



Review

Application of flow injection analysis for determining sulphites in food and beverages: A review

Ruiz-Capillas Claudia *, Jiménez-Colmenero Francisco

Department of Meat and Fish Science and Technology, Instituto del Frío, (CSIC), C/ Jose Antonio Novais, 10, Ciudad Universitaria, 28040 Madrid, Spain

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ABSTRACT

Sulphites have been widely used as preservatives in food and beverages. Preservative determination is essential for the purposes of legislation and consumer safety. Among the analytical methodologies available, those based on flow injection analysis (FIA) can be highlighted. FIA offers interesting advantages such as versatility, accuracy, low cost, speed and automation, among others. These advantages make FIA an important alternative to conventional methods. This review considers the present status of published FIA methodology for determining sulphites in food and beverages. A detailed analysis of the technique is done, stressing its impact on the aspects of the extraction, separation, detection and quantification procedures in different matrices.

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1. Introduction

“Sulphites” or “sulphating agents” are general terms used to describe sulphur-based substances that have been in widespread use for many years in a large variety of food and beverages (fish, potatoes, wine, etc.). As a food additive they include sulphur dioxide, sodium sulphite, sodium and potassium bisulphite, and sodium and potassium metabisulphite, among others (Table 1). Although sulphites have various permitted uses, their primary function is as a preservative or antioxidant to prevent or reduce spoilage (Fazio & Warner, 1990; FDA, 2000; Ruiter & Bergwerff, 2005; Taylor, Hingle, & Bush, 1986; Wood, Foster, Damant, & Key, 2004). Sulphites

are also used because of their food technology effects, they help stabilize product colour and inhibit discolouration, thereby improving the appearance and flavour of many foods during preparation, storage and distribution. Furthermore, other substances such as sodium dithionite may also yield residual sulphite in food products. Therefore, many commonly consumed food and beverages contain varying amounts of preservatives, including sulphites. These varying amounts of preservatives in food and beverages is because they have been added during processing or conservation, because the ingredients have been treated with sulphites, or because they are a natural ingredient in the food, as is the case of certain vegetables (Adams, 1997; FDA, 2000; Taylor et al., 1986).

Generally, the use of sulphites in normal or general conditions is not a problem for the consumer. However, sulphites have been associated with allergic reaction and food intolerance symptoms.

* Corresponding author. Tel.: +34 915445607; fax: +34 915493627.

E-mail address: claudia@if.csic.es (C. Ruiz-Capillas).

Table 1
Sulphites additives approved by the European Union for use in food and beverages

E numbers	Names	
E-220	Sulphur dioxide	SO ₂
E-221	Sodium sulphite	Na ₂ SO ₃ , Na ₂ SO ₃ · 7H ₂ O
E-222	Sodium hydrogen sulphite	NaHSO ₃
E-223	Sodium metabisulphite	Na ₂ S ₂ O ₅
E-224	Potassium metabisulphite	K ₂ S ₂ O ₅
E-226	Calcium sulphite	CaSO ₃ , CaSO ₃ · 2H ₂ O
E-227	Calcium hydrogen sulphite	Ca(HSO ₃) ₂
E-228	Potassium hydrogen sulphite	KHSO ₃

An SO₂ content of not more than 10 mg/kg is not considered to be present.
Food additive legislation: European Parliament and Council Directives (Directives 95/2/EC and 2006/52/EC).

Adverse reactions can be experienced by sensitive individuals when they consume foods containing sulphites or unexpectedly large amounts of sulphites (Adams, 1997; FDA, 2000; Taylor et al., 1986). The most frequent sulphite-reaction symptoms are of the asthmatic and allergic type, such as difficult breathing, wheezing and hives, as well as gastrointestinal distress (Taylor et al., 1986). Therefore, sulphites are potentially toxic, and that is why they can be considered a hazard to human health. Moreover, when sulphite is added as a preservative it degrades thiamine (vitamin B1), which affects the nutritional quality of food (e.g., meat is a good source of it) containing it. The acceptable daily intake (ADI) of sulphite (expressed as SO₂) is 0.7 mg/kg body weight (FAO/WHO, 2007).

