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Rijkscentrum voor Landbouwkundig Onderzoek - Gent

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Directeur: Dr. P. HOVART

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W. VYNCKE

Paper presented at the 14 th meeting of the Working Group on Analytical Methods of the West-European Fish Technologists' Association (WEFTA), Nantes, April 1991

Mededelingen van het Rijksstation voor Zeevisserij (C.L.O.-Gent)
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ABSTRACT.

Two basic techniques are used to liberate sulfite from the sample matrix. The first is based on distillation of sulfur dioxide from an acidified aqueous suspension of the product in an absorbing solution. The second is based on the extraction of sulfite from the sample with water or alkali.

The most commonly used of the distillation techniques is the Monier-Williams method, where the sulfur dioxide evolved by acidification is displaced by a stream of inert gas into hydrogen peroxide to convert it to sulfuric acid which is quantitated by titration with base. However, the need for a more rapid and sensitive assay for sulfite has led to considerable effort in searching for suitable alternatives.

Methods involving jodometric titration after steam distillation, colorimetry with p-rosaniline, polarography or ion chromatography are described.

After direct extraction of sulfite, ion chromatography , flow injection analysis, enzymatic determination and gas-liquid chromatography have recently been proposed.

The choice of method will depend mainly on two factors : the number of samples to be analyzed in a certain period of time and the required sensitivity (detection limit).

SAMENVATTING

Bepaling van het totaal sulfietgehalte in garnalen : een overzicht van de methodologie.

Twee basistechnieken worden gebruikt om sulfiet uit de monstermatrix vrij te maken. De eerste is gebaseeerd op de destillatie van zwaveldioxyde uit een aangezuurde waterige suspensie van het product in een absorberende oplossing. De tweede is gebaseerd op de extractie van sulfiet uit het monster met water of alkali.

De meest gebruikte destillatietechniek is deze volgens Monier-Williams, waar de door aanzuren vrijgemaakte zwaveldioxyde door een stroom inert gas wordt overgebracht in een waterstofperoxyde-oplossing waar het tot zwavelzuur wordt omgezet. De concentratie hiervan wordt door titratie met een base bepaald. De noodzaak om over een snellere en meer gevoelige methode te beschikken heeft evenwel tot een aanzienlijke reeks onderzoekingen aanleiding gegeven om geschikte alternatieven te vinden.

Methoden die betrekking hebben op jodometrie na stroomdestillatie, colorimetrie met p-rosaniline, polarografie of ionchromatografie worden beschreven.

Na directe extractie van sulfiet werden ionchromatografie, flow injection analyse, enzymatische bepaling en gas-vloeistofchromatografie in recente tijden voorgesteld.

De keuze van de methode zal vooral van twee factoren afhangen : het aantal monsters dat in een bepaalde tijdsspanne moet worden ontleed en de vereiste gevoeligheid (detectielimiet).

1. Introduction. (*)

The defect known as "blackspot" (melanosis) in raw crustaceans (especially shrimp) can be avoided by the addition of sulfite which inhibits the enzymatic oxidation of monophenols to melanine (Faulkner et al.1954). Recent work has concluded that sulfite represents the most effective and practical control agent as compared to a large variety of chemical alternatives (Orwell and Marshall 1986). Although sulfiting agents have a long history of use, recent health-related problems in persons sensitive to sulfites have prompted renewed scrutiny by regulatory agencies for their presence in foods (Taylor and Bush 1986). Hence, interest has increased in methods for determination of sulfite in foods at levels not previously regarded as relevant from a food safety standpoint. In view of the increasing number of assays, these methods should be fast, straightforward, low-cost, accurate and precise (De Vries et al. 1986).

When sulfur dioxide is used as an additive, part of it combines with naturally occurring components of the food such as aldehydes, disulphide groups in proteins and part of it remains free in the inorganic state as sulfurous acid and bisulfite ions. The equilibrium between SO_2, H_2SO_3, HSO_3^- and SO_3^{2-} is also depending on the pH (Green 1976).

Consequently, there are two analytical aspects : the determination of free and of total sulfur dioxide (sulfite) the difference between the two being the bound form.

The food technologist will be interested not only in the total sulfite but also in the free form which is widely recognized to be the effective agent. The enforcement analyst on the other hand is mainly if not only interested in the total content.

This review deals only with the latter aspect. Only methods which have been reported to give reliable results with shrimp are described. It should be mentioned however that a more general review on analytical methodology was recently published (Fazio and Warner 1990).

Two basic techniques are used to liberate sulfite from the sample matrix. The first is based on distillation of sulfur dioxide from an acidified aqueous suspension of the product in an absorbing solution. The second is based on the extraction of sulfite from the sample with water or alkali.

2. Distillation methods.

(*) Sulfites are also used in other crustaceans such as Norway lobster (Nephrops norvegicus, molluscs, salted fish, canned fish. With the exception of one report mentioning results on clams (De Vries et al. 1986), all papers dealing with the determination of sulfite in fishery products found in the open literature were related to shrimp species only.

2.1. Monier-Williams method.

Numerous methods have been investigated for sulfite residue analysis. The most commonly used of these is the Monier-Williams method (1927), where the sulfur dioxide evolved by acidification is displaced by a stream of inert gas into hydrogen peroxide to convert it to sulfuric acid which is quantitated by titration with base.

The AOAC analytical methods book (Williams 1984) describes a modified Monier-Williams method incorparating modern glassware and acidometric titration of the absorbing solution (neutralised hydrogen peroxide) to a methyl red end-point (annex 1). On the other hand, the modification due to Tanner (1963) is preferred as a general method by EEC and the Codex Alimentarius Commission (FAO/WHO) (annex 2).

The Monier-Williams method has been proved to be reliable with a variety of foods including shrimp (Mitsuhashi et al 1979, Warner et al.1986). It has however several disadvantages : (a) it is time consuming (1,75 h) and laborious ; (b) it is subject to interference by other sulfur compounds present (Wedzicha and Bindra 1980) ; (c) the distillation requires a rather large sample size (50 g) and (d) it is relatively insensitive (detection limit of 10 ppm).

The need for a more rapid and sensitive assay for sulfite has led to considerable effort in searching for suitable alternatives.

2.2. Jodometric titration after steam distillation.

Kjeldahl-type rapid distillation units can be used for the determination of sulfite (De Vries et al. 1986, Aberg and Person 1988). Iodometric titration is preferred because it is more selective for sulfur dioxide and avoids interference from volatile acids present.

The method shows a detection limit of 10 ppm, a relative standard deviation of 7,5 % (compared with 10,4 % for the Monier-Williams method) and recoveries of 97,9 ± 6,4 %.

Total distillation time by this technique is only 6 min.

The analytical technique is described in annex 3.

