Spectrophotometric Flow-Injection Determination of Sulphite in White Wines Involving Gas Diffusion through a Concentric Tubular Membrane

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A flow-injection system is proposed for the spectrophotometric determination of sulphite in white wines. The method involves analyte conversion to SO₂, gas diffusion through a Teflon® semi-permeable membrane, collection into an alkaline stream (pH 8), reaction with Malachite green (MG) and monitoring at 620 nm. With a concentric tubular membrane, the system design was simplified. Influence of reagent concentrations, pH of donor and acceptor streams, temperature, timing, surfactant addition and presence of potential interfering species of the wine matrix were investigated. A pronounced (ca. 100%) enhancement in sensitivity was noted by adding cetylpyridinium chloride (CPC). The proposed system is robust and baseline drift is not observed during 4 h operating periods. Only 400 µL of sample and 0.32 mg MG are required per determination. The system handles 30 samples per hour, yielding precise results (r.s.d. < 0.015 for 1.0 - 20.0 mg L⁻¹ SO₂) in agreement with those obtained by an alternative procedure.

Keywords: concentric tubular membrane, sulfite, spectrophotometric flow analysis, micellar medium, gas diffusion

Introduction

Gas diffusion is a very effective means to enhance selectivity in analytical chemistry, and was implemented in a flow-injection system in the pioneer work dealing with the development of an improved procedure for determination of total CO₂ in plasma.¹ Since then, the strategy has been often exploited, as it is efficiently accomplished in flow systems.²,³ Generally, a gas-permeable membrane is placed between two liquid streams, one of them - the donor stream - containing the sample under processing. The analyte is converted to a volatile chemical species that is removed from the sample matrix and collected by other stream - the acceptor stream.

Sulphite is an important preservative in the food and beverage industry, especially with regard to wine production⁴-⁸ and its content should be strictly controlled in view of its potential toxicity.⁶-⁸ The maximum allowable concentrations of the sulphite species in foodstuffs are defined in specific legislation, in accordance to the type of wine and the sugar contents.⁸

For the determination of sulphite in wines, addition of citric acid to the processed sample is normally carried out...
and the released SO₂ is collected by an alkaline solution. In flow-injection analysis, the step of gas separation takes place only at a defined site of the manifold whereas the other involved processes take place in a continuous manner along the entire analytical path. The drawback can be circumvented by taking advantage of the concentric tubular reactor, whose potentialities and limitations were recently discussed. With this tube-in-tube configuration, the gas diffuses through the wall of the inner tube towards the external tube, and the process becomes continuous. The approach was applied to the determination of free chlorine in natural waters, dissolved inorganic carbon in river waters and ethanol in spirits. Its usefulness for monitoring purposes was also demonstrated.

The aim of this work was then to develop a flow-injection system for the spectrophotometric determination of sulphite in white wines, using a tubular membrane for in-line gas diffusion. The method is based on AOAC and Malachite green is the main reagent. Considering that a micellar medium is interesting for modifying the analyte/reagent interactions, thus improving sensitivity and/or selectivity, the establishment of an organised medium was also investigated.

**Experimental**

**Reagents, standards, samples**

All solutions were prepared with analytical-reagent quality chemicals and freshly distilled/deionised water. The 1000 mg L⁻¹ sulphite stock solution was weekly prepared by dissolving 0.197 g Na₂SO₃ in 100 mL of water. Working standard solutions (0.0 - 20.0 mg L⁻¹ SO₂) were freshly prepared by diluting the above stock with a solution containing the chemical species commonly present as macro-constituents of most of the commercially available wines (wine matrix solution): 0.7 % m/v citric acid, 0.3 % m/v sucrose, 0.2 % m/v glycerol, 0.4 % m/v tartaric acid, 2.2 x 10⁻³ mol L⁻¹ phosphoric acid and 10 % (v/v) ethanol. The sample carrier stream (R₁ - Figure 1) was a 0.5 mol L⁻¹ citric acid solution. Reagent R₂ was a 0.3 mol L⁻¹ phosphate plus 1.0 x 10⁻³ mol L⁻¹ cetylpyridine chloride (CPC) solution that was prepared by dissolving 26.1 g K₂HPO₄ plus 1.8 g CPC in about 450 mL of water, adjusting the pH to 8.0 ± 0.1 with 0.3 mol L⁻¹ phosphoric acid, and filling the volume up to 500 mL with water. The reagent R₃ was stored under 5 °C and equilibrated to room temperature immediately before use.

