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# Development of a Gas Diffusion Multicommuted Flow Injection System for the Determination of Sulfur Dioxide in Wines, Comparing Malachite Green and Pararosaniline Chemistries

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A flow system based on the multicommutation concept was developed for the determination of free and total sulfur dioxide in table wines, exploiting gas diffusion separation and spectrophotometric detection. The system allowed the comparison of malachite green and pararosaniline chemistries, using the same manifold configuration. Free and total $SO_2$ were determined within the ranges 1.00−40.0 and 25.0−250 mg $L^{-1}$, at determination throughputs of 25 and 23 $h^{-1}$, respectively. Employing the malachite green reaction, detection limits of 0.3 and 0.8 mg $L^{-1}$ were attained for free and total $SO_2$, respectively. Pararosaniline chemistry provided detection limits of 0.6 mg $L^{-1}$ for free $SO_2$ and 0.8 mg $L^{-1}$ for total $SO_2$. Relative standard deviations better than 1.8 and 1.4% were obtained by the malachite green and pararosaniline reactions, respectively. With regard to the two tested chemistries, 18 wines were analyzed and the results achieved by the pararosaniline reaction compared better with those furnished by the recommended procedure.

**KEYWORDS: Multicommutation; gas diffusion; spectrophotometry; sulfur dioxide; wines; malachite green; pararosaniline**

## INTRODUCTION

Sulfiting agents have been added to foods and beverages as preservatives to prevent detrimental phenomena such as oxidation and microbiological growth, as well as to control enzymatic reactions during production and storage. In the winemaking industry, sulfur dioxide content is often monitored before and after its addition, first to determine if it is necessary to proceed with addition and then to be sure of the correct added amount (*1*). Nevertheless, this adjustment can be a complex task as insufficient sulfite concentration might not ensure total microbiological wine stability and excessive concentrations will interfere with wine aromas and can cause adverse effects on human health (*2*). For this reason, $SO_2$ levels in wines are strictly regulated in several countries (*3*).

$SO_2$ may be present in wines in the free form, as $SO_2$ and as $H_2SO_3^-$, or bound to carbonyl group containing compounds. The recommended method for $SO_2$ determination, known as the Ripper procedure, is based on the iodometric titration using starch for the end-point detection (*4*). However, this method can suffer from lack of accuracy due to the reaction of iodine with other oxidizable substances such as phenols and from the difficult visual detection of the end-point, especially in red wines. To overcome these limitations and acting in response to the demand of simple and rapid methods to control this parameter, several flow methodologies incorporating both free and total $SO_2$ determinations in wines have been proposed in recent years. Within these methods, spectrophotometric (*5*−*12*), amperometric (*13*−*18*), potentiometric (*19*), conductometric (*20*) or chemiluminescent (*21*) detections were employed. Separation devices such as gas diffusion (*5*, *7*, *9*−*13*, *16*−*21*), microdistillation (*6*), or pervaporation (*8*) was employed to separate the liberated sulfur dioxide from the matrix. The majority of the described methodologies required offline treatments such as sample dilution and/or hydrolysis (*7*−*9*, *12*−*19*, *21*). However, sample handling and treatment can represent a source of error, because equilibrium variations may occur, leading to the possible liberation and loss of free or weakly bound $SO_2$ from the sample before analysis.

Among the spectrophotometric methodologies described for free and/or total $SO_2$ in wines, reactions of sulfite with malachite green (*22*−*25*) and with pararosaniline (*8*, *11*, *12*, *26*−*28*) have been often used due to their high sensitivity. The first method relies on the instantaneous decolorization of malachite green in the presence of neutral sulfite solutions. This color change is due to the destruction of the quinoidal structure of the dye by the sulfurous acid (*29*). The second assay is based on the monitoring of the red-violet color produced in the mixture of pararosaniline, hydrochloric acid, and formaldehyde in the presence of sulfite (*30*). For the works based on the malachite green reaction, only free $SO_2$

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determination was performed except in one case, when it was applied to determination of total $SO_2$ content in white and red wines, providing successful results in the analysis of white wines, but low $SO_2$ recoveries for red wines (*23*). In fact, this method was later recommended by the AOAC (*31*) as the official method for total sulfite in foods and beverages, but the applicability was not extended to red wines. On the other hand, pararosaniline methods were applied to free (*26*−*28*) or free and total $SO_2$ determinations (*8*, *11*, *12*). With regard to the latter ones, the incorporation of the necessary hydrolysis step for total $SO_2$ determination was challenging, requiring an offline digestion step (*12*) or a long reactor to provide long residence times for the inline hydrolysis step (*8*) or the introduction of an additional peristaltic pump for continuous sample digestion during the whole analytical cycle (*11*).

Although flow injection (FIA) is the most exploited flow methodology for this determination, sequential injection (SIA) (*11*, *18*) and multisyringe flow injection (MSFIA) (*12*) systems were also proposed. Whereas the FIA concept is based on the continuous flow of solutions, in SIA the reagent consumption is reduced through the selection of the precise amounts of the reagents needed for the determination. However, in SIA there is a lower mixing efficiency due to the limited overlapping of the reagents and sample plugs, which is frequently referred to as a drawback. The more recently described multicommuted flow concept (*32*) (in which multisyringe systems can be included, because both comprise a flow network in which solutions can be accessed by controlling the position of the solenoid valves) combines the advantages of the preceding flow methodologies through the combination of the reagent addition in confluence furnished by FIA with the possibility of selecting the reagent quantities provided by SIA systems. A multicommuted flow injection system (MCFIA) is composed of an array of solenoid valves; the programmed actuation of these devices controls the flow path of sample and reagents. The analytical performance of these systems can be further improved by placing the propulsion unit before detection (*33*).

In this work, the first application a multicommuted flow injection system to the determination of free and total $SO_2$ in white and red wines is proposed, without the need to carry out any offline sample treatment. Malachite green (MG) and pararosaniline (PRA) spectrophotometric reactions were compared in the flow methodology because replacement of pararosaniline by malachite green for the determination of free and total $SO_2$ in white and red wines could be interesting as the lower toxicity of the latter makes it an environmentally friendly option.