2.3. Colorimetric determination.

Ogawa et al. (1979) reported that replacement of the alkaline titration by pararosaniline colorimetry lowered the absolute detection limit from 30 µg (titration method) to 2 µg. These authors proposed a modified Rankine-distillation apparatus, which gives results comparable to these obtained with the Monier-Williams method but is more convenient and rapid to

use.

The technique is described in annex 4. With shrimp, a recovery of 94,7 ± 1,9 % was obtained with the colorimetric determination and only 86,8 ± 3,1 % with the titrimetric method.

2.4. Polarographic methods.

A differential pulse polarographic (DPP) method was recently developed (Holak and Patel 1987). The method is outlined in annex 5.

The rationale for this approach has been to adapt the time-tested Monier-Williams procedure for isolating SO_2 from the sample, i.e., by purging with nitrogen, but using a simplified apparatus with a sensitive and specific mode of detection. The sample is blended with 5 % alcohol to minimize the oxidation of sulfite by atmospheric oxygen.

Total sulfite is released from a strongly acid solution (<pH 1) with an application of heat (100° C) by purging with nitrogen for 10 min SO_2 is trapped in a pH 5,2 acetate buffer, containing alcohol, in which it is then polarographed. The apparatus is quite simple and can be easily constructed by any laboratory.

The method has been submitted to a collaborative study including shrimp. It appeared to be suitable for as low as approximately 10 ppm. Recoveries were comparable to those for the official Monier-Williams method at high levels and were superior at low levels.

Stonys (1987) used the classical Monier-Williams method (1984) followed by polarographic detection by DPP or square wave voltametry (SWV). The trapping buffer and supporting electrolyte is 1 M ammonium acetate-acetic acid. The methods appeared to be very sensitive and specific for SO_2. Screening levels of less than 1 ppm total sulfite were achieved. Recoveries from fortified shrimp averaged 98 %.

Both methods showed the same results but Stonys recommends square wave voltammetry. The attractive features of this technique are : (a) better sensitivity than for differential pulse polarography, (b) speed (analysis time measured in the order of seconds) and (c) use of the single drop technique (no stirring problems as associated with the DPP procedure).

Microprocessor-based systems are now commercially available which makes the square wave technique accessible to a routine analytical laboratory (e.g. EG & G Parc, Princeton, N.J., U.S.A.)

The main drawback of the proposed method is the unchanged lengthily distillation time (1,75 hr) of the Monier-Williams

method.

2.5. Ion chromatography.

Sullivan and Smith (1985) and Anderson et al. (1986) combined the chemical approach of the Monier-Williams technique with the superior detection system available with ion chromatography. The original apparatus was modified (annex 6) to provide more rapid analysis (10 min. distillation time). The trapping solution consists of 0,1 NaOH and 0,1 % formaldehyde. A conductivity detector or electrochemical detector can be used. Chromatography time is 10 min. The method appeared to be very sensitive and selective for sulfite. The detection limit was 1 ppm. An average recovery of 98,3 % was obtained with fortified shrimp.

Using the same method Cooper et al. (1986) however found recoveries of only 50-65 % with dip-treated shrimp. They concluded the 10 min. phosphoric acid distillation procedure to be insufficient to extract free and reversible bound forms of sulfite from the shrimp matrix. The Monier-Willimas method provides a much more severe heat and acid treatment which is capable of extracting a greater portion of the residual sulfite present.

These authors obtained better results with extraction procedures (see 3.1).

Instead of the Monier-Williams apparatus or the above mentioned modification, steam distillation can be used. The trapping solution then consists of H_2O_2 and NaOH. The sulfate formed is determined by ion chromatography (Tecator 1987)

3. Extraction methods.

3.1. Ion chromatography.

Cooper et al. (1986) applied a water-dichloromethane extraction procedure followed by anion exchange chromatography. It should be remarked that sulfite is very soluble in water. Results compared very well with the Monier-Williams method It was considered to be a time saving alternative.

Kim et al. (1986, 1987) extracted shrimp samples in a homogenizer with Na_2HPO_4 - D-mannitol solution (pH 8,9) followed by anion exclusion chromatography and electrochemical detection. The procedure is outlined in annex 7. D-mannitol was used to minimize oxidation of sulfite. The whole analysis, starting with the sample preparation can be carried out with in 10 min, which compares very favourably with other methods for sulfite determination. The detection limit is 0,1 ppm in the extract, allowing determination far below the 10 ppm level in food. It was claimed to be more selective, i.e. presenting less interference, than the method proposed by Cooper et al. (1986).

Finally the ion chromatographic method is versatile in the sense that it enables one to measure free sulfite separately, by using a extraction of pH 2 instead of pH 8,9.

Sulfite analysers based upon the procedure described above are commercially available (e.g. Waters, Milford M.A., U.S.A. and Bio-Rad, Richmond C.A., U.S.A.).

Lawrence and Chadha (1988) determined sulfite in shrimp and other foods by headspace -ion chromatography. The samples were mixed with a solution containing mannitol, $FeSO_4$ and Na_2HPO_4 adjusted to pH 11, and left to stand for 15 min at room temperature. An aliquot of the mixture was place in a headspace vial and mixed with 50 % H_3PO_4. After 15 min, a portion of the headspace was removed with a syringe containing LC mobile phase without acetonitrile. The syringe was shaken and an aliquot of the solution was analyzed on an anion exchange column with a mobile phase of 0,03 M methane sulfonate (pH 10.8) containing 5 % acetonitrile. Sulfite was detected amperometrically (glassy carbon electrode) at +0.7 V. The method was successfully compared to the Monier-Williams procedure for a variety of foods, including shrimp. Minimum detectable levels were about 1 ppm, based on a 15 g sample.

3.2. Flow injection analysis (FIA).

Ruzicka and Hansen (1980),Sullivan et al. (1986) and Aberg and Persson (1988) described a method based on the decolorisation of malachite green by SO_2, which is isolated from the flowing sample stream by means of a gas diffusion cell (see annex 8). Shrimp are extracted with dilute tetrachoromercurate reagent. This compound forms a strong complex with sulfite. The detection limit is 2 ppm and the precision of replicate injections 1-2 %. Results compared favourably with those obtained by the Monier-Williams method.

The FIA-method is very rapid (50 samples/h) and sensitive, the instrumentation is relatively simple and is easily automated. Turbid or pigmented sample can be injected with no sample cleanup.

The complete set-up op the FIA-method is commercially available (e.g. Tecator, Höganäs, Sweden).

3.3. Enzymatic determination.

Sulfite oxidase converts sulfite to sulfate with the production of hydrogen peroxide. The latter products is subsequently reacted with NADH in the presence of peroxidase and the disappearence of NADH is followed spectrophotometrically at 340 nm. A commercial kit is available (Boehringer, Mannheim, Germany). De Witt (1988) reported favourable results with this method. The most effective method for extracting SO_2 from shrimp involved treatment with an alkali at

pH 11,66. Recoveries varied from 64 to 91 % with shrimp piked

with 50-200 ppm SO_2.

