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A spectrophotometric method for quantification of sulphite ions in environmental samples

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This study described an alternative method developed for the quantification of sulphite ions in environmental samples. The method was based on results of an investigation of the reaction of excess pentacyanidonitrosylferrate(II) popularly known as nitroprusside (NP) and the sulphite anion. $NP-SO_3^{2-}$ reaction product by use of zinc-ethylenediamine complex cation(s) was stabilized. The $NP-SO_3^{2-}$ reaction product was stabilized for 30 minutes by use of zinc ethylenediamine complex cation(s) and the absorbance was enhanced, making determination of sulphite possible. The method has a limit of quantification of 2.321 µg SO_3^{2-} mL^{-1}. Good accuracy was achieved for samples spiked with SO_3^{2-} in the range from 1 to 10 µg SO_3^{2-} mL^{-1}, which demonstrated the validity of the proposed procedure. The repeatability (CV) was not more than 2.37% and the limit of detection was estimated at 0.99 µg SO_3^{2-} mL^{-1}. The method was applied to determine the concentration of sulphite ions in sugar and wine brands sold in local market. Comparable results were obtained between this method and an iodometric procedure for determination of sulphite in environmental samples.

Key words: Determination of sulphite ions, wine, sugar, environmental samples.

INTRODUCTION

Sulphite anion (SO_3^{2-}) is a major species of sulphur in which oxidation state IV is expressed. The detection of the anion has long held the interest of the analytical community, because of the large number of roles that it can play within environmental and physiological systems (Isaac et al., 2006). It may occur in boilers and boiler feed waters treated with it for dissolved oxygen control, in natural waters or wastewaters as a result of industrial pollution, and in treatment plant effluents dechlorinated with sulphur dioxide. Sulphites or sulphiting agents are the most common preservatives used in winemaking (Koch et al., 2010), and are also important additives in many food products, because they inhibit development of both enzymatic and non-enzymatic browning in a variety of processing and storage situations (American Public Health Association, 1998; Claudia and Francisco, 2009). The sulphite ion is a very effective microbial inhibitor in
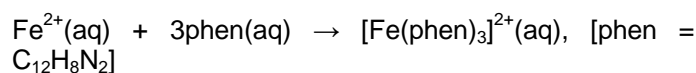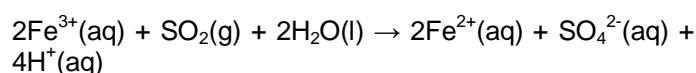
acid or acidified foods.

However, excess sulphite in boiler waters is deleterious, because it lowers the pH and promotes corrosion. Control of SO_3^{2-} in wastewater treatment and discharge may be important environmentally, principally because of its toxicity to fish and other aquatic life and its rapid oxygen demand (American Public Health Association, 1998). Sulphites as additives can cause an asthmatic reaction; presence of excessive amounts of sulphites is respon-sible for off flavour in food products (McFeeters and Barish, 2003; Machado et al., 2008). Some of it added to foods often disappears as a result of reversible and irreversible chemical reactions. Thus, it is often important to measure both free and bound forms of sulphite that are present in foods.

Several techniques have been developed to quantify the sulphite anion alone or in combination with other

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sulphur species like sulphate, thiosulphate and dithionate. These include: titrimetric methods with potassium iodide-potassium iodate (American Public Health Association, 1998), copper sulphate (Shahine and Ismael, 1979), cerium(IV), mercury(II) (Crompton, 1996), spectro-photometric methods with 1,10-phenanthroline (American Public Health Association, 1998), Fuchsin N solution (Badri, 1988), mercuric chloranilate (Humphrey and Hinze, 1971), mercuric thiocyanate and Fe^{3+}, electro-chemical methods with mercury(I) chloride-mercury(II) sulphite electrodes (Marshall and Midgley, 1983) and the sulphite oxidase enzyme electrode (Smith, 1987). Other methods include molecular emission spectrometry (Schubert et al., 1979), high performance liquid chromatography (HPLC) with ultraviolet (UV) detection (McFeeters and Barish, 2003), ion-exchange chroma-tography (Edmond et al., 2003), chemiluminescence methods (Al-Tamrah, 1987; Koukli et al., 1988) and flow injection analysis techniques (Thanh et al., 1994; Araujo et al., 1998; Xiaoli and Wei, 1998; Atanassov et al., 2000; Hasson and Spohn, 2001; Claudia and Francisco, 2009).

