ELECTROCHEMICAL METHOD FOR SULFITE DETERMINATION IN WINES BY ELECTROCHEMICAL RESPONSE USING A MEMBRANE ABSORBER SYSTEM

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ABSTRACT

This research demonstrates how the sulfite content can be measured by cyclic voltammetry using a previously reported membrane absorber system, which separates efficiently sulfite present in wine. Results obtained show notably similar values to those obtained for the same wine samples using modified Monier-Williams method (aspiration method) and Ripper method. The membrane absorber system allows the release of the free SO_2, and can be used to determine the sulfur dioxide present in juices and other foods that contain high concentrations of phenols, polyphenols and other structurally related compounds that act as interferers in the electrochemical oxidation of sulfite. The absorber solution allows a direct measurement without change in pH or added electrolyte, facilitating the determination of great amounts of samples from diverse wines using only one calibration curve. In this way, a system that allows the detection of sulfite and that can be used in vineyards is obtained.

Finally, the method was assessed on linearity, sensitivity, accuracy, reproducibility and repeatability, obtaining values that account for the applicability of the method.

Keywords: wines, sulfite, membrane contactor, aspiration method, ripper method, electrooxidation.

1. INTRODUCTION

Sulfite is a commonly used antiseptic in wine, juices and foods. Sulfite can be found free (fulfilling its antiseptic role) or combined with phenols, aldehydes and other organic compounds[1]. High concentrations in sulfite (10 $mg \cdot L^{-1}$) produce toxic effects, such as pain when breathing in asthmatics, hypotension and gastrointestinal problems[2]. For these reasons, it is useful to determine the concentration of sulfite in a fast, precise and reproducible manner, using techniques that require simple and low-cost equipment and that entail easy implementation.

The sulfite (SO_3^{-2}) in aqueous solution is in equilibrium with bisulfite (HSO_3^-) and with sulfur dioxide (SO_2), where the respective concentrations will depend on the pH. The equilibrium between these species is the following[3]:

$$H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \quad (1)$$
$$HSO_3^- \rightleftharpoons H^+ + SO_3^{2-} \quad (2)$$

There are several methods for the determination of SO_2 in industry. These methods include the modified Monier-Williams method (aspiration method), which, as one of the most precise methods, is used as standard[4] and control method in this study. The Ripper method[5] is also used in the determination of sulfite and as a second control in this work. This research has been focused on the electrochemical determination of sulfite present in wines by cyclic voltammetry in which, after passing the wine through a system of membranes, the SO_2 is extracted by an absorber solution (0.02 $mol \cdot L^{-1}$ NaOH) that, in addition to extract the SO_2, acts as the sole electrolyte[6]. The foundation of the aspiration method for the determination of free sulfite in wines[5] consists in the removal of the SO_2 present in the wine by passing a current of air or inert gas (nitrogen) through the wine, which is previously acidified to displace the equilibrium from neutral sulfite and bisulfite to sulfur dioxide. Subsequently, the SO_2 is recovered in a solution of hydrogen peroxide, where it turns into sulfuric acid (eq. 3), which, finally, is titrated with a standard solution of NaOH.

$$SO_2 + H_2O_2 \rightarrow H_2SO_4 \quad (3)$$

The determination of combined sulfite was performed under strong acid conditions and at temperatures of approximately 80ºC in order to dissociate sulfite-polyphenol adducts, and convert it into SO_2. The total sulfur dioxide is then determined by the aspiration method applied to heated and strongly acidified wine. The combined sulfite is determined by the difference between the total and the free sulfite. The Ripper method consists in a redox titration in which iodine is used to titrate the total or free SO_2 of a sample. This method requires the previous bleaching of the red wine, which generates losses of SO_2, which is absorbed by the bleaching agent when combined with phenols. The free sulfite is directly titrated with iodine. To determine the total sulfite, the sample is initially treated with sodium hydroxide to displace the equilibrium towards SO_3^{2-}, dissociating the bisulfite-acetaldehyde adducts and other molecules and, thus, directly titrating the total sulfite, free sulfite + released sulfite[5].

This study demonstrates that sulfite content can be measured by cyclic voltammetry enhanced by a membrane absorber system described in a previous work[6, 7]. Membrane absorber efficiently separates the sulfite contained in wine and avoids the presence of other compounds, which can interfere these measurements. Sulfite can be quantified by square wave voltammetry using one calibration curve[8, 9, 10, 11], obtaining very similar values to those obtained for the same sample by the modified Monier-Williams method (aspiration method) and the Ripper method.