Williams et al (1990) on the other hand found the method to be unsatisfactory for analysis of sulfite in prawns. In view of the good results shown by the enzyme assay for standard solutions, the poor recoveries indicated a major interference from the prawn matrix in the determination. It should be noted however that the extraction of sulfite was made with water at 60°C instead of alkali, as applied by De Witt (1988).

3.4. Gas-liquid chromatography.

Mitsuhashi et al (1979) used head-space gas-liquid chromatography with flame photometric detection. The sample is treated with an alkaline extractant containing potassium-sodium tartrate and iron II sulfate (deoxidant) to liberate total sulfite. This is then released from the extract by phosphoric acid and an aliquot of the head-space gas is injected in a gas chromatograph.

Results were comparable to those obtained with the Monier-Williams technique and the colorimetric determination mentioned previously (see 2.3). With shrimp a recovery of 96,1 % was reported.

4. Choice of method.

The choice of method will depend mainly on two factors : the number of samples to be analyzed in a certain period of time and the required sensitivity (detection limit).

If the sulfite concentration is expected to be relatively high (e.g. clearly above 10 ppm) and the number of analyses is rather low, steam distillation followed by titrimetry or colorimetry seems to be adequate. The equipment is reasonably cheap.

If on the other hand emphasis is put on the toxicological aspects and detection limits below e.g. 2 ppm are desirable, or if many samples have to be dealt with per day, modern sophisticated methods with high output are recommended such as polarography, ion chromatography and FIA.

The main drawback of these techniques are the rather high price of the equipment.

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Annex 1

Modified Monier-Williams Method (Williams 1984)

20.123 Reagents

(a) Hydrogen peroxide solution 3%. Check 30% ACS reagent to ensure compliance with sulfate specification. Determine H_2O_2 content by $KMnO_4$ titration dilute to ca 6% H_2O_2, neutralize to Methyl red, (b), and dilute to calculated volume to give 3.0%.
(b) Methyl red indicator. -0.25% in alcohol. Adjust to transition color.

20.124 Apparatus

See Fig 1. Connect 3 neck (24/40) 1 l distilling flask at an outer neck to 30 cm Allihn condenser in reflux position. (Condenser must condense all HCl but none of SO_2). Place inner joint adapter with right angle hose connection in condenser and connect through piece of ¼ x 6" silicone tubing preboiled in HCl (1+20) and rinsed with H_2O, to set of 2 U-tubes of 20 mm tubing, ball joint 35/20, 55±5 mm center to center and 150±5 mm long, connected with cross-over tube, ball joint 35/20, 55±5 mm center to center and 115±5 mm long. To each U-tube add 2 ca 25 mm lengths of solid glass tube, 10 ml mm glass beads at exit side, and 10 mL 3% H_2O_2 containing drop Methyl red. Attach either curved gas inlet tube for outer neck or straight tube for center neck of distilling flask with tip reaching nearly to bottom.

Alternatively substitute apparatus shown in Fig 2. for U-tubes.

Connect right angle hose connection to 30-50 ml bulb (D) and fritted cylindrical gas dispersion tube (A). Suspend fritted end near bottom of Kuderna-Danish Evaporative Concentrator (B, C) (Kontes Glass Co.), part B, vol. 500 ml, 24/25 lower joint; part C, ca 15 ml, 24/25 joint) containing 10-12 ml 3% H_2O_2 and drop Methyl red Diameter of C should provide min. gas scrubber path of 10 cm with 10 ml H_2O_2.

Grind 4.5 g pyrogallol with 5 ml H_2O in small mortar and transfer slurry to 250 ml 24/40 gas washing bottle. Repeat grinding and transfer with two 5 ml portions H_2O. Pass H_2O-pumped N_2 from tank through 2-stage regulator into gas washing bottle to flush out air and add to bottle, through long stem funnel, cooled solution of 65 g KOH in ca 85 ml H_2O. (Prepare complete solution fresh daily.). Turn off N_2, and attach ¼ x 6" silicone tubing, preboiled in HCl (1+20) and rinsed with H_2O, to exit end and to gas inlet tube of distilling flask. Clamp off both ends of washing bottle.

Attach 125 mL separator through 24/40 joint to third neck of distilling flask. Attach piece of rubber tubing to short U-tube inserted through rubber stopper in neck of separator.

Blow into rubber tubing, and close separator stopcock. Let stand for few min. to check for leaks shown by liquids leveling in U-tubes.

Place distilling flask in heating mantle controlled by variable transformer.

20.125 Determination

Place sample, containing ≥ 45 mg SO_2, in distilling flask, using H_2O for transferring, if necessary. Dilute to ca 400 ml with H_2O. Add 90 ml HCl (1+2) to separator and force HCl into flask with gentle pressure. Start N_2 flow at slow steady stream of bubbles. Heat flask to cause refluxing in 20-25 min (ca 80 volts on 7 amp transformer). When steady refluxing is reached, apply line voltage and reflux 1.75 hr. Turn off H_2O in condenser and continue heating until inlet joint of first U-tube shows condensation and slight warming. Remove separator, and turn off heat. When joint at top of condenser cools, remove connecting assembly and rinse into second U-tube. Attach cross-over tube to exit joint of first U-tube, rotate until open ends touch, add drop Methyl red, and titrate with 0.1N NaOH just to clear yellow, mixing with gentle rocking. 1 ml 0.1N NaOH = 3.203 mg SO_2. Titrate second U-tube similarly. If alternative apparatus is used, disconnect, and rinse bulb D and tube A with few ml H_2O into Kuderna-Danish app. B, C. Add 2 drops Methyl red and titrate with 0.1N NaOH.

Gravimetric determination may be made after titration by rinsing tubes into 400 ml beaker. Add 4 drops 1N HCl and excess of filtered 10% $BaCl_2$ soln, and let stand overnight. Wash precipitate by decantation 3 times with hot H_2O through weighed gooch. Wash with 20 ml alcohol and 20 ml ether, and dry at 105-110°.
mg $BaSO_4$ x 274.46/g sample = ppm SO_2.
Determine blank on reagents, both by titration and gravimetrically, and correct results accordingly.