The Malachite green (MG) stock reagent was prepared by dissolving 20 mg of the dye [oxalate form, C₄₉H₅₀N₄O₄.2C₂H₂O₄, M = 927.02 g mol L⁻¹] plus 0.85 g KH₂PO₄ in 100 mL of water, letting it to stand overnight and filtering through a 0.45 µm cellulose membrane. This stock reagent was stable for at least one month if stored at 5 °C. For preparation of the MG working reagent (R₃ - Figure 1), a 30-fold water dilution of the above stock was performed.

White wine samples were purchased from a local supermarket and analysed without any prior treatment.

**Apparatus**

The flow system comprised a model 432 Femto spectrophotometer with a tubular flow cell (ca. 18-mm optical path, 200 µL illuminated volume) connected to a model 111 Kipp & Zonen strip-chart recorder, a model IPC-8 Ismatec peristaltic pump, a model 100 Fanem thermostat water-bath and accessories. The injector was similar to that used in earlier work.

The sampling loop, reactors and transmission lines were build-up with i.d. 0.7 mm polyethylene tubing of the non-collapsible wall type. For transferring the gaseous species from the donor to the acceptor stream, a concentric tubing reactor similar to the model TB-21-05 Sumitome Electric was used. It comprised an inner tubular Teflon membrane (i.d. = 1.0 mm, wall thickness = 0.5 mm) inside a polyethylene tube (i.d. = 4.0 mm, wall thickness = 1.0 mm). Connection was done as in earlier work.

**Flow diagram**

The flow system for sulphite determination in white

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**Figure 1.** Flow diagram. S = sample injected by means of a 80 cm (ca.. 400 µL) sampling loop; R₁ = 0.5 mol L⁻¹ citric acid (0.8 mL min⁻¹); R₂ = 0.3 mol L⁻¹ phosphate buffer, pH 8.0 (0.8 mL min⁻¹); R₃ = 6.67 mg L⁻¹ MG (1.6 mL min⁻¹); R₉ = concentric tubing reactor (15 cm); B₁ and B₂ = transmission lines (30 cm); B₃ = cooling reactor (50 cm); D = detector (620 nm). Dashed line = components inside the water-bath (35 °C). For details, see text.
wines is outlined in Figure 1 that indicates the injector in the sampling position. The sample is aspirated to fill the 80 cm sampling loop, and the selected volume (400 µL) is further intercalated into the sample carrier stream (R₁, 0.8 mL min⁻¹ citric acid). With this design, the sample plug is not inserted into a chemically inert carrier stream, and the Schlieren effect does not manifest itself because the stream is not directed towards detection. Moreover, citric acid is in large excess and sample/carrier mixing is not critical. Thereafter, the processed sample flows through the inner tube of the concentric tubing reactor Rₚ towards waste. During passage through Rₚ, the released SO₂ diffuses through the semi-permeable tubular Teflon® membrane and is collected into the acceptor stream that flows through the external tube of the concentric tubing reactor. The acceptor stream is continuously formed by the confluence of R₂ (0.8 mL min⁻¹) and R₃ (1.6 mL min⁻¹) reagents. The SO₂ collection induces the MG discolouring reaction to proceed inside Rₚ. Thereafter, the sample zone is directed towards the flow cell and its passage through it results in a transient absorbance lowering recorded as an inverted peak with height proportional to the sulphite concentration in the injectate.

The concentric tubing reactor was immersed into the thermostatic water-bath, and the transmission lines B₁ and B₂ were selected as short as possible (30 cm). Reactor B₃ was kept under ambient conditions and its length (50 cm) was enough to provide good cooling conditions.

Procedure

Conditions for MG discoloration were investigated by injecting 0.0 - 20.0 mg L⁻¹ SO₂ solutions into the flow-injection system shown in Figure 1. System optimisation was based on the univariate approach and the figure of merit was the recorded peak height after adjusting baseline absorbance to about 0.9. Repeatability was always checked after triplicate injections.

Influence of acidity of the donor stream was studied by varying the citric acid concentration (0.3 - 0.8 mol L⁻¹) in the sample carrier stream, and the pH of the acceptor stream was studied within 6.0 and 12.0 by adjusting the amounts of the buffer constituents.

Flow rates were individually studied within 0.4 and 1.8 mL min⁻¹. Direct and reverse flows for the donor and acceptor streams were tested. In these experiments, different Rₚ lengths (7.5, 15.0 and 30.0 cm) were used. In addition, the rotation speed of the peristaltic pump was varied within 50 and 200% of the nominal speed (corresponding to flow rates in Figure 1).

Influence of temperature was verified by varying the temperature of the water bath into which Rₚ, B₁ and B₂ were immersed (20 - 60 °C).

