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Determination of sulphite and acetic acid in foods by gas permeation flow injection analysis

C.S. Tavares Araújo, J. Lira de Carvalho, D. Ribeiro Mota,
C.L. de Araújo, N.M.M. Coelho *

Institute of Chemistry, University of Uberlândia, Av. João Naves de Ávila, Uberlândia 2160-38.408.100, MG, Brazil

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Abstract

Two simple, rapid and sensitive gas-diffusion conductimetric methods were used for the determination of acetic acid and sulphite, separately. For sulphite, the method was based on formation of sulphur dioxide in acid medium ($1.5 \text{ mol L}^{-1} \text{ HCl}$). The sulphur dioxide formed diffuses through an hydrophobic permeable membrane into an acceptor stream (de-ionised water). The change in the conductivity of the de-ionised water was measured and referred to sulphite content in the samples. The method is applicable in the range of $1.0\text{--}50.0 \text{ mg L}^{-1}$ sulphite and the limit of detection is $0.03 \text{ } \mu\text{g mL}^{-1}$. The relative standard deviation for 10 replicate determinations of $10.0 \text{ } \mu\text{g mL}^{-1}$ of sulphite is 0.2%. Sulphite was determined by the method of standard addition. Recovery of sulphite from wine samples ranged from 97.3% to 99.3%. The method was successfully applied to the determination of sulphite in wines and compares well with the standard iodometric method. For acetic acid determination, the method was based on the change of the conductivity of acceptor solution (de-ionised water) when acetic acid from the matrix, diffuses via a permeable membrane into the receptor solution. The increase in conductivity of the de-ionised water was proportional to the acid content. A linear calibration graph in the range of $0.010\text{--}0.100 \text{ mol L}^{-1}$ of acetic acid with a relative standard deviation of 0.8% (0.010 mol L^{-1} acetic acid, $N = 10$) was obtained. Sample throughputs of 120 h^{-1} for sulphite and 80 h^{-1} for acetic acid were achieved. The systems were successfully applied for the assays of sulphite in wines and fruit juices and acetic acid content in vinegar, respectively.

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1. Introduction

Sulphite is used as a preservative in beverages to prevent oxidation and bacterial growth (Pearson, 1976). Because of the potential toxicity of sulphite, its content in foods and beverages should be strictly limited. Hence, its determination is important.

The total sulphite content in wines is the sum of the free and bound sulphite (in certain matrix compounds like acetaldehydes, reducing sugars and phenolic com-

pounds). The free sulphite content in wines provide the enologists valuable information about the fermentation process. Then, enologists prefer information about the free sulphite content rather than the total sulphite content. However, the determination of the free concentration appears to be difficult since the equilibrium between bound and free sulphite is rather labile. Any change in composition of the wine, like dilution, will inevitably shift the equilibrium (Decnop-Weever & Kraak, 1997).

The classical method to determine the total concentration of sulphite in wine and other beverages is by iodometric titration after hydrolysis of the various sulphite complexes and sometimes distillation (AOAC,

* Corresponding author. Tel.: +55 34 3239 4143; fax: +55 34 3239 4208.

E-mail address: nmmcoelho@ufu.br (N.M.M. Coelho).

1990; Ough & Amerine, 1980). However, this is a rather time consuming procedure.

For the determination of free sulphite, the sample is acidified and the resulting sulphurous acid is titrated with standard iodate solution, using starch as an indicator. For the determination of total sulphite, the sample is previously treated with sodium hydroxide to release the bound sulphur dioxide. The results should be interpreted with caution because of the poor stability of the solutions, the possibility of occurrence of parallel reactions and additional difficulties associated with the end point observation, particularly in the case of the red wines.

Other problem concerned with the determination of sulphite originates from the instability of this ion the presence of oxygen. Although various compounds have been proposed as stabilisers for sulphite is difficult to find a stabiliser which is compatible with the chosen analytical method (Lindgreen & Cedergren, 1982).

