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Multisyringe flow system: determination of sulfur dioxide in wines

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A multisyringe flow system for the spectrophotometric determination of sulfur dioxide in wines is described. The methodology is based on the well-known reaction among SO_2, formaldehyde and pararosaniline. The proposed manifold also includes a gas diffusion unit in order to prevent the colour interference of red wines in the spectrophotometric measurement. The proposed method was successfully applied to the determination of free SO_2 (2–75 mg l^{-1}) and total SO_2 (10–250 mg l^{-1}) in wines, just by changing a few operating parameters in the controlling software. A sampling-rate of 25–30 samples per hour was achieved with good repeatability for 10 consecutive injections of wine samples (RSD < 3.2%). The results obtained from 10 wine samples for each determination were statistically comparable to those obtained by the recommended procedure.

1. Introduction

Since sequential injection (SI), introduction by Růžička and Marshall in 1990,[1] major efforts were devoted to developing devices for driving liquids. The new technique demanded rigorous control of sample and reagent aspiration, flow reversal, flow halting and channel flushing. All of this could be provided by the proposed cam-driven piston pumps.[2]

Subsequently, the performance of peristaltic pumps was compared with that of the sinusoidal flow piston pumps.[3] It was found that the performance of peristaltic pumps met the strict requirements of SI. This was a valuable observation, since the use of peristaltic pumps presents some advantages. First, the analytical cycle is short as there is no need for wash solution aspiration or syringe filling. Further, peristaltic pumps are easy to handle and widely available.[3,4] Nevertheless, peristaltic pumps have the major disadvantage of the short life of the flexible propulsion tubing. The tubes are also vulnerable to moderately concentrated acids or bases and organic solvents.[4,5] To overcome this shortcoming, a low pressure, constant flow rate piston pump was successfully used to propel the flow in SIA.[6]

Recently, the multisyringe has been proposed for propelling liquids in flow systems.[5] This propulsion system opens up new possibilities, combining the multichannel operation of peristaltic pumps with the constant, pulseless and exactly known volume delivery achieved by piston pumps. Moreover, the use of a two-way commutation valve on each syringe introduces flexibility and reagent savings, since any stream can be connected to the system or disconnected from the reagent vessel when required, without interfering with the other channels. However, it has the same disadvantage as for piston pumps that the forward movement must be stopped to reload the syringes, decreasing the sample frequency. The multisyringe has already been successfully applied for single point titration of protolytes, using on-line dilution.[7]

In this work, the multisyringe was applied to wine analysis. The determination of free and total sulfur dioxide was implemented using the well-known reaction among SO_2, formaldehyde and pararosaniline.[8] Several flow systems have been described previously for the same determination, many of them based on the cited reaction.[9–14] As in previous work, a gas diffusion unit was incorporated in the manifold to prevent the colour interference of red wines in the detection system used.[12,13]

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical-reagent grade and used with no further purification, and de-ionized water with a specific conductance < 0.1 μS cm^{-1} was used throughout. The chromogenic reagent was prepared on-line by mixing two solutions, one containing pararosaniline and the other formaldehyde. To prepare the first solution, suitable aliquots were taken from a concentrated pararosaniline solution (20 ml of ethanol containing 0.10 g of pararosaniline hydrochloride diluted with water to 100 ml); the concentrations used varied between 0.01 and 0.50 g l^{-1}. The second solution was prepared by diluting a suitable volume of 37% m/v formaldehyde to attain concentrations between 0.10 and 3.0 g l^{-1}. Concentrated hydrochloric acid was added to both solutions at 0.06 mol l^{-1}.

The carrier solution was 1.2 mol l^{-1} hydrochloric acid, prepared by appropriate dilution of the commercial solution.

A 500 mg l^{-1} stock standard solution of sulfur dioxide was prepared by weighing 0.250 g of Na_2SO_3 and dissolving it in 250 ml of 0.001 mol l^{-1} EDTA solution;[15] the stock standard solution was standardised daily by iodimetric titration. Working standard solutions were prepared daily from the above solution by rigorous dilution with 0.001 mol l^{-1} EDTA solution.

For the determination of free SO_2, the wine samples were inserted into the system without prior treatment. For the determination of total SO_2, release of the bound SO_2 was required and was carried out according to the rapid assay

method recommended by the Office International de la Vigne et du Vin (OIV).[16] Therefore, 10.0 ml of wine was previously alkalinised with 1.6 ml of 4 mol l^{-1} NaOH solution before introduction into the system.

