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Optical Sensor for Sulfur Dioxide Determination in Wines

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A method for the determination of free and total sulfur dioxide in wines, based on the use of an optical sensor that employs a dichlorobis(diphenylphosphino)methane dipalladium I complex [$Pd_2(dppm)_2Cl_2$] immobilized in a PVC membrane plasticized with *o*-nitrophenyloctylether (*o*-NPOE) is described. A sensing membrane [4.2% $Pd_2(dppm)_2Cl_2$, 20.8% PVC, and 75% *o*-NPOE] was adapted to the tip of a bifurcated optical fiber bundle to perform reflectance measurements at 550 nm. The detection system consisted of two cells (40 mL), which hold the sample solution (plus reagents) and the optical sensor, respectively. For the determination of free SO_2, a wine sample was mixed with H_2SO_4 solution in the sample cell, into which N_2 was bubbled, providing mixing of the solutions and conducting the SO_2 formed toward the detection cell. For determination of total SO_2, a KOH solution was mixed with the wine in the sample cell. Afterward, an H_2SO_4 solution was added to the cell, and then N_2 was bubbled to conclude the measurement. Linear responses up to 50 and 150 mg L^{-1} were obtained for free and total SO_2, with detection limits of 0.37 and 0.70 mg L^{-1}, respectively. The repeatability of the method was evaluated by carrying out 10 measurements using a single wine sample, providing relative standard deviation values of 2.2 and 2.5% for free and total SO_2, respectively. The sensing membrane prepared from 10 μL of the cocktail solution lasted for 80 measurements, whereas those prepared from 200 μL can be used for 250 measurements. The method was applied to free and total SO_2 determination in wines, and the results did not show significant difference from those obtained with the Ripper reference method at a confidence level of 95%.

KEYWORDS: Optical sensor; gas sensor; sulfur dioxide; sulfite; wine

INTRODUCTION

Sulfur dioxide is usually added to wine as a preservative due to its antioxidant and antiseptic properties. Its content is rigorously controlled by legislation in many countries because it is toxic, causing asthma and other allergic reactions in persons hypersensitive to SO_2, besides changing the organoleptic properties of the final product (*1*, *2*).

A classical method for the determination of SO_2 (usually added as sulfite) in wines is based on iodometric titration, known as the Ripper method (*1*−*3*). The free SO_2 amount (inorganic forms, SO_2, SO_3^{2-}, HSO_3^-, and H_2SO_3) is determined by direct titration with a standard iodine solution, whereas total SO_2 is determined after alkaline hydrolysis of the bound forms, followed by acidification and titration. The bound SO_2 (bonded to ketones, aldehydes, and phenolic derivatives) is determined as the difference between total and free sulfur dioxide. The accuracy of the method is affected by iodine-reducing compounds, whereas the precision can be impaired by the low stability of the standard iodine solution (*2*). Another procedure accepted as reference is the Rankine method, which employs a distillation step, followed by alkalimetric titration (*3*). Both methods suffer from the usual problems of manual volumetric methods, such as laboriousness and low analytical frequency, impairing their use for large-scale analysis. To overcome these drawbacks, several automated and semiautomated methods have been proposed for the determination of free and total sulfur dioxide in wines. These methods usually employ a separation step, based on microdistillation or gas diffusion through a membrane, preceding the analytical determination of sulfur dioxide, which is usually performed by spectrophotometry (*4*, *5*), potentiometry (*1*), amperometry (*6*), or conductimetry (*7*). Flow methods (flow injection and sequential injection analyses) have frequently been used to manage the solutions, because they offer a convenient way to separate SO_2 from wine samples, with the use of a permeation cell furnished with a PTFE hydrophobic membrane (*6*−*8*). Other methods include chemiluminescent (*9*) and ICP OES (*2*, *3*) detections.

Optical fiber chemical sensors (optodes) have proved to be a useful analytical tool for solving many problems, because they provide simple and reliable methods for the determination of

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many species of environmental, medical, and industrial importance. Optodes are in agreement with the claims of green chemistry, employing an immobilized reagent phase, which minimizes the production of chemical wastes. As far as the detection of sulfur dioxide or sulfite is concerned, several optodes, based on absorbance (*10*), fluorescence quenching (*11*), chemiluminescence (*12*), and phosphorescence (*13*), have been described in the literature. Recently, Alves et al. (*14*) proposed an optical sensor for the detection of gaseous sulfur dioxide based on the palladium complex dichlorobis(diphenylphosphino)methane dipalladium I [$Pd_2(dppm)_2Cl_2$], which was immobilized in a PVC membrane plasticized with *o*-nitrophenyloctylether (*o*-NPOE). The composition of the membrane was optimized for SO_2 determination, and experiments performed in dry nitrogen demonstrated that the sensing phase responds reversibly to this gaseous species, with a response time of 2−3 min, providing an analytical response range up to 300 ppm_v and a detection limit of 3.5 ppm_v.

