. Larcher, G. Nicolini. Ital. J. Food Sci. 13 (2) (2001) 233.
3. - M.T. Arcos, M.C. Ancín, J.C. Echeverría, A. González, J.J. Garrido. J. Agric. Food Chem., 41 (1993) 2333.
4a. - A. Boutaric, L. Ferre, M. Roy,  J. Annal. Falsific. 30 (1937) 196.
4b. - P. Ribéreau - Gayon, Yves Glories, Alain Maujean,Denis Dubourdieu,"Tratado de Enología". Ed. Hemisferio Sur. Tomo II
5. - C.M.G. van den Berg, J.R. Kramer, Anal. Chim. Acta, 106 (1979) 113.
6a. – G. E. Batley, Anal. Chim. Acta, 189 (1986) 371.
6b. – C.M.S. Botelho, R. A. R. Boaventura, M.L.S. Simoes Gonçalves, Electroanalysis 14 (24) (2002) 1713.
7. I. Esparza, I. Salinas, C. Santamaría, J.M. García-Mina, J.M. Fernández, Anal. Chim. Acta, 543 (2005) 267.

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