

# Electrochemical biosensor for detection of copper ions in wine

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## Abstract

Determination of copper content in wine is of particular importance due to the fact that a relatively high concentration of this element can lead to the development of copper casse. The presence of copper in wine is the results of using copper sulfate solutions in the treatment of vine and for the removal of sulfide odors in wine, and to a lesser extent is due to the accumulation of copper ions from soil and atmosphere.

This paper describes an electrochemical method for the determination of copper traces in wine using a specific biosensor. Electrochemical biosensors are among the instrumental methods with high sensitivity that can be used to determine copper ions. The developed biosensor uses as recognition element an amino acid, namely glycine, and displays the response in the form of a cyclic voltammogram. In the first part of this paper a brief description of the biosensor used for the quantification is given, while the second half of the study comprises the results of the experimental analysis conducted. A comparative study was carried out between the experimental data regarding the detection limit of the proposed biosensor using 10 wine samples and the results of an ICP-MS analysis of the same wine samples.

**Keywords:** glycine, cyclic voltammogram, copper casse, ICP-MS

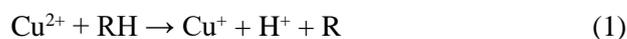
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## 1. Introduction

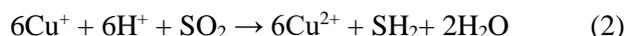
Copper is an element with a considerable notoriety for wine. The current practice of winemaking tends to minimize the amount of copper resulting from wine and vine sources. Despite these efforts, the addition of copper(II), either as sulfate or citrate, to remove sulfide odors can result in a high concentration in wine bottled [1]. During the alcoholic fermentation the copper content is reduced significantly, however, depending on the initial metal concentration, this decrease can not be sufficient to protect the wine from loss of stability [2, 3]. The presence of copper in wine in a high concentration may lead to the copper casse of wine, process which can be induced at concentrations of 0.5 mg/L [4].

Copper casse is indicated by the presence of a reddish-brown precipitate, which appears in wine as a result of the formation and insolubilization of some compounds as an outcome of the reaction between copper and different substances. This form of casse may occur in any wine with a high copper content which has been kept for a long time in the absence of air (bottled wine) and/or is rich in sulfur dioxide, chemical compound which provides a reducing medium. Solar light accelerates the occurrence of this phenomenon by favouring photochemical reduction reactions [2]. The resulting deposits contain copper and copper sulfate, as well as proteins. The mechanism unfolds through the following steps:

(a) Formation of reducing copper ions:



(b) Reduction of sulfur dioxide:



(c) Formation of copper sulfide and SCu flocculation upon contact with proteins:



In another mechanism,  $\text{Cu}^{2+}$  is reduced to  $\text{Cu}^+$ , which partially precipitates as colloidal element, while the remaining  $\text{Cu}^+$  reduces  $\text{SO}_2$  to  $\text{H}_2\text{S}$ , leading to SCu formation. The colloid is then flocculated [5, 6].

The analysis of metallic ions in wine has the following main objectives: contamination monitoring, study of concentration changes of an element during wine making, determination of the legal limits for export, as well as their contribution to the quality of wine aromas and role as markers of the authenticity of the final product. Of the several methods available for the determination of the concentrations in metals, commonly used are atomic spectrometry and the electrochemical techniques [7].

Several papers have reported the use of miniaturized electrodes or miniaturized particles coupled with various detection techniques for the study of heavy metals [8-12].

Electrochemical biosensors are defined by miniaturization, robustness, portability, sensitivity, selectivity, low analyte volumes, and minimal costs. In the last decades, tremendous advances have been made in electro-analytical chemistry through the development of ultra-microelectrodes, adapted interfaces, molecular devices and, intelligent sensors [13, 14].

Voltammetry is the most widely used technique in electrochemistry, partly due to the fact that allows the study of system reversibility [15]. Cyclic voltammetry (CV) is a technique often used for its relative simplicity and the large data that derives from its use [16].

The purpose of this study is to achieve a method of identification and quantification of the copper ion in wine before bottling to prevent the copper casse. For this purpose, glycine has been used as biological recognition element, and the immobilization method is photoreactive with benzophenone.

The principle of the method is based on the property of Cu(II) to form stable complexes with chelating amino acids, and the scientific evidences that glycine is one of the simplest amino acids that can bind copper to a dimeric form [17].

