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Spectrophotometric determination of carbon dioxide and sulphur dioxide in wines by flow injection

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Abstract. Flow injection analysis (FIA) methods for the spectrophotometric determination of CO_2 and SO_2 in wines are described. The determination of CO_2 is based on the colour change of a low capacity buffer (containing an acid-base indicator) due to the dissolved carbon dioxide. The determination of SO_2 is based on the decoloration of malachite green by sulphur dioxide. Two FIA manifolds are presented; one for the determination of CO_2 in sparkling wines and another for the simultaneous determination of CO_2 and SO_2 in table wines. The analytes are isolated inside the manifold from the sample matrix using gas-diffusion units. Regression equations (FIA versus reference methods) showed no statistical difference, at 95 % confidence level, between the two sets of results for both determinations; additionally, for the determination of CO_2, recovery values between 93.5 % and 111 % were found. RSD lower than 4.5 % for SO_2 and 2.4 % for the CO_2 determination were found. The sampling rates achieved were: 30 h^{-1} for the uniparametric system and 40 h^{-1} for the biparametric system. The single determination manifold is applicable in the concentration ranges of 0.5 to 4 g L^{-1} of CO_2, and the simultaneous determination manifold in the range of 0.25 to 3 g L^{-1} of CO_2 and 0.05 to 0.3 g L^{-1} of SO_2.

Key words. Carbon dioxide – sulphur dioxide – wines – flow injection – spectrophotometry.

Introduction

Sulphur dioxide is commonly used in wine processing to inhibit chemical and microbial spoilage. The amount of SO_2 in the final wine product is strictly controlled by legislation. Carbon dioxide can be present in young wines as a product of the fermentation, can indicate spoilage, and in the case of sparkling wines it is added to the wine in high concentration. Both components are important in developing the organoleptic properties of the wines. Therefore, the levels of carbon dioxide and sulphur dioxide are routinely controlled in wineries.

The analytical methods usually used for these determinations are time consuming, often limited in the application range and sometimes affected by the loss of the analyte during the determination [1].

To automate these determinations, some flow injection methodologies have been proposed [2-11]. These methods involve the acidic conversion of the analytes, present in different forms, to CO_2 and SO_2, and subsequent in-line separation of the gaseous species from the sample matrix, resorting to a gas-diffusion process. Afterwards, the analytes could be detected in the acceptor stream using either spectrophotometric [2-7], electrochemical [7-9] or a chemiluminescence detection [10,11]. Spectrophotometric detection of CO_2 is based on the decolorization of an acid base indicator in low capacity buffer stream. Although the reaction is not specific, the use of the gas diffusion process excludes the possible interference of most sample components, except sulphur dioxide, which also diffuses through the membrane. Spectrophotometric determination of SO_2 was based on the colorimetric reaction of SO_2 with formaldehyde and p-rosaniline [2,6,12] or p-aminoazobenzene [5], with iodine [7], or with malachite green [4]. Regarding electrochemical detection, potentiometry [7,9] and amperometry [8] were used.

The only flow injection system proposed for the simultaneous determination of CO_2 and SO_2 in wines was described by Linares *et al.* [12]. A non-specific potentiometric detection (pH measurement affected by both analytes) was combined with a spectrophotometric detection of SO_2, and the concentrations were calculated using an empirical model.

In this work the objective was to develop one flow injection system for the spectrophotometric determination of CO_2 in Portuguese sparkling wines (Vinhos Verdes), avoiding the interference of sulphur dioxide, and another one capable of determining the two analytes with a single manifold. Spectrophotometric detection was preferred as it is more robust and it is usually available in routine control laboratories.