## MATERIALS AND METHODS

**Reagents and Solutions.** All reagents used were of analytical grade, and deionized water (conductivity $< 0.1\ \mu S\ cm^{-1}$) was used throughout.

For the malachite green reaction, acceptor solution was obtained inline by mixing a solution containing this reagent and potassium dihydrogen phosphate with a dipotassium hydrogen phosphate solution. Malachite green stock solution was prepared by dissolving 200 mg of malachite green oxalate (Fluka) and 8.5 g of potassium dihydrogen phosphate (Merck) in 1000 mL of water, followed by filtration using a 0.45 $\mu$m cellulose acetate membrane filter (Whatman). Working solution was prepared daily by appropriate dilution of the stock solution in deionized water. Dipotassium hydrogen phosphate solution was prepared by dissolving 16.4 g of the respective anidrous solid (Merck) in 1000 mL of water.

In the pararosaniline reaction, the acceptor stream was generated inline by mixing this reagent with formaldehyde, both with an equal hydrochloric acid concentration of 0.06 mol $L^{-1}$. Pararosaniline stock solution was obtained by dissolution of 0.500 g of pararosaniline hydrochloride (Sigma) in 100 mL of ethanol, followed by volume adjustment to 500.0 mL with water. Pararosaniline solution was prepared daily by dilution in water of 25.00 mL of the previous solution plus 5.0 mL of HCl 3 mol $L^{-1}$ in a 250.0 mL volumetric flask. To prepare the second reagent of the acceptor solution, 2.5 mL of formaldehyde 37% (Merck) and 2.5 mL of HCl 37% (Merck) were diluted in 500.0 mL of deionized water.

Sodium hydroxide solution 2 mol $L^{-1}$ was used as the hydrolysis solution. Sulfuric acid solutions were obtained by appropriate dilution of the commercial solution 95−98% (m/v) (Merck).

A 500 mg $L^{-1}$ stock standard solution of sulfur dioxide was prepared by dissolving 0.2522 g of $Na_2SO_3$ in ethylenediaminetetraacetic acid (EDTA) 0.001 mol $L^{-1}$ (*34*), and the final volume was adjusted to 250.0 mL. EDTA solution was obtained by dissolving 0.3722 g of the respective solid (Merck) in 1000 mL of deionized water. Working standard solutions were daily prepared from the above solution, by dilution in EDTA 0.001 mol $L^{-1}$, corresponding to sulfur dioxide concentrations of 1.00, 5.00, 10.0, 20.0, 30.0, and 40.0 mg $L^{-1}$ for free sulfur dioxide determination and 25.0, 75.0, 150, and 250 mg $L^{-1}$ for total sulfur dioxide determination.

**Wine Samples.** Various table wines were purchased in local supermarkets, being representative of ordinary table wines. Source data including harvest year, region, and style, as well as some analytical parameters (ethanol, dry extract, residual sugars, and volatile and total acidities) are presented in **Table 1**.

All samples were introduced in the flow system without any previous treatment. Wines from the same bottle were analyzed in the optimized flow system first using the pararosaniline reaction and then using the malachite green reaction. Samples were frozen between the two analyses. To compensate for $SO_2$ losses during storage and defrosting, each sample was analyzed by the reference procedure on the same day of the flow assessment.

**Instrumentation.** A Minipuls 3 multichannel peristaltic pump (Gilson, Villiers-le-Bel, France) equipped with PVC Gilson and Ismatec (Glattbrugg, Switzerland) pumping tubes was used to propel solutions. All connections were made of PTFE tubing with 0.8 mm i.d. (W025953, Omnifit, Cambridge, U.K.) attached to Gilson end-fittings and connectors. Acrylic laboratory-made Y-shaped joints were used as confluences.

The direction of the solutions was controlled by three-way solenoid valves (NResearch, 161 T031, Caldwell, NJ), operated by means of a power drive (CoolDrive, NResearch). A 486 personal computer (FR-746WW-A9, Digital, Gumi, South Korea), equipped with an interface card (PCL-818 L, Advantech, Taipei, Taiwan) running laboratory-made software written in QuickBasic 4.5 (Microsoft) controlled the switching of the solenoid valves.

The gas diffusion device consisted of two separate acrylic blocks, pressed against each other by six screws, with a diffusion surface area of 1524 $mm^2$ and matching cavities characterized by a zigzag channel configuration (*33*). A hydrophobic membrane (HVHP09050, Millipore Durapore, Madrid, Spain) with a pore size of 0.45 $\mu$m was placed between the two blocks, being replaced weekly.

A UV−vis spectrophotometer (Unicam 8625, Cambridge, U.K.), equipped with a flow-through cell with 18 $\mu$L of internal volume and a 1 cm flow path (Hellma 178.712-QS, Mullheim/Baden, Germany), was used as detection system. Analytical signals were recorded using a chart recorder (Kipp & Zonen BD111, Delft, Holland) connected to the spectrophotometer.