The control and regulation of the use of sulphites in food and beverages is important for effectiveness and basically to ensure consumer safety. In the European Community (Directives 95/2/EC and 2006/52/EC) on food additives other than colours and sweeteners, a maximum level is set (mg/kg or mg/l as appropriate) expressed as SO₂ for the different food and beverages. These levels vary widely depending on the products. Thus, for example, the maximum levels for crustaceous and cephalopods range between 50 and 300 mg/kg (of edible part), meat products 450 mg/kg, dry biscuit between 30 and 50 mg/kg, vegetables between 50 and 2000 mg/kg, and beverages 20 and 2000 mg/kg. Furthermore, in the 1986 FDA legislation it banned the use of sulphites in fruit and vegetables (except potatoes) to be served or presented fresh to the public, and required that the presence of detectable levels of sulphites be stated on food labels, even when these sulphites are used as a processing aid or are a component of another ingredient in the food. The FDA establishes levels of 10 mg/kg sulphite which must be indicated on the label and these compounds are also not permitted for use in meat in the USA (FDA, 2000).

In order to assess that the use of sulphites in the specified food is adequate and according to the amount permitted, it is essential to carry out a rigorous control of their content in many products. Therefore, the use of sensitive, selective, fast and low-cost methods for determining sulphite is an important aspect for food assurance and quality control. Numerous methods have been developed for determining sulphites in food and beverages. These procedures involve titrimetry, electrochemistry, fluorimetry, chemiluminescence spectrometry, colorimetry, gas-liquid chromatography, liquid chromatography, etc. (Fazio & Warner, 1990; Jiménez Colmenero & Blázquez Solana, 2008; Ruiter & Bergwerff, 2005; Wood et al., 2004). The most commonly used techniques are those based on the classical Monier-Williams method. Although this methodology offers sufficient sensitivity, it also has some constraints because of the length of time necessary for the analysis (with a significant impact on the capacity of the analytical laboratory) and the need for an exhaustive control of some analytical stages (e.g., N₂ stream adjustment). Flow injection analysis procedures have been extensively researched to determine sulphite in food

and beverages in an attempt to overcome such limitations and reduce the time and effort needed for these procedures. The FIA is a means of carrying out food analyses based on classical chemistry simply, cheaply, precisely and quickly, with low reagent consumption, small volumes of samples, less use of toxic substances and compatibility with almost any detection principle, using relative simple instrumentation and miniaturisation possibilities (Osborne & Tyson, 1988; Ruiz-Capillas & Jimenez Colmenero, 2008). FIA offers a fast analytical response in real time, which is especially valuable in monitoring schemes. FIA is a very versatile, flexible, and low-cost technique suitable for the routine determination of large numbers of samples. It is also very accurate, fast and easy to handle.

This review considers the present status of the published flow injection analysis methodology for determining sulphites in food and beverages. A detailed analysis of the technique is done, stressing its impact on the aspects of extraction, separation, detection and quantification procedures in different matrices.

2. Analytical procedures

Numerous flow injection analysis procedures have been described for determining sulphites in food and beverages (Table 2). Depending on the type of sample, these procedures generally consist of two phases. The first phase is related the extraction process of the sulphating agent, where this (SO₂) must be transferred into the liquid state prior to analysis using appropriate batch pre-treatment procedures. The second phase involves injecting the liquid extract (containing the extracted sulphur dioxide) into the FIA system (Fig. 1), where the extracted sulphur dioxide (present in the injection sample) is captured in a solution and analysed by a variety of means (Table 2). The two phases are now discussed in detail.

2.1. Sulphite sample extraction

When sulphating agents (Table 1) are added to food and beverages they can combine with some of their components (aldehydes, proteins, ketones, sugars, etc.). Accordingly, they may be present as free, reversibly bound (probably as hydrosulphonate adducts) and irreversibly bound (Adams, 1997). The relative presence of free, reversibly and irreversibly bound sulphites depends on factors linked to composition, processing and conservation. This is why in many instances the determination of sulphites requires some procedures for removing and recovering the sulphites (free and reversibly bound both together are called total sulphite) present in the sample. Liquid solutions such as water, wines, fruit juice, etc., however, are frequently directly injected into the FIA system without any prior treatment (Table 2) (AOAC, 2005; Araujo, Couto, Lima, & Montenegro, 1998; Araujo, de Carvalho, Mota, de Araujo, & Coelho, 2005; Bartroli, Escalada, Jorquera, & Alonso, 1991; Corbo & Bertotti, 2002; Granados, MasPOCH, & Blanco, 1986; Huang, Kim, & Schmid, 1992; Linares, DeCastro, & Valcarcel, 1989; Mana & Spohn, 2001; Safavi & Haghghi, 1997; Su & Wei, 1998). However, in complex matrices (solid samples, some kind of wines, etc.) sample extraction procedures are applied prior to injecting the sample in the FIA system (Table 2) (AOAC, 2005; Atanassov, Lima, Mesquita, Rangel, & Toth, 2000; Bendtsen & Jorgensen, 1994; Frenzel & Hillmann, 1995; León, Santín, Pich, & Centrich, 2004; Linares et al., 1989; MacLaurin et al., 1990; Sullivan et al., 1990). Since during extraction substantial losses of sulphites can occur, different alternative extraction conditions (distillation, acidic or alkaline extractant, etc.) have been tried to obtain free and bound sulphite fractions (Fazio & Warner, 1990; Frenzel & Hillmann, 1995; Sullivan et al., 1990).

