Determination of total and free sulfur dioxide in wine by flow injection analysis and gas-diffusion using p-aminoazobenzene as the colorimetric reagent

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Table IV. Result of Sample Analysis

element	ref value, $\mu g \cdot g^{-1}$	found,[a] $\mu g \cdot g^{-1}$	SD[b]	RSD[c]
Mn	75.4	71.9	1.88	2.61
Fe	431	439	22.98	5.24
Zn	22.8	23.4	0.27	1.17
Cu	10.4	10.1	0.18	1.78
Pd		not found		

[a] Average value of five determinations. [b] Standard deviation. [c] Relative standard deviation.

for the separation. When the concentration of acetate buffer is larger than 10 mmol·L^{-1}, the retention time of the metal complexes will decrease and this experimental result is also an evidence of the previous explanation for the effect of pH upon the retention time. In this paper, 1.0 mL·min^{-1} was used as the flow rate and a 20-μL sample was injected. Chromatograms obtained under these conditions are shown in Figure 5.

Calibration Curves. The calibration curves for the determination of Mn(II), Fe(III), Pd(II), Cu(II), and Zn(II) were linear over the range (0.07–4.4) × 10^{-6} M for Mn, (0.07–4.3) × 10^{-6} M for Fe, (0.04–4.0) × 10^{-6} M for Pd, (0.06–5.0) × 10^{-6} M for Cu, and (0.06–5.0) × 10^{-6} M for Zn; Mn, C (μg·mL^{-1}) = (7.80 × 10^{-6}) × area; Fe, C (μg·mL^{-1}) = (4.00 × 10^{-6}) × area; Pd, C (μg·mL^{-1}) = (3.43 × 10^{-6}) × area; Cu, C (μg·mL^{-1}) = (1.81 × 10^{-6}) × area; and Zn, C (μg·mL^{-1}) = (1.54 × 10^{-6}) × area. The following detection limits (at a signal-to-noise ratio of 2:1) for metals (20 μL injected) were achieved: 160 pg of Mn, 79 pg of Fe, 70 pg of Pd, 36 pg of Cu, and 31 pg of Zn.

Effect of Foreign Ions. The effect of the presence of other ions on the determination of Mn(II), Fe(III), Zn(II), Pd(II), and Cu(II) was examined. It was found that metal ions such as Cd(II), Pb(II), Hg(II), Co(II), and Ni(II) which could react with meso-BrTPPS$_4$ would not interfere with the determination, because they did not form complexes with meso-

BrTPPS$_4$ or the formed metal complexes were not eluted.

Sample Analysis. The peach leaves standard sample (No. 08501) was provided by the Institute of Environmental Chemistry, Academia, Sinica, Beijing. The results are shown in Table IV. The contents of Mn, Fe, Zn, and Cu obtained are in good agreement with the reference values.

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* To whom corespondence shoud be addressed.

Xue-Jun Xu
Hua-Shan Zhang
Chuan-You Zhang
Jie-Ke (Jai-Kai) Cheng*

Department of Chemistry
Wuhan University
Wuhan 430072, China

TECHNICAL NOTES

Determination of Total and Free Sulfur Dioxide in Wine by Flow Injection Analysis and Gas-Diffusion Using p-Aminoazobenzene as the Colorimetric Reagent

Jordi Bartroli,* Manel Escalada, Cecilia Jimenez Jorquera, and Julian Alonso

Departament de Quimica, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

INTRODUCTION

Sulfur dioxide is typically used as an antioxidant and antiseptic in winemaking. As a result, this substance occurs in two different forms in wine: free (aqueous SO_2 and HSO_3, the actual active fraction) and bound to aldehyde and ketone groups (basically acetaldehyde, with which it forms hydroxysulfonates) (1). The total and free SO_2 contents of wine are key analytical parameters for must and wine quality control; the amounts added to wine should ensure adequate protection while complying with prevailing legislation. Processing of a large number of samples calls for the use of automated methods minimizing analytical times and costs in addition to providing major advantages over the typically laborious classical distillation methods (2, 3) and iodometries, which are subject to a number of interferences (2).

So far, sulfur dioxide has been determined by various FIA methods (4–6), even in environmental analysis (7–9). FIA methods for the determination of this compound in wine rely on colorimetric techniques based on the well-known p-rosaniline reaction (10–12) or on potentiometric (13, 14) or chemiluminescence detection (15). None of these methods allows total SO_2 to be determined, and only those including a gas-diffusion separation can be applied to colored wine. However, some commercially available continuous segmented-flow analyzers (SFA) allow the determination of total and free SO_2 in wine. All of them include a gas-diffusion module for sample treatment and use p-rosaniline as reagent.