## 2. Materials and methods

### 2.1. Materials

Glycine, benzophenone and copper sulfate were purchased from Sigma Aldrich. Wine samples were purchased from a wine vault in Suceava County.

### 2.2. Apparatus

Electrochemical measurements were performed with a Metrohm Autolab PGSTAT204 potentiostat/galvanostat controlled by the NOVA 2.1 software.

To verify the method, the same samples of wine were analyzed by the commonly used method, namely ICP-MS. For spectroscopic analysis, the Agilent Technologies 7500 Series spectrometer (Agilent, USA) was used.

### 2.3. Immobilization on the electrode

The glycine solution was immobilized with 5% benzophenone solution in the presence of UV light at the 360 nm wavelength on the working electrode. The electrodes used in this study are of the type of screen printed electrodes, with an working electrode made out of carbon.

Advantages of immobilization with benzophenone include chemical stability, activation in a UV range that does not degrade biological elements and stability to ambient light. Disadvantages are the hydrophobicity and volume increase of the molecule [18].

Benzophenone has been reported to be one of the most effective photophores. The photoprocess of benzophenone includes a 3-phase excitation forming a diradicaloid. The oxygen deficient electron interacts with weak C-H bonds resulting in loss of hydrogen and a recombination of the radical which creates covalent bonds with oligonucleotides and proteins [19].

### 2.4. Preparation of samples for ICP-MS

Approximately 1 ml of each sample of wine was weighed into PTFE containers and dissolved in 9 ml of 65%  $\text{HNO}_3$  and 1 ml of 30%  $\text{H}_2\text{O}_2$ .

Blank solutions were prepared in the same way [20]. The copper concentration was calculated using the following formula:

$$C = \frac{C_m \times V_t}{V_m} \quad (4)$$

C = concentration of the element in the sample;  
 $C_m$  = concentration of the element in the diluted sample;  
 $V_t$  = final volume of solution in ml;  
 $V_m$  = volume of wine used, in ml [21].

### 3. Results and discussions

Most cyclic voltammograms have an anodic and cathodic peak, and their values and other parameters can be calculated.

As shown in Figure 1, when E becomes more positive, the analyte becomes oxidized, and when E becomes more negative, the analyte is reduced. The relationship between  $i_p$  and  $v$  is given by the Randles-Sevcik equation (Eq. 5):

$$i_p = (2.69 \times 10^5) A C D^{1/2} n^{3/2} v^{1/2} \quad (5)$$

Where:

A = surface of the electrode,  
 C = concentration of analyte in solution,  
 D = diffusion coefficient of the analyte,  
 N = the number of electrons involved in the reaction.

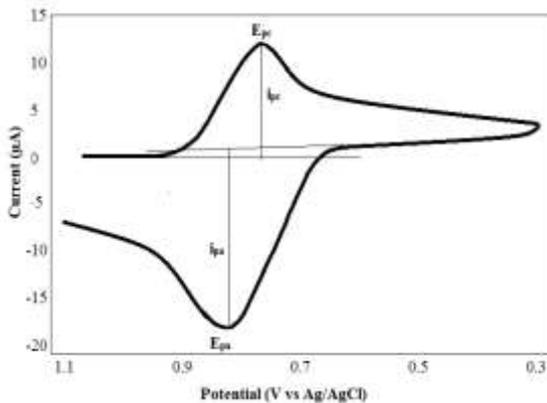


Figure 1. Typical cyclic voltammogram showing significant parameters [22]

The magnitude of  $i_p$  can be applied to determine the analyte concentration and the potential to which the oxidized/reduced analyte can be used for qualitative identification.

Maximum peak current can be improved by redox cycles through which species of interest that are oxidized or reduced to the surface of the electrode are regenerated (and thus repeatedly measured) either chemically, enzymatically or electrochemically [23].

In order to carry out the proposed study, copper solutions of 0.2, 0.4, 0.6, 0.8, 1.0 mg/L were prepared and then analyzed by cyclic voltammetry with an upper vertex potential 0.6 V, a lower vertex potential -0.6 V, and a rate scan of 0.05 V/s (Figure 2).

Considering that carbon electrodes were used, the resulting voltammograms, both for copper solutions and for wine samples, have a hysteresis form. Therefore, the maximum and minimum intensities of the current were used for the interpretation of the results.

Based on the values which result from voltammograms, a linear regression was made (Figure 3.) and from that the unknown copper concentrations in the analyzed samples can be determined.

The form of the cyclic voltammograms is given both by the type of electrode used and the compound analyzed.