Previous studies[12] demonstrate that it is possible to find a potential interval in which SO_3^{-2} shows a response under increasing current that is linear with the concentration at basic pHs, namely, the actual pH of the absorbent solution.

The absorber membrane system allows the release of the free SO_2 and can be used for the determination of sulfur dioxide present in juices and other foods that contain high concentrations of phenols, polyphenols, flavonoids and other structurally related compounds that act as interferers in the electrochemical oxidation of sulfite[13]. The absorber solution allows a direct measurement, without change in the pH or added electrolyte, which facilitates the determination of great numbers of samples from diverse wines using only one calibration curve. In this way, the content of sulfites in wines can be determined in a fast and reliable manner using a three-electrode electrochemical system coupled to a membrane absorber for the instantaneous separation of interferers, thereby offering an integral control solution for the production of export wine. The method demonstrates a linear interval and is sensible, precise, reproducible and repeatable to determine sulfite concentrations in the concentration interval used in wines and juices.

2. EXPERIMENTAL

Membranes absorption system

A membrane absorption system was implemented using a Celgard Liquicel® G542 minimodule. This membrane contactor module contains 7400 polypropelyne (PP) hollow fibers, which represents an effective surface contact area of 0.58 m^2. The hydrophobic hollow fiber contactor was used to contact

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the red wine samples with the receiving solution[6] (0.02M NaOH) in a non-dispersive mode. These solutions were circulated in countercurrent mode using peristaltic pumps, where the wine sample circulated through the shellside; meanwhile the receiving phased was circulated into the lumen of the hollow fiber contactor. This operation configuration is described in the outline reported in **figure 1**.

300 mL of wine sample was contacted with 300 mL of receiving phase. The solutions were circulated at 1.45 L/min into the shellside and at 1.20 L/min into the lumenside. Receiving phase was a NaOH(aq) solution with concentrations ranged from 0.02 to 0.1 M. Initially, red wine sample was acidified using a H_2SO_4 solution to ensure the formation and release of SO_2 [14, 15] at pH<1.0.

The electrochemical measurement system for sulfite quantification is coupled to the membrane absorption system described in figure 1. Thus, the electrode is constantly immersed in the receiving phase.

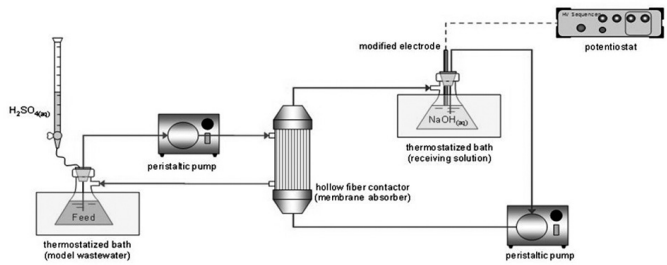

Figure 1: Outline of the membrane absorption coupled to electrooxidation treatment system proposed in this study.

Aspiration Method

Determination of free and combined SO_2 can be done through a same procedure. In the first case, the flask that contains the sample was introduced in an ice bath; and in the second case, the flask was heated with a heating plate[16, 17, 18].

The free SO_2 of a wine was determined (6 samples) by[5] (eq 1).

$$mg \cdot L^{-1}\ SO_2 = \frac{n \times N_{NaOH} \times 32 \times 1000}{Vm}\ [1] \qquad [1]$$

where:
n: Volume of NaOH used in the titration.
V_m: Volume of the sample.

In a second stage, the total sulfite was measured by the same method, but after heating the wine sample[5]. Furthermore, aspiration method was implemented according to the procedure described in the Official Methods of Analysis of AOAC[19].

The same analysis was performed for several samples until obtaining a data set of 6 measurements for each sample measured in cold and of 6 measurements for each sample measured in hot was obtained.

$$total\ SO_2\ (mg \cdot L^{-1}) = bonded\ SO_2\ (mg \cdot L^{-1}) \ + \ free\ SO_2\ (mg \cdot L^{-1}) \qquad [2]$$

Ripper Method[6, 20, 21]*:*

The concentration of free SO_2 (in mg·L^{-1}) can be determined by eq 3.

$$mg \cdot L^{-1}\ SO_2 = \frac{n \times N_{YODO} \times 32 \times 1000}{Vm} \qquad [3]$$

Where:
V_m: Volume of the sample.
The same analysis was performed 6 times for each one of the 6 samples.