Table 2
FIA determination of sulphites in food and beverages

Additives	Sample	Extraction	Hydrolysis	Donor reagent	Separation	Acceptor reagent (colorimetric reagent)	Detection system	Ref.
Total sulphite $\geq 5 \mu\text{g/g}$	White wine and food (shrimp, potatoes, pineapple)	Sodium tetrachloromercurate (TCM)	NaOH	Sulphuric acid	Gas diffusion cell	Malachite green	615 nm	AOAC (No. 990.29), 2005
Free sulphite (also bound sulphite that is labile at pH 2.2)	Wines	No test solution pre-treatment required	–	Citric acid	Gas diffusion cell	Malachite green	615 nm	AOAC (No. 990.30), 2005
Sulphites	White and red wine, apple juice, guacamole, dried apricots, potatoes, pickled onions, gulf shrimp, shrimp, dried apples, lettuce, cabbage	TCM	–	Sulphuric acid	Gas diffusion	Malachite green	615 nm	Sullivan et al. (1986)
Total sulphites	Shrimp, potatoes, dried pineapple, white wine	TCM	NaOH	Sulphuric acid	Gas diffusion	Malachite green	615 nm	Sullivan et al. (1990)
Total sulphite	Wines	No pre-treatment required except for dilution	NaOH	Sulphuric acid	Gas diffusion	H ₂ O ₂ –EDTA/luminol	Chemiluminescence	Huang et al. (1992)
Free sulphite	Wines	No pre-treatment required except for dilution	–	Sulphuric acid	Gas diffusion	H ₂ O ₂ –EDTA/luminol	Chemiluminescence	Huang et al. (1992)
Sulphite	Standards	–	–	–	Immobilized sulphite oxidase	Luminol + Co(II)	Chemiluminescence	Yaqoob et al. (2004)
Sulphite	Wine, fruit juices	Without prior treatment	–	HCL	Gas diffusion	De-ionised water	Conductivimeter	Araujo et al. (2005)
Sulphite	Beverages grape juice and wines	Without prior treatment	–	Sulphuric acid	Gas diffusion	Sodium hydroxide solution	Amperometric sensor	Corbo and Bertotti (2002)
Sulphur dioxide	Wines, sparkling wines	Wines were treated with NaOH	–	Sulphuric acid	Gas diffusion	Malachite green	614 nm	Atanassov et al. (2000)
Sulphite	Water sample and lemon juice	Without prior treatment	–	Sulphuric acid	Gas–liquid separator	–	Spectrophotometry 200 nm	Safavi and Haghghi (1997)
Sulphite	Wines and fruit juices	Without prior treatment	–	Sulphuric acid	Gas diffusion	0.01 M H ₂ O ₂ –0.15 mM H ₂ SO ₄	Impedance sensor	Su and Wei (1998)
SO ₂	White and red wines	Without prior treatment	–	HNO ₃	Gas diffusion	Iodine	Potentiometric detector	Araujo et al. (1998)
Sulphite	Wine	Etanol and KOH	–	Sulphuric acid in 10% ethanol	Gas diffusion	Bromocresol green	620 nm	Decnopweever and Kraak (1997)
Sulphite and sulphur dioxide	Brine, water, atmospheric SO ₂	TCM and formaldehyde solutions	NaOH/HMSA	Sulphuric acid	Gas permeation denuder	4,4'-Dithiodipyridine	324 nm	Frenzel and Hillmann (1995)
Total sulphite	Unstabilized beer	Degassing in the ultrasonic bath	NaOH	Sulphuric acid	Gas diffusion	Malachite green	615 nm	Bendtsen and Jorgensen (1994)
Free sulphite	Unstabilized beer	Degassing in the ultrasonic bath	–	Citric acid	Gas diffusion	Malachite green	615 nm	Bendtsen and Jorgensen (1994)
Free sulphur dioxide	Wine	Without prior treatment	–	HCl	Gas diffusion	<i>p</i> -Aminoazobenzene (PAAB) (and formaldehyde)	520 nm	Bartroli et al. (1991)
Total sulphur dioxide	Wine	Without prior treatment	NaOH	HCl	Gas diffusion	<i>p</i> -Aminoazobenzene (PAAB) (and formaldehyde)	520 nm	Bartroli et al. (1991)
Sulphite	Sulphite stock solution	–	–	–	–	<i>o</i> -Hydroxymercuribenzoic acid	545 nm	Yaqoob et al. (1991)
Sulphite	Sulphite stock solution	–	–	–	–	Cacotheline (nitrobruciquinone)	510 nm	Yaqoob et al. (1991)
Sulphite	Sulphite stock solution	–	–	–	Resin in thiocyanate	–	455 nm	Yaqoob et al. (1991)