**Manifold and Flow Procedure.** The system components were arranged as shown schematically in **Figure 1**. The determination

**Table 1.** Information Relative to the Analyzed Table Wines

| sample | style | harvest year | region | ethanol (%) | dry extract (g $L^{-1}$) | residual sugars (g $L^{-1}$) | volatile acidity (g $L^{-1}$ acetic acid) | total acidity (g $L^{-1}$ tartaric acid) |
|---|---|---|---|---|---|---|---|---|
| 1 | dry red | 2005 | Douro | 13 | NA[a] | NA | 0.5 | 5.1 |
| 2 | dry red | 2005 | Bairrada | 13 | NA | <1.5 | NA | 5.2 |
| 3 | dry red | 2005 | Alentejo | 13 | NA | NA | NA | NA |
| 4 | dry red | 2007 | Alentejo | 13 | 27.7 | NA | 0.57 | 6.16 |
| 5 | dry red | NA | NA | 11.5 | NA | NA | NA | NA |
| 6 | dry red | 2003 | Bairrada | 12.5 | NA | NA | NA | NA |
| 7 | dry red | 2005 | Dão | 12 | NA | NA | NA | NA |
| 8 | dry red | NA | NA | 11.5 | NA | NA | NA | NA |
| 9 | dry white | NA | Douro | 11.5 | NA | NA | NA | NA |
| 10 | dry white | 2007 | Alentejo | 12.5 | 20.4 | NA | 0.26 | 6.12 |
| 11 | dry white | 2007 | Alentejo | 12.5 | NA | NA | NA | 5.5 |
| 12 | dry white | NA | NA | 11.5 | 21 | NA | 0.40 | 5.5 |
| 13 | dry white | 2004 | Douro | 11 | 18.2 | 1.2 | 0.47 | 4.88 |
| 14 | dry white | 2004 | Estremadura | 11.5 | NA | NA | NA | NA |
| 15 | dry white | 2007 | Alentejo | 12 | NA | 5 | NA | 5.1 |
| 16 | dry white | 2007 | Alentejo | 12.5 | NA | <2 | 0.2 | 5.5 |
| 17 | dry white | 2006 | Dão | 12 | NA | NA | NA | NA |
| 18 | dry white | NA | NA | 11.5 | NA | NA | NA | NA |

[a] Not available.

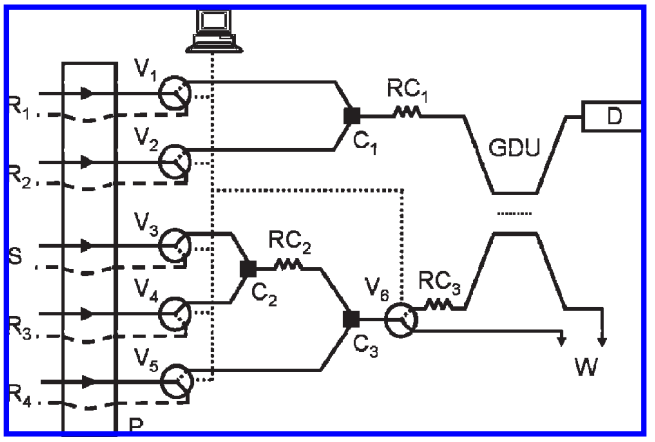


**Figure 1.** Multicommuted flow manifold for the determination of sulfur dioxide in wines using MG- or PRA-based reaction chemistries: $R_1$, 1.0 mL $min^{-1}$, malachite green 20 mg $L^{-1}$ + $KH_2PO_4$ 0.85 g $L^{-1}$ (MG) or pararosaniline 100 mg $L^{-1}$ + HCl 0.06 mol $L^{-1}$ (PRA); $R_2$, 1.0 mL $min^{-1}$, $K_2HPO_4$ 16.4 g $L^{-1}$ (MG) or formaldehyde 1.5 g $L^{-1}$ + HCl 0.06 mol $L^{-1}$ (PRA); S, 1.0 mL $min^{-1}$, sample or standard; $R_3$, 0.3 mL $min^{-1}$, NaOH 2 mol $L^{-1}$; $R_4$, 1.3 mL $min^{-1}$, $H_2SO_4$ 0.75 mol $L^{-1}$ (free $SO_2$) or 3 mol $L^{-1}$ (total $SO_2$); P, peristaltic pump; $V_i$, solenoid valves in the position "on" (continuous line) or "off" (discontinuous line); $RC_i$, reaction coils; $RC_1$ = 60 cm; $RC_2$ = 100 cm; $RC_3$ = 20 cm; $C_i$, confluence points; GDU, gas diffusion unit; D, detector set at 615 nm (MG) or 580 nm (PRA); W, waste.

of free and total $SO_2$ in wines was performed following the protocol sequence presented in **Table 2**.

For the determination of free $SO_2$, some washing steps were necessary when a new sample was introduced in the flow system. Steps 1−3 were performed only when a new sample was analyzed. After these washing steps, the analytical cycle started with introduction of sample (50 and 150 $\mu$L using MG and PRA reactions, respectively) merged with $H_2SO_4$, in order to convert $SO_3^{2-}$ present in the sample into gaseous $SO_2$ (step 4). Then, the acceptor stream was stopped during 40 s in order to concentrate the diffused $SO_2$ in the acceptor solution (step 5) while the carrier stream was transporting the sample through the donor channel. Finally, the acceptor solution with the retained analyte was sent toward the spectrophotometic detector, and the analytical signal was recorded (step 6).

The determination of total $SO_2$ required an alkaline hydrolysis to release the bound $SO_2$ prior to analysis. This procedure was performed inline by mixing the sample with NaOH in reaction coil $RC_2$ for 40 s. In this step, the frontal plug of the formed mixture was discarded to waste to remove the remains of the previous sample (step 7). Then, while the rear part of the mixture of sample plus alkali solution remained in $RC_2$, the connection between $C_3$ and $V_6$ was washed with $H_2SO_4$ (step 8) during 20 s. In the next stage, the digested sample (25 $\mu$L for MG and 75 $\mu$L for PRA) was introduced in $RC_3$, where it reacted with $H_2SO_4$ (step 9), and finally the acceptor solution with the diffused $SO_2$ was sent toward detection (step 10).

**Recommended Procedure.** The results obtained by the developed methodology were compared with those obtained with the procedure recommended by OIV for free and total $SO_2$ determinations in wines. The recommended procedure for free $SO_2$ determination consisted of direct titration with iodine, using starch for detection of the end-point. Determination of total $SO_2$ involved a previous hydrolysis of the bound $SO_2$ with an alkali solution, followed by the same procedure used for free $SO_2$ (*4*).