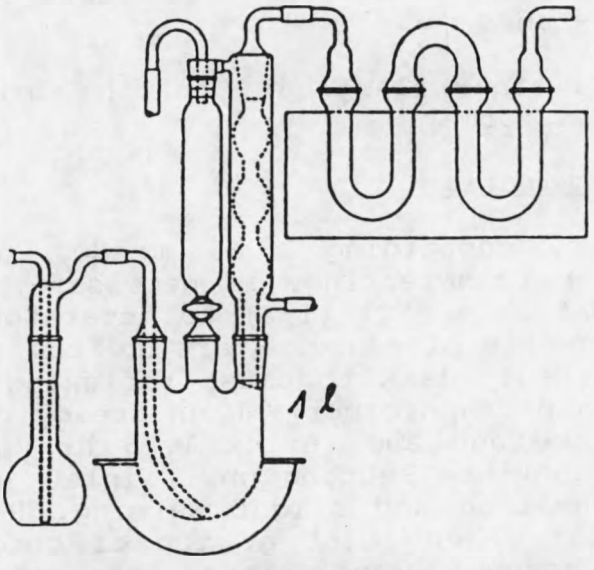


FIG. 1.—Apparatus for modified Monier-Williams method for sulfur dioxide

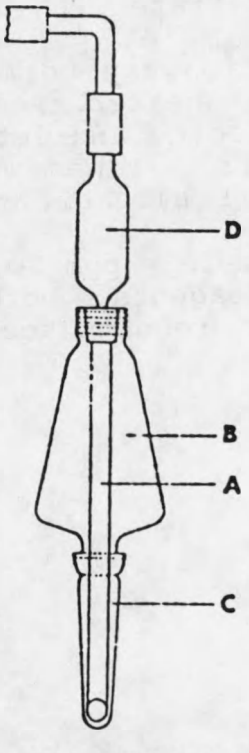


FIG. 2.—Alternative SO_2 absorber

Annex 2

Tanner Method (Tanner 1963)

Apparatus : see fig. 3

Reagents

Phosphoric acid, 88 per cent (d = 1.75).

Hydrogen peroxide solution, 0.2 per cent w/v. Dilute 0.7 ml of 100 vol. hydrogen peroxide to 100 ml.

Prepare as required daily.

Sodium hydroxide solution, 0.01M. Standardise against potassium hydrogen phthalate, dried at 110°C.

Methanol, AR.

Mixed indicator solution. Mix 50 ml of 0.03 per cent ethanolic solution of methyl red with 50 ml of 0.05 per cent ethanolic solution of methylene blue and filter.

Method

Weigh, or pipette, a quantity of sample into the distillation flask as indicated by the table below :

Expected SO_2 content (mg/kg)	Quantity of sample to be taken (g or ml)	Vol of distilled water to be added (ml)
<10	40-50	20
10-100	20-25	30
>100	5-10	40

Add distilled water to the flask as indicated. Add 50 ml of methanol and mix. Introduce into the distillation receiver 10 ml of hydrogen peroxide, 60 ml of distilled water and a few drops of mixed indicator solution. Add a few drops of 0.01 M sodium hydroxide solution to produce a green colour. Add a similar quantity of neutralised hydrogen peroxide solution to the guard wash bottle. Connect up the apparatus and adjust the nitrogen flow to approximately 60 bubbles per minute. Add 15 ml of phosphoric acid to the funnel and run it into the distillation flask. Heat rapidly to boil the mixture and then simmer gently for a total period of 30 min. Detach the receiver from the distillation apparatus and rinse the tube. Titrate the sulphuric acid present with 0.01 N sodium hydroxide solution until the indicator turns green.

Calculation

$$\text{Sulphur dioxide content (mg/kg or mg/l)} = \frac{a \times N \times 32 \times 100}{Q}$$

where a = volume (ml) of sodium hydroxide solution,
N = molarity of sodium hydroxide solution,
Q = weight of sample in g or volume of sample in ml.

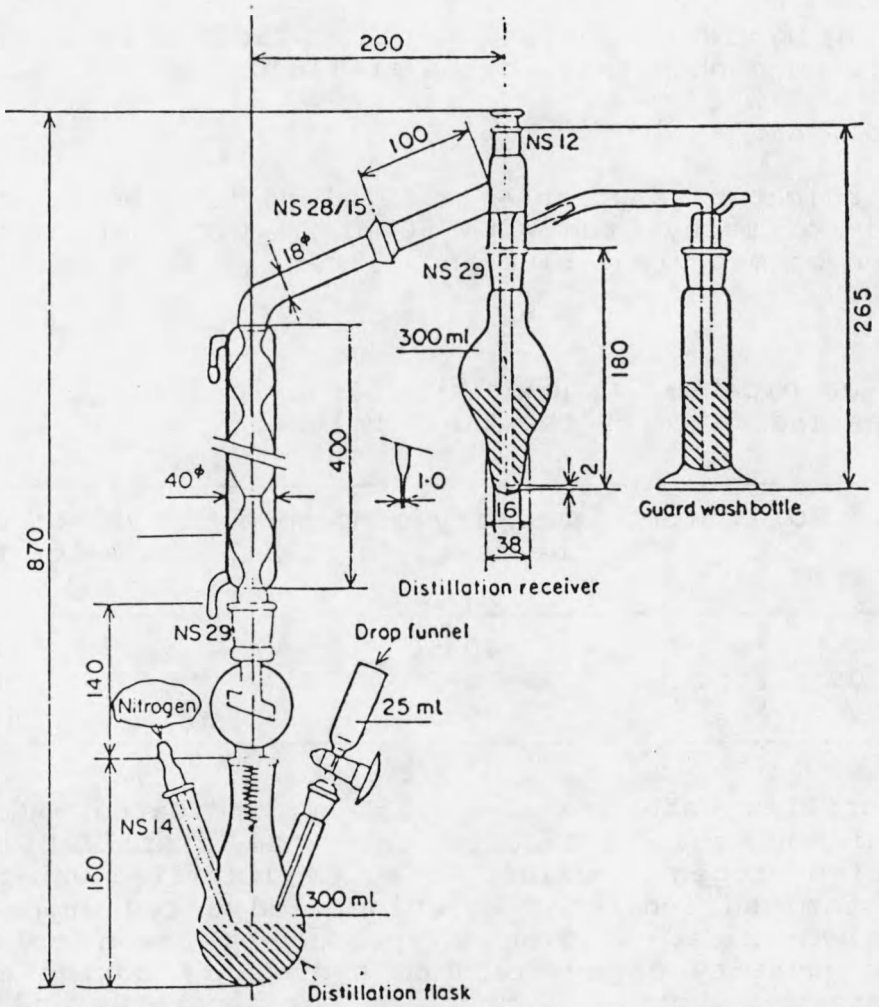


Fig. 3 Distillation apparatus for the determination of sulphur dioxide (Tanner). All dimensions in mm.

Annex 3

Distillation using rapid distillation equipment. (De Vries et al. 1986, Aberg and Persson 1988)

Apparatus : Kjeltec rapid distillation unit. No. 1002 (Tecator) or equivalent modified with appropriate tubing to allow distillate to be received in receiving beaker under buret outside of distillation unit. (fig. 4).