To achieve a higher sample throughput, several flow injection analysis (FIA) methods have been developed for sulphite determinations (Azevedo, Araki, Toma, & Angnes, 1999; Carvalho, Campo-Dall'orto, Lo-Balbo, & Rezzano, 2003).

Flow systems with gas-diffusion can be used successfully to separate the sulphur dioxide from the acidified matrix. With this proposal, the detection in most often performed by spectrometry after mixing with a colour reagent, such as ρ -rosaniline (Cosano, Luque de Castro, & Valcárcel, 1995) and in micellar medium (Yang, Guo, & Zhao, 2002). These systems are far from ideal, since ρ -rosaniline is carcinogenic and its reaction with sulphur dioxide is rather slow.

Alternatively, spectrophotometric pH-detection (Grudpan, Sritharathikhun, & Jakmunee, 1998) can be used for the determination of sulphite based on the change of the absorbance of an indicator solution when sulphur dioxide, liberated from the matrix diffuses via a permeable membrane into the indicator solution and lo-

cally shifts the pH. Other detection schemes in FIA are based on chemiluminescence (Silva, Dias, & Magalhães, 2001) or on enzymatic reactions (Fatibello-Filho & Vieira, 1997).

But in spite of the reliable methods for the determination of acetic acid are fairly rare realised. Commonly used methods such as high-pressure liquid chromatography (HPLC) and gas chromatography (GC) do not give satisfactory results (Becker, Kittsteiner-Eberle, Luck, & Schimdt, 1993). Other method proposed is based on a pH-value determination; it is, however, severely interfered by CO_2 and other acids (i.e., lactate) present in the sample (Lenghor et al., 2002).

Since the use of toxic or expensive reagents is not required in conductimetry, this could be an attractive detection method for sulphite and acetic acid.

The purpose of this paper is to develop a diffusion conductimetric method for the determination of sulphite and acetic acid, separately. This method which is simple, rapid and sensitive, uses only readily available reagents and instrumentation. The proposed procedure has been applied with good results, to the determination of sulphite in wines and fruit juices and of acetic acid in vinegar.

2. Experimental

2.1. FIA manifold

The FIA manifold for determination of sulphite and acetic acid is schematically shown in Fig. 1. All solutions and the carrier were pumped using a Gilson Model Minipuls 3 peristaltic pump (Villiers-le-Bel, France) and Tygon pumping tubes. The connection and mixing tubes were made from 1.0 mm i.d. PTFE tubing. A proportional injection valve (Bergamin, Reis, Jacintho, & Zagatto, 1980) with a sample loop volume of 200 μL , a straight gas-diffusion cell and a flow through cell connected to a Analyser Model 650 conductivimeter