(continued on next page)

Table 2 (continued)

Additives	Sample	Extraction	Hydrolysis	Donor reagent	Separation	Acceptor reagent (colorimetric reagent)	Detection system	Ref.
Sulphur dioxide (carbon dioxide) Sulphite	Wine Standard solution + formaldehyde	Without prior treatment	-	Sulphuric acid Phosphate buffer	Gas diffusion cell Mini-column of sulphite oxidase	<i>p</i> -Rosaniline-formaldehyde solution	578 nm. Amperometric	Linares et al. (1989) Massom and Townshend (1986)
Sulphur dioxide Total sulphur dioxide (free and bound SO ₂) Free SO ₂	Beer Wines (red and whites)	TCM -	NaOH NaOH	Sulphuric acid Sulphuric acid	Gas diffusion cell Gas diffusion cell	Malachite green	615 nm Chronopotentiometry	Leon et al. (2004) Dvorak et al. (2006)
Sulphite	Wines (red and whites) Standard	No test solution pre-treatment required	-	Sulphuric acid	Gas diffusion cell	-	Amperometric	Granados et al. (1986)
Sulphite	Bamboo shoot and rice cake	Grind the sample and add bidistilled water static for 1 h filtered and inject No pre-treatment required	-	Sulphuric acid/MES Solution of Ce(IV) + H ₂ SO ₄	TP-epoxy membrane-based gas dialyzer Reaction area	-	Potentiometric sensor (Ag/AgCl) Chemiluminescence	Hassan et al. (2001) He et al. (2005)
Sulphite/hydrogen sulphite/sulphur dioxide Sulphite and sulphur dioxide	Wines Brine	Lowering the pH to neutral and readjustment to the original value	-	- pH 11.7 buffer	Gas dialysis cell	<i>o</i> -Phthalaldehyde (OPA)/ammonium 5-Dithiobis(2-nitrobenzoic acid)	Fluorescence Exc: 330 nm Em: 390 nm 412 nm	Mana and Spohn (2001) MacLaurin et al. (1990)

HMSA: Hydroxymethanesulphonate; MES: 4-Morpholino-ethanesulphonic.

In the specific case of implementing FIA procedures for sulphite determination different alternative extraction conditions have been tested. Sullivan et al. (1990) reported sulphite loss by alkaline extracting treatment. These authors studied several alternative extractions that included solutions containing acetone, formaldehyde ethanol and tetrachloromercurate. The most efficient extraction procedure in different foods for FIA analysis includes extraction with tetrachloromercurate (TCM) solution (AOAC, 2005; Frenzel & Hillmann, 1995; León et al., 2004; Sullivan, Hollingworth, Wekell, Newton, & Larose, 1986; Sullivan et al., 1990) (Table 2).

TCM helps release the sulphites from solid foods and stabilizes them to form a stable sulphite–mercury complex, providing excellent sulphite recoveries (Ruiter & Bergwerff, 2005; Sullivan et al., 1986). Several concentrations of TCM were studied as the extraction medium. A 0.01 M Hg solution provided the best sensitivity in the FIA system (Bartoli et al., 1991). Since sulphites are usually labile and can be lost during isolation from the sample, they have been stabilized by reaction with formaldehyde to form a stable derivate, hydroxymethyl-sulphonate (Frenzel & Hillmann, 1995). Ultrasonic bath for degassing together with an anti-foaming agent (1-octanol) has also been used as a simple preparation to determine sulphites in beer (Bendtsen & Jorgensen, 1994). Some authors have reported the addition of 10% ethanol to test sample solutions and flushing these solutions extensively with nitrogen (Decnopweever & Kraak, 1997). Moreover, in the experiment by Mana and Spohn (2001) the sample was degassed and equilibrated with nitrogen.