Reagents

(a) Hydrochloric acid-16% (v/v). - For standard glassware distillation : Cautiously add 160 ml concentrated HCl (ACS) reagent grade) to 1 l volumetric flask containing 700 ml water. Mix carefully. Dilute to 1 l and mix.

(b) Hydrochloric acid-33% (v/v). - For rapid distillation : Cautiously add 330 ml concentrated HCl to 1 l volumetric flask containing 500 ml water. Mix carefully. Dilute to 1 l and mix.

(c) Starch indicator solution. - To 20 g ACS grade starch powder, add enough water with stirring to make a paste. Transfer with mixing to 1 l boiling water. Store in refrigerator. Prepare fresh monthly.

(d) Potassium iodide solution. - 1%. Add 1.0 g AR potassium iodide to 100 ml volumetric flask containing 80 ml water. Dissolve and dilute to volume with water.

(e) Sodium thiosulfate solution. - 0.1N. Quantitatively empty entire contents of Certified Sodium Thiosulfate Solution Concentrate with rinsing into 1 l volumetric flask and dilute to volume with water. Prepare as often as the expiration date instructions on label indicate.

(f) Iodine stock solution. - 0.1 N. Dissolve 18.0 g AR potassium iodide and 6.5 g AR iodine, in 500 ml volumetric flask containing 400 ml water. Mix to dissolve and dilute to volume with water. Store in amber glassware protected from light. Solution is normally stable 6 months.

(g) Iodine working standard. - 0.02 N. Pipet 20.0 ml iodine stock solution into 100 ml volumetric flask and dilute to volume with water. Standardization of iodine working standard : Pipet 5 ml 0.1 N sodium thiosulfate solution to beaker containing 3-5 drops of starch indicator and titrate with unstandardized iodine working standard until blue remains 30-45 s.

Distillation

Place tubing which feeds Tecator alkali dispensing unit in appropriate container containing solution of 33% HCl. Prime dispensing unit into empty tube. When dispensing unit is properly primed, turn on water flow to steam generation unit, turn on power to unit, and let warm up 30 min. Place 250 ml

collection beaker under distillation outlet tube, and add to it 75 ml water, ca 1 ml starch indicator solution, 4-5 drops of potassium iodide solution, 3-4 drops of standardized iodine working standard, and magnetic stirring bar. Solution should be blue. Be sure tip of distillation unit outlet is below surface of receiving solution. Fill buret to zero mark and position it over beaker. Weigh appropriate size sample (5-15 g) into distillation tube, add 50 ml water, swirl briefly to disperse sample, and place in distillation unit. Pull dispenser handle one time to dispense 30 ml HCl solution into tube, open steam handle, and begin distillation while simultaneously titrating with standardized iodine solution to maintain blue color in receiving beaker. Distill until no further iodine is consumed and blue color is maintained in receiving solution 30-45 s (ca 100 ml distillate is usually collected). Record ml iodine solution used. Total distillation time by this technique is usually 5 min or less.

Calculations

Normality of iodine working standard :

$$N = 5 \times 0.1/V$$

where N = normality of iodine working standard ; 5 = ml sodium thiosulfate standard ; 0.1 = normality of sodium thiosulfate standard ; V : ml unstandardized iodine working standard necessary to reach end point.

Concentration of sulfite in sample as sulfur dioxide :

$$SO_2 \text{ ppm} = V \times N \times 32 \times 1000/W$$

where V = ml standardized iodine titrant consumed ; N = normality of iodine working standard from above ; 32 = equivalent weight of sulfur dioxide ; 1000 = conversion from mgmoles to gmoles ; W = sample weight in g.

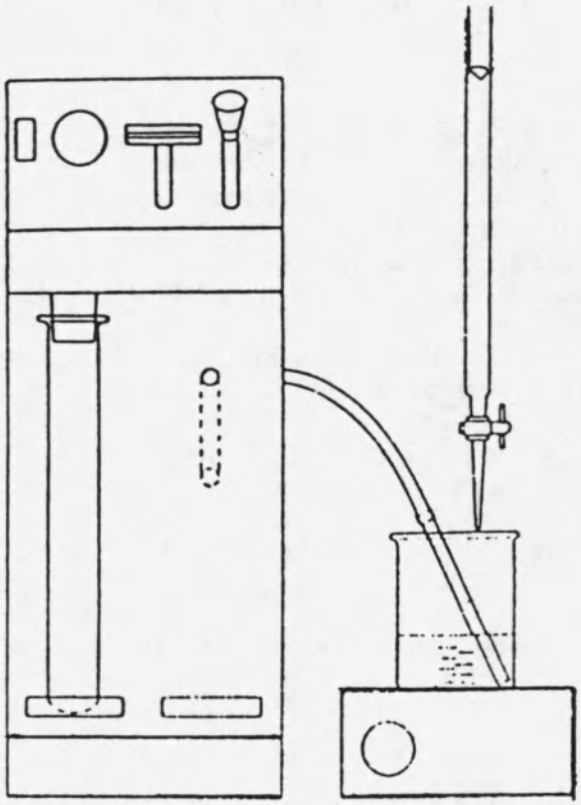


Figure 4. Modified Tecator apparatus for sulfite analysis.

Annex 4

Colorimetric pararosaniline method (Ogawa et al. 1979)

- Reagents

- Pararosaniline-formaldehyde solution (PRF) : 0.2 g of pararosaniline hydrochloride are dissolved in 100 ml of distilled water. After standing for one night the solution is filtered, if necessary. Twenty ml of this solution ware mixed with 6 ml of hydrochloric acid, made up to 100 ml with distilled water, then mixed with 100 ml of 0.2% formaldehyde solution.

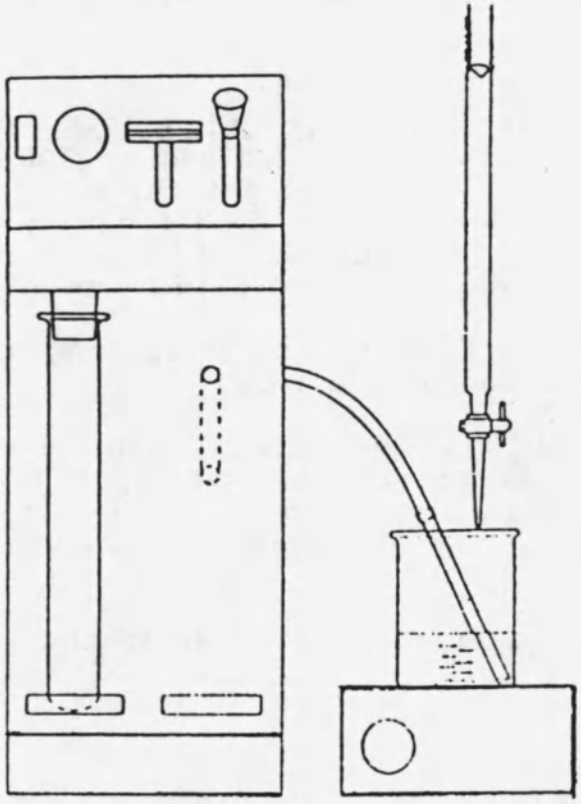
- Acetate buffer : Sodium acetate solution (1 M) adjusted to pH 1.0 with 1 N-hydrochloric acid.