This paper describes the use of the above-mentioned optical sensor for the determination of free and total sulfur dioxide in wine samples. Initially, the effect of humidity on sensor response was investigated, to certify its sensitivity, accuracy, and stability. Afterward, a procedure for the determination of SO_2 was proposed, the main particularity of which is the use of a detection cell separated from the reaction cell, providing a system with easy operation, which also protects the sensing phase from poisoning.

EXPERIMENTAL PROCEDURES

Reagents and Solutions. All reagents used were of analytical grade. High molar mass poly(vinyl chloride) (PVC, Aldrich), *o*-NPOE (Fluka), tetrahydrofuran (THF, Merck), and dichloromethane (Mallinckrodt) were used as purchased. The Pd(I) complex, $Pd_2(dppm)_2Cl_2$ [dichlorobis(diphenylphosphino)methane dipalladium I], was synthesized as previously described by Gimenez and Alves (*15*) and used after proper purification. For studies of interference, 1000 ppm_v gas standard mixtures in nitrogen (SO_2, CO, CO_2, HCl, Cl_2, H_2S, N_2O, and NO_2) were used (supplied by White Martins Gases Industriais S/A, Campinas, Brazil). Sulfuric acid (Mallinckrodt) and potassium hydroxide (Aldrich) were used when necessary for the determination of free and total SO_2. For the iodometric determination of SO_2 (Ripper method), a standard solution of iodine (Mallinckrodt) containing potassium iodide (Merck) was employed (*16*). For standardization of the iodine solution, a standard solution of sodium thiosulfate (Synth) was used, which was previously standardized against potassium iodate (Merck). A solution of starch (Vetec) was employed for endpoint detection in all titrations. Reference solutions in 10% ethanol were prepared from sodium sulfite (Synth) in the ranges of 0−50 and 0−150 mg L^{-1}, expressed as SO_2, for the determination of free and total SO_2, respectively.

Preparation of the Membranes. The membranes were cast from a cocktail solution prepared by dissolving 4 mg of the Pd(I) reagent in 50 μL of CH_2Cl_2 and 20 mg of PVC in 950 μL of THF. After dissolution of the compounds, these solutions were mixed and 69 μL of *o*-NPOE was added, followed by homogenization. The sensing membranes were obtained by manual deposition of 10 μL of the cocktail solution onto polyester films, dried for 24 h, and then stored in a desiccator sheltered from ambient light.

Sensor Design and Instrumentation. The sensing membrane was coupled to a bifurcated optical fiber bundle (Ocean Optics) as previously described (*17*). An acrylic screw, the tip of which was covered with a reflective tape (3M) to improve light reflectance, was used as support for the sensing membrane. An acrylic coupling with two windows to permit air diffusion into the sensor film was adapted to the common end of the bundle. The screw was inserted in the coupling and positioned at a given distance from the tip of the optical fiber bundle to provide the highest reflectance signals, which were measured with an Ocean Optics USB2000 spectrophotometer. An Oriel model *77567* was employed as light source. The detection cell was constructed by inserting the optode into a 40-mL vial, with connections for the inlet and outlet of gas, as shown in **Figure 1**. The spectrum obtained in humid air (or in dry nitrogen) was employed as reference. Spectra were always expressed as the difference between the signal intensities obtained in the reference condition and after exposure of the sensor to the gas sample.

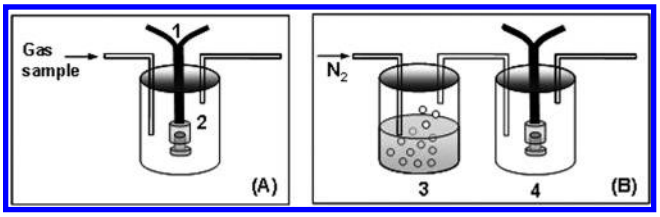

Figure 1. Schemes of the detection cell (**A**) and of the coupled reaction and detection cells (**B**), showing the bifurcated optical fiber bundle (1) and sensor head (2). Vials 3 and 4 have volumes of 40 mL.