2.2. Apparatus

The multisyringe (Crison Instruments, Alella, Spain) has been depicted schematically elsewhere.[7] It is a multiple channel piston pump, driven by a single motor of a common automatic burette and controlled by computer software through a serial port. It was equipped with four syringes with different volumes: 5 ml in position 1, 2.5 ml in positions 2 and 3 and 1 ml in position 4 (Fig. 1). A two-way commutation valve (N-Research, Caldwell, NJ, USA) was connected to the head of each syringe; two extra commutation valves were included in the module used. For all valves, the exchange options were classified in off/on lines. The 'off' line was assigned to the vessels (reagents, carriers or waste) and the 'on' line was reserved for the manifold direction.[7]

An IBM compatible PC was used to control the multisyringe, including the two independent two-way commutation valves; it also performed data acquisition *via* the software package AUTOANALYSIS[17] [the software can be obtained on request from SCIWARE Bank of Programs, Association of Environmental Sciences and Techniques (AEST), Department of Chemistry, Universitat de les Illes Balears, E-07071 Palma de Mallorca, Spain].

A laboratory-made gas diffusion unit (GDU) was also incorporated in the manifold.[18] It consisted of two Perspex blocks, pressed against each other by four screws. The matching cavities drilled in each block were 2 mm wide, 0.5 mm deep and 7 cm long (linear path). A hydrophobic membrane (Millipore, Bedford, MA, USA, ref. GVHP, pore size 0.22 μm) was placed between the two blocks, being replaced weekly.

Absorbance measurements were carried out at 580 nm, using an Ocean Optics (Dunedin, FL, USA), PC 1000 spectrophotometer connected to a 200 μm fibre optic cable and a deuterium and halogen light source from Top Sensor Systems (Eerbeek, The Netherlands). Facing the fibre optic, a Hellma (Müllheim/Baden, Germany) 178.711-QS flow-through cell (30 μl inner volume, 1 cm optical path) was placed in a Ocean Optics CUV-ALL-UV cell support. The detection system was connected to the computer *via* an HPIB interface and was also controlled by AUTOANALYSIS.

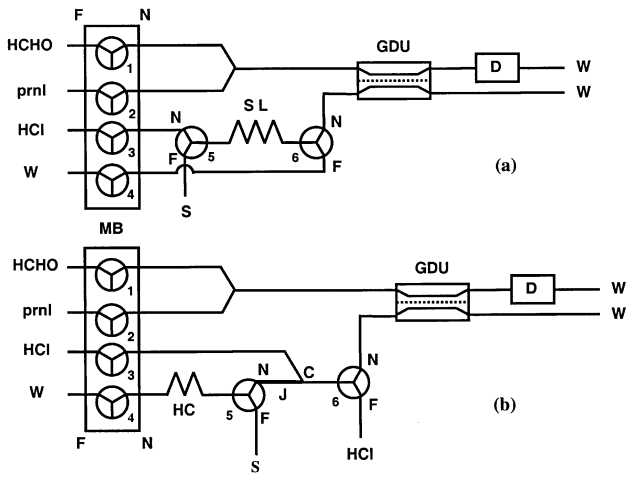

Fig. 1 (a) Manifold initially used for the determination of SO_2 in wines. (b) Final manifold for the determination of free and total SO_2 in wines. MB = multisyringe; GDU = gas diffusion unit; D = detection system; SL = sample loop; HC = holding coil; C = confluence; J = tubing filled with sample after the determination cycle; S = sample or standard; W = waste; N = on position; F = off position; HCHO = formaldehyde solution; prnl = pararosaniline solution; HCl = hydrochloric acid solution.

2.3. Manifold

Manifolds were made from Omnifit (Cambridge, UK) PTFE tubing (0.8 mm id) with Gilson (Villiers-le-Bel, France) end-fittings and connectors. Perspex Y-shaped joints were used as confluences. The system components were disposed as shown schematically in Fig. 1.

The connection between the confluence and the acceptor channel of the GDU was 7 cm long. As valves 5 and 6 were fixed, the minimum sample loop length in the first manifold [Fig. 1(a)] was 25 cm. In the first manifold, the connections between the multisyringe and the valves 5 and 6 were both 20 cm long, as was the connection between valve 6 and the donor channel of the GDU. In the second manifold [Fig. 1(b)], the holding coil length was 100 cm. The total connection length between valves 5 and 6 was 20 cm; the tubing length between valve 5 and confluence C was 10 cm. Other connections remained the same as in the first manifold.