- Sulphite standard solution : 162.5 mg of sodium hydrogensulphite dissolved in water to make 100 ml, then standardised by titration against freshly standardised 0.1 N-iodine to 1 mg SO_2 ml concentration. The solution is to be used within 30 min of preparation.

- Apparatus : The modified Rankine apparatus used is shown in Fig. 5.

- Procedure

(1) Pipette 8 ml of 0.1 N-sodium hydroxide into the flask (A) of Fig. 5, then connect the flask to the apparatus.
(2) Add 1 ml of 5% dimedone-ethanolic solution, 1 ml of 1% sodium azide solution, 2 ml of ethanol, 2 drops of antifoaming reagent (silicone oil) and 10 ml of 25% phosphoric acid into the flask (B). Furthermore, if the sample is solid, add 20 ml of water. Connect the flask to the apparatus.
(3) Turn on the gas supply and allow nitrogen to be drawn down the glass capillary tube (E) at a flow rate of 0.5-0.6 litre/min for 5 min.
(4) Add either 20 g of liquid sample, or suitable amount of solid sample cut into pieces less than 2 mm in lenght, to the flask (B) as quickly as possible.
(5) Turn on the gas supply again and allow nitrogen to be drawn down at a flow rate of 0.5-0.6 litre/min. Continue bubbling for 10 min, heating he flask (B) gently with a flame 4-5 cm high from a microburner and without a wire gauze so that the point of the flame is in contact with the flask.
(6)Turn off the gas supply, disconnect flask (A) and wash down the open end of the bubbler with a small quantity of 0.1 N-sodium hydroxide into the flask (A), then make to 10 ml with the alkaline solution.
(7) Take an aliquot (e.g. 5 ml) of the solution, add 5 ml of a 1 M-sodium acetate-hydrochloric acid buffer (pH 1.0) and PRF mixture (4:1), shake vigorously, stand for 35 min at room temperature, then measure the optical density of the solution at 560 nm with reference to that of a sample blank, run in the same way without the addition of sample.



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(8) Calculate the sulphite content as SO_2 from the calibration graph prepared previously.

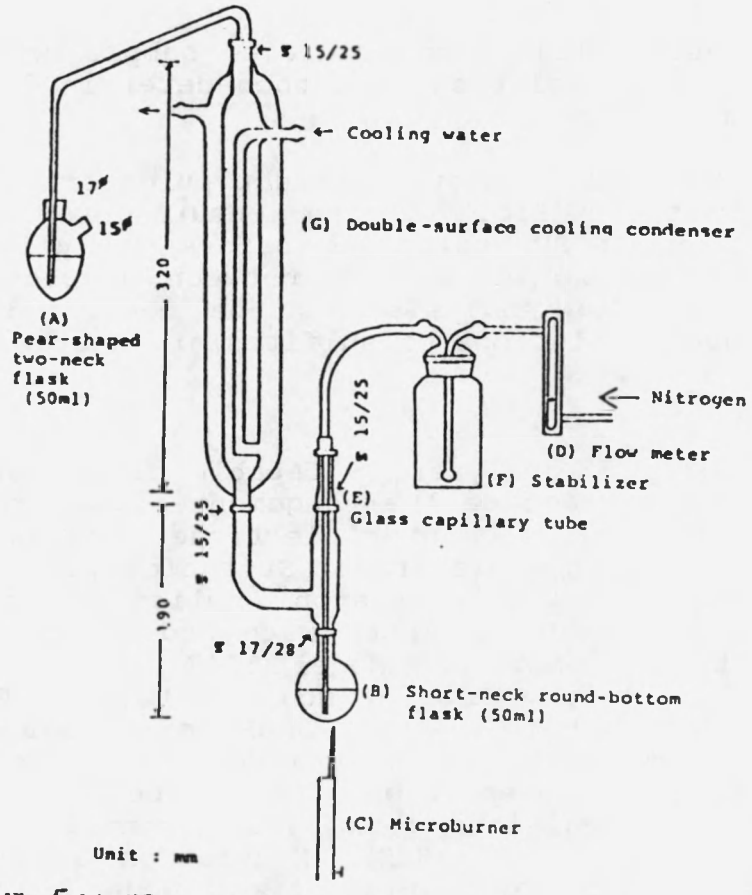

Fig. 5. Modified Rankine Apparatus

Annex 5

Differential Pulse Polarographic Method (Holak and Patel 1987).

(Applicable to determination of ≥10 μg total SO_2/g in shrimp).

Principle

SO_2 is purged with N_2 from acidified sample collected in electrolyte-trapping solution, and then determined by differential pulse polarography.

Notes. - (1) Analyst must construct purge-trap apparatus similar to that in Fig. 6, and ensure proper operation by analyzing aquous SO_2 solutions before analyzing samples. (2) System must be purged with N_2 between samples to remove any residual SO_2. (3) Analysis must be completed without undue delay ; aquous solutions of sulfites are unstable.

Apparatus

(a) Polarographic analyzer. - Capable of DPP analysis. Equipped with 3-electrode cell arrangement, i.e. dropping Hg electrode, platinum wire counter electrode, and saturated calomel or Ag-AgCl reference electrode. Suitable systems are (1) Model 174 or 264A (EG & G Princeton Applied Research, Box 2565, Princeton, NJ 08540) equipped with Model 303 static Hg dropping electrode (SMDE) or Model 1747 drop timer, and Model RE0089 X-Y recorder. Or. (2) Model 384 (EG & G Princeton Applied Research) with Model 303 SMDE and HIPLOT digital plotter (Houston Instrument, 8500 Cameron Rd, Austin, TX78753). Equivalent instrumentation may be used. Suggested polarographic conditions : initial potential, -0.45 V: final potential, -0.80 V (vs SCE). Modulation amplitude, 50 mV : scan rate, 5 mV/s ; drop time, 1 s ; mode, differential pulse ; range, 10 μamp (Models 174 and 264A)

(b) Purge-trap apparatus. - Connect directly to polarographic cell through 1 hole of cell top 'see Fig. 20:C1). Use standard N_2 purging tube to direct N_2 flow over solution during polarography. Add ca 10 ml H_2O to 25 x 200 mm tube and to polarographic cell, and purge system with N for ca 3 min before use. Check for leaks.

(c) Tank nitrogen. - Oxygen-free.

(d) Homogenizer. - Polytron (Brinkmann Instruments, Inc.), or equivalent

(e) Glass tubes. - Borosilicate, 25 x 200 mm.