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Materials and methods

Reagents and solutions

Reagents with analytical grade and deionized water were used. A 10.0 g L^{-1} stock solution of carbon dioxide was prepared by dissolving 9.55 g of $NaHCO_3$ in 500 mL of previously boiled water. The standard solutions of carbon dioxide in the range of 0.5 to 5 g L^{-1} were prepared by rigorous dilution of the stock solution. The standard solutions for the simultaneous determination were prepared as combinations of CO_2 with SO_2 in the following compositions: 3 g L^{-1} with 0.05 g L^{-1}, 2 g L^{-1} with 0.1 g L^{-1}, 1 g L^{-1} with 0.15 g L^{-1}, 0.5 g L^{-1} with 0.2 g L^{-1} and 0.25 g L^{-1} with 0.3 g L^{-1}, respectively. The standard solutions also contained 0.325 M of NaOH. The working standard solutions and the SO_2 stock solution of 1 g L^{-1} were prepared daily and the concentration of the stock solution of SO_2 was determined by iodometric titration. The reagent solutions used in the flow injection system were: 20 mg L^{-1} malachite green in 6.25 10^{-3} M KH_2PO_4; 0.094 M K_2HPO_4; 0.2 M H_2SO_4; 300 mg L^{-1} of H_2O_2 in 0.06 M H_2SO_4; 60 mg L^{-1} of H_2O_2 in 0.2 M H_2SO_4 and a 28 mg L^{-1} of bromothymol blue in 0.1 mM carbonate buffer at pH = 7.8.

Instrumentation and flow injection procedure

The flow injection systems depicted in figure 1 were composed of Gilson Minipuls 3 peristaltic pumps, a Rheodyne 6 port rotary valve, Unicam 8625 UV/Vis spectrophotometers equipped with Hellma 178011 flow cells and a Kipp & Zonen BD 112 recorder. The flow channels were constructed using Gilson poly-tetrafluorethilene (PTFE) tubing (i.d. 0.8 mm), Omnifit end-fittings and connectors and a Y shaped confluence. Gas diffusion units [13] with straight flow channels ($35 \times 2 \times 0.5$ mm and $70 \times 2 \times 0.5$ mm) were used. The applied gas diffusion membranes were made of PTFE and poly-vinylidene fluoride (PVDF) (Millipore, GVHP09050).

Flow injection procedure for the determination of CO_2 (Fig. 1A)

The sample was injected into a water carrier stream (Q_2) and merged with the acid stream (Q_3) containing hydrogen peroxide. The two streams were mixed in the R_1 reactor. When the sample plug reached the gas diffusion unit, part of the free carbon dioxide diffused to the acceptor stream (Q_1) of carbonate buffer containing the bromothymol blue. Inside the R_2 reactor the analyte produced a decrease in the pH of the buffer stream and the corresponding colour change of the acid base indicator was measured at the flow cell.

Flow injection procedure for the simultaneous determination of SO_2 and CO_2 (Fig. 1B)

The sample was injected into a carrier stream of water (Q_3) and subsequently was mixed with a solution of sulphuric acid (Q_4) to convert all forms of the analytes to CO_2 and SO_2. The SO_2 that diffused through GDU_1 to the acceptor stream ($Q_1 + Q_2$) reacted with malachite green and caused the colour change of the solution.

In parallel, the portion of the sample, which remained in the donor channel, was mixed with the solution of H_2O_2 to eliminate the remaining SO_2, which would interfere in the determination of carbon dioxide. The CO_2 then diffused (GDU_2) to the channel Q_6 and caused an alteration of the pH of the solution and consequently a change in the colour of the acid base indicator.

Sample treatment

Before introduction in the flow system, the wines were treated with hydroxide to allow the determination of total SO_2 and to fix the concentration of CO_2. A 20 mL of 50 % w/w NaOH was added to bottles containing 750 mL wine.

Reference determination

The reference determinations [1] used for evaluating the quality of the results obtained by the FIA methods were: Ripper method for total sulphur dioxide, and a titration of alkalized CO_2 with H_2SO_4 between pH 8.6 and 4.0 for carbon dioxide.

Results and discussion

The different parameters of the systems were studied in order to achieve a good sensitivity in the expected determination range. After preliminary experiments to set approximate values for the manifold parameters, an univariate optimization procedure was followed. The values found are presented in figure 1.

CO_2 determination

The manifold was optimized to be able to perform the determination of carbon dioxide in the range of 0.5 to 4 g L^{-1} for sparkling wines.

The injection volume was varied between 50 and 240 µL. Sensitivity increased with increasing volumes, but the sampling rate decreased due to the longer time necessary to return to the baseline. Therefore, the volume of 100 µL was selected for further experiments.