2.4. Procedure

Preliminary experiments were performed in the manifold shown in Fig. 1(a), where the two additional commutation valves were connected in such a way that they contained a sample loop, replacing the injection valve. Initially, the commutation valves 1 and 2 were in the on position; the other valves were in the off position. The formaldehyde placed in syringe 1 and the pararosaniline placed in syringe 2 were then driven until the confluence, where they were mixed, forming the chromogenic reagent. This mixture was further propelled, filling the acceptor channel of the GDU. Subsequently, valves 3, 5 and 6 were changed to the on position and syringe 3 dispensed the carrier through the previously filled sample loop. The sample was propelled through the donor channel of the GDU towards the waste; the SO_2 present in the sample passed across the membrane, combining with the reagent placed on the acceptor channel. The reaction product formed was then propelled to the detector where the absorbance was measured at 580 nm, while the donor channel was washed with carrier. The syringes and sample loop loading were performed simultaneously, with all valves in the off position, except valve 4. However, this configuration presented some problems. First, the sample volume was determined by the sample loop length. On the other hand, to provide efficient passage of the SO_2 across the membrane, a suitable mixture of the sample and HCl was required.[15] Therefore, the connections of valves 5 and 6 were changed and a confluence was introduced in the connection between them.

Using this new configuration [Fig. 1(b)], the determination of free and total SO_2 in wines was performed according to the protocol sequence given in Table 1.

With this new configuration, the operations were similar to those described previously. However, the sample was aspirated to a holding coil placed between syringe 4 and valve 5. At a suitable time, the sample was introduced into the system and mixed with the carrier in confluence C. By controlling the time that valve 4 was in the on position, it was also possible to inject different sample volumes into the system. The ratio between sample and carrier was 1:2.5, accounting for the syringe volumes. After the operations described previously (SO_2 diffusion across the membrane, product detection and donor channel washing), the connection between valves 5 and 6 and the holding coil were still filled with sample. To rinse them, the carrier was aspirated through valves 5 an 6 (on and off, positions respectively). After this, the sample was aspirated as described

above, and the system was ready for a new determination cycle.

3. Results and discussion

3.1. Development of the multisyringe flow system

The following studies were performed using the configuration depicted in Fig. 1(b). After an initial assessment to select approximate values for each parameter, optimisation of the variables was carried out by the univariate method. The chemical and system related parameters studied are given in Table 2, and also the value range studied and the values chosen. The results obtained are discussed below.

The reagent volumes were set to 100 μl of formaldehyde solution and 50 μl of pararosaniline solution; both solutions contained HCl at the same concentration. The concentrations of the three reagents were studied by establishing calibration curves using the conditions for the determination of total SO_2 (Table 1) and standards with concentrations between 25 and 300 mg l^{-1}.

The HCl concentration was studied with the formaldehyde and the pararosaniline concentration set to 0.25 and 0.10 g l^{-1}, respectively. The sensitivity achieved was the same until 0.06 mol l^{-1}; at higher concentrations, the sensitivity diminished.

The influence of the pararosaniline concentration was evaluated keeping the HCl concentration at 0.06 mol l^{-1} and the formaldehyde concentration at 0.25 g l^{-1}. As the pararosaniline concentration was increased, the sensitivity increased, and also the blank signal. Hence the chosen concentration of 0.10 g l^{-1} was a compromise between these two effects.

The formaldehyde concentration was studied while the pararosaniline and HCl concentrations were maintained at 0.10 g l^{-1} and 0.06 mol l^{-1}, respectively. The results obtained indicated a significant increase in sensitivity until 2.0 g l^{-1}, accompanied by an increase in the blank signal.