The sulphite solution patron used in FIA applications may also present considerable instability problems. The oxidation of sulphite in solution has been a major problem in the determination of this compound. The presence of iron and other metal ions in trace amounts in analytical grade Na₂SO₃ (used for the preparation of standard solutions) may be responsible for the fast degradation of unstabilized standard sulphite solutions. Massom and Townshend (1986) showed those 2 h after the first injection of standard solutions, the peak high decreased 25–30%. In order to limit the instability problem of sulphite in solution, stabilizing agents have been used for the preparation of standard solutions and the conservation of aqueous samples. Various stabilizers have been proposed such as EDTA, TCM, formaldehyde, glycerol, isopropanol (Bendtsen & Jorgensen, 1994; León et al., 2004; Massom & Townshend, 1986; Safavi & Haghghi, 1997). In any case, it is crucial that the test or standard solutions are prepared quickly to minimise the oxidation of SO₂ by atmosphere O₂ or other compounds, and the process of its entry into the FIA system.

2.2. Separation/detection systems

Next, the extracting solution obtained from food and beverages containing the sulphating agents is injected into the FIA system where the SO₂ separation process takes place followed by the corresponding detection process.

Although some authors (MacLaurin et al., 1990; Yaqoob, Siddiqui, & Masoom, 1991) have done a determination of sulphite by a simple flow system analysis without any separation system, based only on a colour reaction in a reaction coil, generally the determination of sulphites consists of a sulphite release process which is carried out based on a gas diffusion (GD), gas–liquid system, dialysis, ion-exchange or enzymatic techniques (Table 2). Among them, the most widely used is based on a gas diffusion cell (Fig. 1) (AOAC, 2005; Araujo et al., 1998, 2005; Atanassov et al., 2000; Bartoli et al., 1991; Bendtsen & Jorgensen, 1994; Corbo & Bertotti 2002; Decnopweever & Kraak, 1997; Dvorak et al., 2006; Frenzel & Hillmann, 1995; Granados et al., 1986; Huang et al., 1992;

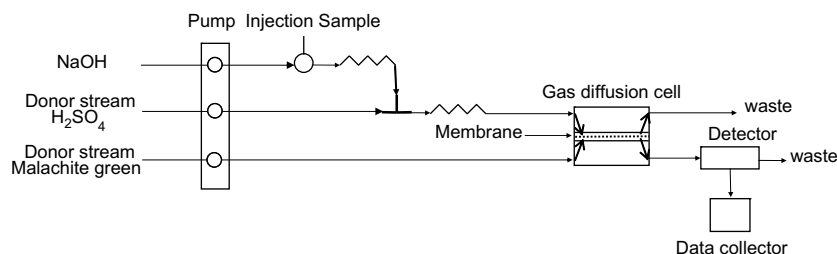


Fig. 1. Diagram of gas diffusion-flow injection analyzer for sulphite determination.

León et al., 2004; Linares et al., 1989; Mana & Spohn 2001; Su & Wei, 1998; Sullivan et al., 1986, 1990).

2.2.1. Gas diffusion system

In general, the gas diffusion unit (Fig. 1) consists of two liquid streams, a strong acidic donor solution (the acid medium enables the release of SO_2 gas from the stream with the test solution) and an acceptor solution (that collects the released SO_2) containing an acid–base indicator separated by a membrane permeable (generally of Teflon) to only gases. The membrane is not only necessary to separate the strong acidic donor solution from the acceptor solution but also forms a barrier for potential interferences in the sample. Therefore, when a sample is injected into the strong acidic donor solution, the released sulphur dioxide diffuses through the membrane and dissolves into the acceptor solution. GD-FIA can be used for determining sulphite in liquid or beverages and solid samples. It has the advantage that it can be used for food extracts, and even strongly coloured red wines can be processed without sample pre-treatment. An other inherent advantage of GD-FIA, which is of special importance with respect to the foregoing, is the possibility of optimising the sample treatment conditions in the donor stream without affecting the detection chemistry in the acceptor stream (Frenzel & Hillmann, 1995).