The phenanthroline method is currently adopted as the standard one for determination of sulphite in water and wastewaters, while the sulphite in foods and beverages is determined by the traditional AOAC Official Method 990.28 (AOAC, 1995; Cunniff, 1995). The phenanthroline method requires elaborate technical specification and user expertise and as such, can incur substantial running costs. It involves purging of an acidified sample with nitrogen gas and trapping the liberated sulphur dioxide gas (SO_2) in an absorbing solution containing Fe^{3+} ion and 1,10-phenanthroline. The Fe^{3+} ion is reduced to Fe^{2+} by SO_2, producing the orange tris(1,10-phenanthroline)iron(II) complex as illustrated in the following reaction schemes:

$$2Fe^{3+}(aq) + SO_2(g) + 2H_2O(l) \rightarrow 2Fe^{2+}(aq) + SO_4^{2-}(aq) + 4H^+(aq)$$

$$Fe^{2+}(aq) + 3phen(aq) \rightarrow [Fe(phen)_3]^{2+}(aq), [phen = C_{12}H_8N_2]$$

In solution, the sulphite anion reacts with pentacyanidonitrosylferrate(II) ion to form an unstable, red sulphite-nitroprusside ion ($[Fe(CN)_5(NOSO_3)]^{4-}$) (Fogg et al., 1966; Andrade and Swinehart, 1972; Leeuwenkamp et al., 1984; Araujo et al., 2005). In our laboratory, we succeeded in stabilizing both the sulphate (Mbabazi et al., 2011) and the sulphite anions through a series of spectrophotometric tests. The red sulphite-nitroprusside reaction product was stabilized by ethylenediamine complexes of zinc; the general reaction scheme for this behavior being;

$$[Fe(CN)_5(NOSO_3)]^{4-}(aq) + [Zn(en)_x]^{2+}(aq) \rightarrow [Fe(CN)_5(NOSO_3)][Zn(en)_x]^{2-}(aq)$$
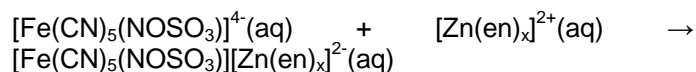
The main objective of this study therefore was to utilize the sulphite-nitroprusside stabilized product and describe an alternative spectrophotometric procedure for the quantification of the sulphite ion in solution. Our method has been compared with an iodometric titration method for determining the concentration of sulphite ions in wine and other beverages such as sugar that are commonly sold in local markets.

MATERIALS AND METHODS

Apparatus

Weighing was done on an AAA 160DL dual range balance (Adam Equipment Co. Ltd UK). The absorption spectra were recorded on a UV-VIS Shimadzu UV-1700 CE double beam spectrophotometer (Shimadzu Corporation, Japan) and absorbance measurements at a fixed wavelength were made with the same instrument in the photometric mode. pH measurements were done with a Corning Pinnacle 555 pH/ion meter (Corning Incorporated Life Sciences Corning, New York, 14831 USA). The addition of aqueous nitroprusside (NP) to aqueous SO_3^{2-} was carried out using Transferpette micro pipettes (BRAND GMBH + CO KG Postfach, 11 55 97877 Germany).

All chemicals used were of analytical grade. The solutions used were prepared as subsequently described.

Starch indicator solution

The starch indicator solution was prepared by dissolving analytical grade soluble starch (2 g) and salicylic acid (0.2 g) in hot deionized water (100 mL).

Standard potassium iodate solution

Potassium iodate (0.0021 mol/L) solution was prepared by dissolving the solid (812.4 mg) in a minimum amount of deionized water and diluted to 1000 mL.

Standard sodium thiosulphate

Sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$, 6.204 g) was placed in a volumetric flask (1000 mL) and dissolved in deionized water (50 mL). Sodium hydroxide (0.4 g) was added and the solution made to the mark with deionized water. The solution was then standardized using a standard potassium hydrogen iodate solution as follows. Potassium iodide (2 g) was dissolved in an Erlenmeyer flask with deionized water (150 mL). Concentrated sulphuric acid (3 drops) and starch solution (20 mL, 0.0021 mol/L) were added, respectively. The solution was diluted to 200 mL and the liberated iodine was titrated against the sodium thiosulphate titrant with starch as the indicator. The concentration of sodium thiosulphate was found to be 0.025 mol/L.

