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ELECTROCHEMICAL BIOSENSOR BASED ON THE USE OF SPE FOR THE DETECTION OF IRON CONTENT IN WINE

***Liliana NOROCEL[1], Gheorghe GUTT[1]**
[1]*Faculty of Food Engineering, Ştefan cel Mare University of Suceava, Romania*
liliana.norocel@fia.usv.ro
*Corresponding author

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Abstract: *The purpose of this paper is to develop a new method for iron analysis in wine. The determination of iron in winemaking products is particularly important because a content higher than 10 mg/L can lead to turbidity or a change in color, which causes ferric casse. The new method is based on the use of screen printed electrodes (SPE), previously immobilized with Protein A-Agarose and connected to a potentiostat which displays a cyclic voltammogram. To verify the method, the samples of wine were also analysed by a reference method, namely AAS. In order to visualize the deposition of the iron ions on the electrode, surface analyses were perfomed by a microscope which combines confocal microscopy with white light interferometry. The main features of this sensor are simplicity of operation, good sensitivity and low limit of detection.*

Keywords: *AAS, cyclic voltammetry, iron casse, surface analysis*

1. Introduction

The analysis of trace metals in wine is of great importance for the quality of a product, and also for the authenticity control of wine. The content of mineral elements in wine can be attributed to natural sources or to contamination during the wine-making process [1, 2].

It is known that there are certain species that significantly contribute to the destabilization of wines and their oxidative evolution. These species include the oxygen molecules, which is a process initiator, polyphenols as oxidative matter, and certain metal ions, such as Fe, Cu and Mn as process activators, which are also present [3]. At low concentration, iron ions have an important role in metabolism and fermentation processes as stabilizer, enzyme activator and functional component of proteins. At a higher concentration, it plays other roles such as altering redox systems of the wine in favour of oxidation, affecting sensory characteristics and participates with chemical compounds (tannins and phosphates) to instabilities. At concentrations above 10 mg/L iron forms insoluble suspensions which are known as ferric casse [4].

The analysis of iron in wine before bottling, can prevent the casse formation through the addition of potassium hexacyanoferrate(II), which eliminates part of iron content and significantly decreases its concentration [5].

The most commonly used technique for determining iron in wine is atomic absorption spectrometry (AAS) due to its precision [6]. Also, many papers have been published reporting the use of ICP-MS methods for the determination of mineral elements in wine fingerprints [7, 8]. The proposed methods for the analysis of metals in wine by these techniques usually involves sample preparation [9], thus it is necessary to develop a simple and easy to prepare method with effective costs. The use of quick and cheap biosensors for the detection of mineral elements represents an important requirement in the wine sector

[10]. Several studies have been reported on the use of miniaturized electrodes coupled with various detection techniques in the study of trace metals. Electrochemical biosensors bring a number of significant advantages such as miniaturization, sensitivity, portability, selectivity, low sample volumes and effective costs. In the last decades, advances have been made in electro-analytical chemistry through the development of ultra-microelectrodes, molecular devices and intelligent sensors [11]. Cyclic voltammetry (CV) is very popular electrochemical technique commonly used to investigate the reduction and oxidation processes of molecular species [12].

In many papers are studied the interactions between iron and proteins in wine. A simple, cost-effective method of iron is accomplished by using lactoferrin as part of a biosensor to detect iron in samples [13]. Normally, iron entraps a variety of protein transports such as transferrin and lactoferrin and is stored in proteins such as ferritin and hemosiderin [14].

The purpose of this study was to develop a simple biosensor for iron determination in wine, through the use of protein-A as biological element which was immobilized on screen printed electrodes.

2. Materials and methods

Protein A-Agarose and iron sulphate were purchased from Sigma Aldrich (Germany) and the 15 wine samples used for iron analysis were bought from wine cellars in Suceava (Romania).

Preparation of screen printed electrodes: 1µL of Protein A-Agarose was immobilized on SPE and used in the iron analysis with a potentiostat which displays a cyclic voltammogram.

Voltammetric measurements were carried out with a PGSTAT204 Autolab at a minimum potential of -0.6 V and a maximum of 0.6V.

Preparation of wine samples for AAS: the wine samples for AAS analyses were prepared according to [15]. Thus the alcohol was evaporated from the wine by reducing the sample volume to half the original quantity using a rotary evaporator (50 to 60°C), and then it was made up with distilled water to the initial volume. The absorbance was read at 248.3 nm.

Spectrometric measurements were carried out with an Atomic Absorption Spectrometer 6300 Shimadzu.

The microscopic analysis of the working electrode surface after immobilization and use in the wine was performed with the MahrSurf CWM 100 microscope, which combines confocal microscopy with white light interferometry.

3. Results and discussion

In this study, immobilized protein (Protein A - Agarose) was used as biological element. In Fig. 1 is given the calibration curve with maximum and minimum intensities of the artificial samples and Fig. 2 illustrates the cyclic voltammogram of the five iron concentrations, respectively 1, 2.5, 5, 7.5, 10 mg/L, analyzed at 120 seconds after immersion.