Although most of the GD-FIA methods have used sulphuric acid as a donor reagent, other acids such as HCl (Araujo et al., 2005), HNO_3 (Araujo et al., 1998) or citric acid (AOAC, 2005; Bendtsen & Jorgensen, 1994; León et al., 2004) have also been used. However, in the case of citric acid it was observed that the calibration curve of the system is non linear in the high range (>5 mg/kg), as well as the low range (<2 mg/kg), and the results are a dynamic detection range of only 2–5 mg/kg. The non-linearity is probably the result of the lower driving force of citric acid compared with that of sulphuric acid and the resulting limited transfer of SO_2 gas across the membrane (Bendtsen & Jorgensen, 1994).

Various compounds have been used as an acceptor solution. Their choice is closely associated with the detection system (spectrophotometric, fluorometric, amperometric, etc.) (Table 2). Spectrophotometric detection is preferred as it is more robust and usually available in routine control laboratories. The acceptor reagent most frequently used in the FIA system with a gas diffusion unit is a malachite green solution (AOAC 2005; Atanassov et al., 2000; Bendtsen & Jorgensen 1994; Sullivan et al., 1986, 1990). The degree of discolouration of malachite green is proportional to the amount of sulphite in the test solution and it is measured spectrophotometrically at 615 nm. Atanassov et al. (2000) used this system for the simultaneous determination of two analytes, CO_2 and SO_2 , in wine without sample pre-treatment. Iodine was used as a sulphur dioxide acceptor agent in food with photometric detection at 620 nm (Araujo et al., 1998). *p*-Rosaniline–formaldehyde solution was also used as a colour reagent. It yields coloured compounds with SO_2 with a maximum absorption at 578 nm (Linares et al., 1989). This system allows the simultaneous determination of sulphur dioxide and carbon dioxide in wines. However, the

application of this reagent has some serious limitations. The complexation of *p*-rosaniline and sulphur dioxide is rather slow, the reproducibility has been found to be poor, the reagent is toxic and carcinogenic and the detection limit of sulphur dioxide with this technique is lower. *p*-Aminoazobenzene has also been used as the colorimetric reagent for determining total and free sulphur dioxide in wine by flow injection analysis and gas diffusion detected at 520 nm (Bartoli et al., 1991). The reaction of sulphite and *p*-aminoazobenzene shows very slow reaction kinetics that requires extremely long reaction coils to obtain sufficient sensitivity. Huang et al. (1992) used another reagent colour such as luminal with chemiluminescence detection. Decnopweever and Kraak (1997) also proposed bromocresol green as an indicator, because it is a less toxic reagent and the kinetics is faster with maximum absorption at 620 nm. However, this method cannot be applied for sparkling wines and beers because carbon dioxide interferes with photometric detection. To solve this problem, some stabilization steps could be done in the sample (Decnopweever & Kraak, 1997). Frenzel and Hillmann (1995) also proposed 4,4-dithiodipyridine as an acceptor solution of the FIA manifold. The reaction of sulphite and 4,4-dithiodipyridine proceeds rapidly and quantitatively in the pH range 4–7. It yields a thiol anion complex applied in water and brine which is detected at 324 nm. MacLaurin et al. (1990) also used 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) in brine, the reaction was between the sulphite with the DTNB to produce the chromophoric species 2-nitro-5-mercaptobenzoic acid that is monitored spectrophotometric at 412 nm. More recently, *o*-phthalaldehyde (OPA)/ammonium reagent and fluorescent detection have been used. The highest sensitivity was achieved at an excitation wavelength of 330 nm and emission wavelength of 390 nm at pH 6.5 (Mana & Spohn, 2001).

As an essential process for determining total sulphite prior to the separation of SO_2 in the gas diffusion cell, a treatment aimed at releasing most of the bound sulphite with some sample component is applied. In this sense, the hydrolysis process has generally been carried out with NaOH (AOAC 2005; Atanassov et al., 2000; Bartoli et al., 1991; Frenzel & Hillmann, 1995; Huang et al., 1992; León et al., 2004; Sullivan et al., 1990), KOH (Decnopweever & Kraak, 1997) and hydrosylmethanesulphonate (HMSA) (Frenzel & Hillmann, 1995). Although this treatment could be carried out during the sample preparation as a prior step to injection in the system, it is generally carried out in the FIA system (Fig. 1). In this instance, an additional flow channel is installed through which sodium hydroxide solution or other hydroxide solutions are introduced. Moreover, in some systems a pre-hydrolysis unit forming part of the FIA system allows this step to be automated. The pre-hydrolysis unit consisted of a well-stirred chamber with two channel inlets and a net volume in which the sample was continuously mixed with a stream of NaOH (prior to acidification and the release of SO_2 gas). After this point the procedure involved the same steps as for the determination of free SO_2 . Automation of this step will allow the sample manipulation process to be simplified and sample throughput to be increased with respect to the classical methods.

