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Electroanalytical Determination and Fractionation of Copper in Wine

A. Sánchez Misiego,*,† R. M. García-Moncó Carra,†
M. P. Ambel Carracedo,† and M. T. Guerra Sánchez-Simón§

Departamento de Química Analítica y Electroquímica, Universidad de Extremadura, 06071 Badajoz, Spain, and EUITA, Centro Universitario Sta Ana. Almendralejo, 06200 Badajoz, Spain

Eight different bottled wines (six red wines and two white ones) were studied for copper determination and fractionation. For this purpose, copper determination by square wave voltammetry (SWV) and potentiometry (PSA) stripping analysis using Hg electrodes (drop and film, respectively) were carried out. Two direct procedures for the determination of total copper in wine are proposed; in both cases, drastic treatment of samples is not necessary, the procedures are very fast (estimated time to carry out an analysis is <10 min) and require no deaeration. Fractionating treatment consists of various HCl additions followed by the addition of ethylenediamine. Precision (RSD < 3%) and accuracy (recovery > 98%) data justify that both methods proposed are valid for total copper determination in wine. The wines studied displayed similar behaviors regarding fractionation: the percentages of total copper fractionated in each step are statistically similar: differences are lower than 2 S.

KEYWORDS: Copper; voltammetry; potentiometry; fractionation; wine

INTRODUCTION

The degree of usefulness of analytical information usually depends to an important extent on how easily it can be obtained; this includes aspects such as the simplicity of the analytical techniques used, the instrumentation and execution costs, and equipment mobility. On the other hand, the interest in the knowledge, effects, and control of oligoelements justifies the increasing relevance of analytical chemistry (in this facet of its activity), regarded as the science dedicated to the design of new procedures for their analysis and monitoring. Bringing both reflections together one concludes that, in this field of biomaterials, attention must be focused on the development of analytical methodologies sufficiently accessible (cost, size, facilities, etc.) and sufficiently sensitive. With respect to these determining factors, electroanalytical techniques often present significant advantages, especially in routine analyses (*1*).

Copper contained in wine can have its origin both in the initial must or as an additive/contaminant during the manufacturing process. Copper in must may also come from two different sources: the biological fluid itself or as a result of some type of treatment (anticryptogamic, for instance). In a similar way, during the vinification copper can be added both intentionally (through additives, clarifiers, etc.) and nonintentionally (as a result of contact with piping, vessels, and other copper-containing materials in the cellar). All of the above justify the presence of a variable, yet important, amount of copper in wine. Both during and after the vinification, copper can evolve in two different directions: as a precipitate, mainly with sulfur, and as soluble complexes of very dissimilar strengths; inorganic and mostly organic ligands participate in the latter complexes in the form of peptides, proteins, anthocyans, etc. However, between the two directions already mentioned, there is a third intermediate—one characterized by its colloidal nature. As a result, not only does the stability of complexes become important in those physical−chemical equilibria in which copper participates but also redox processes and phenomena related to colloidal systems (adsorptive, peptization, etc.). Besides, this phenomenology tends to be slow (*2*, *3*).

Due to all of this, detailed speciation of copper in wine is not possible nowadays. Furthermore, and as is the case with elements other than copper and biological matrixes other than wine, the research carried out actually refers to the fractionation (rather than the speciation) in groups of chemical forms of copper present in the wine. Several authors have designed fractionating protocols to provide an increasing amount of information related to the topic mentioned (*4*−*11*). The latter information is very useful to satisfy the growing demand regarding the copper−wine binomial in scientific, technological, commercial, toxicological, and cultural fields. For these reasons, in the present work, copper has been selected in the matrix wine.

These authors have studied the copper content in various fluids (*12*−*14*) but never before in a vegetal matrix. The aim was to design direct procedures for the determination of copper in wine, as well as to establish different fractionation means. Anodic stripping techniques were employed: square wave voltammetry (SWASV) and potentiometry (PSA) with static

* Author to whom correspondence should be addressed (e-mail amisiego@unex.es; telephone 924289393; fax 924274244).
† Universidad de Extremadura.
§ Universitario Sta Ana. Almendralejo.