With the chromogenic reagent composition defined, some system parameters were studied. The sample volume was studied under the conditions described previously, except for the donor stream flow rate, which was changed to 2.2 ml min^{-1}. Values between 100 and 300 μl were tested; for a 100 μl volume it was not possible to establish a calibration curve since the absorbance values were constant for the concentration range studied. The sensitivity increased with increase in the sample volume and the detection limit decreased. For instance, the 5 mg l^{-1} standard gave absorbance values different from the blank signal when the sample volume used was 300 μl. For 200 and 250 μl volumes, the 10 mg l^{-1} solution was the first standard to give a signal above the blank. The donor stream flow rate was the second variable studied as it restricted the contact time between the sample and gas diffusion membrane. Three different flow rates were used; for the higher values tested, a waiting period was also added before sending the reaction product towards the detector. In this way the time between sample passage and product detection was the same (70 s) for the three flow rates. The results are presented in Fig. 2. As established in previous studies,[19] the results indicated that an increase in donor flow rate decreased the peak height response, probably owing to a shorter contact time between the sample and the gas diffusion membrane. However, the sensitivity increased at the higher flow rates when the waiting period was added. In this way, when the contact time was the same, the sensitivity was increased by higher flow rates, associated with a stop period in the donor stream flow.

As the previous results indicated a significant influence of the stop period in the donor stream flow after the sample passage through the GDU, this variable was studied. The conditions used were as stated above, except for the flow rate (1.2 ml min^{-1}); two different sample volumes were used (200 and 250 μl), aiming at two different concentration ranges. The chosen conditions for the determination of free SO_2 were a 250 μl sample volume and a stop period of 20 s, as a compromise between sensitivity and sampling rate. For the determination of total SO_2, the chosen sample volume was 200 μl and the stop period was 5 s, as an increase in this value did not cause an increase in sensitivity. Finally, the hydrochloric acid concentration in the carrier was studied. This parameter is important since the sample must be acidified to promote the formation of gaseous SO_2 and its passage across the permeable membrane.[15] The study comprised the establishment of calibration curves, using the conditions for the determination of total

Table 2 Range of values used in the study of system variables and chosen conditions for operation

Parameter	Range	Chosen value
HCl concentration in chromogenic reagent/mol l^{-1}	0.01–0.30	0.06
Pararosaniline concentration/g l^{-1}	0.01–0.50	0.10
Formaldehyde concentration/g l^{-1}	0.10–3.0	2.0
Flow rate at GDU donor channel/ ml min^{-1}	0.6–2.2	1.2
Sample volume/μl	100–300	200/250[a]
Stop period after passage through GDU donor channel/s	0–30	5/20[a]
HCl concentration in the carrier/ mol l^{-1}	0.06–1.20	1.20

[a] Parameters with different values for determination of total and free SO_2, respectively.

Table 1 Protocol sequence for the determination of SO_2 in wines. The indicated values for flow rate and volume are referred to syringe 1. N and F represent on and off position, respectively

	Position of the commutation valves							
Description	1	2	3	4	5	6	Volume/μl	Time/s
Piston adjustment	F	F	F	F	F	F	1800	3.0
Placement of the chromogenic reagent in the acceptor channel of the GDU	N	N	F	F	F	F	100	4.3
Sample passage through the donor channel of the GDU, with previous HCl addition in the confluence	F	F	N	N	N	N	1000/1250[a]	34.7/43.4[a]
Stop period, during which sample was in contact with the GD membrane							—	5/20
Propulsion of the reaction product towards the detector and simultaneous wash of the donor channel	N	F	N	F	F	N	2000	55.6
HCl aspiration for washing the HC and J tubing	F	F	F	N	N	F	1200	2.0
Sample/standard aspiration to the HC for a new determination cycle	F	F	F	N	F	F	3700	12.6

[a] Parameters with different values for determination of total and free SO_2, respectively.

SO_2, and the injection of wine samples, previously digested as indicated above. For HCl concentrations < 0.3 mol l^{-1}, the wine signal was lower than the blank signal; for concentrations > 0.6 mol l^{-1}, the wine samples produced signals which gave concentration values comparable to those from the reference method. To ensure sample acidification, the HCl concentration used was 1.2 mol l^{-1}.

The detection limit was calculated as the concentration corresponding to the blank signal plus three times the standard deviation of 10 consecutive blank injections. The blank signal was obtained by injecting solutions with the same composition as the standards, except for the sulfur dioxide. For the free SO_2 determination, the calculated detection limit was 1.0 mg l^{-1}; for the total SO_2 determination, the detection limit was 5.6 mg l^{-1}.

The sample frequency was different for each determination: 25 determinations per hour for free SO_2 and 30 determinations per hour for total SO_2.