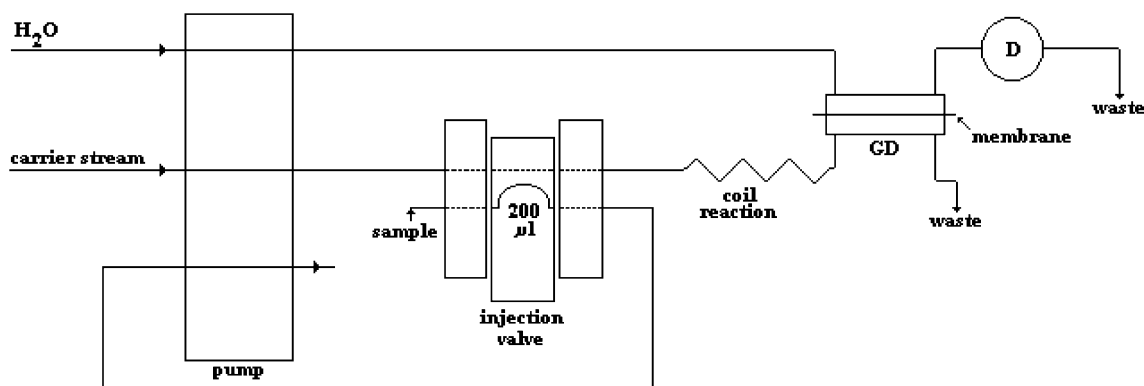


Fig. 1. Schematic diagram of the flow injection system used for determination of gaseous species. GD, gas diffusion; D, detector; Carrier stream: 1.5 mol L^{-1} HCl and flow rate of 2.8 mL min^{-1} for sulphite determination and de-ionised water and flow rate of 1.0 mL min^{-1} for acetic acid determination.

(São Paulo, Brazil) were used and both cells were home made (Pasquini & Faria, 1987). Gas-diffusion cell is constructed of two plexiglass blocks, each with a groove of 10.0 cm length, 4.0 mm width and 0.4 mm depth, with a gas-permeable PTFE membrane of 50 μm thickness clamped between. Each groove has an inlet and an outlet in order to pass the donor and the acceptor streams. In the final set-up a 20 cm \times 1 mm i.d. mixing coil was used between the injection valve and the diffusion module and a 10 cm \times 1 mm i.d. connection tubing was used between the diffusion module and the detection cell. The system consists of two liquid streams, a strong acidic donor solution (1.5 mol L^{-1} HCl) and an acceptor stream containing de-ionised water, separated by a PTFE membrane permeable for only gasses. Some different Teflon membranes were tested in the diffusion unit. The stripe usually employed as stopper in pipes junction (fabricated by Akros – Amanco Brazil S.A., Brazil) was preferred. As this membrane is inexpensive and can be replaced very easily, during this work. Commonly, the same membrane was used for three weeks, without apparent variations. When a sample is injected in the 1.5 mol L^{-1} HCl stream, the liberated sulphur dioxide diffuses through the membrane and permeates into the acceptor solution. The change in the conductivity of the de-ionised water is measured and referred to sulphite content in the sample.

The same configuration manifold as for acetic acid determination was utilised, except with standard/sample and reagent as shown in Fig. 1.

2.2. Chemicals and solutions

All chemicals were of analytical reagent grade and de-ionised water (resistivity 18.0 $\text{M}\Omega\text{ cm}$) was used for prepared solutions. To stabilise the sulphite, solutions were prepared in 10% (v/v) ethanol and de-aerated with nitrogen prior to use. A standard solution with a concentration of approximately 1000 mg L^{-1} was prepared from sodium sulphite (Vetec – São Paulo, Brazil) and titrated with iodine and amperometric detection to determine the exact concentration. Working standard solutions were prepared fresh daily and diluted as appropriate directly before use. Acetic acid, hydrochloric acid and ethanol were obtained from Vetec (São Paulo, Brazil). Standard acetic acid solution (20% m/v) was prepared by weighting 50.00 g of glacial acetic and diluting it to the mark with de-ionised water into a 250 mL volumetric flask.

2.3. Interference

The effect of the presence of anions in the sample was studied by injecting into the conductimetric manifold 10.0 mg L^{-1} standard solutions of sulphite containing from 0.0 to 1000.0 mg L^{-1} of carbonate, phosphate

and acetate solutions, separately. Five replicate measurements were made at each level of potential interference.

2.4. Samples

Bottled wine, fruit juices and vinegar samples were obtained on the local market. For determination of sulphite, the bottles of wines and fruit juices were opened immediately before the procedures and 200 μL of the samples were injected directly in flow injection system without a prior pre-treatment. For determination of acetic acid, the vinegar samples were diluted appropriately with de-ionised water and 200 μL of the samples were injected in flow injection system. In order to determine the reliability of the developed FIA method, recovery studies were investigated. In this case, the red wine and orange juice samples were spiked at concentration of 5.0, 10.0 and 15.0 $\mu\text{g mL}^{-1}$ for sulphite.