Procedure. For evaluation of the effect of humidity and other interfering species, a gas blender assembled in the laboratory with four mass flow controllers (Aalborg) was employed. To generate air samples with different relative humidity values, streams of 1000 ppm_v of SO_2 in dry nitrogen and 100% humid air were mixed, totaling a flow rate of 1000 mL min^{-1}. For interference studies, a third stream with the interfering species was employed to generate the proper gas mixture.

For the determination of sulfur dioxide in wine samples, a second 40-mL vial was employed as reaction cell and connected to the detection cell with the aid of Tygon tubing, as shown in **Figure 1**. For the determination of free SO_2, 10 mL of a wine sample were mixed with 2.5 mL of 2.0 mol L^{-1} H_2SO_4 solution in the sample cell, into which N_2 was bubbled at a flow rate of 600 mL min^{-1}, providing the mixing of the solutions and the conduction of the SO_2 formed toward the detection cell. A transient signal was obtained, with maximum intensity after ≈1 min. For determination of total SO_2, 2.0 mL of 2.5 mol L^{-1} KOH solution was mixed with 5.0 mL of wine in the sample cell. After 30 s, 6.0 mL of a 2.0 mol L^{-1} H_2SO_4 solution was added to the cell, and then N_2 was bubbled to conclude the measurement. After each measurement, the baseline signal was recovered by substituting sample solution with water and flushing with nitrogen for 6 min.

The free and total SO_2 contents in wine samples were also determined according to the Ripper reference method (*16*).

RESULTS AND DISCUSSION

The composition of the membrane employed in this work was previously optimized (*14*), showing good performance in dry nitrogen and maximum reflectance variations at 550 nm. It was initially observed that signal intensities increased with the continuous use of the sensor, reaching a maximum value after about six measurements. **Figure 2** illustrates this behavior, showing the reflectance signals obtained for consecutive measurements performed with 500 ppm_v of gaseous SO_2. Therefore, this procedure was adopted as a means of conditioning the sensing membrane, being carried out when a new sensing phase was placed in the measuring cell. A similar result is obtained when SO_2 is generated from a sulfite solution to perform the membrane conditioning.

The analytical signal, that is, the variation in the reflectance intensity, is due to the insertion of the SO_2 molecule into the metal−metal bond of the complex (*14*). The humidity of air obviously affects signal intensities, as SO_2 reacts with water, forming sulfurous acid and, therefore, interfering with the reaction of this species with the Pd(I) complex immobilized in the PVC matrix. **Figure 3** shows the sensitivity of the sensor measured in air samples with different values of relative humidity. As can be seen, water significantly affects the response of the sensor when the measurements are performed in dry air

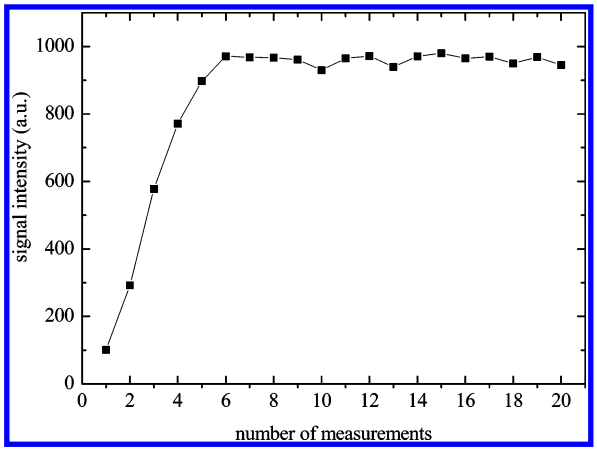

Figure 2. Effect of the number of measurements on the sensor response for 500 ppm_v of gaseous SO_2.

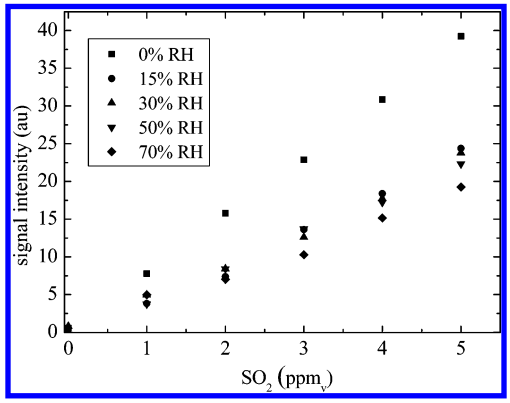

Figure 3. Effect of the relative humidity (RH) on the sensor response (error bars are smaller than the symbols used to represent the points).

and in 30% relative humidity. However, this effect is less significant when the relative humidity is increased from 30 to 70%. Therefore, the effect of water on signal intensity can be minimized by performing measurements at a given value of relative humidity or within a narrow range. This condition was accomplished by taking the reference spectrum for the determination of SO_2 in wines with water in the reaction cell, into which nitrogen was bubbled for 6 min to reach a steady-state signal.