The maximum intensities obtained had a  $R^2=0.801$ , while the minimum intensities had an insignificant value and therefore could not be considered in estimating the concentration of copper ions.

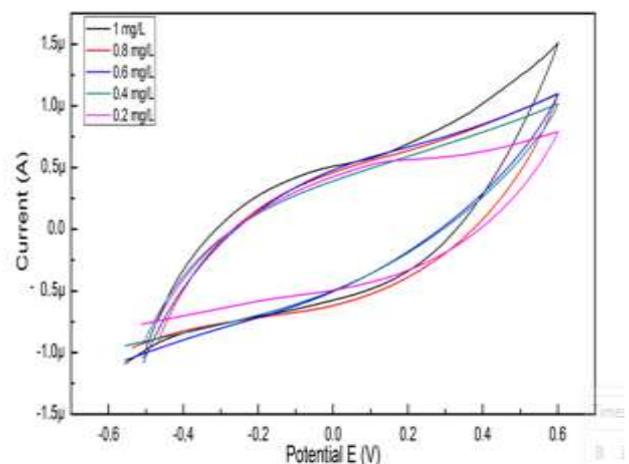


Figure 2. The cyclic voltammogram resulting from the analysis of the five copper solutions

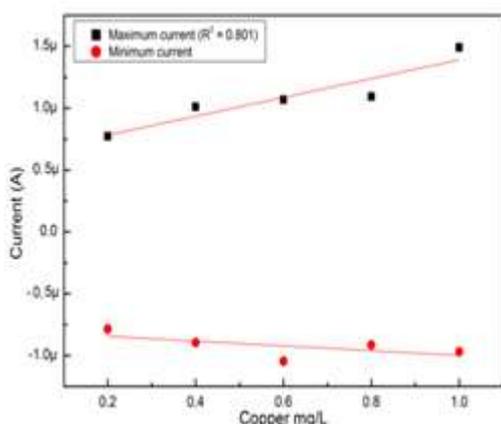


Figure 3. The maximum and minimum intensities of the five copper solutions

In order to measure the analytical performances of the biosensor, sensitivity and detection limit values were calculated and the results are presented in Table 1.

Table 1. Biosensor characteristics

Characteristic	Value
Slope	7.595E-07
Sensitivity	2.4187E-06
Limit of detection	0.187 mg/L
Response time	30 sec

Regarding the detection limit, this biosensor does not have low enough values to detect the smallest copper concentrations in wine, however, it can analyze wine samples and can provide values close to the maximum cutoff limit that can stop the casse phenomenon; based on these values can be assessed the need to add various substances that can precipitate copper (sodium sulphide or potassium ferrocyanide).

From the point of detection limit, ICP-MS is superior to the biosensor, with a value of 10-12 ppb.

The biosensor response time is relatively small, approximately 30 seconds.

In recent years, a series of papers that report on the use of ICP-MS methods for the determination of trace metals and rare mineral elements of wine have been published [24]. That set the basis for selecting the ICP-MS detection method as a mean to compare the results gave by the developed biosensor, as it can be observed in Figure 4.

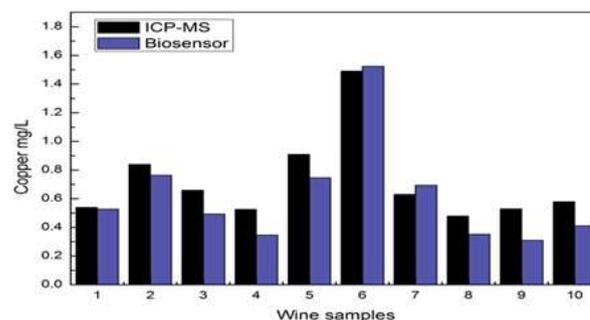


Figure 4. Copper content resulting from the two methods

As shown, the copper content resulting from the analysis of wine samples with the biosensor does not have a general tendency in evolution, some values being higher than those obtained by ICP-MS, while others are lower than those measured through the spectroanalytical technique. The values obtained in this paper with the developed method are generally lower by 10.72% than the values obtained with the other method, namely ICP-MS.

#### 4. Conclusions

The comparative study shows some variations in concentration indicating that the developed biosensor is suitable for use in copper analysis.

In conclusion, the electrochemical biosensor can be applied for a non-specialized, fast, simple, and inexpensive analysis, while the ICP-MS method can be used for a more accurate analysis of both copper ions and many other mineral elements.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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