The feasibility of sensitivity enhancement through the establishment of a micellar medium was investigated by adding the cetylpyridinium chloride (CPC), or dodecyltrimethyl ammonium bromide (DTAB) surfactants at different concentrations (1.0 x 10⁻⁴ - 1.0 x 10⁻¹ mol L⁻¹) to reagent R₃.

After system dimensioning, the main analytical characteristics were evaluated. Repeatability was estimated in terms of relative standard deviations of results obtained after ten-fold processing of typical white wine samples, and accuracy was checked by running samples already analysed by an alternative flow-injection procedure.

Results and Discussion

Concentration of citric acid in the sample carrier stream is of paramount relevance as it influences the acidity for sulphite liberation. Loss of sensitivity was observed for concentrations within 0.1 and 0.3 mol L⁻¹, and no analytical signal was recorded in the extreme situations of citric acid concentrations < 0.1 mol L⁻¹. A pH value of about 2 is recommended, and this was attained with 0.5 mol L⁻¹ citric acid in Rₚ.

Regarding alkalinity of the acceptor stream, R₂ + R₃, experiments were carried out under different conditions (6 < pH < 12) and the results are shown in Figure 2. Analysis of this figure reveals that this was also a very important parameter in the system design and that better SO₂ collection was accomplished under pH 8. This matches the pH value of 8.8 recommended by Sullivan and was attained with 0.3 mol L⁻¹ K₂HPO₄ in the acceptor stream.

The Malachite green concentration also manifested itself as a very important parameter, as it defines the temperature of the water bath into which Rₚ, B₁ and B₂ were immersed (20 - 60 °C).

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![Figure 2. Influence of alkalinity of the acceptor stream. Figure refers to 8.0 mg l⁻¹ SO₂ injected into the system of Figure 1. The pH value corresponds to the solution leaving the flow cell. ∆Abs = transient negative variation in absorbance.](image-url)
baseline. Improved measurement conditions were attained for 6.67 µg L⁻¹ MG that means a 30-fold water dilution of the MG reagent stock. The corresponding baseline absorbance was then about 0.9.

Regarding the concentric tubing reactor, similar results were obtained for tube lengths of 15 and 30 cm, although a 20% deterioration in sampling rate was noted when using the longer reactor. Increasing the reactor length only slightly increased the recorded peak height because of the simultaneous modifications in available time for development of the physicochemical processes and in sample dilution. As losses in sensitivity (40 - 50%) were noted for a too short (7.5 cm) reactor, the length of the concentric tubing reactor was set as 15 cm.

Individual variations in flow rates of the donor and acceptor stream showed a tendency similar to that theoretically expected. In addition, increasing the rotation speed of the peristaltic pump led to a lowering in analytical signal due to the reduction of the mean sample resident time inside Rc. The increase in sensitivity with the decrease in rotation speed followed an asynthetic behaviour. The rotation speed however could not be reduced indefinitely in view of the compromise between sampling rate and sensitivity. Moreover, the sample processing time could not be increased at will, because only the determination of the labile form of the sulfite is aimed. The flow rates specified in Figure 1 were then selected to provide a sample resident time inside the main reactor similar to that recommended by AOAC. With these selected flow rates, the linear speed of donor and acceptor streams were about the same. Finally, it should be reported that only slight modifications in sensitivity were noted when the direction of the acceptor stream was inverted.

Temperature of the water bath should also be taken into account for system design, as a 30% enhancement in sensitivity was verified when it was raised from 20 to 35 °C (Table 1). For T > 45 °C, air bubbles in the analytical path were eventually noted, leading to a deterioration in repeatability and to a baseline drift. The temperature was then set as 35 °C.

The establishment of a micellar medium proved to be relevant to enhance sensitivity (Table 2). Best results were obtained when the cationic surfactants dodecyltrimethyl-ammoniumbromide (DTAB) or CPC were added with enhancements in analytical signal of up to 100%. Although any of these surfactants could be used, CPC was selected in view of the more pronounced influence in sensitivity. When it was added to all reagents, problems with base line were observed. Therefore, CPC was added only to R₂ reagent and a stable baseline was noted. With the selected concentration of 0.01 mol L⁻¹ CPC, there as a guarantee that the critical micellar concentration inside the inner tube of the concentric reactor was surpassed. At this point, it should be stressed that addition of anionic or non-ionic surfactants could not be recommended. In fact, when the anionic surfactant sodium dodecylsulphate, SDS, was added (0.001 mol L⁻¹), losses in analytical signal of about 90% were noted whereas concentrations beyond 0.01 mol L⁻¹ led to a suppression of the analytical signal. The non-ionic surfactant Triton X-100 presented similar characteristics as the SDS, yet less pronounced. With Triton X-100 at concentrations > 1.0% air bubbles were formed inside the analytical path, impairing the measurement.