The vinegar samples for acetic acid determination and wine samples for sulphite determination were also analysed by standard titrimetric method (AOAC, 1990) for referee purposes.

3. Results and discussion

3.1. Evaluation of the experimental parameters

The principle of the proposed method for sulphite determination is the injected sample in the strong acidic donor solution liberates sulphur dioxide that diffuses through the membrane and dissolves into the acceptor solution. Due to equilibrium ($2\text{H}_2\text{O} + \text{SO}_2 \rightleftharpoons \text{HSO}_3^- + \text{H}_3\text{O}^+$), the conductivity water increases. The detection of sulphite is based on the increase of the conductivity.

In order to find a compromise between sensitivity and sampling frequency, the effects of the flow rate, coil length and injection volume were investigated. The analytical signal for consecutive injections of sulphite 5.0, 10.0 and 20.0 $\mu\text{g mL}^{-1}$ increased proportionally with the injected volume (50, 100, 150 and 200 μL). Higher volumes were not used in this proposed method because, in this case, there is a large time for the analytical signal to return the base line and to maintain the compromise with the analytical frequency, sample volumes of 200 μL were used for further experiments.

The effect of the mixing coil length was also evaluated. A decrease of the signal was observed, when the length was gradually increased from 20 to 120 cm, reflecting a proportional dispersion of the sample zone. In spite of the flow injection system allows a lower reaction coil length led to the highest signal, however the response was very unstable. A good compromise between

Table 1
The FI conditions for sulphite and acetic acid determination

Parameter	Sulphite		Acetic acid	
	Studied range	Selected conditions	Studied range	Selected conditions
Injected volume (μL)	50–200	200	50–200	200
Flow rate (mL min^{-1})	2.3–5.0	2.8	1.0–6.0	1.0
Reaction coil (cm)	20–120	20	10–100	20
HCl concentration (mol L^{-1})	0.5–2.5	2.0	–	–

signal intensity and stability was obtained by adjusting the coil length to 20 cm. Analogously, increasing flow rate values in both channels, varying from 2.3 to 5.0 mL min^{-1} decrease the signal. A best compromise for flow rate of both solutions (donor and acceptor) was obtained at 2.8 mL min^{-1} . Appropriate results were obtained with a flow rate of 2.8 mL min^{-1} , a coil length of 20 cm and an injection volume of 200 μL .

In order to obtain sufficient sensitivity, the concentration of the donor solution was investigated. In this case, the injected sample in the strong acidic donor solution liberates sulphur dioxide that diffuses through the membrane and dissolves into the acceptor solution. The concentration of acid to achieve maximum signal (conversion of sulphite to SO_2) was found to be 2.0 mol L^{-1} . The conductivity increases with HCl concentration. Highest hydrochloric acid concentration are not recommended due problems with the performance membrane.

The pH of wine is about 4.5, at this pH value, the sulphur dioxide is mainly present as hydrogen sulphite and thus liberation of sulphur dioxide is not likely. According Decnop-Weever & Kraak (1997), the ethanol have been proposed as stabilisers for sulphite. In addition, the ethanol content in wines appeared to vary about 10.0–18.0% (v/v) ethanol. On the basis of this assumption, we studied the ethanol effect in the determination of sulphite. The effect of varying the ethanol concentration on the signal analytical from sulphite was evaluated for 2.5%, 5.0%, 10.0%, 15.0% and 20.0% (v/v) ethanol. The signals related to sulphite not change significantly. We decided to add always 10% (v/v) ethanol to all standard solutions.

The performance of gas-diffusion module was not tested. Since gas-diffusion and conductivity meters are a temperature dependent process, the temperature was kept at 21 ± 1 °C.