(f) Block heater. - Multi-Blok No. 2090 (Lab-Line Instruments. Inc. 15th & Bloomingdale Aves. Melrose Park. IL60160), or Al block with thermometer, placed on hot plate.
(g) Micropipets. - 50μl.

Reagents

(Use ACS Reagent Grade chemicals unless otherwise indicated, and distilled or deionized H_2O).

(a) Alcohol. - 5% v/v.

(b) Sulfuric acid. - H_2SO_4 (1+1).

(c) Sodium sulfite ($Na2SO_3$)std. - Determine purity as follows : Accurately weigh ca 250 mg Na_2SO_3, into exactly 50 ml 0.1 N iodine solution in g-s flask. Let stand at room temp. 5 min. Add 1 ml HCl, and titrate excess iodine with 0.1 N $Na_2S_2O_3$, using 1% aquous starch solution as indicator (1 ml 0.1 N iodine consumed = 6.302 mg Na_2SO_3).

(d) Sulfite standard solution - 200 μg SO_2/ml. Dissolve 0.1968 g Na_2SO_3, adjusted for % purity, in 500 ml % alcohol (0.1968 x 100/x, where x = % assay). Prepare fresh daily.

(e) Ammonium acetate buffer. - 2M. Add 77.1 g NH_4 acetate to 500 ml graduated cylinder. Add ca 400 ml H_2O, and mix to dissolve. Add 57 ml HOAc. Then add H_2O to 500 ml and mix.

(f) Electrolyte-trapping solution - Dilute 2M NH_4 acetate buffer with equal volume 5% alcohol.

(g) Silicone defoamer. - Dow Corning Antifoam A, or equivalent.

Sample Preparation

Use open-pan balance (sensitivity to 10 mg per division) to weigh representative sample ($\leq$10.00 g) into 200 ml erlenmeyer. Add ca 0.5 g antifoam and then add 5% alcohol so total weight of mixture is 100.00 g. Stopper and shake erlenmeyer, or if necessary homogenize to obtain fine suspension. Complete sample preparation quickly to minimize oxidation of SO_2 by atmospheric O_2.

Determination

Shake prepared sample and immediately weigh aliquot of suspension ($\leq$10 g) containing $\leq$60 μg SO_2 into 25 x 200 mm tube and, if necessary, add 5% alcohol so total weight of mixture is ca 10 g. Add 10.0 ml electrolyte-trapping solution to dry polarographic cell and assemble apparatus as in Fig. 6.

(a) Total SO_2. - Add to sample tube 0.5 ml 2M NH_4 acetate buffer and 0.2 ml H_2SO_4(1+1) to adjust pH to ca 1.5. Purge with O_2-free N or 10 min at 1 l/min (Fig. 6 (a)). Stop N_2 flow, add 2 ml H_2SO_4 (1+1) to sample tube, and place tube in block heater preheated to 100° (Fig. 6 (b)). Purge with N_2 for 10 min at 1 l/min. Stop N_2 flow and lift exit tubing from electrolyte-trapping solution in polarographic cell (to prevent solution from

backing up). Offset pen slightly and obtain polarogram under conditions given in Apparatus. To verify complete transfer of SO_2 from sample, reinsert exit tubing into electrolyte-trapping solution, purge again for 5 min, and obtain polarogram. Repeat if necessary.

(b) Reagent blank. - Prepare reagent blank in same manner as for sample, and carry through analysis.

Calibration Curve

Prepare calibration curve at time of sample analysis as follows : Pipet 10.0 ml electrolyte-trapping solution into dry polarographic cell and add 50 µl 200 µg/ml standard solution Bubble N_2 through solution for 4 min and obtain polarogram as for sample. Repeat 5 times with additional 50 µl aliquots of standard, bubbling N for 30 s after each addition. Construct calibration curve, representing 10, 20, 30, 40, 50, 60 µg SO_2 in cell.

Calculation

Obtain total amount SO_2 (µg) in cell from calibration curve by using highest peak currents produced by sample. Calculate SO_2 in sample in µg/g correcting for reagent blank if necessary.

Alternative Trapping Technique

Add 10.0 ml electrolyte-trapping solution to 25 ml graduated cylinder and insert exit tubing (Fig. 6, items 2 and 7). Add to sample tube 0.5 ml 2M NH_4 acetate buffer and 0.2 ml H_2SO_4 (1+1) to adjust pH to ca 1.5. Purge with O_2-free N_2 for 10 min at 1 l/min. Stop N_2 flow, add 2 ml H_2SO_4 (1+1) to sample tube, and place sample tube in block heater preheated to 100°. Purge with N_2 for 15 min at 1 l/min. Stop N_2 flow, and transfer solution to polarographic cell, deaerate, and obtain polarogram. Dilute aliquot with electrolyte-trapping solution if too concentrated Calculate total SO_2 by using calibration curve. Purging time given should be sufficient to completely transfer of SO_2 from most samples. Verify complete transfer by additional purging. e.g., 5 min, using 10.0 ml fresh electrolyte-trapping solution. Allow longer purging time if required.