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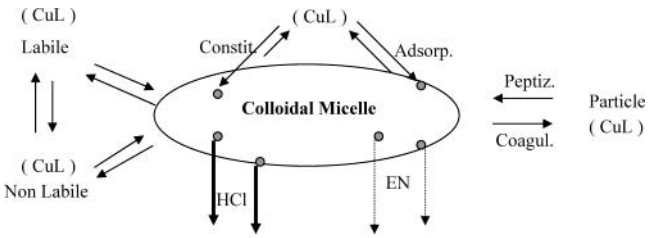
drop and mercury film electrode, respectively. In previous works, direct determinations were carried out using ethylenediamine (EN) as the only medium modifier in matrixes with increasing amounts of organic matter (OM): water, seawater, cerebrospinal fluid (LCR), and blood plasma; both the electroanalytical techniques used for stripping and the working medium (EN) provided a sensitivity that allowed a high enough dilution to avoid problems caused by Hg^0 (electrode) ↔ OM (matrix) interactions; another feature of the mentioned publications is that the total amount of copper contained in the samples was determined in all of them; this information is normally sufficient. However, experimental data prove that when EN is used as the fractionating agent (in order to obtain additional and very interesting information from both technological and commercial points of view), the corresponding results obtained are too erratic. The latter implies the necessity to find not only the reason for this phenomenon but also some solution to the problem.

Theoretically, in biological systems copper forms complex species that participate in equilibria and appear in different types of dispersions: solution, colloidal, and suspension.

$$(Cu^{2+})aq + L \rightleftharpoons (CuL)diss \rightleftharpoons (CuL)coll \rightleftharpoons (CuL)part$$

In the case of wine, the last two above-mentioned situations originate unwanted phenomena: turbidity and development of a precipitate, respectively. In general, the processes are slow and their kinetics establish the rate of the phenomenology observed in wine. EN is highly capable of destabilizing the CuL species, and it has an influence on the final equilibrium conditions.



In theory, an exclusive use of electroanalytical techniques (combined with the reagent EN) allows copper fractionation in biological fluids to various degrees. Among voltammetric modes, SW was selected because it is the fastest one and also because it allows work to be performed under open-atmosphere conditions. Due to these reasons PSA techniques were also used. The following table briefly shows a feasible option applied to copper in wine:

technique (SWASVor PSA)	Cu fraction measured in wine
with EN (added after accum)	"labile": hydrated ion and other complexes with Cu reducible over Hg^0 at –1.0 V: nearly the total Cu in the true solution
with EN (added before accum)	exchangeable with EN: free, labile, and nonlabile fraction scavenged
with EN (added after mineralization)	total Cu (any possibility)

This general outline leads to the assumption that the use of different EN concentrations allows similar fractionation structures to be obtained. Nevertheless, preliminary studies provided nonreproducible results, even for the same type of wine. Everything consistently indicates that the studied phenomenology is mainly controlled by a kinetic component which prevails over the thermodynamic component, in a way similar to that described by Mackey and Zirino (*15*) in seawater. To overcome this inconvenience (obviously complex and mainly of kinetic nature), a second agent was added: HCl. Just like EN, HCl has the ability to destabilize CuL species and, furthermore, act with greater speed than EN. Thus, under the described conditions, HCl fractionation speed and EN electroanalytical properties can be made the most of.

MATERIALS AND METHODS

Materials and Reagents. The materials to be analyzed were eight different bottled wines (six red wines and two white ones): seven commercially available Spanish wines and one obtained from the enologic laboratory of the University of Extremadura. Once opened, each wine was kept at 2 °C until analyzed.

Mercury chloride, hydrochloric acid, and nitric acid (all of "Suprapur" grade) were purchased from Merck. Distilled nitric acid was obtained from a quartz distillation device (Kürner).