Determination of acetic acid was based on fact that acetic acid diffuses through the membrane and dissolves into the acceptor solution. Due to equilibrium ($\text{CH}_3\text{COOH} + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{H}_3\text{O}^+$), the conductivity water increases. The detection of acetic acid is based on the increase of the conductivity. The physical configuration of the FIA system was the same as above for determination of sulphite. The donor solution was changed (see Fig. 1). A similar optimisation as for sulphite was carried out for the determination of acetic acid in

a range of 0.010–0.100 mol L^{-1} . The studied ranges and the selected conditions are summarised in Table 1.

3.2. Interference

The effect of carbonate, phosphate and acetate ions on the determination of sulphite was studied. A range of solutions was prepared containing 10.0 mg L^{-1} of sulphite and 10.0, 50.0, 100.0, 250.0 and 1000.0 mg L^{-1} of possible interfering anions. The solutions containing the sulphite sample plus the potential interference anion were analysed by the proposed method. The response was compared with those obtained from an uncontaminated sulphite solution. The results obtained are summarised in Table 2. As expected, carbonate caused an enhancement effect on sulphite signal. This effect is due to evolution of carbon dioxide which changes the conductivity of the de-ionised water.

3.3. Analytical figures of merit

The limit of detection based on the $3\sigma/m$, where σ is the standard deviation of 10 measurements of a blank and m is the slope of the calibration graphs, was 0.03 mg L^{-1} sulphite and the quantification limit ($10\sigma/m$) was 0.10 mg L^{-1} . Under the optimised conditions the injection frequency is about 120 samples h^{-1} . The relative standard deviation of ten replicate determinations of 10.0 $\mu\text{g mL}^{-1}$ of sulphite is 0.2%. The typical mathematical equation of the calibration curve obtained for sulphite standard solutions was $Y = 11.411 [\text{SO}_3^{2-}] + 1.443$, where Y was the analytical signal (conductivity) and $[\text{SO}_3^{2-}]$ expressed as mg L^{-1} . The correlation coefficient was 0.9990. The selected conditions were judged from a good slope and linearity of the calibration graph obtained with a reasonable analysis time.

Table 2
Potential Interference (%) of species on analytical signal

Interferent concentration (mg L^{-1})	CO_3^{2-}	CH_3COO^-	PO_4^{3-}
10	90	5	-15
50	110	10	-10
100	120	15	-15
250	125	30	-15
1000	140	30	-15

Sulphite: 10 mg L^{-1} .

Under the conditions, a linear calibration graph for 0.010–0.100 mol L⁻¹ acetic acid standard solutions was $Y = 7429.4 [\text{CH}_3\text{COOH}] + 81.604$ and $R = 0.9987$, where Y was the analytical signal (conductivity) and $[\text{CH}_3\text{COOH}]$ expressed as mg L⁻¹ and R was the correlation coefficient. Relative standard deviations for 10 replicate determination of 0.010 mol L⁻¹ acetic acid was 0.8%. Sample throughput of 80 h⁻¹ was achieved. For acetic acid determination, the limit of detection and quantification limit were 5.0×10^{-6} and 1.7×10^{-5} mol L⁻¹, respectively.

3.4. Samples

The total sulphite content in wines is the sum of the free and bound sulphite. Then, information about the free sulphite content rather than the total sulphite content is preferred. However, the determination of the free concentration appears to be difficult since the equilibrium between bound and free sulphite is rather labile. Any change in composition of the wine, like dilution, will inevitably shift the equilibrium. In this paper, we decided to focus our research on the determination of the free sulphite content in wine. Attention is given to the determination of sulphite immediately after opening bottles of wine and fruit juices.