Cyclic voltammetry

Initially, the material was washed and the electrodes were cleaned. The cleaning of the glassy carbon electrodes (GCE) (A = 0.07 cm^2) used was performed by immersing them in a mixture of H_2SO_4:H_2O_2 (3: 1 v/v) for two minutes. Then, the electrodes were rinsed with abundant distilled water. Subsequently, GCE (A = 0.07 cm^2) was polished to a mirror finish on a felt pad using alumina slurries (3 mm). The Pt counter electrode was placed under flame for its activation. The reference electrode, Ag/AgCl, was kept immersed in a KCl 3 mol·L^{-1} solution in a compartment coupled to the working electrode through a Luggin capillary in the three-electrode conventional electrochemical cell, and, before and during the measurements, all the solutions were purged with nitrogen. A potentiostat CHI900B manufactured by CH Instruments, Inc. (USA) was used, connected to an interface with a PC to store the electrochemical data.

Cyclic voltammetry was performed with the following parameters: From -1.0 to 1.0 V vs. Ag/AgCl with scan rate of 0.1 V·s^{-1}, during one, two or more cycles for sulfite electrooxidation.

Subsequently, a calibration curve was prepared with a set of 6 solutions of sodium sulfite at different concentrations ranged from 1.0*10^{-4} mol·L^{-1} to 1.0*10^{-3} mol·L^{-1} in a NaOH 0.02 M solution, exactly the same as the absorber solution.

Statistical Analysis

The statistical analysis applied in this work is detailed described by Box and coworkers[22] in order to validate the proposed method. This validation involves calculating linearity (calibration curve, r^2), accuracy (standard deviation and relative standard deviation) and sensitivity (detection limit and quantification limit).

3. RESULTS AND DISCUSSION

Sulfite electrooxidation on glassy carbon

Figure 2 shows the comparison of the voltammetric responses of the glassy carbon electrode in the absence and in the presence of sulfite 1mM. The electrocatalytic activity of the GC electrodes for the oxidation of sulfite is observed as an oxidation wave with a foot-of-wave potential of approximately 0.5 V vs. Ag/AgCl.

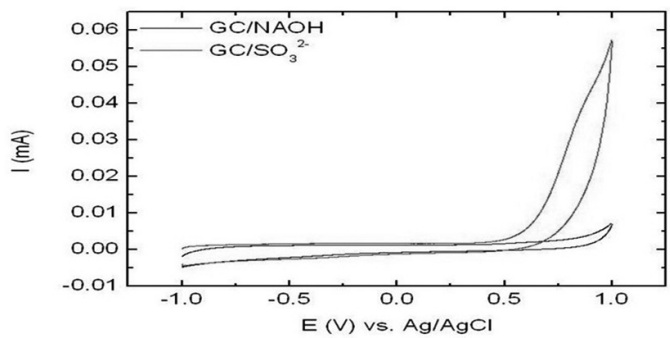

Figure 2: Profiles of comparative voltammetry of GC electrode for the electrooxidation of sulfite in 0.02 NaOH mol·L^{-1} solution. v: 100 mV·s^{-1}. Cycle 1.

The eventual transfer of ethanol and other volatile compounds through the membrane does not seem to affect the sulfite electrochemical response. There is no significant difference between measurements done in presence and absence of ethanol.

Evaluation of the electrode + membrane absorber integrated system.

The effect of changing the pH in the electrochemical response of the absorber solution that contains the pH was determined. As expected, the best response was found at pH 12 because at that pH the fraction of species in aqueous solution is 0.998; that is, almost 100% of this species is present. The oxidation peak actually corresponds to sulfite, which is not present at other pH values.

Subsequently, the voltammetric profiles were obtained in a solution of NaOH 0.02 mol·L^{-1} at different sulfite concentrations. From these profiles, the oxidation current at a fixed potential of 0.75 V, I, where the current is principally faradaic, was plotted as a function of sulfite concentration. Thus, figure 2 shows an increase of the sulfite oxidation current, which is found with the increment of the analyte concentration. This increase of oxidation current is directly proportional to the sulfite concentration in the ranges used in this work.

Validation of the method: measurements in wines.

The study of the analytical parameters of sulfite oxidation involves the assessment of linearity, limit of detection (LOC), limit of quantification (LOQ) and accuracy of the method using a GC electrode.

Linearity. Six different calibration curves were obtained at 6 days and resulted in a mean linear regression of:

I(A) = (0.00537 ± 4.10203E-4)[SO_3^{2-}] + (9.71926E-6 ± 2.48841E-7), with a regression coefficient of 0.99135 (n=5) (Figure 3).

All curves were undertaken in a range of concentrations between 1E-4 mol·L^{-1} and 1E-3 mol·L^{-1} from various voltammograms obtained. These data demonstrate a good relation between I and the SO_3^{2-} concentration with RSD values of 2.49E-5 % and 4.10E-2 % for the intercept and slope, respectively. **Figure 3** shows an obtained calibration curve; it can be observed that its correlation coefficient (R^2) is close to 1, giving evidence of the reasonable linearity of the method.