EN solution was prepared from Merck−Schuchardt reagent for synthesis and kept below 5 °C in a light-protected room.

Copper stock solution (1 g L^{-1}) was prepared from a "Titrisol" standard (Merck) and acidified to pH 2 with HCl. Diluted standards were prepared for daily use. Glassware, quartz, polyethylene, and Teflon materials used were cleansed by keeping them at 60 °C in an HCl (analytical reagent grade)−H_2O 1:10 bath for 3 days and then in an HNO_3−H_2O 1:10 bath for another 3 days.

The water used to prepare all of the solutions and to rinse all of the materials was of "Suprapur" grade [$R = 18$ MΩ purified via a Mill-Q system (Millipore)].

Apparatus and Electrodes. Voltammetric and potentiometric stripping measurements were carried out by employing an apparatus and electrodes the characteristics of which have already been described in a previous work (*14*). Film preparation is described in a previous publication (*12*).

When necessary, the samples were digested in an Ethos Plus Microwave Lavestation, with varying power, which permits the simultaneous digestion of 10 samples in Teflon vessels (HPR 1000-10) at controlled temperature and time. The software chosen to control the process was Easy Wave 3.30.

Instrumental Parameters. *SWASV:* Eac, −1000 mV; Tac, 60 s; stirring rate, in position 3 of stand (1500 rpm); square wave frequency, 50 Hz; anodic sweep, from −700 to −200 mV; wave amplitude, 50 mV; potential step, 2.4 mV. The analytical signal was measured versus the baseline.

PSA: Eac, −1200 mV; Tac, 60 s; rest time, 30 s; stirring rate, 1500 rpm. The oxidation curve was recorded from −1200 to −100 mV by means of the chemical oxidants in solution (oxygen and Hg^{2+}) and applying a positive constant current of 1.00 μA. Previous studies (*14*) have proved that the simultaneous use of chemical (O_2 and Hg^{2+}) and electrochemical (current) oxidants provides more accurate results.

Methods. From each of the selected wines, nine aliquots of 2 mL were taken and modified by adding different amounts of concentrated HCl: 0, 10, 20, 35, 50, 100, 150, 200, and 250 μL. The copper content in each aliquot was determined using simultaneously two anodic stripping electroanalytical techniques: square wave voltammetry (SWV) with static mercury drop and potentiometry (PSA) with mercury film electrode.

In both techniques the operative procedure consisted of preparing a blank solution with 6 mL of water, 10 μL of concentrated HCl, and 2100 μL of EN 0.15 mol L^{-1}; one addition of 125 μL of wine sample modified (2 mL of wine with 100 μL of concentrated HCl) and two additions of standard copper solution (each addition increased the copper concentration in the solution by ∼1.2 μg L^{-1}). All values shown in the tables and figures were corrected for the dilution effect. This addition sequence has been repeated on some occasions (see **Figure 2**).

The analytical signal was obtained after each addition, and the copper fraction measurable in wine was calculated. All work was performed