## RESULTS AND DISCUSSION

**Development of the Flow System.** Chemical and other system-related parameters were studied by the univariate method, considering the required concentration range (1.00−40.0 mg $L^{-1}$ for free $SO_2$ and 25.0−250 mg $L^{-1}$ for total $SO_2$), maximum sensitivity, accuracy, and sample throughput.

The influence of reagent concentration of the acceptor solution in the malachite green system was evaluated through the study of malachite green and $K_2HPO_4$ concentrations, setting $KH_2PO_4$ concentration to 0.85 g $L^{-1}$. Malachite green concentration was studied within the range of 5.0−20 mg $L^{-1}$. Both baseline absorbance and sensitivity increased with the malachite green concentration. Higher values were not tested because the required working range was already attained with the highest concentration tested. Therefore, a malachite green concentration of 20 mg $L^{-1}$ was selected for further experiments. Study of $K_2HPO_4$ concentration reflected the pH study of the acceptor solution. $K_2HPO_4$ concentrations of 0.17, 1.64, 16.5, and 164 g $L^{-1}$ originated in the acceptor solution pH values of 6.2, 7.2, 8.2, and 9.2, respectively. Better sensitivity was obtained with

**Table 2.** Protocol Sequence for the Spectrophotometric Determination of Free and Total Sulfur Dioxide in Wines

| | | position of the commutation valves[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| step | description | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | time (s) |
| | free $SO_2$ | | | | | | | |
| 1 | wash connection between $V_3$ and $V_6$ with sample | N | N | N | F | N | N | 50 |
| 2 | wash connection between $C_3$ and $V_6$ with $H_2SO_4$ | N | N | F | F | F | N | 10 |
| 3 | wash acceptor and donor channels | F | F | F | F | F | F | 20 |
| 4 | sample introduction and stop acceptor stream | N | N | N | F | F | F | 2.9[b]/8.6[c] |
| 5 | stop acceptor solution flow | N | N | F | F | F | F | 37.1[b]/31.4[c] |
| 6 | propel acceptor toward the detector; signal registration | F | F | F | F | F | F | 80 |
| | total $SO_2$ | | | | | | | |
| 7 | fill $RC_2$ with sample and NaOH; hydrolysis | F | F | N | N | N | N | 40 |
| 8 | wash connection between $C_3$ and $V_6$ with $H_2SO_4$ | F | F | F | F | F | N | 20 |
| 9 | introduction of the hydrolyzed sample and reaction with $H_2SO_4$ | F | F | N | N | F | F | 1.4[b]/4.3[c] |
| 10 | propel acceptor toward the detector; signal registration | F | F | F | F | F | F | 90 |

[a] N and F represent positions on and off, respectively. [b] MG [c] PRA.

a pH of 8.2, so a solution containing 16.5 g $L^{-1}$ of $K_2HPO_4$ was chosen for further work.

In the pararosaniline method, the study of the acceptor solution comprised assessment of pararosaniline and formaldehyde concentrations, maintaining a fixed concentration of HCl at 0.06 mol $L^{-1}$. Pararosaniline and formaldehyde concentrations were studied within the ranges of 0.020−0.20 and 0.50−3.0 g $L^{-1}$, respectively. In both cases, higher concentrations provided better sensitivity values. However, using high pararosaniline or formaldehyde concentrations, analytical signals became too high, leading to baseline instability, mainly in the more concentrated standard solutions, resulting in longer analytical cycles to maintain baseline stability. For this reason, 0.10 g $L^{-1}$ of pararosaniline and 1.5 g $L^{-1}$ of formaldehyde were selected as a compromise between the sensitivity and the determination frequency.

The study of $H_2SO_4$ concentration needed for free $SO_2$ determination was carried out by testing $H_2SO_4$ concentrations between 0.10 and 2.0 mol $L^{-1}$ in the malachite green reaction. $H_2SO_4$ concentrations under 0.20 mol $L^{-1}$ gave rise to almost imperceptible analytical signals. Sensitivity increased 83% when the $H_2SO_4$ concentration was increased from 0.20 to 0.30 mol $L^{-1}$. Then, a rise of 12% on the sensitivity was noted up to 0.75 mol $L^{-1}$, maintaining constant for higher values. Hence, $H_2SO_4$ 0.75 mol $L^{-1}$ was chosen for additional studies in free $SO_2$ determinations.

The influence of the temperature, flow rate, and stop time of the acceptor solution was studied in the flow system, using the analytical cycle for free $SO_2$ determination and malachite green reaction. Results were evaluated through comparison of sensitivity values obtained by the linear part ($SO_2$ concentrations between 0 and 5.00 mg $L^{-1}$) of calibration curves (second-order polynomial) established using $SO_2$ concentrations ranging between 0 and 20.0 mg $L^{-1}$.

Temperature influence of acceptor and donor solutions was studied by introducing the reaction coils $RC_1$ and $RC_3$, respectively, in a thermostatic bath. The temperature of the acceptor solution was evaluated in a range between 25 °C (room temperature) and 55 °C. An increase of 11% in the sensitivity was observed when the temperature of the acceptor solution was raised from 25 to 40 °C, maintaining constant for higher values. Donor solution temperature was varied from 25 to 70 °C. The sensitivity increased by 15% with rising temperature of the donor stream from 25 to 55 °C, remaining stable for higher temperatures. However, the temperature increase of the donor or acceptor streams promoted air bubble formation inside the flow system, compromising the accuracy of the results. Thus, room temperature was chosen for further work.

The flow rate of the acceptor solution was evaluated between 1.6 and 2.8 mL $min^{-1}$, keeping a flow rate of 2.6 mL $min^{-1}$ for donor solution. Constant sensitivity values were obtained using flow rates ranging from 1.6 to 2.0 mL $min^{-1}$, decreasing 14% when the higher flow rate was employed. For this reason an acceptor flow rate of 2.0 mL $min^{-1}$ was chosen for the following experiments.