Standard iodine solution

Potassium iodide (25 g) was dissolved in a minimum amount of water and iodine (3.2 g) was added. After dissolution of the iodine, the solution was diluted to 1000 mL and standardized against $Na_2S_2O_3.5H_2O$ (0.025 mol/L) using starch solution as indicator.

Sodium sulphite stock solution

Fresh sodium sulphite stock solutions were prepared by weighing sodium sulphite (0.5 g) and dissolving it in deionized water to make

100 mL of aqueous solution. Determination of the sulphite ion concentration was carried out iodometrically.

Aqueous sodium nitroprusside (SNP)

A fresh stock solution of SNP (0.02 mol/L) was used. This was prepared by dissolving SNP crystals (6 g) in deionized water and the volume was made up to 1000 mL.

Aqueous zinc acetate solution

Zinc acetate (220 g) was weighed into a volumetric flask and dissolved in deionized water. The solution was made up to 1000 mL.

Aqueous ethylenediaminetetraacetic acid (EDTA) solution

The sodium salt of EDTA (0.0372 g) was dissolved in deionized water and the solution was made up to 10 mL.

Spectrophotometric procedure for determination of sulphite ion

This was done as per our validated stabilization procedure. SNP (4.5 mL, 0.02 mol/L) was pipetted into in a 50 mL volumetric flask; EDTA (100 µl, 0.01 mol/L) was added followed by ethylenediamine (30 µl, 13.66 mol/L) and ethanol (10 mL). A solution of 0.025 g of gelatin in 5 mL of deionized water was added followed by perchloric acid to lower the pH to 6.5. An aliquot of intermediate standard aqueous SO_3^{2-} followed by zinc acetate solution (0.5 mL, 1 mol/L) was added; the mixture was diluted to the mark by addition of an appropriate amount of deionized water and uniformly mixed for 5 s. The resultant red solution was immediately transferred to cuvettes which were inserted in the spectrophotometer for scanning its spectrum. A stable absorbance at λ_{max} 482 nm was read off and then using a pre-prepared absorbance versus concentration calibration curve, the concentration of the sulphite ion was determined.

Spectrophotometric determination of total sulphite in wine

Wine was treated to release matrix bound SO_3^{2-} as follows: sodium hydroxide solution (1.6 mL, 4.0 mol/L) was added to 10 mL of the sample to release the bound SO_3^{2-}. The mixture was left to stand for 5 min and the pH of the solution was adjusted to 8.5 using sulphuric acid. SNP solution (4.5 mL, 0.02 mol/L) containing EDTA (4.5 µl, 0.01 mol/L) was pipetted into four different 50 mL volumetric flasks. Ethylenediamine (30 µl, 13.66 mol/L) was added to each flask followed by ethanol (10 mL) to stabilize SO_3^{2-} in aqueous solution. A solution of 0.025 g of gelatin in 5 mL of deionized water was added (to avoid bubbling in the ethanol stabilized solutions) followed by treated wine (200 µl). Three of the flasks were then spiked with 6, 8 and 10 µg SO_3^{2-} mL^{-1}. Zinc acetate solution (0.5 mL, 1 mol/L) was added; the mixture was diluted to the mark with deionized water and was uniformly mixed for 5 s. The resultant red solution was immediately transferred to a cuvette which was inserted in the spectrophotometer to read the absorbance. The concentration of the sulphite anion in the sample that was not spiked was obtained from absorbance versus concentration curve.