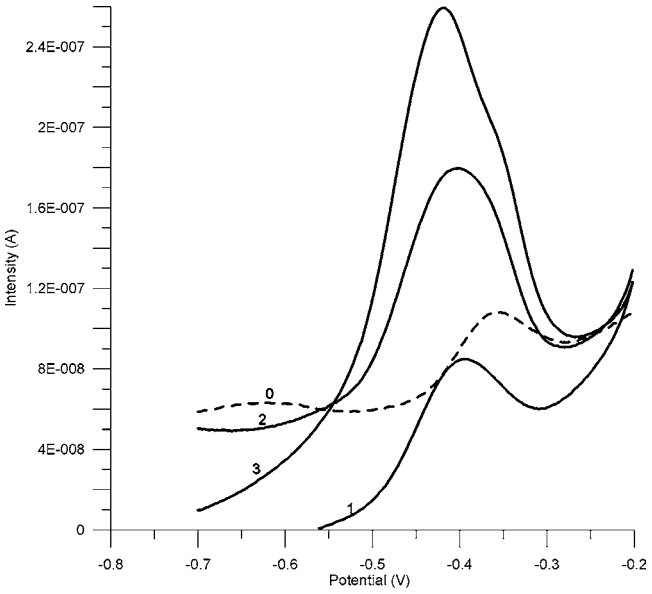


Figure 1. Example of voltammograms of Cu determination: (curve 1) blank solution with 125 μL of modified wine; (curves 2 and 3) curve 1 with 100 and 200 μL of Cu (100 μg L^{-1}), respectively; (curve 0) curve 1 without EN.

at room temperature, which was 25 ± 2 °C, and the solutions were never deareated.

Sample digestion was used only to investigate the accuracy of the proposed procedures. Approximately 1 g of wine was accurately weighed in a Teflon vessel, and 2 mL of distilled nitric acid was added. The vessel was closed and inserted in the microwave system. The digestion program comprised a fast temperature ramp to 85 °C (2 min), a 4 min ramp from 85 to 140 °C, a 5 min ramp from 140 to 230 °C, and 10 min at 230 °C. The power varied between 0 and 1000 W during the process. The vessels were then opened, and the colorless solution was evaporated to dryness. The residue was dissolved in 2 mL of water acidified to pH 2 with HCl.

RESULTS AND DISCUSSION

According to the procedures specified, wine present in the cell (as well as its OM) is diluted ~70 times and copper concentration is of the order of 1 μg L^{-1}. A typical set of voltammograms obtained along one copper determination is shown in **Figure 1**. Sometimes (voltammogram 3) a small shoulder appears in the voltammogram corresponding to the second addition of standard copper solution. This phenomenon (which does not modify significantly the result) indicates that the concentration of available EN is starting to become insufficient; in such cases, a new addition of 100 μL of EN suppresses the aforementioned shoulder.

The first experiments confirmed that the ethanolic fraction of the solvent does not lead to significant changes in the analytical signal with respect to that obtained in totally aqueous media. Possible interferences caused by OM were studied as well; those experiments designed for this purpose consist of six measurements that correspond, successively, to one addition of previously modified wine (adding 100 μL of concentrated HCl to 2 mL of sample) and two additions of standard copper solution (points 1, 2, and 3) and repetition of the same sequence (points 4, 5, and 6). The straight lines configured with these data have linear correlation coefficients (r) >0.99. **Figure 2** shows the results obtained with two different wines (red and white); both the great similarity of the slopes and the coherence between X-intercept values can be observed. This proves that there is no interaction between the OM from the wine and the

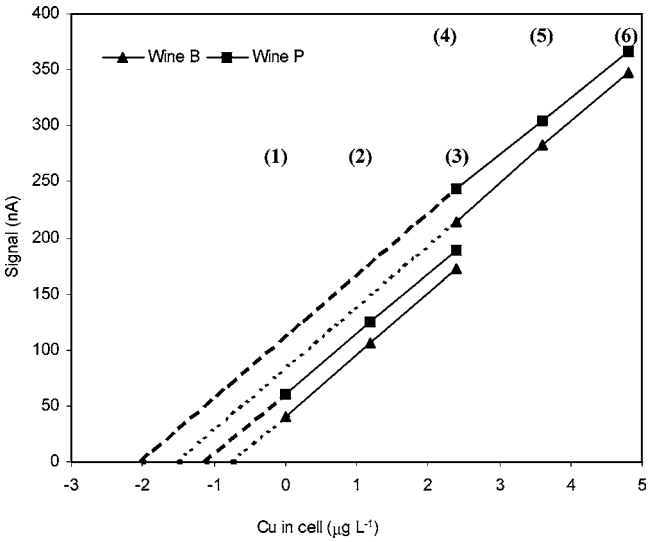


Figure 2. SWASV copper determination in wines P (red) and B (white): (1) blank solution with modified sample; (2 and 3) (1) spiked with 1.2 and 2.4 μg L^{-1} of Cu, respectively; (4, 5, and 6) the sequence (1), (2), and (3) repeated.