The FIA methods reported herein involve a preliminary separation by means of a gas-permeable membrane, which makes it more selective and applicable to any type of wine,
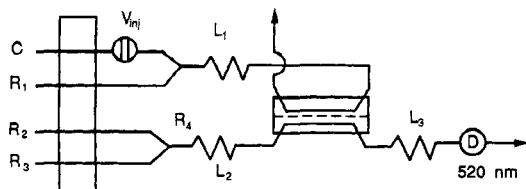
Figure 1. Scheme of the FIA configuration used for the determination of free SO_2. Conditions: flow rate of C, R_1, R_2, and R_3, 0.58 mL/min; V_{inj} = 300 μL; L_1, 30 cm $\times$ 0.5 mm i.d.; L_2, 200 cm $\times$ 0.7 mm i.d.; L_3, 250 cm $\times$ 0.5 mm i.d.; C and R_1, 0.6 M HCl; R_2, 1% HCHO in 0.2 M HCl; R_3, 0.004% PAAB.
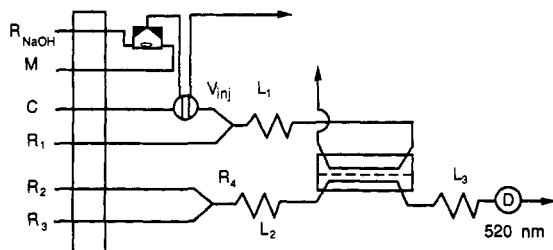

Figure 2. Scheme of the FIA configuration used for the determination of total SO_2. Conditions: flow rate of C, R_1, R_2, and R_3, 0.83 mL/min; flow rate of R_{NaOH}, 0.6 mL/min; V_{inj} = 50 μL; volume of mixing chamber, 1 mL; L_1, 30 cm $\times$ 0.5 mm i.d.; L_2, 200 cm $\times$ 0.7 mm i.d.; L_3, 200 cm $\times$ 0.8 mm i.d.; M, sample; R_{NaOH}, 4 M NaOH; C, 2 M HCl; R_1, 6 M HCl, R_2, 1% HCHO in 0.2 M HCl; R_3, 0.004% PAAB.

whatever its color. For the determination of total SO_2, the sample is continuously hydrolyzed within the FIA system prior to analysis in order to release all bound SO_2. Automation of this step will allow the sample manipulation process to be simplified and sample throughput to be increased with respect to the classical methods.

The analytical basis for the method is the reaction between SO_2 and p-aminoazobenzene (PAAB) and formaldehyde in an acid medium, which is monitored colorimetrically. PAAB is chemically similar to p-rosaniline but containing only one amino group. Thus, we can assume that the reaction between PAAB and SO_2 is similar to the p-rosaniline one which involves formation of an aminocarbinol as the first intermediate, followed by nucleophilic substitution by bisulfite (*16*).

Unlike p-rosaniline, which is commercially unavailable in a pure state and must thus be purified quite often to prepare solutions (*17, 18*), PAAB had not yet been used for the determination of SO_2 in wine, but only for the determination of SO_2, formaldehyde, and methanol in environmental samples (*19–22*).

EXPERIMENTAL SECTION

Reagents. All reagents were prepared from analytical-reagent grade chemicals. The stock solution of PAAB (0.02% in 0.08 M HCl containing 4% ethanol) was found to be stable for at least 1 year. Aqueous solutions of formaldehyde were made daily.

Sulfur dioxide standards were prepared in deaerated water from a stock solution of sodium sulfite containing 500 ppm SO_2 stabilized in 0.1 M NaOH with 5% glycerin as stabilizer (*23*), which was found to be superior to Hg_2Cl_4 (*18*)—only a 1% loss was observed after 1 week's storage at 4 °C.

Apparatus. Figures 1 and 2 show the schemes of the proposed FIA configurations. They included a Gilson Minipuls-2 peristaltic pump, an Omnifit FIT-1106 six-way valve, and an LKB-Novaspec II spectrophotometer with a bandwidth of ±12 nm and a precision of ±1 mUA, equipped with a Hellma flow-cell (10 mm path length, 18 μL inner volume). The diffusion module was a methacrylate block with a straight channel (7 cm long, 2 mm wide) provided with a Millipore GVHP 09050 polyvinylidene fluoride gas-permeable membrane. The well-stirred mixing chamber used was also made of methacrylate and had an inner volume of 1 mL.