With the aim of increasing the sensitivity of free $SO_2$ determination, holding times of the acceptor solution between 0 and 60 s were studied. A 40% increase in the sensitivity was observed by rising the stop time of the acceptor solution to 40 s, increasing 20% further with a stop time of 60 s. However, longer stop periods led to a longer analytical cycle and consequently to a lower sampling rate. Thus, a stop period of 40 s for free $SO_2$ determination was selected as a compromise between the sensitivity and the sampling rate.

All of the subsequent parameters were studied using the analytical cycle for total $SO_2$ determination and considering the recovery ratio of total $SO_2$ in wine samples, expressed as (total $SO_2$ obtained by the flow methodology/total $SO_2$ obtained by the reference method) × 100. For this study, two white and two red wines were analyzed.

The study of donor solution flow rate was accomplished by testing individually several flow rates of the sample, the $R_3$ (NaOH) and the $R_4$ ($H_2SO_4$) streams. Sample and NaOH flow rates were studied within the ranges of 1.0−1.7 and 0.30−0.80 mL $min^{-1}$, respectively. Higher sample and NaOH flow rates gave rise to lower total $SO_2$ recoveries in the wine samples, probably due to shorter residence periods in $RC_2$ and consequently less time for hydrolysis. Flow rates of 1.0 and 0.30 mL $min^{-1}$ were selected for sample and NaOH solution, respectively. The influence of the $H_2SO_4$ flow rate was evaluated between 1.3 and 2.7 mL $min^{-1}$. Although higher $H_2SO_4$ flow rates provided better sensitivity values, total $SO_2$ recovery ratio decreased with $H_2SO_4$ flow rate increase, probably due to higher sample dilution factors attained with higher $R_4$ flow rates. A flow rate of 1.3 mL $min^{-1}$ was established for the $H_2SO_4$ solution.

A length of 60 cm was set for $RC_1$ to provide adequate mixing of the two reagents needed for inline acceptor generation. The $RC_2$ is where hydrolysis occurs, so the efficiency of inline hydrolysis was studied by varying $RC_2$ lengths between 50 and 400 cm. Besides noting a poor repeatability

for the longer reactors (300−400 cm), the recovery of total $SO_2$ in wine samples increased by 7% when $RC_2$ was increased from 50 to 100 cm, remaining stable for longer lengths. Hence, 100 cm was the length selected for reaction coil $RC_2$. For $RC_3$ study, lengths of 20, 50, and 100 cm were tested. Although similar sensitivities were obtained using all tested lengths, higher recoveries of total $SO_2$ were achieved with the shortest length, probably due to lower dispersion of the sample. Therefore, a $RC_3$ length of 20 cm was chosen for further work.

Sample parameters (introduction mode and sample volume) were tested individually by applying both tested reactions to free and total $SO_2$ determination.

The sample introduction mode in the flow system was a critical parameter in the total $SO_2$ determination. In a first approach, a volume of sample was propelled by the alkaline solution toward reaction coil $RC_3$, where $H_2SO_4$ was added. Using this method, no analytical signal was obtained in the analysis of red wines by the pararosaniline reaction, and recoveries of total $SO_2$ under 83% for white wines and 57% for red wines were achieved by the malachite green chemistry. These results may be explained by the high dispersion of sample along $RC_2$. With the aim of minimizing sample dispersion, the method of sample introduction was modified to an approach that consists of passing continuously the mixture of sample plus NaOH through $RC_2$ and then propelling a part of this mixture to the flow system. In previous experiments the flow rate ratio for sample/NaOH was set to 3.5; in addition to this, by use of the new injection method the sample dispersion along $RC_2$ was minimized, and total $SO_2$ recoveries close to 90 and 100% were attained by the malachite green and pararosaniline reactions, respectively.

Sample volume was varied by changing the propulsion time in steps 4 and 9 of **Table 2**. When the malachite green reaction was applied, sample volumes ranging from 25 to 100 $\mu$L were tested using the free $SO_2$ cycle. Although sensitivity enhancement was observed with the increase of sample volume, the correlation coefficient of the calibration curve became poorer, compromising the applicable concentration range. As a consequence, a sample volume of 50 $\mu$L was selected for further work. In relation to the total $SO_2$ determination, sample volumes between 20 and 40 $\mu$L were evaluated, 30 $\mu$L being chosen because this volume provided 85% of the sensitivity attained using the maximum volume tested. With regard to the pararosaniline reaction, sample volumes between 50 and 200 $\mu$L and between 25 and 100 $\mu$L were evaluated for free and total $SO_2$, respectively. For free and total $SO_2$ determinations, sample volumes of 150 and 75 $\mu$L yielded 82 and 85% of the sensitivity achieved with the higher tested volumes, so these volumes were chosen for the following studies.

**Study of Interferences.** The study of potential interfering species was performed by considering the usual composition of wine samples. This study was carried out by adding known concentrations of the possible interfering compound to a standard solution containing $SO_2$ 20.0 mg $L^{-1}$. The apparent $SO_2$ content was calculated by interpolation of the obtained analytical signal in the second-order equation obtained for the free $SO_2$ determination. The compounds were considered to interfere if the originated apparent concentration had a relative deviation above 5% (*35*) from the standard containing 20.0 mg $L^{-1}$ $SO_2$. The relative deviations presented in **Table 3** reveal that most of the species, tested at concentrations expected in this kind of sample, did not interfere with either of the reactions employed. However, concentrations higher than those indicated in the table for lactic acid, glycerol, and acetaldehyde interfered in the methodology, using both chemistries.