The decrease of the flow rate in the acceptor stream (Q_1) from 3.3 to 1.2 mL min^{-1} produced a higher sensitivity but lowered the sampling rate. Therefore, a compromise value of 1.7 mL min^{-1} flow rate was used.

The length of the reactor (R_1) was increased up to 50 cm to allow sufficient mixing. The effect of the length of the R_2 reactor was studied in the range of 30 to 130 cm. The sensitivity improved up to 120 cm, and decreased for longer ones due to higher dispersion.

Two types of gas diffusion membrane materials, PTFE and PVDF, were tested. When the PTFE membrane was

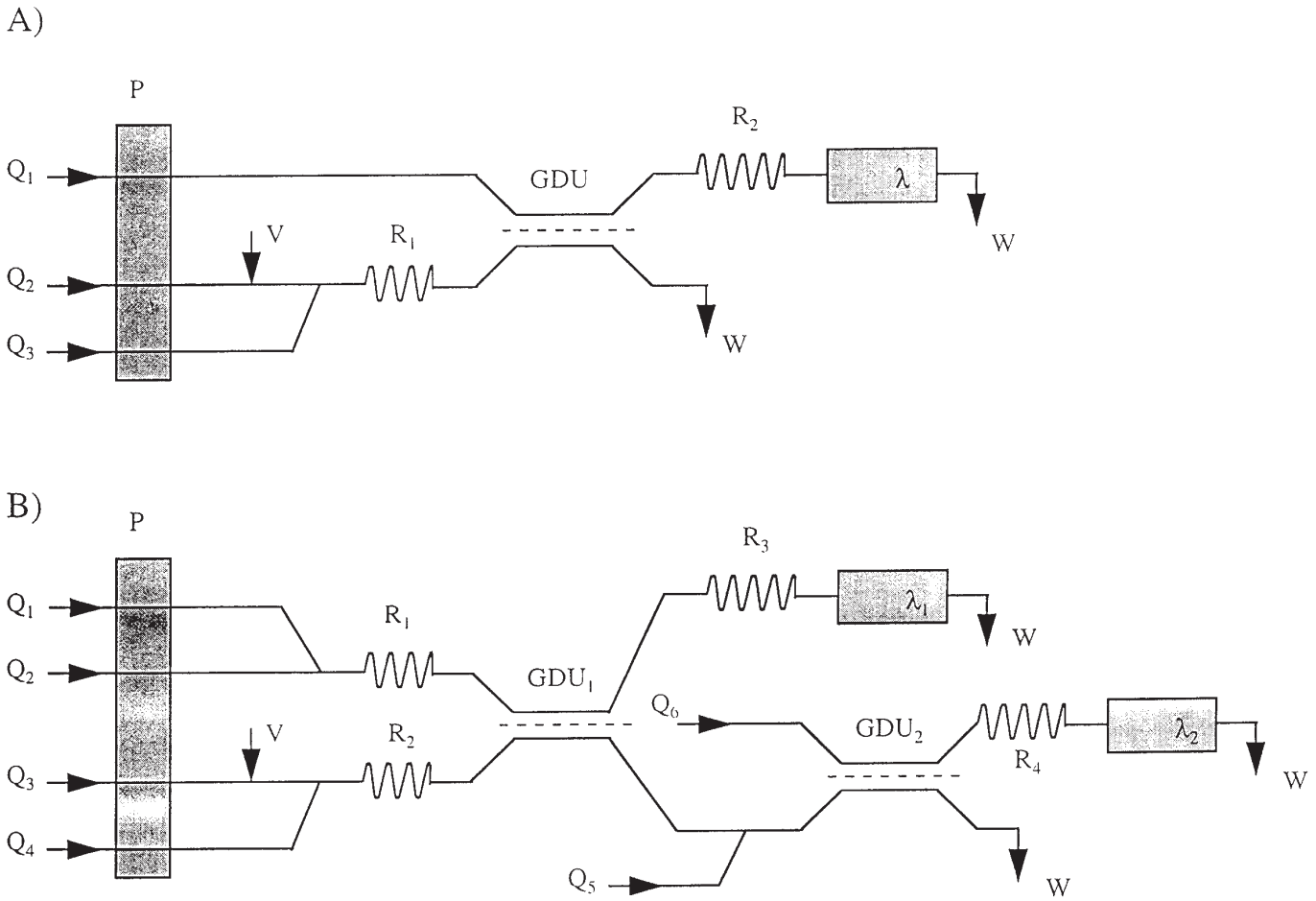

Fig. 1. A. Flow injection manifold developed for the determination of carbon dioxide in wines, P: peristaltic pump; V: injection valve (100 µL); Q_i: reagent streams; Q_1 (1.7 mL min^{-1}): 28 mg L^{-1} bromothymol blue in 0.1 mM carbonate buffer; Q_2 (0.8 mL min^{-1}): H_2O; Q_3 (1.4 mL min^{-1}) 60 mg L^{-1} H_2O_2 in 0.2 M sulphuric acid solution; R_i: reactors; R_1: 50 cm; R_2: 120 cm; GDU: gas diffusion unit; λ: spectrophotometer (614 nm); W: waste. **B.** Flow injection manifold developed for the simultaneous determination of SO_2 and CO_2 in wines, Q_i: reagent streams; Q_1 (0.8 mL min^{-1}): 20 mg L^{-1} malachite green in 6.25 10^{-3} M KH_2PO_4; Q_2 (0.8 mL min^{-1}): 9.4 10^{-2} M K_2HPO_4; Q_3 (0.8 mL min^{-1}):H_2O; Q_4 (1.3 mL min^{-1}): 0.2 M H_2SO_4; Q_5 (0.3 mL min^{-1}): 300 mg L^{-1} H_2O_2 in 0.06 M H_2SO_4; Q_6 (1.7 mL min^{-1}): 28 mg L^{-1} bromothymol blue in 0.1 mM carbonate buffer; λ_i: spectrophotometers, λ_1: 614 nm, λ_2: 617 nm; R_i: reactors; R_1, R_2, R_4: 50 cm; R_3 120 cm; other designation are the same as for system in Fig. 1.A.

used the sensitivity at the low concentration range (up to 1.5 g L^{-1}) was higher than the one achieved with the PVDF membrane. However, the PVDF membrane presented higher resistance to the pressure difference between the two channels; therefore this type of membrane was chosen to guarantee better repeatability.

