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Determination of Free and Total Sulfites in Wine using an Automatic Flow Injection Analysis System with Voltammetric Detection

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An automated Flow Injection Analysis (FIA) system based on a initial analyte separation by gas-diffusion and subsequent determination by square-wave voltammetry (SWV) in a flow cell is proposed for the determination of total and free content of sulphur dioxide (SO₂) in wine. The proposed method was compared with two iodometric methodologies (the Ripper method and a simplified method commonly used by the wine industry). The developed method shown repeatability (RSD lower than 6%) and linearity (between 10 to 250 mg·L⁻¹) as well as suitable LOD. (3 mg·L⁻¹) and LOQ (9 mg·L⁻¹). A major advantage of this system is that SO₂ is directly detected by flow SWV.

Keywords: Wine; Sulphur dioxide; Sulfites; Gas-diffusion; Square-wave voltammetry; Flow injection analysis (FIA); Food analysis; Food additives; Analytical chemistry

Introduction

SO₂ and its source compounds, such as sulfite salts, are widely used as preservatives in the food industry. They are added to foods and beverages, in order to prevent undesirable microbial growth, discoloration and oxidation processes, consequently improving the quality and final appearance of the products. In wine, sulphur dioxide is usually found either free (as HSO₃⁻, SO₃²⁻ and SO₂, depending on the sample's pH), or bound to carbonyl and unsaturated compounds and/or phenol derivatives (Barbe et al. 2000).

The relevance of SO₂ determination in food analysis is widely established; in the particular case of wine, its control is essential not only because at high levels it produces an unpleasant aroma and taste but also because of health hazards especially for hypersensitive people (Decnop-Weever et al. 1997). SO₂ levels should be checked before bottling in order to be adjusted for a suitable aging and at the end-product to comply with existing legal limits (Segundo et al. 2001). In the EU the maximum level of total SO₂ permitted is 160

mg·L⁻¹ for red wines and 210 mg·L⁻¹ for white and rosé wines (EC 1999).

There are many alternative methods proposed for the determination of free and total SO₂ in wine. Nevertheless, voltammetric methodologies appear to be the most accurate ones (Bruno et al. 1979, Cardwell et al. 1991, Sarudi et al. 1998). The EU recommended method (EC 1990) is based on the classical Monier-Williams procedure (Monier-Williams 1927) which involves a sample distillation under an inert atmosphere (e.g. nitrogen). Then the analyte is collected in a hydrogen peroxide solution producing sulphuric acid, which can be determined by titration using sodium hydroxide. In spite of its general applicability, the long time required for the analysis precludes a more extensive use of the Monier-Williams method, especially in routine analysis. The Ripper method is a direct iodometric titration which is prone to interferences from iodine-reactive compounds and has been strongly criticized (Maquieira et al. 1993). In general, iodometric methods (rather common simplifications of the Ripper method) are preferred by the wine industry (Azevedo et al. 1999). For the determination of free SO₂, the sample is acidified and is titrated with a standard iodine solution, using starch as the indicator. For the determination of total SO₂, the sample is previously treated with sodium hydroxide to release the bound SO₂. The results should be interpreted with caution because of the solutions' poor stability, the possible occurrence of parallel reactions and additional difficulties associated with the end point observation, especially in the case of the red wines (Azevedo et al. 1999).

During the last years FIA methods have been developed (Dvořák et al. 2006, Oliveira et al. 2009, Ruiz-Capillas et al. 2009). To overcome the problems caused by the colored complex matrix and interferences, some authors proposed manifolds where it is possible to separate the analyte from the wine, by means of gas-diffusion (Araújo et al. 2005, Chinvongamorn et al. 2008, Silva et al. 1998), microdistillation (Maquieira et al. 1993), pervaporation (Mataix et al. 1998) or by using a gas generating and purging device (Zhi et al. 1995), followed by a wide variety of detection methods (Isaac et al. 2006).

The aim of the present work was the development and validation of a rapid and reliable procedure for identification and determination of total and free SO₂ in wine samples based on an automatic flow voltammetric system. The direct voltammetric determination of SO₂ in wine is difficult because of matrix interference. To develop an on-line analytical method, a separation step has to be employed. Gas-diffusion was used as the separation technique. In this technique there are two distinct chambers separated by a gas permeable hydrophobic membrane. The volatile or semi-volatile analytes are released from the donor chamber, go through the membrane and are collected in the upper chamber where a proper acceptor solution takes them to the instrumental detection. Detection was performed in a patented flow voltammetric cell (Figure 1) that has several advantages, being the most important one the possibility of using high frequency square-waves in flow without oxygen interference

(Barros et al. 1999).