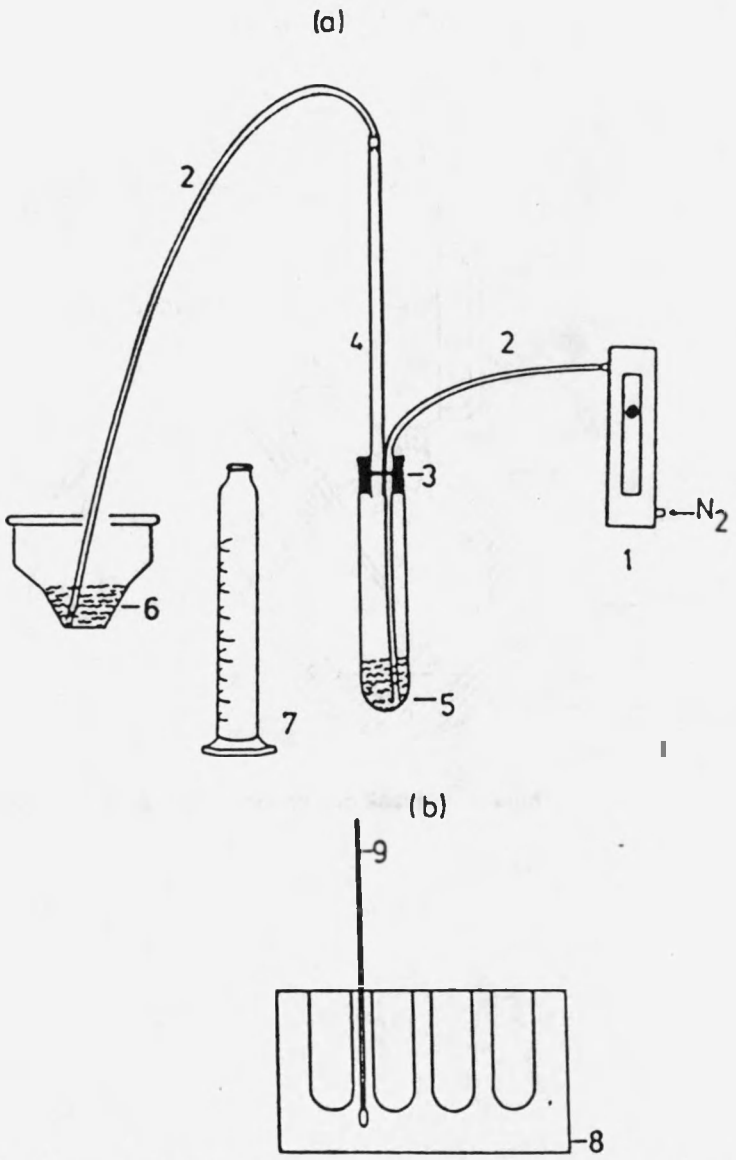

FIG. 6. —Purge-trap apparatus for SO_2 determination: (a) 1, flow meter; 2, Teflon tubing, 2 mm id; 3, rubber stopper; 4, glass tubing, 1.0 cm id × 40 cm; 5, sample test tube, 25 × 200 mm; 6, polarographic cell, connected to polarograph in usual way, containing 10.0 mL electrolyte-trapping solution; 7, graduated cylinder, containing 10.0 mL electrolyte-trapping solution. (b) 8, block heater; 9, thermometer.

Annex 6

Modified Monier-Williams distillation apparatus
(Sullivan and Smith 1985 ; Anderson et al. 1986)

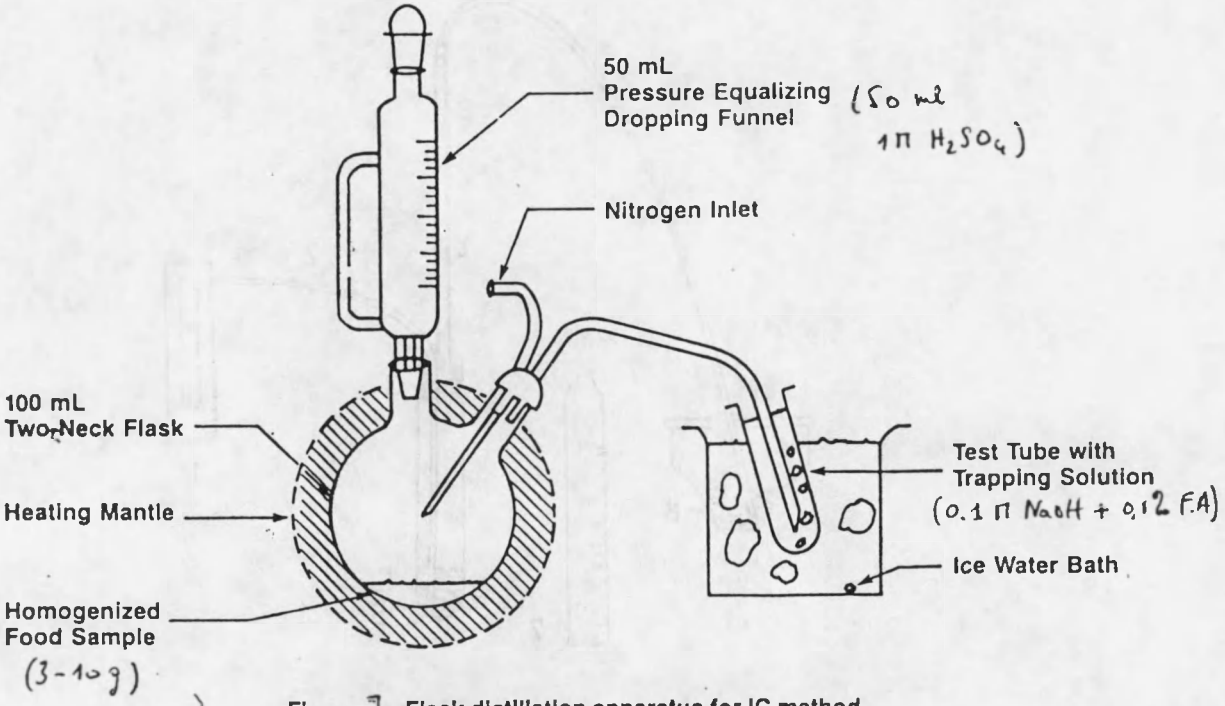

Figure 7. Flask distillation apparatus for IC method.

Annex 7

Ion exclusion chromatography (Kim et al. 1986, 1987).

1. Extraction

1 min with homogenizer
pH 8.9 for total sulfite
(30 ml 20 mM Na_2HPO_4, 10 mM D-mannitol)

2. Filtration

0.22-μm membrane filter

3. Anion exclusion chromatography

High-speed column (4.6 x 100 mm)
20-μl injection loop
6 mM H_2SO_4 solution as eluant

4. Electrochemical detection

Platinum electrode
+0.4 V vs Ag/AgCl electrode

5. Printer integrator

Prints the chromatogram
Calculates SO_2 concentration

Annex 8

Flow injection analysis (Sullivan et al. 1986)

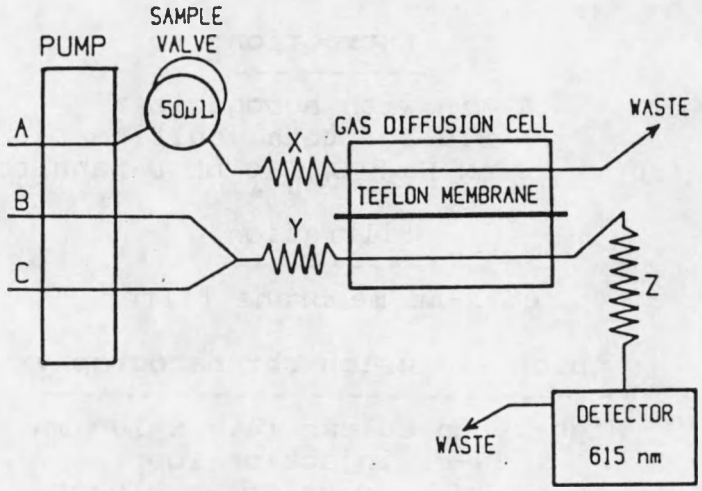

Figure 8. Flow diagram for flow injection analyzer. Reagents: A, 0.15M H_2SO_4; B, dilute malachite green reagent; C, phosphate buffer. Mixing coils: X (0.5 mm id × 60 cm); Y (0.5 mm id × 60 cm); Z (0.5 mm id × 260 cm).