Table 1. Analytical Quality of the Results Obtained Using in Data the Mean of the Two Values of Cu Found When 100 and 125 μL of HCl/mL of Wine Were Added[a]

	DL	precision RSD (%)	accuracy[b] recovery (%)
SWASV	<0.06 μg L^{-1} of Cu in cell	1.85	98.1
PSA	<0.04 μg L^{-1} of Cu in cell	2.90	98.2

[a] Average values from eight determinations. [b] Reference material used: the same (wine) previously mineralized with HNO_3.

mercury electrode and that only the first sequence (points 1−3) is necessary for the determination of the labilized copper. On the other hand, there is evidence that, under the described working conditions, all of the added copper spike forms complexes with the EN and it is not complexed with the OM from the wine. Parallel experiments carried out with hydroalcoholic solutions provide signals similar to those obtained with real samples; this behavior is similar to that observed with seawater (*16*): the high rate of complexation of the added copper with the EN does not allow the formation of any other complex of copper.

Direct Determination of Total Copper. By applying the general procedures already described, the total copper content has been determined in eight different wines (six red and two white). In all cases, 2 mL aliquots were taken and 250 μL of concentrated HCl was added. The analyses conducted at various time intervals (from 0 to 60 min) between the addition of HCl and the addition of EN show significantly equal results; this confirms the great speed of HCl as well as the slowness of EN under similar conditions. Each analysis was carried out twice, and the average value was used for calculations. Information regarding the accuracy of the results was obtained using as a reference value for each type of wine—the content of copper found in the same wine previously mineralized with HNO_3 in the microwave digestor; to compare voltammetric and potentiometric results, a set of Cu analyses was performed by AAS on a graphite furnace with Zeeman correction. **Table 1** summarizes the results (quality parameters) obtained when the two analysis methods employed (voltammetric and potentiometric) were applied. Overall, they present very similar char-

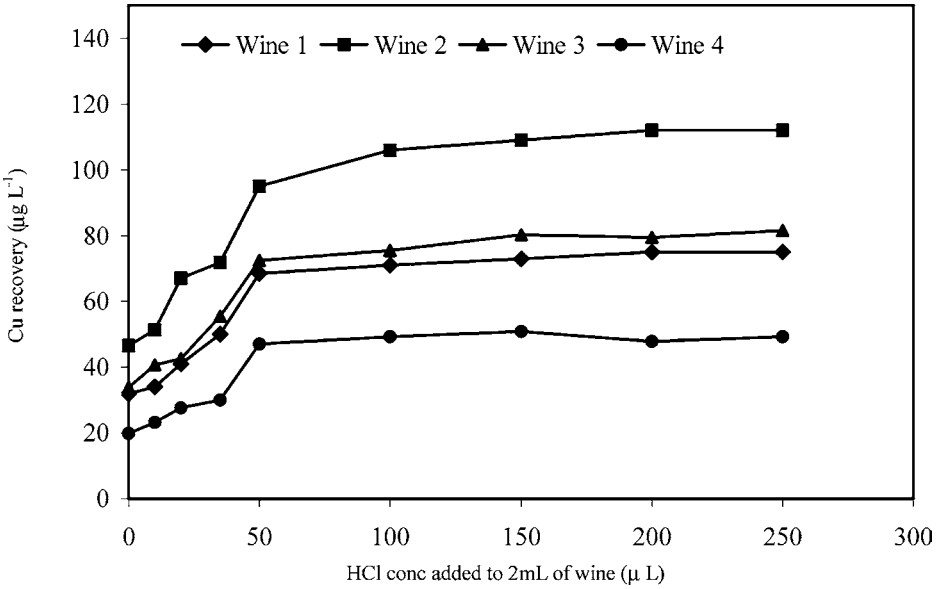


Figure 3. Fractionation curves (SWASV): influence of HCl added to sample in Cu fraction measured in wines 1, 2, and 3 (red) and 4 (white).