FIA System for the Determination of Free SO_2. As can be seen from Figure 1, the sample (300 μL) was injected into
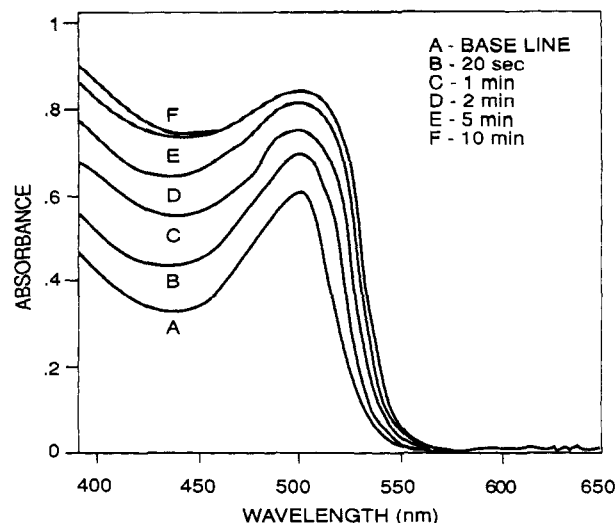

Figure 3. Absorption spectrum showing the course of the reaction between SO_2, PAAB and formaldehyde in an acid medium.

channel C and then merged with stream R_1, which provided a sufficiently acidic medium for the SO_2 to diffuse across the membrane. Acceptor stream R_4, a mixture of PAAB and formaldehyde, collected the SO_2 and transported the resulting reaction product formed in L_3 to the detector, where its absorbance was measured at 520 nm.

FIA System for the Determination of Total SO_2. The configuration depicted in Figure 2 was constructed from that shown in Figure 1 by including a prehydrolysis unit consisting of a well-stirred chamber with two channel inlets and a net volume of 1 mL in which the sample (50 μL) was continuously mixed with a stream of 4 M NaOH. The flow rate was adjusted so that the residence time (1 min) was long enough for the reaction to complete.

The stream emerging from the chamber was used to fill the loop of the injection valve, and the sample was injected into a strongly acidic stream. After this point, the procedure involved the same steps as described above for the determination of free SO_2.

RESULTS AND DISCUSSION

The kinetics of the reaction between SO_2 and PAAB is rather slow: complete formation of the product takes about 10 min (Figure 3). This was a serious constraint to its implementation in an FIA system, where the time of contact between the analyte and the reagent is usually less than 1 min. We thus optimized the chemical and hydrodynamic parameters of the system in order to adapt it to the concentration range required by the method while minimizing analysis times.

Optimization of Hydrodynamic Parameters. Previously, in order to assure the robustness of the overall flow system, the carrier and reagent flow rates were set in such a way as to avoid overpressure on either side of the membrane and obtain analysis times of about 1 min. With this prefixed parameter, the others were performed by using the univariate approach.

Obviously, the length of L_3 determined the extent to which the analytical reaction would take place. As can be seen from Figure 4, the behavior is inconsistent with what one would expect solely from dilution (*24*). The kinetic component prevailed at reactor lengths up to 250 cm, as the reaction rate was quite high at such a short reaction times (*25*). On the other hand, dispersion prevailed after the maximum of the curve. In such a way, the coil length for the total SO_2 system proved to follow the same behavior.

Consequently, the injected volume was selected to obtain a proper linear working range for both systems.

Optimization of Chemical Parameters. According to Figure 5, the analytical reaction must have a complex kinetics
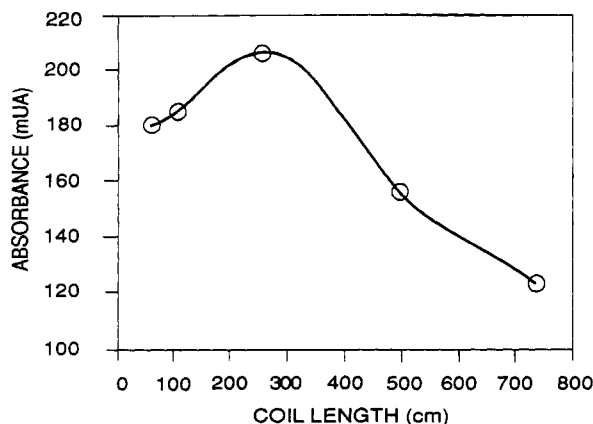
Figure 4. Variation of the response of the system for the determination of free SO_2 as a function of the length of coil L_3 (0.5 mm i.d.).