The sensitivity increased by decreasing the concentration of the carbonate buffer from 0.2 to 0.1 mM. The lower concentration was more difficult to handle because of limited stability of the low capacity buffer, due to dissolution of atmospheric carbon dioxide, causing a considerable baseline drift. Therefore, nitrogen was bubbled through the freshly prepared acceptor solution, and subsequently maintained in a closed bottle. This way, no significant sensitivity change was observed for one working day.

By increasing the bromothymol blue concentration in the acceptor stream from 10 to 30 mg L^{-1} the sensitivity augmented. However, when a 30 mg L^{-1} concentration was used, the baseline absorbance became too high (> 1.8), decreasing dramatically precision. The 28 mg L^{-1} concentration was used in the subsequent experiments, yielding a stable baseline reading at around 1.5 of absorbance.

The effect of the acid concentration (Q_3) on the sensitivity was measured by increasing the concentration of the sulphuric acid in the range of $5\ 10^{-3}$ M to 0.5 M. Over 0.2 M the sensitivity in the desired concentration range (up to 5 g L^{-1}) did not change. Therefore, for the further experiments a 0.2 M solution was used.

The reaction on which the determination is based is about 5 times more sensitive for SO_2 than for CO_2. Therefore, even in the presence of a small amount of SO_2 in the wine, the CO_2 result can overestimated. To overcome this problem, hydrogen peroxide was used to oxidise SO_2 to SO_4^{2-}. As sulphate does not pass through the gas diffusion membrane to the acceptor stream, the interference is avoided. To assess the necessary amount of hydrogen peroxide, its concentration was increased until there was no peak recorded for the injection of 0.3 g L^{-1} SO_2 standard solution, as this should be the maximum amount of this analyte in the wine samples [14]. It was found that a 60 mg L^{-1} concentration of hydrogen peroxide was sufficient for this purpose. As the peaks recorded for the CO_2 standards had the same height in the presence or the absence of hydrogen peroxide, it indicated that this amount of hydrogen peroxide had no effect on the CO_2 determination.