Spectrophotometric determination of sulphite ion in sugar

SNP solution (4.5 mL, 0.02 mol/L) containing EDTA (4.5 µl, 0.01 mol/L) was pipetted into four different 50 mL volumetric flasks; ethylenediamine (30 µl, 13.66 mol/L) was added followed by ethanol (10 mL) to stabilize SO_3^{2-} in aqueous solution. A solution of 0.025 g of gelatin in 5 mL of deionized water was added followed by a sugar solution (200 µl). Three of the flasks were then spiked with 6, 8 and 10 µg SO_3^{2-} mL^{-1}. Zinc acetate solution (0.5 mL, 1 mol/l) was added; the mixture was diluted to the mark by addition of an appropriate amount of deionized water and was uniformly mixed for 5 s. The resultant red solution was immediately transferred to cuvettes which were inserted in the spectrophotometer to read the absorbance. The concentration of the sulphite anion in the sample that was not spiked with SO_3^{2-} was obtained from absorbance versus concentration curve.

Iodometric determination of sulphite ion

Standard iodine (10 mL, 0.0125 mol/L) was measured from a burette into a 250 mL conical flask. Sodium sulphite solution (2 mL) was added, the excess iodine was back titrated against standard $Na_2S_2O_3.5H_2O$ (0.025 mol/L) using starch as the indicator.

Iodometric determination of sulphite ion in sugar

Sugar (1.0 g) was dissolved in deionized water to make 10 mL of solution. Starch (0.5 mL) was added and the resultant mixture was titrated against standard iodine solution.

Iodometric determination of sulphite ion in wine

Wine (10 mL) was pipetted into a conical flask. Sodium hydroxide solution (1.6 mL, 4 mol/L) was added to release the bound SO_3^{2-}. The mixture was left to stand for 5 min. Sulphuric acid (1.7 mL, 10% v/v) was added followed by starch solution (0.5 mL). The resultant mixture was titrated against standard iodine solution.

Method validation

A validation was carried out on the developed method and the following characteristics were evaluated; working range and linearity, accuracy, precision, selectivity and detection limits.

Working range and linearity

The linearity of the method was evaluated by using calibrators in the entire working range of 1 to 10 µg mL^{-1} for the analyte. The curve was a plot of the absorbance of the sulphite-nitroprusside reaction product against concentration. The regression equation with the slope, intercept and correlation coefficient (r^2) was generated using Microsoft Excel. Limit of quantification formed the lower end of the working range. The linearity was established using the square of correlation coefficient value (r^2) of the line of best fit.

Accuracy and precision

Accuracy was determined by calculating the mean recovery of the seven portions spiked with standard SO_3^{2-} solutions at three concentration levels, all within the working range. The accuracy was then expressed as;

$$Accuracy = \frac{y}{z} \times 100\%$$

Table 1. The accuracy and precision of the developed nitroprusside method at three concentration levels.

Nominal concentration (μg SO_3^{2-} mL^{-1})	Concentration measured (μg SO_3^{2-} mL^{-1})*(mean ± SD)	CV (%; n = 7)
1.521	1.466 ± 0.035	2.368
2.500	2.457 ± 0.046	1.888
5.031	4.846 ± 0.098	2.028

*Mean ± SD, at 95% confidence interval.

where y was the mean value of the concentration of the seven replicates and x was the spiked (nominal) concentration. The intra-batch variability in the measurement of the sulphite ion concentration (precision) was calculated from seven repeat determinations of spiked samples and was expressed as a coefficient of variation (CV).

Selectivity

Several cationic and anionic species such as sulphide, thiosulphate, hydrogen phosphate, chloride, copper (II), iron (II) potassium and sodium ions were tested for interference. Different amounts of the ionic species were added to a solution containing sulphite ions of known concentration and the extent of interference caused by each species was investigated.

Detection and quantification limits

The limit of detection (LoD) was defined as the lowest concentration of sulphite ion in a sample that could be detected but not necessarily quantified under stated conditions of the developed method. In this work, LoD was determined by analyzing 10 independent sample blanks for sulphite ion amount and the standard deviation (SD) among the values determined was calculated. LoD was then expressed as the sulphite ion concentration corresponding to the mean sample blank value + 3SD

The limit of quantification (LoQ) was defined as the lowest concentration of sulphite ion that could be determined with an acceptable level of repeatability, precision and trueness. In this work, LoQ was determined by analyzing 10 independent sample blanks for sulphite ion amount and SD was calculated. LoQ was then expressed as the sulphite ion concentration corresponding to the mean sample blank value + 10SD.

RESULTS AND DISCUSSION

A comparison of sulphite content in wine and sugar using both NP and an iodometric titration method was made and the results are shown in Tables 3 and 4.