3.2. Application to wine samples

The proposed system allowed the determination of free and total sulfur dioxide in wines by changing the parameters introduced in the controlling software. To accomplish this, two second-order calibration curves were established, defining two concentration ranges, one for each determination. The concentration of standards varied between 2 and 75 mg l^{-1} and between 10 and 250 mg l^{-1}, respectively. For the free SO_2 determination, the sample was introduced directly into the system; for the total SO_2 determination, the sample was introduced after previous hydrolysis of bound SO_2 with an alkaline solution.

The proposed system was applied to the determination of free and total sulfur dioxide in 15 table wines. The results (C_p) were compared with those furnished by the recommended procedure[16] (C_r) and are presented in Table 3.

For comparison purposes, a linear relationship ($C_p = C_0 + SC_r$) was established. The equation parameters and the 95% confidence limits are presented in Table 4. From these figures it is clear that the estimated slope and intercept do not differ significantly from 1 and 0, respectively. Hence, there is no evidence for systematic differences between the two sets of results[20] obtained by the proposed methodology and the recommended procedure, for both determinations.

The precision of the developed methodology was estimated by calculating the relative standard deviation from 10 consecutive injections of wine samples. The relative standard deviations were $< 2.1\%$ for the determination of total SO_2 and $< 3.2\%$ for the determination of free SO_2.

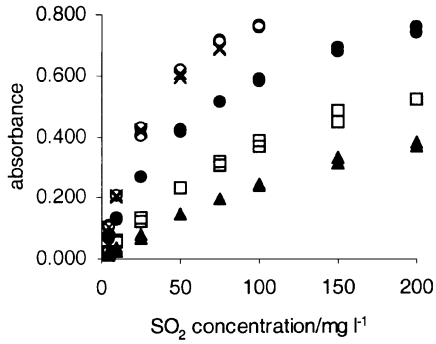

Fig. 2 Results obtained from the study of the flow rate in the gas diffusion unit donor stream. The following values in parentheses are flow rate/ml min^{-1} and stop period/s: ● (0.60; 0); □ (1.2; 0); ▲ (2.2; 0); ○ (2.2; 51), × (1.2; 35).

4. Conclusion

The proposed system allowed the determination of free and total SO_2 in wines. The inclusion of the multisyringe makes it suitable for continuous process control, as it is robust and requires little maintenance. Hence its application during wine production is recommended, as SO_2 determinations are performed during the must fermentation. On the other hand, SO_2 concentration limits are legally imposed in several countries and the free SO_2 levels must be adjusted before bottling, so a large number of determinations are also carried out in the final product. The proposed system is also suitable for quality control in wines, with a higher sample frequency and lower reagent consumption compared with the recommended procedure.

The multisyringe has other advantages such as a propulsion system that can be applied to any flow system. First, it has a driving capability equivalent to four automatic piston pumps working simultaneously. This arises because all syringe pistons are driven by the same motor, moving at the same time in the same direction. However, this simultaneous movement of the pistons does not imply the driving of liquids from all the syringes into the system. As each syringe is equipped with a two-way commutation valve, it is possible to propel the liquid inside the syringe into the manifold or to send it towards the waste or back to its own vessel, saving reagents.

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Table 3 Results (mg l^{-1}) obtained by the proposed methodology (C_p) and by the recommended procedure (C_r) for the determination of free and total SO_2

Total SO_2		Free SO_2	
C_r	C_p	C_r	C_p
79.3	80.4	11.8	11.1
128.8	124.1	9.4	10.5
154.7	144.7	25.7	24.4
91.7	82.2	9.4	8.2
79.1	76.2	7.5	7.1
63.3	56.8	12.4	14.1
87.3	87.8	40.0	38.4
108.9	101.1	4.3	4.5
63.3	53.7	4.8	5.7
117.7	111.3	6.3	7.6
127.9	119.2	28.9	28.0
50.7	52.9	23.7	21.8
53.4	48.8	23.8	25.6
102.5	93.3	18.2	19.3
150.3	151.5	23.7	24.3

Table 4 Parameters of the equation $C_p = C_0 + SC_r$ for comparing the results (mg l^{-1}) obtained by the proposed methodology (C_p) and by the recommended procedure (C_r)

	C_0	S	R^a
Free SO_2	0.909 (±1.257)[b]	0.948 (±0.064)[b]	0.994
Total SO_2	−2.110 (±7.852)[b]	0.970 (±0.077)[b]	0.991

[a] Correlation coefficient. [b] The values in parentheses are the limits of the 95% confidence intervals.

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