**Table 3.** Study of Interfering Species in the Proposed Flow System, Using Malachite Green (MG) and Pararosaniline (PRA) Reactions

| | | relative deviation (%) | |
|---|---|---|---|
| species studied | concn tested | MG | PRA |
| glucose | 10 g $L^{-1}$ | −4.3 | −4.9 |
| fructose | 10 g $L^{-1}$ | −1.2 | 0.8 |
| citric acid | 7 g $L^{-1}$ | −1.8 | −2.4 |
| tartaric acid | 10 g $L^{-1}$ | −2.0 | −2.0 |
| ascorbic acid | 2 g $L^{-1}$ | −2.6 | −0.7 |
| lactic acid | 4 g $L^{-1}$ [a] | −3.1 | 3.3 |
| malic acid | 10 g $L^{-1}$ | −2.4 | −4.2 |
| acetic acid | 5 g $L^{-1}$ | −0.3 | 0.4 |
| $K_2SO_4$ | 5 g $L^{-1}$ | −3.0 | 0.2 |
| ethanol | 20% | −2.0 | −1.9 |
| $CO_2$ | 3 g $L^{-1}$ | −2.7 | 4.6 |
| glycerol | 1 g $L^{-1}$ [a] | −4.2 | −4.9 |
| acetaldehyde | 1 mg $L^{-1}$ [a] | −4.9 | −3.8 |

[a] Maximum concentration tolerated.

**Figures of Merit.** The developed methodology allowed the determination of free and total $SO_2$ in wine samples, based on decolorization of malachite green and on color change of pararosaniline. In both chemistries, second-order calibration curves were obtained for $SO_2$ concentrations between 1.00 and 40.0 mg $L^{-1}$ of free $SO_2$ and between 25.0 and 250 mg $L^{-1}$ of total $SO_2$.

The detection and quantification limits were calculated from the least-squares linear regression parameters using the linear part of calibration curves attained with low concentrations of $SO_2$. The detectable absorbance limit ($Y_{LD}$) was assessed as $Y_{LD} = b + 3S_{y/x}$, where $b$ is the intercept and $S_{y/x}$ is the standard error of the linear regression. The detection limit, $C_{LD}$ was calculated by interpolation of $Y_{LD}$ on the equation $Y_{LD} = mC_{LD} + b$, where $m$ corresponds to the slope of the regression. Detection limits of 0.3 and 0.6 mg $L^{-1}$ for free $SO_2$ and 0.7 and 0.8 mg $L^{-1}$ for total $SO_2$ were obtained with the malachite green and pararosaniline reactions, respectively. The quantification limit, $C_{LQ}$, was calculated by interpolation of $Y_{LQ}$ on the equation $Y_{LQ} = mC_{LQ} + b$, where $Y_{LQ}$ was achieved through the equation $Y_{LQ} = b + 10S_{y/x}$ (*35*). Quantification limits of 1.1 and 1.8 mg $L^{-1}$ for free $SO_2$ were achieved using the malachite green and pararosaniline reactions, respectively. For total $SO_2$ determination, 2.5 mg $L^{-1}$ was the quantification limit attained by both chemistries. The determination frequency was estimated as the sum of the time elapsed in each step of the analytical cycle. Determination rates of 25 and 23 $h^{-1}$ were achieved by free and total $SO_2$ cycles, respectively. Reagent consumption and effluent generation per determination are presented in **Table 4**.

**Application of the Flow System to Wine Samples.** To evaluate the accuracy of the method, 18 table wines were analyzed by the proposed system with both chemistries and by the recommended procedure. The results and the corresponding relative deviations are presented in **Tables 5** and **6**.

From the comparison of the obtained results by the developed flow system and those provided by the recommended procedure, a relationship of the type $C_s = C_0 + SC_r$ (where $C_s$ is the result of the proposed methodology and $C_r$ represents the results of the recommended method) was

**Table 4.** Reagent Consumption and Effluent Generation per Determination

| | free $SO_2$ | total $SO_2$ |
|---|---|---|
| malachite green[a] (mg) | $3.33 \times 10^{-2}$ | $5.05 \times 10^{-2}$ |
| $KH_2PO_4$[a] (mg) | 1.42 | 2.14 |
| $K_2HPO_4$[a] (mg) | 27.3 | 41.3 |
| pararosaniline[b] (mg) | $1.67 \times 10^{-1}$ | $2.57 \times 10^{-1}$ |
| formaldehyde[b] (mg) | 2.50 | 3.86 |
| HCl[b] (mg) | 73.0 | 112 |
| NaOH (mg) | 0 | 16.6[a] |
| | | 17.7[b] |
| $H_2SO_4$ (g) | $2.39 \times 10^{-1}$ | $7.10 \times 10^{-1a}$ |
| | | $7.29 \times 10^{-1b}$ |
| effluent generated (mL) | 6.91[a] | 8.34[a] |
| | 7.01[b] | 8.58[b] |

[a] MG. [b] PRA.

established. The equation parameters and the 95% confidence interval limits (*35*) are presented in **Table 7**. The repeatability of the flow methodology was assessed from 10 consecutive injections of 2 white and 1 red wine sample. Relative standard deviations lower than 1.8 and 1.4% were achieved with the malachite green and pararosaniline reactions, respectively.

These results demonstrate a good agreement between the proposed methodology using both reactions and the recommended method, because the slope is close to unity and the intercept is close to zero. However, with the pararosaniline reaction, the correlation coefficient is closer to unity and the 95% confidence interval limits of the estimates are narrower than the ones obtained with results from the malachite green reaction. These results demonstrate lower dispersion of data, revealing a better linear regression for pararosaniline