Materials and methods

Instrumentation

Voltammetric measurements (parameters are described in Table 1) were performed with a Metrohm 663 VA Voltammetric Stand (Herisau, Switzerland) equipped with a hanging mercury drop electrode with glassy carbon auxiliary electrode and reference AgCl/Ag (3M KCl) electrode. The system was connected to an Autolab PGSTAT 10 Voltammetric System (Eco Chemie, Utrecht, The Netherlands). All measurements were made at room temperature.

The voltammetric flow cell was manufactured in our laboratory and has been described previously (Barros et al. 2001, 2002, Rodrigues et al. 2001, Rodrigues et al. 2002).

A home-made Perspex gas-diffusion module was used with PTFE membranes, 5.0 µm pore size and 47 mm diameter, from Pall Corporation (Ann Arbor, U.S.A.).

The FIA system is depicted in Figure 2: it includes several three way solenoid valves, a peristaltic pump and PTFE tubing. The sample flow reacts on-line with an acid solution, in the “Y” connection in order to turn all sulfites into SO₂; then it goes through a thermostated coil that has the function of pre-heating the donor flow before entering the gas-diffusion module; the SO₂ passes through the hydrophobic membrane and is received by the acceptor flow (acetate buffer) that takes it to be instrumentally detected.

The FIA system was incorporated in a prototype (Figure 3). This equipment works automatically with its own purposely designed control software. A GPES 4.9 Software project was developed for automatic voltammetric data acquisition, data saving, and signal processing. It was possible to interconnect both software using a signal trigger transmission.

Chemicals and wine samples

All reagents used were of p.a. grade and were used without further purification. Ultra-pure water from a Millipore Simplicity 185 water purification system (Millipore, Billerica, U.S.A.) was used. The supporting electrolyte (acceptor solution) consisted of acetate buffer pH 4.0 (0.1 mol·L⁻¹), sodium acetate was purchased from Merck (Darmstadt, Germany). The buffer’s pH was adjusted with hydrochloric acid, 6 mol·L⁻¹, from Prolabo (Fontenay-sous-Bois, France). Na₂SO₃ (Sigma, St. Louis, U.S.A.) was used as a source of SO₂. Ethanol was also purchased from Merck.

Some of the wine samples were bought in regular supermarkets in Porto while others were kindly supplied by the Instituto dos Vinhos do Douro e Porto (IVDP).

Sample preparation

For the determination of free SO₂ no sample preparation was required. For the determination of total SO₂ an alkaline pre-treatment was essential to destroy the adducts (SO₂ bounded to several aldehydes and ketones) formed in wine (Sarudi et al. 1998). For that purpose 10 g of sodium hydroxide were added per liter of sample. The sample was homogenized and left to rest for about 20 min.

Results and discussion

On-line acidification

The wine sample must be acidified prior to gas diffusion in order to turn all sulfites into SO₂, and the pH must be very low (pK_{a1} = 1.77 and pK_{a2} = 7.22). On-line acidification with sulfuric acid was achieved using a “Y” shaped connection that helped mixing together the sample flow with the acid flow.

The acid concentration was optimized, knowing that it should be as low as possible to save chemical resources and to prevent the equipment's corrosion but still strong enough to accomplish its objective. After several experiments (Figure 4) concentration chosen was 1 mol·L⁻¹.

Voltammetric analysis of SO₂

Research on the electrochemical reduction of SO₂ in aqueous solution with dropping mercury electrode has reported two waves in acidic medium and no waves in neutral or slightly alkaline media. There has been much disagreement in the interpretation of polarographic reduction of aqueous sulphur dioxide at pH 4 (Streeter et al. 2005). A one-electron reduction of HSO₃⁻ has been suggested (1) (Reynolds et al. 1986) along with the possibility that HSO₃⁻ becomes SO₂ at the drop's surface, and it is this species reduced in the electrode (2) (Tolmachev et al. 1999).

Due to matrix effect, standard additions were used to quantify the level of SO₂ in the different wine samples (Figure 5). Since the linearity was quite good for routine analysis one single addition was enough.

Two separate determinations must be done for each wine, one for the free sulphur content and another for the total, the difference lays on previous sample treatment with sodium hydroxide for the second case.

Study of interferences

The level of ethanol in wine varies to a large degree and consequently the influence of

ethanol concentration was studied. With that purpose, two SO₂ calibrations curves (10 to 100 mg·L⁻¹) were compared: one in water and the other one in ethanol 10% (v/v), replicating ethanol concentration in wine. As can be perceived in Table 2, ethanol showed no influence in the analysis.