Table I. Results Obtained in the Analysis for Total SO_2 from a Calibration with Aqueous Standards and by the Standard Addition Method[a]

	wine sample			
	white 1	white 2	rosé 1	red 1
direct method	63 ± 5	56 ± 5	45 ± 5	11 ± 6
std addition method	63 ± 2	57 ± 3	45 ± 4	9 ± 7

[a] All concentrations expressed in ppm.

Table II. Figures of Merit of the FIA Methods for the Determination of Total and Free SO_2

	linear range, ppm	det limit, ppm	repeatability (rsd), %	sensitivity, mUA/ppm	sampling rate, samples/h
total SO_2	5–300	2	0.4	2.7 ± 0.1	20
free SO_2	2–35	0.2	0.4	24.2 ± 0.3	50

dependent on the PAAB, formaldehyde, and HCl concentrations. While an exhaustive study of the kinetics of this reaction was beyond the scope of this work, we believed it of interest to investigate the influence of the above chemical parameters in order to find the working conditions ensuring optimal performance of the FIA system.

Thus, we studied the effect of acidity by varying the HCl concentration in channel R_2. A concentration of about 0.2 M was found to result in the highest sensitivity and hence in the highest reaction rate. On the other hand, as can be seen from Figure 5, at a moderately low PAAB (0.004%) concentration minimizing baseline drifts, the reaction rate can be increased by increasing the formaldehyde concentration.

The optimal values of the different chemical parameters finally chosen for use with the two FIA configurations are reflected in Figures 1 and 2.

Interferences. The chemical complexity of the wine compelled us to check that their matrices would have no effect on the accuracy of the method in performing a calibration with aqueous SO_2 standards. For this purpose, total SO_2 was determined by FIA in various wines by using two different methods: direct determination by interpolation on a cali-
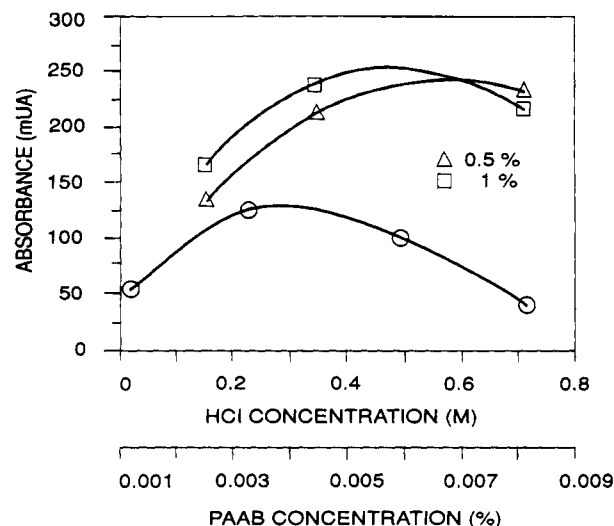

Figure 5. Variation of the response of the system for the determination of free SO_2 as a function of (O) the HCl concentration in the formaldehyde solution (R_2) and (△, □) the PAAB concentration at different formaldehyde concentrations.
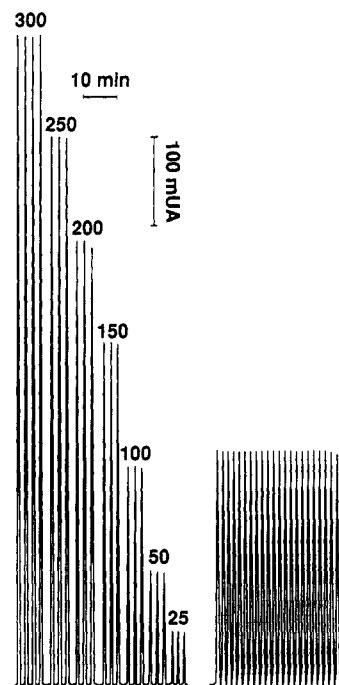

Figure 6. FIA recording showing the response of the system for the determination of total SO_2 obtained by calibration with aqueous standards and the evaluation of repeatability with 20 injections of a 100 ppm standard. The numbers on the peaks are SO_2 concentrations in ppm.

bration graph run from aqueous standards and by standard additions of different volumes of an SO_2 standard at a constant sample volume. The results revealed the absence of interferences from the assayed samples (Table I).