Simultaneous determination of SO_2 and CO_2

Based on the optimized system for CO_2, the injection volume, the flow rate and the concentration of the acid stream was kept constant. The part of the system corresponding to the CO_2 determination was not changed.

The implemented method for the determination of SO_2 was based on the one described by Sullivan *et al*. [4] and modified to achieve the desired working concentration range (between 0.05 and 0.3 g L^{-1} SO_2).

The effect of the concentration of the malachite green was studied between 2 and 24 mg L^{-1}. The sensitivity increased with the concentration of the reagent; however, the intense colour of the solution did not allow using higher concentrations. The 20 mg L^{-1} solution provided a stable baseline reading at around 1.5 of absorbance.

The effect of the acceptor stream flow rate ($Q_1 + Q_2$, $Q_1 = Q_2$) was studied in the range of 1.0 to 2.6 mL min^{-1}, sensitivity increased with lower flow rates therefore, 1.2 mL min^{-1} was chosen as a compromise value.

The configuration of the gas diffusion unit was selected to provide a good sensitivity. The sensitivity increased with the length of the flow channel; meanwhile when the zigzag configuration was used, the calibration curve became more affected by the other (CO_2) component diffusing through the membrane.

The length of the reactor R_3 was tested in the range of 30 to 200 cm. Since no significant change in sensitivity occurred between 50 and 150 cm, a 50 cm length, which produced higher sampling rate, was chosen.

Application to wine analysis

Under the selected conditions, the performance of the flow injection methods was tested, and some of the important characteristics of the systems are presented in table I.

Determination of CO_2 in wine

The proposed method (A) was applied to the determination of CO_2 in 13 samples of carbonated Portuguese wines, and the values (in g L^{-1}) were compared with those obtained by the reference method, involving titration of alkalized CO_2 with H_2SO_4 between pH 8.6 and 4.0. A linear relationship ($C_{FIA} = C0 + S \times C_{Reference}$) was established, and the values for intercept (C_0), slope (S) and the correlation coefficient were 0.018 (± 0.230), 0.990 (± 0.086) and 0.9916 respectively, where the values in parenthesis are the limits of the 95 % confidence intervals [15]. These figures demonstrate a good agreement between the two methods.

Recovery studies were also carried out to assess the accuracy of the developed method in a wider application range. Three wine samples whose carbon dioxide concentration corresponded to three different ranges of the analysed samples were used. The CO_2 was added to the wines in the form of $NaHCO_3$ corresponding to three different levels of CO_2 concentration: 0.5, 1.0 and 1.5 g L^{-1}. The results obtained are presented in table II. When statistical test (*t* test) was used the results showed that the added and the recovered amounts were not different at 95 % significance level; the calculated *t* value was 1.368 corresponding to a critical *t* value of 2.306.

Table I. Some figures of merit of the developed systems.

	CO_2 system	CO_2 / SO_2 system	
		CO_2	SO_2
Repeatability [a] (RSD %)	1.91 (0.61 g L^{-1}) 0.82 (2.33 g L^{-1}) 1.06 (2.74 g L^{-1})	1.5 (0.81 g L^{-1}) 2.4 (1.4 g L^{-1})	4.5 (0.079 g L^{-1}) 3.9 (0.084 g L^{-1})
Determination range (g L^{-1})	0.5-4.0	0.25-3.0	0.05-0.3
Sampling rate (h^{-1})	30	40	

[a] Calculated from 10 consecutive injections of wine samples. Values in parentheses are the mean concentrations.

Table II. Recoveries obtained when 0.5, 1.0 and 1.5 g/L of CO_2 was added to three different wines.

No addition, g L^{-1}	Recovery, % 1st add. a)	2nd add. b)	3rd add. c)
0.61	100	98.0	98.8
2.27	98.0	102	103
2.74	96.0	96.7	94.7

Addition of a) 0.5 g L^{-1}, b) 1.0 g L^{-1} and c) 1.5 g L^{-1} CO_2 in the form of sodium carbonate.