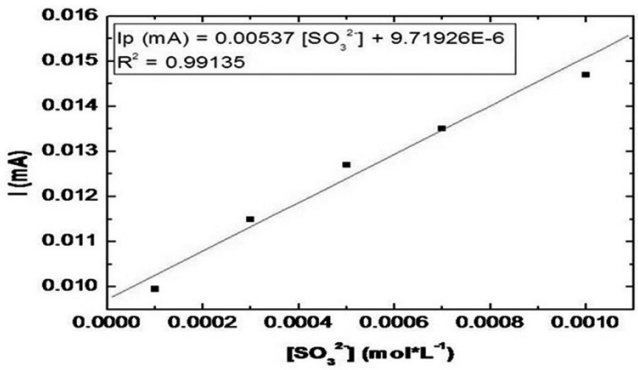

Figure 3: Calibration curve: I at E=0,75V versus sulfite concentration.

LOD and LOQ were estimated through the calibration curve, in which these values were calculated using the value of the slope of the curves obtained:

LOD = (3·SD) / slope
LOQ = (10·SD)/slope

After obtaining these values for all the curves, both parameters were calculated, obtaining average values of 1.60E-4 mol·L^{-1} and 5.34E-4 mol·L^{-1} for LOD and LOQ, respectively.

The parameter of accuracy was studied at two levels: replicability and repeatability. For repeatability studies, the measurement days of sulfite determinations were varied.

- *Replicability*

For this study, 6 aliquots of sulfite solution 1E-3 mol·L^{-1} were measured at the same day. The statistical parameter obtained as average is 1.41E-5, the standard deviation (SD) is 5.27E-7 and the relative standard deviation (RSD) is 5.27E-5.

- *Repeatability*

As well as it was done for the study of replicability; the study of repeatability was done only changing the days of measurements between different aliquots. The result obtained as average is 1.45E-5, the standard deviation (SD) is 6.95E-7 and the relative standard deviation (RSD) is 6.95E-5.

To obtain the concentration in the sample of real red wine that was passed through the membrane and the recovered sulfites, oxidation current in the receiving solution is interpolated. The average value of free sulfite concentration was 36.43 ± 0.21 mg·L^{-1}, for an informed 30 ppm of free sulfite for the wine because at pH=12 sulfite is the predominant specie and bisulfite is combined with phenolic compounds, as reported in the literature[23].

That value is close to the informed 30 ppm value compared to the Ripper and the Aspiration methods (Table 1) and shows data with better repeatability and reproducibility than both methods, indicating that the electrochemical method is reliable in regard to its analytical parameters[24]. The error in the standard measurement methods depends on the matrix[25], and therefore the free sulfite values are different for each type of wine. The difference in the value obtained by electrochemical method in comparison to the Ripper and the Aspiration methods indicates that these two methods determine a value lower than the real value because, in both cases, the measurement is direct. Only the electrochemical method corresponds to an interpolation in a calibration curve that was tested with samples that were prepared specifically to determine the validity of the analysis.

Table 1: Comparative results of the free, combined and total sulfite concentration on samples of red wine and receiving solution using standard methods of measurement.

Sample	Ripper Method			Aspiration Method			Electrochemical Method
	Free Sulfite (mg·L^{-1})	Combined Sulfite (mg·L^{-1})	Total Sulfite (mg·L^{-1})	Free Sulfite (mg·L^{-1})	Combined Sulfite (mg·L^{-1})	Total Sulfite (mg·L^{-1})	Free Sulfite (mg·L^{-1})
Receiving Solution	21.7 ± 5.5	20.7 ± 5.8	42.4	22.4 ± 2.9	22.1 ± 2.2	44.5	36.4 ± 0.2
Red Wine	20.6 ± 4.5	32.7 ± 2.5	53.3	20.4 ± 3.1	39.9 ± 2.6	60.3	

In conclusion, the electrochemical method coupled to the membrane absorption system can be easily applied to red wines and, eventually, to white and pink wines. This method is free of interferences from the other components of the wine, consumes small amounts of samples and is faster than the iodometric Ripper and Aspiration methods. Another possible advantage of the coupled methods proposed in this study is the feasibility for online measurements, which could be implemented for wines or other beverages.