**Table 5.** Results Obtained for the Determination of Free and Total $SO_2$ in Wines by the Proposed Flow System (MCFIA) and the Recommended Procedure (Ref Method) and Corresponding Relative Deviations, Using the Pararosaniline Reaction

| | free $SO_2$ | | | total $SO_2$ | | |
|---|---|---|---|---|---|---|
| sample | ref method[a] (mg $L^{-1}$ $SO_2$) | MCFIA[a] (mg $L^{-1}$ $SO_2$) | DR (%) | ref method[a] (mg $L^{-1}$ $SO_2$) | MCFIA[a] (mg $L^{-1}$ $SO_2$) | DR (%) |
| 1 | 31.0 ± 1.2 | 30.1 ± 0.3 | −2.9 | 118.8 ± 0.7 | 115.4 ± 1.5 | −2.9 |
| 2 | 21.8 ± 0.9 | 21.7 ± 0.1 | −0.5 | 84.6 ± 1.5 | 86.0 ± 1.6 | 1.6 |
| 3 | 26.2 ± 1.5 | 26.2 ± 0.8 | 0.0 | 92.4 ± 0.5 | 91.3 ± 2.5 | −1.2 |
| 4 | 19.1 ± 0.4 | 18.2 ± 0.1 | −4.7 | 100.7 ± 2.1 | 102.4 ± 0.2 | 1.7 |
| 5 | 22.1 ± 0.6 | 22.9 ± 0.2 | 3.6 | 127.3 ± 0.2 | 128.5 ± 1.6 | 0.9 |
| 6 | 11.0 ± 0.9 | 10.7 ± 0.1 | −2.7 | 95.8 ± 1.5 | 91.3 ± 0.5 | −4.7 |
| 7 | 18.0 ± 0.7 | 18.4 ± 0.2 | 2.2 | 75.6 ± 1.1 | 78.3 ± 0.3 | 3.6 |
| 8 | 21.0 ± 0.7 | 20.1 ± 0.6 | −4.3 | 125.8 ± 1.5 | 120.3 ± 1.1 | −4.4 |
| 9 | 24.0 ± 1.5 | 22.7 ± 0.3 | −5.4 | 131.5 ± 1.6 | 124.1 ± 0.3 | −5.6 |
| 10 | 23.7 ± 1.9 | 24.4 ± 0.3 | 2.9 | 106.3 ± 4.5 | 105.5 ± 2.7 | −0.8 |
| 11 | 2.0 ± 0.2 | 2.1 ± 0.0 | 5.0 | 63.4 ± 1.6 | 67.0 ± 0.4 | 5.7 |
| 12 | 5.5 ± 0.2 | 5.8 ± 0.0 | 5.4 | 97.3 ± 2.1 | 102.5 ± 1.6 | 5.3 |
| 13 | 8.5 ± 0.4 | 8.8 ± 0.1 | 3.5 | 66.4 ± 0.9 | 70.2 ± 0.3 | 5.7 |
| 14 | 21.1 ± 0.4 | 21.4 ± 0.2 | 1.4 | 99.7 ± 1.6 | 99.9 ± 1.8 | 0.2 |
| 15 | 27.2 ± 0.9 | 27.0 ± 0.3 | −0.7 | 94.5 ± 0.9 | 95.9 ± 1.1 | 1.5 |
| 16 | 16.8 ± 0.4 | 16.5 ± 0.2 | −1.8 | 65.6 ± 1.5 | 64.8 ± 0.4 | −1.2 |
| 17 | 31.3 ± 0.6 | 30.9 ± 0.3 | −1.3 | 163.3 ± 1.0 | 166.5 ± 0.4 | 2.0 |
| 18 | 26.0 ± 0.7 | 26.3 ± 0.1 | 1.2 | 89.1 ± 2.1 | 93.2 ± 0.3 | 4.6 |

[a] Average ± standard deviation of three determinations.

**Table 6.** Results Obtained for the Determination of Free and Total $SO_2$ in Wines by the Proposed Flow System (MCFIA) and the Recommended Procedure (Ref Method) and Corresponding Relative Deviations, Using the Malachite Green Reaction

| | free $SO_2$ | | | total $SO_2$ | | |
|---|---|---|---|---|---|---|
| sample | ref method[a] (mg $L^{-1}$ $SO_2$) | MCFIA[a] (mg $L^{-1}$ $SO_2$) | DR (%) | ref method[a] (mg $L^{-1}$ $SO_2$) | MCFIA[a] (mg $L^{-1}$ $SO_2$) | DR (%) |
| 1 | 22.9 ± 0.9 | 23.3 ± 0.1 | 1.8 | 95.4 ± 0.8 | 88.9 ± 1.0 | −6.8 |
| 2 | 19.9 ± 0.7 | 15.6 ± 0.2 | −21.6 | 74.9 ± 0.8 | 65.6 ± 0.8 | −12.4 |
| 3 | 16.5 ± 0.2 | 17.4 ± 0.4 | 5.4 | 86.3 ± 1.9 | 74.6 ± 0.6 | −13.6 |
| 4 | 18.3 ± 1.6 | 11.7 ± 0.1 | −36.1 | 87.2 ± 2.1 | 77.2 ± 0.1 | −11.5 |
| 5 | 17.2 ± 0.6 | 17.6 ± 0.2 | 2.3 | 104.1 ± 1.4 | 107.0 ± 1.3 | 2.8 |
| 6 | 9.4 ± 0.4 | 9.4 ± 0.1 | 0.0 | 86.3 ± 1.4 | 75.1 ± 0.6 | −13.0 |
| 7 | 16.3 ± 0.8 | 16.9 ± 0.2 | 3.7 | 71.2 ± 1.5 | 58.3 ± 0.7 | −18.1 |
| 8 | 17.8 ± 0.8 | 17.3 ± 0.3 | −2.8 | 104.5 ± 5.8 | 102.1 ± 1.1 | −2.3 |
| 9 | 20.7 ± 0.9 | 16.0 ± 0.1 | −22.7 | 121.3 ± 2.4 | 100.7 ± 0.6 | −17.0 |
| 10 | 16.0 ± 0.9 | 16.9 ± 0.1 | 5.6 | 101.4 ± 1.1 | 88.0 ± 1.9 | −13.2 |
| 11 | 1.5 ± 0.0 | 1.2 ± 0.1 | −20.0 | 53.2 ± 3.2 | 44.6 ± 0.6 | −16.2 |
| 12 | 3.6 ± 0.4 | 3.6 ± 0.0 | 0.0 | 71.2 ± 1.5 | 71.3 ± 0.5 | 0.1 |
| 13 | 6.2 ± 0.0 | 6.0 ± 0.0 | −3.2 | 51.6 ± 1.8 | 49.0 ± 0.3 | −5.0 |
| 14 | 17.6 ± 0.4 | 18.2 ± 0.1 | 3.4 | 80.5 ± 0.8 | 88.8 ± 0.4 | 10.3 |
| 15 | 21.2 ± 0.9 | 22.1 ± 0.1 | 4.2 | 84.4 ± 0.8 | 84.0 ± 1.2 | −0.5 |
| 16 | 14.2 ± 0.4 | 13.6 ± 0.0 | −4.2 | 59.6 ± 1.1 | 54.7 ± 0.2 | −8.2 |
| 17 | 24.0 ± 0.8 | 25.3 ± 0.3 | 5.4 | 146.6 ± 1.9 | 129.7 ± 0.6 | −11.5 |
| 18 | 20.5 ± 0.4 | 20.9 ± 0.1 | 2.0 | 73.3 ± 2.1 | 81.7 ± 0.6 | 11.5 |