Carbon dioxide is normally found in wine in the concentration range of 1 to 2 g·L⁻¹ due to the fermentation process (Santos et al. 2006). Previous studies showed that this compound does not interfere in the analytical procedures that make use of gas-diffusion (Santos et al. 2006, Segundo et al. 2001).

Method comparison

The determination of sulfite concentrations in the different wine samples by the developed flow procedure was compared with the reference “Ripper” method and its simplified version generally used in the wine industry (Figure 6).

The “Ripper” method was performed according to the Portuguese regulation (IPQ 1987) based in a procedure from the Organisation Internationale de la Vigne et du Vin (OIV 2007).

The wine industry normally uses a simple iodometric methodology for routine analysis: instead of the four titrations in the “Ripper” method (one for the free SO₂, two for the total SO₂ and one for the interferences) only two are carried out (one for the free and another one for the total SO₂). The methodology consists on iodometric titrations of an acidified wine sample. For the free SO₂ determination, 5 mL of 1:4 H₂SO₄ are added immediately before the titration. To determine the total SO₂ content, 25 mL of 1 mol·L⁻¹ NaOH are added 15 minutes prior to analysis, in order to perform alkaline hydrolysis, and then 10 mL of 1:4 H₂SO₄ just before the titration.

Method performance characteristics

Method performance was established by plotting a calibration curve with eight different standards, from 10 to 250 mg·L⁻¹, with six repeated measurements of each point (n = 6) (Table 3). LOD and LOQ (Eurachem 1998) were adequate to wine analysis, though they can be easily decreased with a slight time-consuming change in the procedure. The buffer solution can be stopped and isolated at the upper chamber by this way allowing an incremented quantity of SO₂ passage to the upper chamber and consequently an increase in the analytical signal, i.e. the LOD can be lowered by increasing the time needed for each analysis (Figure 7). This makes it possible to analyze matrices with low sulfite content such as beer, juices and other food products. In the present configuration the prototype is able to analyze 15 samples per hour.

Conclusions

In this paper a new automatic FIA system for the determination of SO₂ in wine is described and evaluated. The developed system shows several advantages when compared to other analytical methodologies: voltammetry directly measures SO₂ which means a more accurate result; it is especially advantageous with red wines when compared to all the iodometric procedures; it is very versatile, i.e. it can be used with other matrices like beer or juices among others. This analytical procedure was characterized by a good precision, an ample linear application range, an adequate limit of detection and quantification which can be easily lowered if necessary. Good agreement was observed between the proposed new methodology and the one currently used in wine quality control laboratories.

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265 **Table 1.** SWV parameters.

Initial potential	- 400 mV	Final potential	- 850 mV
Step potential	2.5 mV	Amplitude	25 mV
Frequency	300 Hz	Drop size	0.024 mm ³

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268 **Table 2.** Evaluation of the possible interference of ethanol.

	water		ethanol 10% (v/v)	
	$y = mx + b$	r^2	$y = mx + b$	r^2
Day 1	$y = 4.7x - 7$	0.995	$y = 4.6x - 16$	0.995
Day 2	$y = 4.6x + 8$	0.998	$y = 4.5x - 5$	0.999

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Table 3. Method performance characteristics, $I = f([SO_2])$.

Linear dynamic range	RSD (n = 6)	r^2	LOD	LOQ
10 to 250 mg·L ⁻¹	6%	0.9999	2.7 mg·L ⁻¹	9.2 mg·L ⁻¹

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Figure 1. Voltammetric flow cell (Barros et al. 2001, 2002).

Figure 2. Flow system scheme. A – acceptor circuit; B – donor circuit; solid lines – permanent pathway; dashed lines – load step pathway (2 min.); dotted lines – injecting step pathway (2 min.); PP – peristaltic pump (all with flow rates of 1 mL·min.⁻¹); V – three-way solenoid valve; H – heating element (both working at 90 °C).

Figure 3. FIA system prototype. V – three-way solenoid valves; PP – peristaltic pump; H – heating elements; GDM – gas-diffusion module.

Figure 4. Signal variation with the sulphuric acid concentration. The sample used in this assay contained a high sulfite level and had endured an alkaline pre-treatment, i.e. reproducing the determination of the total SO₂ content.

Figure 5. Voltammograms obtained in the analysis of several standard solutions of SO₂. Inset: the calibration curve plotted with the voltammograms' peak height.

Figure 6. Method comparison for the determination of free and total sulphites, three replicates were performed for each method.

Figure 7. Signal variation with the stopped flow time in the upper chamber of the gas-diffusion module. Results were acquired with a standard 20 mg·L⁻¹ SO₂ solution.

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