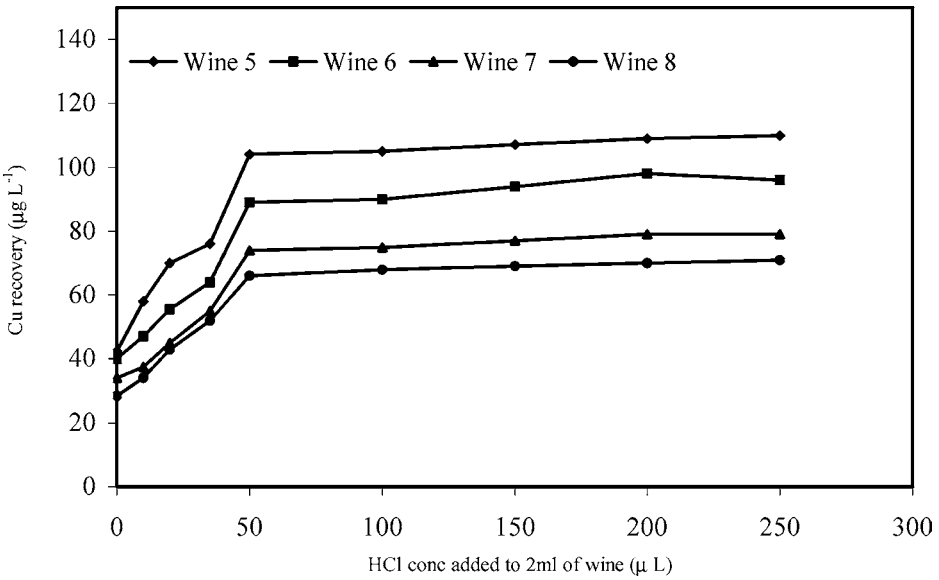


Figure 4. Fractionation curves (PSA): influence of HCl added to sample in Cu fraction measured in wines 5, 6, and 7 (red) and 8 (white).

acteristics. PSA has a small advantage versus SWASV regarding DL, which is compensated by the latter with a slightly better precision. Accuracy data justify in both cases that the two methods are valid for the determination of total copper in wine.

Fractionation of Copper in Wine. First, the destabilizing power of HCl was used, mainly based on the interaction of its H^+ ions with those ligands that stabilize the copper ions and yield complex species that are either soluble or form part of colloidal micelles (as adsorbed forms or as part of the micelles themselves). This process is very fast when compared to the slow performance of EN. Depending on HCl concentration, these species will evolve to a certain extent into soluble forms that will quickly react with EN to form $Cu(EN)_2^{2+}$, electroreducible at −1000 mV. Four different types of wine were studied using SWASV; growing quantities of HCl were added to them and, subsequently, the fraction of labilized copper was determined. Results corresponding to the wines studied can be seen in **Figure 3**, which shows similar curves that tend, in every case, to significantly constant values when the volume of HCl added is >100 μL; for each wine, these values statistically coincide (differences lower than 2 S: S deduced from 20 measurements) with the amount of copper recovered from the same wine previously mineralized (considered as total copper content). **Figure 3** allows as well to establish three quantities of HCl for which the percentage of copper measured (with respect to the total content) remains significantly constant for all four wines: 0.0 μL of HCl and intermediate values from the two portions of the curves that present the minimum slope: 20−35 μL of HCl and over 100 μL of HCl added to 2 mL of wine; consequently, 0.0, 30, and 200 μL of HCl added to 2 mL of sample (see **Table 2**) were the working quantities selected. Parallel studies using PSA provide very similar results. **Figure 4** and **Table 3** show the results corresponding to four wines different from those presented for SWASV. These results prove that the total copper share in wine follows parameters that are fairly common to all wines, particularly when certain fractions are considered and HCl is used as the differentiating agent. Vasconcelos et al. (*11*) concluded that both qualitative and quantitative distribution of OM is, generally, fairly uniform in most of the wines. According to this, the results obtained in the present paper are consistent, considering the HCl−EN binomial will act similarly on different wines.