The interference studies showed that NO_2, Cl_2, HCl, and H_2S (10 ppm_v) react irreversibly with the Pd complex, poisoning the sensing phase. CO_2 and N_2O (10 ppm_v) do not interfere, whereas NH_3 interferes significantly. Similarly, the sensor responds to CO when it is in concentrations >1.0% (v/v). This study also indicates that hydrochloric acid and nitric acid cannot be used for the production of sulfur dioxide, as an excess of acid is necessary to react with the sulfite contained in wines.

The proposed method consists of the separation of the SO_2 from wine by the addition of an excess of sulfuric acid to the sample in the reaction cell. When the acid solution is added to the sample, the mixture needs to be stirred in a constant and reproducible manner to produce gaseous SO_2, which also needs to be impelled toward the detection cell. The more appropriate way to perform both tasks, that is, mix the solutions and carry the SO_2 to the detection cell, consisted of bubbling N_2 into the sample cell at a constant flow rate. Studies employing different flow rates (100, 200, 300, and 600 mL min^{-1}) indicated that higher signals were obtained for lower flow rates, whereas faster responses were obtained for higher flow rates. As sensitivity

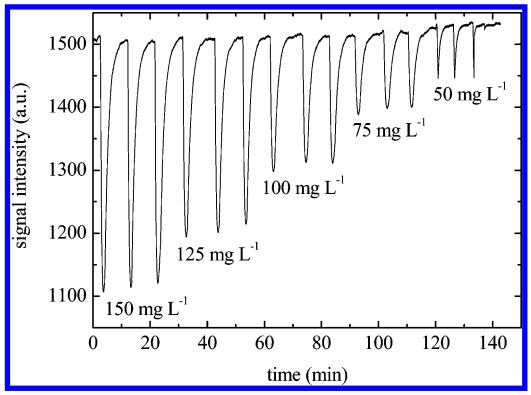

Figure 4. Sensor response to SO_2 produced after acidification of sulfite solutions (N_2 flow rate = 600 mL min^{-1}).

was not the bottleneck of the method, a flow rate of 600 mL min^{-1} was employed, as it improves analytical frequency. The concentrations and volumes of the reagents were also evaluated to adjust properly the sensitivity for the determinations of free and total SO_2. For free sulfur dioxide, good performances were obtained by adding 2.5 mL of 2.0 mol L^{-1} H_2SO_4 solution to 10.0 mL of wine, whereas for total sulfur dioxide 5.0 mL of wine was mixed with 2.0 mL of 2.5 mol L^{-1} KOH, followed by the addition of 6.0 mL of 2.0 mol L^{-1} H_2SO_4. **Figure 4** shows the sensor response for measurements made with 50, 75, 100, 125, and 150 mg L^{-1} SO_2 solutions (prepared from sodium sulfite). It can be noted that SO_2 readily reacts with the sensing phase, whereas the regeneration of the membrane, carried out by passing humid nitrogen through the detection cell, takes a longer interval of time. The maximum difference in the reflectance signal occurs after ≈1 min of bubbling nitrogen into the sample solution. Once the maximum signal is reached, the reflectance signal returns very slowly to the baseline. To accelerate this step, the sample solution is replaced by water in the reaction cell. With this only 5 min is necessary to recover the baseline.

To verify the effect of ethanol and sugar contents on the signal intensity, reference solutions (0−50.0 mg L^{-1}) were prepared in 10 and 20% ethanol, with and without 100 g L^{-1} of sucrose. The sensitivity (expressed as the slope of the analytical curve) decreased 4% when the ethanol content was increased from 10 to 20%. Therefore, reference solutions prepared with ethanol content close to those found in wines are adequate to avoid systematic errors. In this work, reference solutions were prepared in 10% ethanol, as also employed in several other works (*2*, *7*). The addition of 100 g L^{-1} sucrose to these solutions also reduced the sensitivities of the analytical curves (16 and 10% for reference solutions prepared in 10 and 20% ethanol, respectively), indicating the necessity of preparing reference solutions with similar sugar compositions for the determination of SO_2 in sweet wines, such as Port wine.