A lot of attention has been drawn to the concentration of sulphite ions in environmental systems because of their associated health effects. A method that is simple but accurate is therefore essential for monitoring the levels of the sulphite ions in such systems.

Method development and validation

The optimum conditions chosen for this work were those that had been previously validated in our laboratory (Musagala, Busitema University, unpublished); in which ethylenediamine in the presence of zinc was found to stabilize the sulphite-nitroprusside reaction product for a period of 30 min. The range of ethylenediamine concentration used was 5.5 to 22 mmol/L, above this concentration the absorbance was further enhanced but the product was found to be very unstable. The spectrophotometric determination of the SO_3^{2-} from its reaction with NP was carried out in aqueous slightly acidic media by using ethylenediamine and zinc concentrations of 11 and 10 mmol/L, respectively. This was done without alkali metal cations. Absorbance readings were made after a period of 5 to 10 min.

Mbabazi et al. (2011) noted that an excess of NP not greater than ten-fold is sufficient for analysis since the absorbance increases with NP concentration. A similar observation was also noted in this study. Again, it was noted that temperature, pH and the ratio of excess NP to SO_3^{2-} exerted a significant effect on stability of the product of the NP-SO_3^{2-} reaction. An optimum pH of 6.5 was found to be satisfactory for our purpose.

Working range and linearity

The working range was found to lie between 1 and 10 μg SO_3^{2-} mL^{-1}; above the upper limit, the decomposition of the product was relatively fast and often characterized by precipitation with time. The linearity of the method as measured by the correlation coefficient of inter-assay linear regression curves (r^2) was better than 0.99 in all cases in the measured range of 1-10 μg SO_3^{2-} mL^{-1} which was indicative of a good linear relationship between SO_3^{2-} concentration and absorbance.

Accuracy and precision

A comparison of the nominal concentration of the spiked solutions for seven replicates at three concentration levels with the concentration measured at each of the levels showed very good accuracy at all the three concentration levels (Table 1), an indication that the developed method was fit for the intended purpose. The precision of the method was also very good (CV not more than 2.368) in all cases, again suggesting that the method was precise in the concentration levels considered.

Selectivity

The effect of some cations and anions on the method

Table 2. The effect of a number of anions and cations on the nitroprusside method for SO_3^{2-} in the presence of ethylenediamine, using 3.10 µg SO_3^{2-} mL^{-1} for testing.

Foreign ion added	Interference concentration (µg/mL)	SO_3^{2-} recovered (µg/mL)*
S^{2-}	1000	ND
$S_2O_3^{2-}$	1000	ND
HPO_4^{2-}	1000	ND
Cl^-	1000	3.05 ±0.02
Cu^{2+}	100	ND
Fe^{2+}	100	ND
K^+	1000	3.05 ±0.02
Na^+	1000	3.05 ±0.02

*Mean ± SD, at 95% confidence interval; ND = Not detected.

Table 3. Comparison of the developed nitroprusside method to iodometric titration method for the determination of sulphite in wine.

Type of wine	Concentration of SO_3^{2-} µg^{-1} mL^{-1}	
	Nitroprusside method	Iodometric titration
	65.0 ± 6.6	70.0 ± 8.4
	68.0 ± 6.6	70.0 ± 8.4
Baron de Vignon semi-dry white wine	58.0 ± 6.6	60.0 ± 8.4
	60.0 ± 6.6	60.0 ± 8.4
	50.0 ± 6.6	50.0 ± 8.4
	47.0 ± 8.4	50.0 ± 8.4
	68.0 ± 8.4	70.0 ± 8.4
Bellingham dry white wine	58.0 ± 8.4	60.0 ± 8.4
	60.0 ± 8.4	60.0 ± 8.4
	50.0 ± 8.4	50.0 ± 8.4
	69.0 ± 6.9	70.0 ± 7.4
	50.0 ± 6.9	50.0 ± 7.4
King fisher strawberry fruit red wine	63.0 ± 6.9	65.0 ± 7.4
	60.0 ± 6.9	60.0 ± 7.4
	59.0 ± 6.9	60.0 ± 7.4

*Mean ± SD, at 95% confidence interval.