Simultaneous determination of CO_2 and SO_2 in wine

The developed method (B) was applied for the determination of CO_2 and SO_2 in Portuguese wines. The results of the SO_2 determination were compared with the results obtained by the Ripper method, while the result of the CO_2 determination were evaluated by a two level recovery study. The results obtained for the analysis of SO_2 in six red and white wines are summarized in table III. The comparison of the results (in mg L^{-1}) obtained for the determination of SO_2 with the developed FIA method (C_{FIA}) and with the reference method ($C_{Reference}$) showed a good agreement as can be perceived from the parameters of the regression equation: $C_{FIA} = -0.718\ (\pm\ 12.3) + 1.002\ (\pm\ 0.110) \times C_{Reference}$, $R = 0.997$. The values in parentheses are the limits of the 95 % confidence intervals. The recoveries obtained for the CO_2 determination showed acceptable accuracy as well. A repeatability study is presented on figure 2.

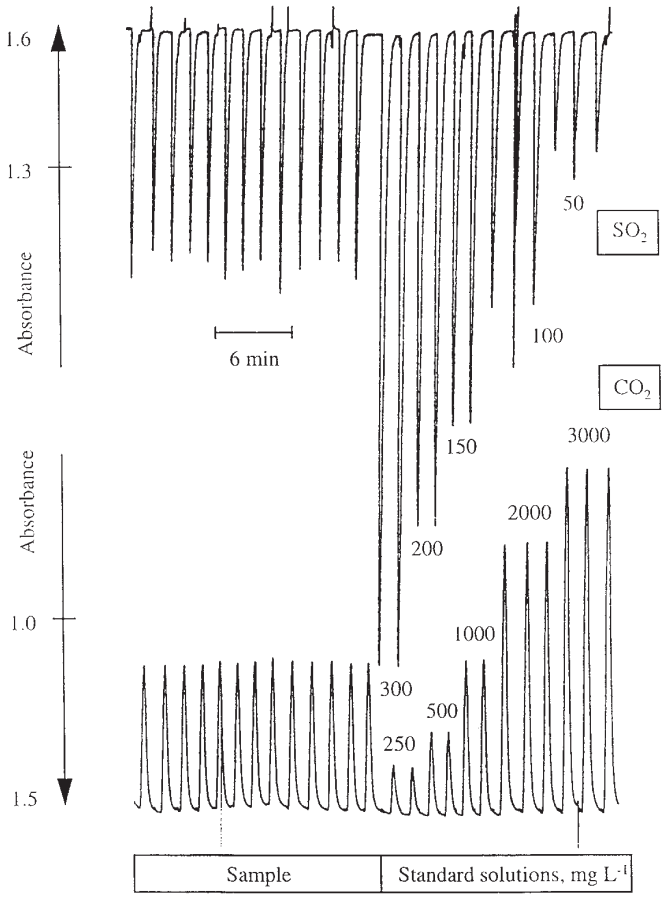

Fig. 2. Recorder output of the simultaneous determination of CO_2 and SO_2, corresponding to the injection of a set of standards and a sample injected 13 times.

Conclusions

The developed method for the determination of CO_2 and SO_2 can be quite useful for wine companies, as its allows to monitor both components with the same manifold. No sample pre-treatment is required, which is an advantage over a previous work [12]. It should also be enphasized the possibility of monitoring spectrophotometrically CO_2 in the presence of SO_2, just by adding hydrogen peroxide to the samples inside the flow system.

Table III. Simultaneous determination of CO_2 and SO_2 levels in wines by the developed manifold.

No addition mg L^{-1}	CO_2 Recovery % 1st add. a)	2nd add. b)	FIA mg L^{-1}	SO_2 Ripper meth. mg L^{-1}	RD % c)
432d)	96.6	93.5	111	112	–0.89
820d)	108	97.4	102	108	–5.5
475d)	102	99.6	87.4	84.8	3.1
288e)	101	99.8	94.6	93.6	1.1
801e)	110	111	64.6	65.3	–1.1
			176e)	175	0.06

a) Addition of 500 mg L^{-1}, b) addition of 1000 mg L^{-1} CO_2 in the form of carbonate; c) relative deviation; d) White table wines; e) Red table wines

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