Different types of wines (red, white and rose) were chosen in order to evaluate the adequacy of the present method for the analysis of samples with various compositions and colours. After setting the optimised conditions, a systematic determination of sulphite in white and red wines and orange juice samples was carried out using the method of standard additions. Results (Table 3) were ranged from 20.8 to 25.4 and 1.3 to 2.0 mg L⁻¹ for wines and fruit juices, respectively. Recoveries of 97.3–99.9% of sulphite from three samples ($N = 5$), were obtained using the proposed method (Table 4). In this study 5.0, 10.0 and 15.0 µg mL⁻¹ of sulphite were added to each sample. This is good evidence of the accuracy of the method. The results (Table 5) demonstrate also a good accuracy comparable with those obtained using the standard iodometric method (AOAC, 1990). It can be seen from the results that the method can be satisfactorily employed for the determination of sulphite.

Table 3
Sulphite determination in wine and fruit juices samples by proposed method

Samples ^a	Sulphite concentration (µg mL ⁻¹)
Dry white wine (Diamante)	20.8 ± 0.2
Dry red wine (Village)	25.4 ± 0.2
Orange juice (Del Valle)	2.0 ± 0.1
Grape juice (Del Valle)	1.7 ± 0.1
Pineapple juice (Del Valle)	1.3 ± 0.1

^a $N = 5$, confidence level 95%.

Table 4
Experimental recovery on sulphite determination by diffusion–conductivity method in wines and orange juice samples spiked with 5.0, 10.0 and 15.0 µg mL⁻¹ of SO₃²⁻

Sample ^a	Sulphite (µg mL ⁻¹)		Recovery (%)
	Added	Found	
Sweet white wine (Château Duvalier)	5.0	4.9	98.0
	10.0	9.9	99.0
	15.0	14.9	99.3
Dry red wine (Challise)	5.0	4.9	98.0
	10.0	9.7	97.0
	15.0	14.6	97.3
Orange juice (Del Valle)	5.0	4.8	96.0
	10.0	9.9	99.9
	15.0	14.7	98.0

^a $N = 5$, confidence level 95%.

Table 5
Comparison of methods for determination of sulphite in wine samples

Sample ^a	Sulphite (mg L ⁻¹)	
	FIA method	Iodometric method
Sweet red wine (Chapinha)	19.0 ± 0.2	17.1 ± 2.0
Sweet red wine (Sangue de Boi)	21.6 ± 0.2	19.8 ± 2.0
Dry rose wine (Canção)	19.9 ± 0.2	18.5 ± 2.0
Dry white wine (Diamante)	20.8 ± 0.2	19.0 ± 2.0

^a $N = 5$, confidence level 95%.

Table 6
Comparison of methods for determination of acetic acid in vinegar samples

Sample ^a	% Acetic acid (m/v)	
	FIA method	Titrimetric method
White vinegar (Castelo)	4.6 ± 0.2	5.0 ± 0.5
Red vinegar (Castelo)	4.2 ± 0.2	4.9 ± 0.5
Red vinegar (Belmont)	4.4 ± 0.2	4.8 ± 0.5

^a $N = 5$, confidence level 95%.

The optimised procedure was applied to vinegar samples. Comparative method was titration of the acid with sodium hydroxide using phenolphthalein an indicator. The acetic acid contents obtained from this procedure and titrimetry are presented in Table 6. The results from the two methods were not significantly different (judged by t -test at 95% confident interval).

4. Conclusion

The FI procedure proposed here for the determinations of sulphite could be employed as an inexpensive alternative to those procedures using spectrophotometric and/or classical methods of analysis. As can be seen, the proposed method is very favourable for rapid and

precise quantification of sulphite in wines and not needs pre-treatment of the samples, particularly the elimination of the wine colour. The disadvantages of the iodometric method, mainly lack from precision because of its deficient selectivity, somewhat slow reaction, dissolved oxygen interference and difficulties for the evaluation of the end point, especially in case of red wines, apparently disappear when the proposed method is applied. The proposed method requires simple parts which are easy to build, involves only one gas separation procedure and the conductimetric detection is simple. Additional advantages are the relatively low consumption of reagents, the use of an inexpensive Teflon membrane and other low cost and ease to improve components. The proposed methodology can be easily automated and has all the advantages of the FIA methods.

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