was studied in detail by adding different amounts of ionic species as shown in Table 2. The greatest anionic interference to this method would be expected to come from S^{2-} that reacts with NP in a similar manner to produce a red product ($[Fe(CN)_5(NOS)]^{4-}$) with λ_{max} 538 nm at pH above 10. Sulphide also forms a sparingly soluble precipitate with Zn^{2+} in the reaction mixture. Other ionic species were also found to interfere during the determination of sulphite as indicated in Table 2. The presence of oxidizable species such as sulphide, thiosulphate, phosphate, and iron may lead to high levels of sulphite. Copper is known to catalyse oxidation of sulphite to sulphate when the sample is exposed to air

leading to low results.

However, the effect of copper can be avoided by adding a complexing agent such as EDTA during sample collection as this would inhibit copper (II) catalysis and promote oxidation of iron (II) to iron (III) before analysis. Sulphide can be removed by adding about 0.5 g of zinc acetate and thereafter analyzing the supernatant of the settled sample while thiosulphate can be determined independently using a simple iodometric titration.

It is therefore recommend that the method be employed mainly for the determination of sulphite in wine and sugar, but its application could be extended to relatively clean waters as is always the case with the iodometric

Table 4. Comparison of the developed nitroprusside method with iodometric titration method for the determination of sulphite in sugar.

Type of sugar	Concentration of SO_3^{2-} mg^{-1} kg^{-1}	
	Nitroprusside method	Iodometric titration
Kinyala white sugar	28.0 ± 5.4	30.0 ± 5.5
	29.0 ± 5.4	30.0 ± 5.5
	28.0 ± 5.4	30.0 ± 5.5
	18.0 ± 5.4	20.0 ± 5.5
	19.0 ± 5.4	20.0 ± 5.5
Kakira white sugar	18.0 ± 5.9	20.0 ± 5.5
	27.0 ± 5.9	30.0 ± 5.5
	29.0 ± 5.9	30.0 ± 5.5
	30.0 ± 5.9	30.0 ± 5.5
	18.0 ± 5.9	20.0 ± 5.5
Kenya Brown Sugar	30.0 ± 5.0	30.0 ± 5.5
	21.0 ± 5.0	20.0 ± 5.5
	19.0 ± 5.0	30.0 ± 5.5
	28.0 ± 5.0	20.0 ± 5.5
	29.0 ± 5.0	30.0 ± 5.5

*Mean ± SD, at 95% confidence interval.

method for the determination of sulphite in environmental samples (American Public Health Association, 1998).

Fogg et al. (1966) reported that several organic compounds, including thiols, amines, and ketones were also known to form coloured compounds with the NP ion, but usually only in strongly alkaline solution. However, at pH of 6.5 used for the nitroprusside-sulphite reaction, the aforementioned compounds did not interfere. Tin(II), ferrocyanide and arsenates gave white precipitates with the reagents, but these also did not interfere with the sulphite determination.

Detection and quantification limits

The LoD and LoQ for the $NP-SO_3^{2-}$ reaction method were found to be 0.99 μgSO_3^{2-} mL^{-1} (Blank + 3SD) and 2.321 μgSO_3^{2-} mL^{-1} (Blank + 10SD), respectively. The obtained detection limits showed that the method could be applied in the detection and quantification of SO_3^{2-} concentrations as low as 1 μg mL^{-1}.

Applicability of the developed method for determination of sulphite ion in wine and sugar

The total sulphite content in wines is the sum of the free and bound sulphite. Usually, information about the free sulphite content rather than the total sulphite content is preferred. Araujo et al. (2005) noted that the equilibrium between bound and free sulphite was rather labile and any change in composition of the wine like dilution

inevitably shifted the equilibrium making the determination of free sulphite difficult. In this work, relatively high values of sulphite were obtained for red wine and this was attributed to the colour of the wine.

Conclusion

A manual spectrophotometric method has successfully been developed and validated for the quantification of micro quantities of sulphite ions based on the modified reaction conditions of the nitroprusside-sulphite ion ($NP-SO_3^{2-}$) reaction.

The method in the determination of sulphite ions has also been applied in selected environmental systems such as wine and sugar. The results obtained by the developed method compared well with those obtained using an iodometric titration method for SO_3^{2-} determination.

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