4. CONCLUSIONS

The electrochemical method for the quantitative determination of sulfite at basic pH values efficiently induced the electrooxidation of the free sulfite from a receiving solution coming from a real wine sample treated with a membrane absorber of 7400 polypropylene fibers and an effective contact area of 0.58 m^2. The conventional or standard methods used for the determination of free sulfite yielded results with poor precision and low repeatability and replicability. The results from these methods are similar to those of the electrochemical method. However, the electrochemical method presents good precision, high repeatability and high replicability. Furthermore, it is more efficient than the standard measurement methods when the integrated system comprised of a membrane absorber and glassy carbon electrode is used because, regarding precision and the reproducibility of the values obtained, values of repeatability and reproducibility on the order of 10^{-5} are obtained. This combined system, on one hand, can isolate the content of free sulfite in commercial wines and, on the other hand, can quantitatively determine the concentration of sulfite and only sulfite. These results are novel and should be of interest for the food industry and environmental organizations.

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REFERENCES

1. I. Hornsey The Chemistry and Biology of Wine Making. The Royal Society of Chemistry. Thomas Graham House, Cambridge, 2007.
2. H. J. Schwartz J. Allergy Clin. Immunol. **71**, 487, (1983)
3. I. Streeter, J. Wain, A. J. Davis, R. Compton J. Phys. Chem. B **109**, 18500-18506, (2005)
4. Food Safety and Inspection Service Office of Public Health Science CLG-SFT1.00, Determination of Sulfites, Department of Agriculture, USA, 2006.
5. B. Zoecklein, K. Fugelsang, B. Gump, F. Nury Análisis y Producción de Vino, Dióxido de azufre y ácido ascórbico, Ed. Acribia, S.A, Zaragoza, 2001.
6. A. Hasanoğlu, J. Romero, A. Plaza, W. Silva Desalination and Water Treatment **51**, 5649-5663, (2013)
7. A. Plaza, J. Romero, W. Silva, E. Morales, A. Torres, M. J. Aguirre, Food Science and Technology International. DOI: 10.1177/1082013213494900, (2013)

8. A. J. Bard & L. R. Faulkner, Electrochemical methods, Electroactive layers and modified electrodes, Wiley, New Jersey, 2001.
9. L. Zhu, L. Xu, B. Huang, N. Jia, L. Tan, S. Yao Electrochim. Acta **15**, 471-477, (2014)
10. G. Bia, L. Borgnino, P. I. Ortiz, V. Pfaffen Sensors and Actuators B: Chem. **203**, 396-405, (2014)
11. D. Skoog, J. Holler, T. Nieman, Principios de Análisis Instrumental, Mc Graw Hill, Columbus, 2001.
12. M. Lucero, G. Ramírez, A. Riquelme, I. Azócar, M. Isaacs, F. Armijo, J. E. Förster, E. Trollund, M. J. Aguirre, D. Lexa J. Mol. Catalysis A: Chem. **221**, 71-76, (2004)
13. B. Molinero-Abad, M. A. Alonso-Lomillo, O. Domínguez-Renedo, M. J. Arcos-Martínez Analytica Chim. Acta **812**, 41-44, (2014)
14. A. Hasanoğlu, J. Romero, B. Pérez, A. Plaza, Chem. Engineering J. **160**, 530-537, (2010)
15. R. Arce, P. Márquez, F. Herrera, M. J. Aguirre, J. Romero J. Chilean Chem. Soc. **58**, 1982-1985, (2013)
16. G. Monier-Williams Rep. Publ. Health Med. Subj. **43**, 1, (1927).
17. Appendix A. Official Methods of Analysis of AOAC, Section 20.123–125, 1984.
18. B. C. Rankine, K. F. Pocock Brewing Spirit Review. Australian Wine 40, (1970)
19. P. Iland, A. Ewart, J. Sitters, Techniques for Chemical Analysis of Grape Juice and Wine, Patrick Iland Wine Promotions, Adelaide, 1993.
20. E. Bordeu, J. Scarpa Análisis Químico del Vino. Antisépticos, Pontificia Universidad Católica de Chile, Chile, 1998.
21. J. M. Vahl, J. E. Converse J. Assoc. Off., *Anal. Chem*. **63**, 194, (1980)
22. G. E. P. Box, W. G. Hunter, J. S. Hunter, Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building, John Wiley & Sons, Hoboken, 1978.
23. J. Henríquez, G. Ramírez, B. Matsuhiro, L. Mendoza, M. Isaacs, C. Arévalo, M. J. Aguirre J. Quantum Chemistry, Sent.
24. C. Montes, J. H. Vélez, G. Ramírez, M. Isaacs, R. Arce, M. J. Aguirre The Sci. World J. doi: 10.1100/2012/168148, (2012)
25. S. McLeod, D. E. Davey Analytica Chim. Acta **600**, 75, (2007)