Hydrogen sulfide was found to be the only gas species interfering with the reaction (16). However, its effect was only

Table III. Repeatability of the Calibration Graphs for the Determination of Total and Free SO_2

day	total SO_2				day	free SO_2			
	slope, mUA/ppm	intercept, ppm	r^2	n		slope, mUA/ppm	intercept, ppm	r^2	n
1	2.7 ± 0.1	3 ± 13	0.9993	7	1	24.2 ± 0.3	1.0 ± 6.4	0.9998	7
2	2.6 ± 0.1	6 ± 13	0.9992	7	2	24.2 ± 0.5	-2 ± 7.7	0.9999	5
3	2.7 ± 0.1	8 ± 10	0.9997	6	3	24.1 ± 0.4	-2 ± 6.4	0.9999	5

Table IV. Results of the Statistical Treatment Performed on the Pairs of Data Obtained by Using the Proposed FIA Systems and the INCAVI SFA System[a]

| | FIA linear regression versus SFA | | | student's t | |
	slope	intercept	r^2	t_{calc}	t_{tab}
total SO$_2$	1.1 ± 0.1	−10 ± 15	0.9258	1.73	2.069
free SO$_2$	1.0 ± 0.1	0.1 ± 0.5	0.9497	0.6	2.069

[a] Confidence limit = 95%; N = 24.

significant above 1 mM—the maximum tolerated concentration in wine is 10^{-5} M (1).

System Features. The figures of merit of the FIA methods proposed for the determination of total and free SO$_2$ are summarized in Table II. While the total SO$_2$ analysis time was 1 min, adding the time required for the steady state to be reached in the mixing chamber gives a sample throughput of 20 samples/h.

As an example, Figure 6 shows the calibration graph of the FIA system for the determination of total SO$_2$ and an evaluation of repeatability. As can be seen from Table III, both methods proved to be highly reproducible over time. This allows calibrations to be more spaced in time and hence resulted in greater operativeness. In addition, the response was very stable, with no baseline drift.

Moreover, the two methods afford much higher precision than classical methods (±10 ppm), so they more than comply with the demands for methods used to control SO$_2$ levels in wine.

By the use of smaller injected volumes, the linear determination range could be extended to 400 ppm for total SO$_2$ and 200 ppm for free SO$_2$ in order to cover samples containing concentrations beyond the legal limits.

The results obtained by analyzing samples of different wines were compared with those provided by a continuous segmented-flow analyzer (SFA) used at an enological station (Institut Català de la Vinya i el Vi) for the routine analysis for total and free SO$_2$ in large numbers of samples. As can be seen from Table IV, which summarizes the results of the statistical treatment applied to each pair of values, there were no significant differences between the two methods in this respect.

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Bulk-Electrolysis Flow-Cell System for UV–Visible and X-ray Absorption Spectroelectrochemical Analysis

David H. Igo,[1] **R. C. Elder,*** and **William R. Heineman***

Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172

Howard D. Dewald

Department of Chemistry and Condensed Matter and Surface Science Program, Ohio University, Athens, Ohio 45701-2979

INTRODUCTION

Recent studies have shown the utility of electrolysis as a practical alternative to chemical generation of specific oxidation states of metal complexes in solution for X-ray spectroelectrochemical analysis (1–6). Electrochemistry is an inherently clean process because the reducing or oxidizing agent is an inert electrode which provides or removes electrons without introducing possible interfering agents. For extended X-ray absorption fine structure (EXAFS) measurements,

potential control of a static solution may be necessary to maintain a specific oxidation state of the metal complex being observed. This is due to the highly reducing environment generated by secondary electrons produced by the intense synchrotron radiation.

Solution cells used in EXAFS analysis generally utilize a thin-layer configuration which enables rapid and exhaustive electrolysis of a specific analyte. Either gold minigrid (7) or reticulated vitreous carbon (RVC) (1) has served as the working electrode material, due to their transparency.

Recent efforts have focused on the development of a more versatile spectroelectrochemical system having an efficiency equivalent to that of the RVC cell previously developed (1).

* To whom all correspondence should be addressed.
[1] Current address: Glaxo, Inc., Five Moore Drive, Research Triangle Park, NC 27709.