From both the voltammogram and the calibration curve it can be easily observed that the values extracted can provide good results and can be used to calculate the iron concentration of the solution.

The performance of the developed biosensor was determined through the sensitivity and limit of detection. The results of these characteristics are presented in Table 1.

Table 1

Parameters of performance

Characteristics	**Results**
Time of immersion (s)	120
Sensitivity	1.48E-06
Limit of detection (ppm)	0.900

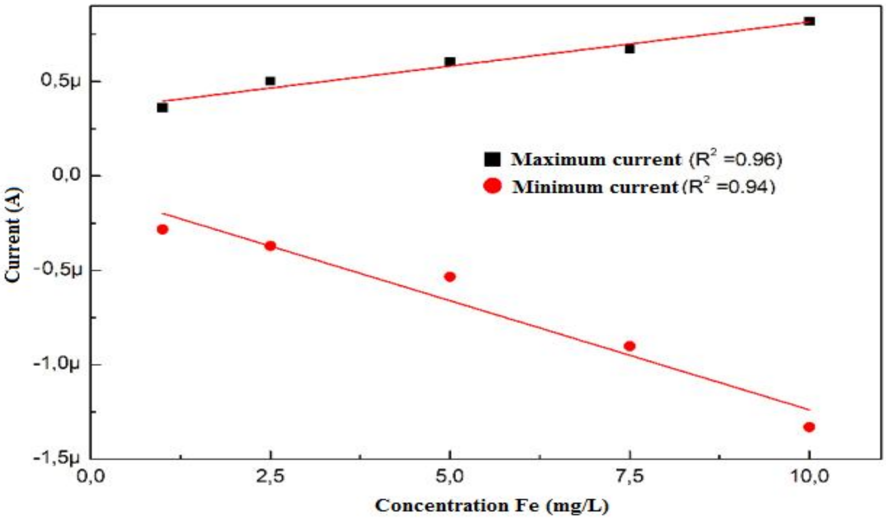

Fig. 1 Calibration curve

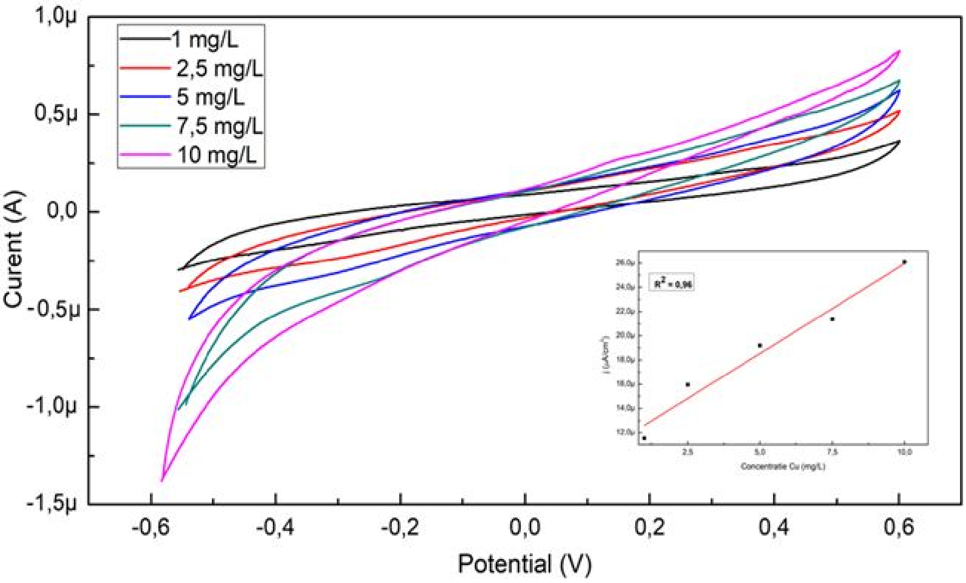

Fig. 2 Cyclic voltammogram of the five iron concentration

Sensitivity was calculated as slope of calibration curve on the surface electrode area. Regarding the limit of detection, this biosensor does not have low enough values to detect the smallest iron concentrations in wine; however, it can analyze wine samples and provide values up to the limit that can stop the ferric casse phenomenon.

For testing the developed method, the results obtained with the biosensor were compared to those obtained with the AAS method, which was used as reference method. For this purpose, 15 samples of wine were analized and the values obtained are presented in Fig. 3.