[a] Average ± standard deviation of three determinations.

**Table 7.** Parameters of the Equation $C_s = C_0 + SC_r$ for Comparison of the Results (Milligrams per Liter of $SO_2$) Obtained by the Developed Method ($C_s$) and the Recommended Procedure ($C_r$), and Values of the Relative Standard Deviation (RSD) Obtained from 10 Consecutive Analyses of 2 White Wines and 1 Red Wine, Respectively

| reaction | | $C_0$[a] | $S$[a] | $R$[b] | RSD[c] (%) |
|---|---|---|---|---|---|
| malachite green | free $SO_2$ | 0.254 (±3.059) | 0.960 (±0.180) | 0.943 | 1.4 (12.8)<br>1.8 (22.6)<br>1.2 (14.9) |
| | total $SO_2$ | 5.95 (±14.64) | 0.859 (±0.164) | 0.941 | 1.3 (54.3)<br>1.0 (128.6)<br>0.9 (99.0) |
| pararosaniline | free $SO_2$ | 0.255 (±0.776) | 0.981 (±0.036) | 0.998 | 0.6 (14.7)<br>1.4 (27.9)<br>0.8 (18.6) |
| | total $SO_2$ | 5.18 (±6.96) | 0.951 (±0.068) | 0.991 | 1.3 (57.7)<br>1.1 (151.6)<br>1.2 (116.5) |

[a] The values in parentheses are the limits of the 95% confidence intervals for the estimated parameters. [b] Correlation coefficient. [c] The values in parentheses are the tested sample concentrations, expressed in mg $L^{-1}$ of $SO_2$.

results when compared with the recommended procedure. Moreover, as we can observe in **Table 6**, 80% of the total sulfur dioxide results provided by the proposed methodology using the malachite green reaction are lower than those obtained by the recommended method. This clear tendency for negative relative deviation values may be explained by interference of acetaldehyde in the malachite green reaction. Acetaldehyde has a strong affinity for $SO_2$, resulting in the product bisulfite−acetaldehyde, which represents the majority of the total $SO_2$ in wines (*36*). Besides releasing bound $SO_2$, alkaline hydrolysis also promotes acetaldehyde liberation. As described previously, addition of aldehydes to a malachite green decolorized solution causes the color to reappear (*29*). Additionally, low recoveries could also be a consequence of possible recombination of the hydrolyzed $SO_2$ with aldehydes in acidic conditions. To test this hypothesis, standard solutions containing 100 mg $L^{-1}$ of $SO_2$ with the addition of different concentrations of acetaldehyde were analyzed in the proposed flow system employing the analytical cycle of total $SO_2$, for the two reactions evaluated in this work.

Results, depicted in **Figure 2** demonstrate that total $SO_2$ recovery is clearly affected by the presence of acetaldehyde in the malachite green reaction. Contrarily, in the pararosaniline reaction, total $SO_2$ recoveries were not affected by the presence of acetaldehyde. These results allow us to conclude that low recoveries were achieved by the malachite green reaction due to the negative interference of acetaldehyde in the decolorization reaction, whereas the pararosaniline reaction does not seem to be affected by acetaldehyde.

In conclusion, the proposed methodology allowed the determination of free and total $SO_2$ in wine samples by two spectrophotometric reactions without the need to carry out any sample treatment. Better accuracy was achieved with the pararosaniline reaction, probably due to the negative interference of acetaldehyde liberated during hydrolysis in the malachite green decolorization process. This explains the need to carry out offline sample dilution of previous works describing $SO_2$ determination in wines with the malachite green reaction (*23*) as well as the preference for the

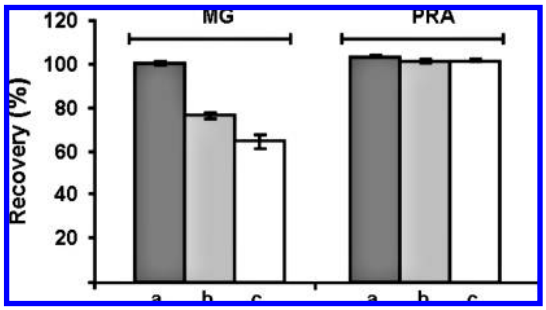


**Figure 2.** Evaluation of acetaldehyde influence on total $SO_2$ determination by the malachite green and pararosaniline reactions, using $SO_2$ 100 mg $L^{-1}$ (a), $SO_2$ 100 mg $L^{-1}$ + acetaldehyde 50 mg $L^{-1}$ (b), and $SO_2$ 100 mg $L^{-1}$ + acetaldehyde 100 mg $L^{-1}$ (c).

pararosaniline reaction among the spectrophotometric methods. The method described herein could be a reliable alternative to be adopted in wineries, because it uses low-cost instrumentation, has high sample throughput, and is easily manipulated. Additionally, in the presented method, reagents are propelled to the flow system when required for the determination, returning to their respective flasks during the rest of the analytical cycle. This feature provides lower reagent consumption and reduction of generated effluents.

**Supporting Information Available:** Analytical features of flow methodologies developed for free and total $SO_2$ determinations in wine samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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