The optimized conditions were employed for the analysis of wine samples purchased on the local market. For free SO_2, a linear response from 0−50.0 mg L^{-1} was obtained (reflectance signal = 3.60 + 4.89 [SO_2], r^2 = 0.999), whereas for total SO_2 the upper limit was 150 mg L^{-1} (reflectance signal = 3.09 + 2.57 [SO_2], r^2 = 0.998), with detection limits (3 times the standard deviation of the blank divided by the sensitivity) of 0.37 ± 0.01 and 0.70 ± 0.01 mg of SO_2 L^{-1}, respectively. The repeatability of the system was evaluated by performing measurement of 10 replicates of a wine sample, providing relative standard deviations of 2.2% (79.6 ± 1.7 au) and 2.5% (151.7 ± 3.7 au) for free and total SO_2, respectively. **Table 1**

Table 1. Values Obtained for Free and Total SO_2 in Wines Employing the Proposed Sensor and the Ripper Reference Method (*17*)

	free SO_2 (mg L^{-1})		total SO_2 (mg L^{-1})	
sample	sensor[a]	reference[b]	sensor[a]	reference[b]
1	15.4 ± 0.1	15.2 ± 0.5	65.2 ± 1.4	62.8 ± 1.8
2	41.9 ± 0.4	40.5 ± 0.8	110.3 ± 0.4	109.7 ± 2.7
3	17.9 ± 0.8	19.9 ± 0.5	80.0 ± 3.0	82.9 ± 0.0
4	21.3 ± 0.2	22.9 ± 1.1	78.8 ± 0.6	80.9 ± 1.5
5	22.7 ± 0.0	24.0 ± 0.4	52.1 ± 0.1	49.3 ± 3.1
6	17.7 ± 0.4	18.3 ± 0.5	113.3 ± 1.4	112.5 ± 2.0
7[c]	23.9 ± 0.2	24.0 ± 0.5	114.6 ± 1.2	117.0 ± 3.1

[a] Average of two determinations ± mean deviation. [b] Average of three determinations ± standard deviation. [c] White wine.

lists the results obtained for these determinations, employing the proposed method and the reference method (iodometric titration, Ripper method), which do not differ significantly at a confidence level of 95%. For free SO_2, a plot of the results against those obtained by the Ripper method provided a relationship of sensor = (−2.51 ± 1.31) + (1.08 ± 0.05) × Ripper, $r^2 = 0.994$. It must be noted that sample 2 (**Table 1**) presents a high weight on the curve fitting, as its value is much higher than the other values. If this value is not considered, a regression of sensor = (1.10 ± 2.36) + (0.90 ± 0.11) × Ripper, $r^2 = 0.970$, is obtained, indicating the good correlation between the methods. For total SO_2 an equation equal to sensor = (3.64 ± 3.21) + (0.96 ±0.04) × Ripper, $r^2 = 0.997$, also demonstrates that there is no systematic error in the proposed method, as the linear coefficient and the slope are close to zero and one, respectively.

The membrane prepared from 10 μL of cocktail solution can be used for ≈80 determinations, without loss of sensitivity. Subsequent measurements showed a decrease of ≈1.1% in the signal intensity after each measurement, and the membrane became ineffective after ≈100 determinations. To improve the durability of the sensor, thicker membranes were prepared from 50, 100, 200, 300, 400, and 500 μL of stock solution. Membranes made from 50 and 100 μL presented inadequate mechanical properties, as they needed to be cast in a proper template and suffered rupture when detached from the template to be placed on the screw. On the other hand, membranes prepared from 300, 400, and 500 μL showed response times >30 min, which impair the analytical throughput of the method. The membrane cast from 200 μL showed the most intense reflectance signal, with a response time of ≈6 min. However, signal intensity was increased after each measurement performed with 500 ppm_v of SO_2, becoming constant after 10 full cycles (N_2 for 6 min followed of 500 ppm_v of SO_2 for 6 min). This membrane lasts for at least 250 measurements, providing a linear response up to 5.0 ppm_v for SO_2 in air, with a detection limit of 130 ppb_v. This value for the detection limit makes the sensor also useful for monitoring SO_2 in air, as it fits the limits imposed by Brazilian legislation (*18*).

The results obtained in this work indicate the usefulness of the $Pd_2(dppm_2)Cl_2$−PVC membrane for SO_2 sensing, allowing the development of a fast, reliable, and simple method for the determination of this species in wines, with the advantage of making the measurements in the gas phase, minimizing interferences and risks of poisoning of the sensing phase. When compared with the methods described in the literature, the proposed method is simpler but maintains practically the same sample throughput. In addition, a dedicated photometer can be constructed by employing a LED as light source and a photodiode as detector (*19*), providing miniaturized instrumentation useful for process control.

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