Table 2. Fractionation Parameters (SWASV): Influence of the Volume (Microliters) of HCl Concentrate Added to 2 mL of Wine Prior to the EN Addition

wine	mineralized wine total Cu (μg L^{-1})	Cu found (ppb) (effect of HCl volume added)				% of total Cu fractionated			
		0	30	100	200	0	30	100	200
1 (red)	76.3	32.0	47.0	71.0	75.0	41.9	61.6	93.1	98.3
2 (red)	114	46.5	70.0	106	112	40.8	61.6	93.0	98.2
3 (red)	82.3	33.8	51.2	75.5	79.4	41.1	62.2	91.7	96.5
4(white)	49.3	19.8	29.3	49.3	47.8	40.2	59.4	100	97.0
mean						41.0	61.2	94.5	97.5
MD						0.5	0.9	2.8	0.75
RMD (%)						1.2	1.5	3.0	0.8

Table 3. Fractionation Parameters (PSA): Influence of the Volume (Microliters) of HCl Concentrate Added to 2 mL of Wine Prior to the EN Addition

wine	mineralized wine total Cu (μg L^{-1})	Cu found (ppb) (effect of HCl volume added)				% of total Cu fractionated			
		0	30	100	200	0	30	100	200
5 (red)	111	42.4	74	105	109	38.2	66.7	94.6	98.2
6 (red)	98	40	61	90	98	40.8	62.2	91.8	100
7 (red)	80	34	52	75	79	42.5	65.0	93.8	98.8
8 (white)	71	28.5	49	68	70	40.1	69.0	95.8	98.7
mean						40.4	65.7	94.0	98.9
MD						1.3	2.1	1.2	0.53
RMD (%)						3.1	3.2	1.3	0.5

Conclusions. The present study provides two new procedures for copper determination in wine. They are both based on electroanalytical stripping techniques: square wave voltammetry (SWASV) and potentiometry (PSA). In both cases direct procedures were used (without drastic treatment of the sample), which can also be considered very fast because they do not need treatment, require only a small accumulation time (60 s), and operate in open atmosphere (no deaeration), in addition to the typical quickness of these techniques; estimated time to carry out an analysis is <10 min.

Those results provided under Fractionation of Copper in Wine allow one to conclude that total copper determination is feasible by measuring the percentage of copper recovered when adding 0.0, 30, or 200 μL of HCl to 2 mL of sample, prior to the measurement.

Fractionation studies allow procedures to be proposed to establish different fractions of copper in wine; each of these fractions corresponds to a common behavior in the presence of HCl. The presented fractionation (labilization + determination) methodology is based on the differential labilization of complex Cu species, in which two factors intervene: (a) the chemical composition of the ligands (grouped in families of substances) and their thermodynamic constants (acid−base and Cu complexation); (b) the chemical−physical constants of the medium and the process kinetics at the moment in which Cu reaches the situation that it presents in the wine. All of this suggests new studies (already initiated by these authors) in relation with the reincorporation of the Cu previously labilized with HCl (just by recovering the initial pH of the wine) or of the Cu added ex novo. The latter conclusion opens new investigating routes, very interesting from a technological point of view. For instance, these authors are developing different techniques for clarifying wine (and their corresponding efficiencies), with the purpose of establishing optimization procedures in such an important phase in enology as the clarification. The study of curves such as those from **Figures 3** and **4** will become of great importance for assessing the efficiency of the clarification process, as well as for diagnosing the state of any wine with respect to its possible turbidity. On the other hand, it is very likely that the behavior described in this paper is not exclusive to the Cu−HCl binomial but that it is also present in other oligoelements and with other fractionating reagents. In such a case, a very interesting field of investigation would be open.

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