Table 2. Influence of surfactant concentration. Concentration refers to reagent R₂ (Figure 1) and expressed in % v/v (Triton X-100) or mol L⁻¹ (DTAB and CPC). Variations in analytical signal expressed in % of the signal obtained without surfactant

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Concentration</th>
<th>Variation in analytical signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton X-100</td>
<td>0.02</td>
<td>-95</td>
</tr>
<tr>
<td>0.2</td>
<td>-77</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>DTAB</td>
<td>0.001</td>
<td>100</td>
</tr>
<tr>
<td>0.01</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>CPC</td>
<td>0.001</td>
<td>90</td>
</tr>
<tr>
<td>0.01</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

* = loss in repeatability (r.s.d. > 0.2).

The macro constituents usually present in matrix wine plays an important role in system performance. When the matrix concentration was halved or doubled, positive or negative variations in peak height (about 20%) were observed. It is interesting to report that these variations were not caused by modifications in the ethanol content. Therefore, it was decided to add the wine matrix to all working standard solutions. With this strategy, variations in sample matrix did not interfere when the concentration of the main components were modified by + 50 or - 50%. This confidence interval is compatible with the natural variation expected in the white wine samples.

<table>
<thead>
<tr>
<th>Temperature/°C</th>
<th>Baseline 6.0 mg L⁻¹</th>
<th>20.0 mg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.730</td>
<td>0.512</td>
</tr>
<tr>
<td>25</td>
<td>0.711</td>
<td>0.408</td>
</tr>
<tr>
<td>30</td>
<td>0.640</td>
<td>0.271</td>
</tr>
<tr>
<td>40</td>
<td>0.602</td>
<td>0.248</td>
</tr>
<tr>
<td>50</td>
<td>0.562</td>
<td>0.231</td>
</tr>
<tr>
<td>60</td>
<td>0.485</td>
<td>0.190</td>
</tr>
</tbody>
</table>
Conclusions

The proposed system is very robust and stable and yields precise results, the relative standard deviations of results being usually < 0.03. For a typical white wine with 12.3 mg L\(^{-1}\), it was estimated as 0.015 after eleven successive replications. With the concentric tube reactor, measurement repeatability was superior in relation to the system with a planar membrane. Also, an important characteristic of the cylindrical membrane separator when compared with the laminar membrane is that the cylindrical shape improves mixing conditions, thus avoiding the need for a long mixing coil between the diffusion unit and the detector. After using the system for several weeks, it was realised that variations in hydrodynamic pressure affects the tubular Teflon\(^\circ\) membrane in a lesser extent that it does in relation to the planar Teflon\(^\circ\) membrane. Moreover, the probability of leaking is lower relatively to other alternatives already proposed for gas diffusion in flow analysis\(^{19}\) that usually require several screws and include an inner chamber that should be eventually opened and closed. The approach is then attractive to be used in combination with distillation, pervaporation and related techniques.

With an organised reaction medium, sensitivity is improved without deteriorating the analytical characteristics of the flow-based procedure.

The analytical curve is linear within the 1.0 – 20 mg L\(^{-1}\) SO\(_2\) range \((r > 0.999; n = 6)\). About 30 samples are run per hour meaning 0.32 mg MG per determination. Detection limit was estimated as 0.4 based on the 3\(\sigma\) above the mean level for a field blank \((\sigma\text{ Blank} = \text{estimated standard deviation of the field blanks})\).\(^{20}\)

The proposed flow system is not directly applicable to red wines.\(^{8}\) Analysis of Table 3 permits one to conclude that fairly accurate results are obtained. Finally, it should be stressed that the system has been applied to routine analysis during the last four weeks, and no major troubles (or impregnation of the inner tube walls) has been noted.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AOAC</th>
<th>This work</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.29 ± 0.55</td>
<td>15.31 ± 0.31</td>
</tr>
<tr>
<td>2</td>
<td>1.72 ± 0.07</td>
<td>1.74 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.398 ± 0.013</td>
<td>0.399 ± 0.010</td>
</tr>
<tr>
<td>4</td>
<td>17.03 ± 0.25</td>
<td>17.58 ± 0.30</td>
</tr>
</tbody>
</table>

Table 3. Comparative results. Sulfite contents in white wines as determined with the reference, AOAC\(^{5}\) and proposed procedures. Data in mg L\(^{-1}\) SO\(_2\).

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