In the case of determining SO₂ by GD-FIA, the interferences will only be those that are capable of passing through the membrane, including some relevant species such as nitrite, sulphite and cyanide (Frenzel & Hillmann, 1995). No interferences were noted when the nitrite was present in concentration levels up to three times higher than that of sulphite. Sulphite exhibits severe positive interference with about the same response as sulphite on the base of equimolar solutions. Cyanine interference became apparent only when present at concentrations above 10 mg/L (Frenzel & Hillmann, 1995). Safavi and Haghghi (1997) reported that the introduction of EDTA helps prevent the interference of some cations and anions, but also increases the sensitivity of the sulphite determination about twofold.

2.2.2. Other systems connected to FIA

Apart from GD, other systems connected to FIA have been described for determining sulphites. Corbo and Bertotti (2002) reported the determination of sulphite in beverages, based on amperometric detection of the analyte using a copper electrode in an alkaline medium in a flow through FIA configuration. The use of inexpensive copper electrodes makes the base FIA method more attractive. Furthermore, a potentiometric sensor involving a hydrated titanium oxide anion exchanger as electroactive material and an epoxy resin as a matrix membrane have been characterised and successfully applied (Hassan, Marei, Badr, & Arida, 2001).

For an aqueous solution such as lemon juice, Safavi and Haghghi (1997) used a stream of distilled water, (gas-liquid separator). The absorbance of the gaseous phase was measured at 200 nm using a UV/Vis spectrophotometer. Yaqoob et al. (1991) reported the use of a mini-column of an ion-exchange resin in thiocyanate form which was incorporated into an FIA system for sulphite determination spectrophotometrically. The thiocyanate rapidly forms a red complex with iron(III) which is measured at 455 nm in a flow through spectrophotometer. Other authors such as Massom and Townshend (1986) employed an enzyme immobilised on controlled-pore glass incorporated into a flow injection manifold using a mini-column of sulphite oxidase immobilised on controlled-pore glass, with amperometric detection of the hydrogen peroxide produced. The reaction is based on sulphite oxidation (in the sulphite oxidase column) to sulphate with the production of hydrogen peroxide which is detected in the amperometric flow cell.

He, Zhang, and Huang (2005) showed a chemiluminescence microflow injection analysis system on a chip for the determination of sulphite in food (bamboo shoot and rice cake), based on the chemiluminescence reaction between a solution of Ce(IV) and sulphite sensed by Rh6G and tween 80 in an acid medium. Yaqoob, Nabi, Weseem, and Massom-Yasinzai (2004) also reported a FIA method using on-line covalently bound immobilized sulphite oxidase packed in a mini-column, which was mixed downstream and detected via cobalt(II)-catalysed chemiluminescent oxidation of luminol.

3. Conclusions

FIA procedures have been extensively researched for determining sulphites in food and beverages in an attempt to overcome the limitations and reduce the time and effort needed for the traditional procedures based on the classical Monier-Williams method. Sulphite determination with FIA gives a fast analytical response in real time, which is especially valuable in monitoring schemes and it also has several advantages over traditional procedures that are usually used in control laboratories. Moreover, this methodology presents important potential industrial applications.

The FIA method enables simple, precise and fast determination of small amounts of sulphite with low reagent consumption when

small volumes of samples are available, using relative simple instrumentation and miniaturisation possibilities. Moreover, this method can be used in all types of matrices, liquids and solids. However, during the determination of sulphites with FIA, the experimental conditions in the donor stream, acceptor stream, colour reagent and separation system have to be considered and standard solutions for the calibration and detection system have to be properly chosen.

The low interference in these methods opens up possibilities for their application to a large number of matrices where other methodologies have presented problems like garlic. Furthermore, it could be interesting to study this methodology in new products where their compositions have been modified and this modification could interfere with sulphite determination.

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