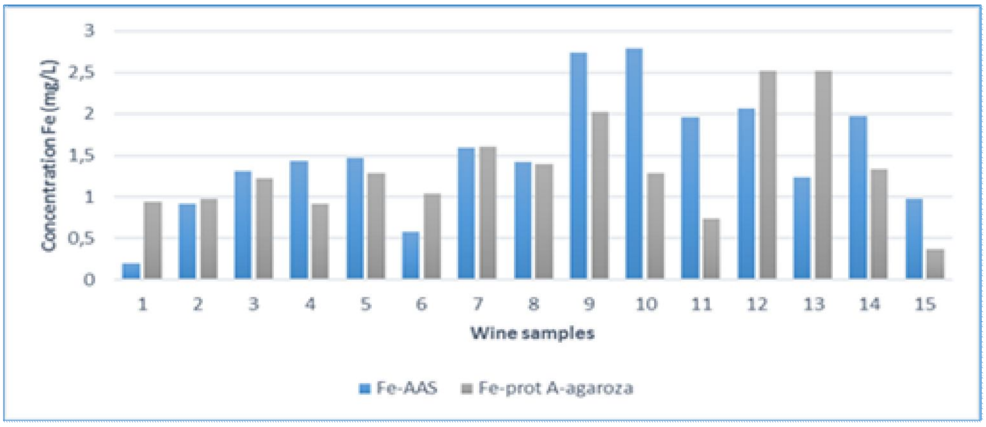

Fig. 3 Iron concentration

From this chart it can be observed that there is no general trend, some values being higher than those obtained with the reference method and others being lower than the reference values.

In order to visualize the sample surface analysis, microscopic images of the unused electrode were captured and analyzed by software in terms of profile.

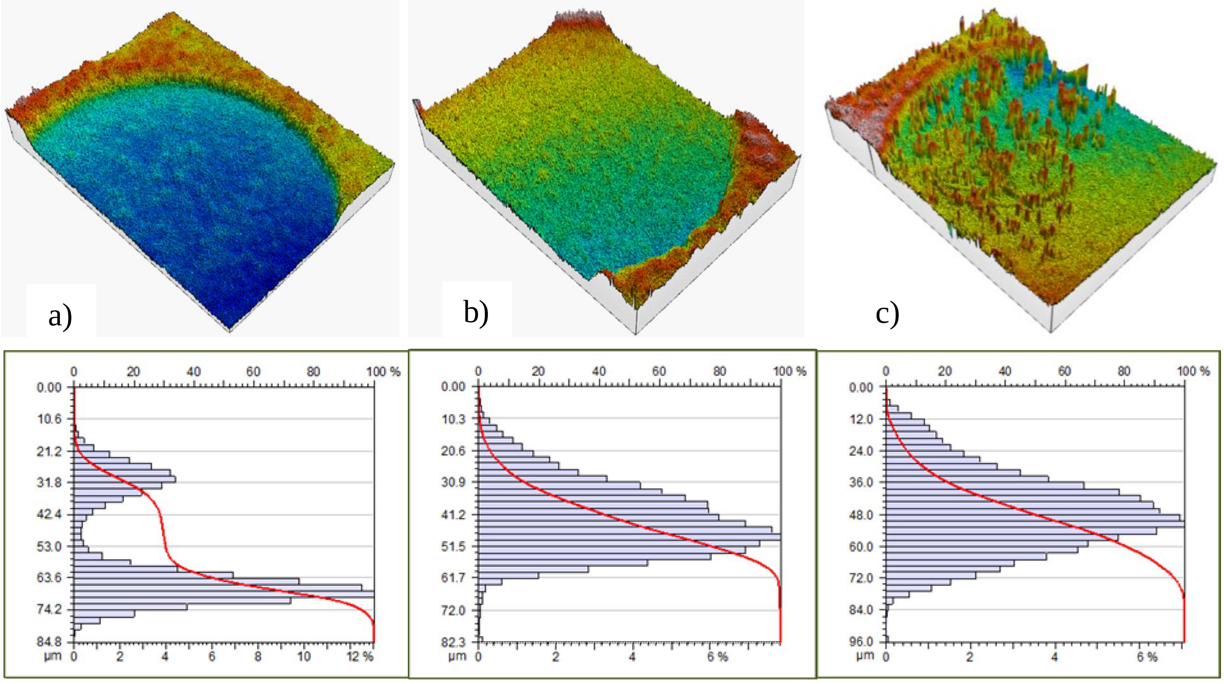

Fig. 4 3D images of the SPE and Skewness and Kurtosis histograms for electrodes a) unused, b) immobilized, c) used in wine

It was also analyzed the surface of the immobilized electrodes and the SPE after the analysis of the iron content in wine.
The results are shown in the 3D figures presented below (Fig. 4).

As can be observed, the electrode used in the analysis of the iron content of wine has deposits, which leads to a higher average height than the immobilized electrode, analyzed from the profile.

The coefficient of Skewness and the coefficient of Kurtosis have lower values for the electrode used in wine compared to the immobilized electrode, obtained by profile analysis, and the immobilized electrode has lower values of these two coefficients than the unsed electrode.

4. Conclusion

The biosensor proposed in this study provided excellent results in artificial iron solutions, both in terms of sensitivity and detection limit and also for R^2 of the calibration curve.

In the case of the wine samples the results don’t have a general trend, some values being higher than those obtained with the reference method and other values being lower than the ones obtained through AAS method.

In the future, the selectivity of the biosensor for the determination of iron ions will be studied on the mineral elements present in wine which can interfere.

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