

## **Kinetic Spectrophotometric Method for Determination of $\text{SO}_3^{2-}$ Using Methylene blue**

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### **ABSTRACT**

The present work reports a kinetic spectrophotometric method for the determination of trace amounts of sulphite that is based on inhibitory effect of sulphite on the oxidation of Methylene blue (MB) in acidic media. The reaction is followed spectrophotometrically by monitoring the decrease in absorbance of MB at 664 nm with a fixed time of 0.5-4.5 minutes. It was found that sulphite exert a strong inhibitory effect on this reaction. The method is applicable in the range of 0.05-120 mg L<sup>-1</sup> sulphite and the lower limit of detection is 0.04 mg L<sup>-1</sup> (3 $\sigma$ ). The relative standard deviation for 6 replicated determinations was 0.9 and 0.5 % for 4.0 and 20.0 mg L<sup>-1</sup>, respectively. This method was successfully applied for the determination of sulphite in real samples.

**Keywords:** Sulphite; Kinetic; Spectrophotometric; Methylene blue; Bromate.

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### **INTRODUCTION**

Sulphiting agents (sulphur dioxide, sodium sulphite, sodium bisulphite, sodium metabisulphite and calcium sulphite) are extensively used as food additives. They are active as antimicrobial agents, enzyme inhibitors, antioxidants and structure modifiers in the control of enzymatic and non enzymatic browning reactions with stabilizing and conditioning functions. Therefore, determination of sulphite is of great interest in industrial, environmental fields and food products such as dried fruits, dehydrated vegetables, biscuits, jellies, mustard and wine [1, 2]. The useful properties of sulphiting agents are generally due to the nucleophilicity of the sulphite ion that may react by addition to

carbonyl groups, carbon-carbon double bonds, quinones, heterocyclic nitrogen compounds or by cleaving disulphite bonds. They are chemically equivalent compounds in foods since they are converted to the same ionic or nonionic species at a given pH, ionic strength and non electrolyte concentration. Nowadays due to the reported harmful effects towards hypersensitive people, in many countries, the sulphite and sulphur dioxide content in food and beverages has been strictly limited [3]. In addition, sulphite may occur in boilers and boiler feed waters treated with sulphite for dissolved oxygen control, in natural waters or waste waters as a result of industrial pollution, and in treatment of plant effluents dechlorinated

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with sulphur dioxide. Excess of sulphite ion in boiler waters is deleterious because it lowers the pH and promotes corrosion. Control of sulphite in waste water treatment and discharge may be important environmentally, mainly because of its toxicity for fish and other aquatic life and its rapid oxygen demand [4]. Although numerous useful methods are available to determine sulphite and sulphur dioxide, [5-10] still new methods appear in the recent literature. HPLC-enzyme coupling [11], exclusion chromatography with electrochemical detection [12], optical sensors [13-17] and chemiluminescence detection [18] are techniques that used to detect sulphite but spectrophotometric methods as a simple, sensitive and selective method are widely used [19,20].

In this work, a kinetic spectrophotometric method for the determination of trace amounts of sulphite is proposed based on inhibitory effect of sulphite on the oxidation of Methylene blue (MB) by bromate in acidic media. The reaction is followed spectrophotometrically by monitoring the decrease in absorbance of MB at 664 nm with a fixed time of 0.5-4.5 minutes. We found in acidic bromate solution, sulphite has an inhibitory effect on the oxidation of MB. Therefore, rate of reaction on the presence of sulphite (sample) is much lower than MB oxidation on the absence of sulphite (blank). In our investigation, a rapid, sensitive and selective kinetic spectrophotometric method for determination of sulphite has been presented which has experimental detection limit of  $0.04 \text{ mg L}^{-1}$  ( $3\sigma$ ).

## EXPERIMENTAL

### Reagents and Standard Samples

All chemicals used were of the highest purity available and used without any further purification. Doubly distilled water was used throughout the experiment. 100 mL standard stock sulphite solution of  $1000 \text{ mg L}^{-1}$ , was prepared by dissolving 0.1575 g of sodium sulphite (Merck) in water. Sulphite solutions are rather sensitive towards oxygen. In order to stabilize such solutions several additives are recommended such as the toxic mercuric

chloride<sup>21</sup> and ascorbic acid<sup>4</sup> as preservative. We used a few drops of  $1.0 \text{ g L}^{-1}$  ascorbic acid. This solution was remained stable at least for one month. After this period, turbidity could be observed in the solution and must be replaced. Working solutions were prepared by appropriate dilution with water.

Potassium bromate stock solution of  $2 \times 10^{-2} \text{ mol L}^{-1}$  was prepared by dissolving 0.8350 g of  $\text{KBrO}_3$  (Merck) in appropriate amount of water and diluted to 250 mL.

MB solution ( $1.5 \times 10^{-4} \text{ mol L}^{-1}$ ) was prepared by directly dissolving 0.0135g of MB in appropriate amount of water and diluted to 250 mL in a volumetric flask. The MB solution was stored in dark at  $4^\circ\text{C}$  and replaced every month.

### Apparatus

The spectrophotometric measurements were made with a Shimadzu A-160 UV-Vis double-beam spectrophotometer utilizing 1 cm glass cell. A Heidolph thermostated water bath was used for fixing temperature. A stop watch was used for recording the reaction time.

### General Procedure

For optimizing of effective factors on the rate of reaction, different volumes of studying factor and 1.0 ml of other reagents was added to a 10 ml standard volumetric flask. The stop watch was started immediately after the bromate solution was added. The solution was diluted to the mark with double distilled water and mixed well.

Then, the reaction mixture was immediately transferred to a cuvette and the absorbance was recorded as a function of time. The catalyzed as well as uncatalyzed reaction was followed spectrophotometrically by monitoring the decrease in absorbance at 664 nm. Absorbance measurement was started exactly after 0.5 minute of initiation up to 4.5 minutes to use as fixed time measurement of initial rate. In order to eliminate any traces of metal ions coming from the glass surface, the glassware used for this work was cleaned scrupulously. Cuvettes were cleaned after use by immersion in nitric acid (1:2) for 15 minutes to remove traces of MB absorbed on the walls.

## RESULT AND DISCUSSION

MB is a well known redox indicator and is susceptible to oxidation irreversibly by bromate ion in acidic media leading to formation of colorless product.

Sulphite ion retards the reaction between MB and oxidant at trace amounts. The reaction is monitored by measuring the decrease in absorbance of the characteristic band of MB at 664 nm. The absorbance change at  $\lambda_{\max}$  is quite high where there is no absorption at all by other species existing in the reaction. The optimization of reaction variables and further determination were therefore carried out at 664 nm.

### Effect of Variables

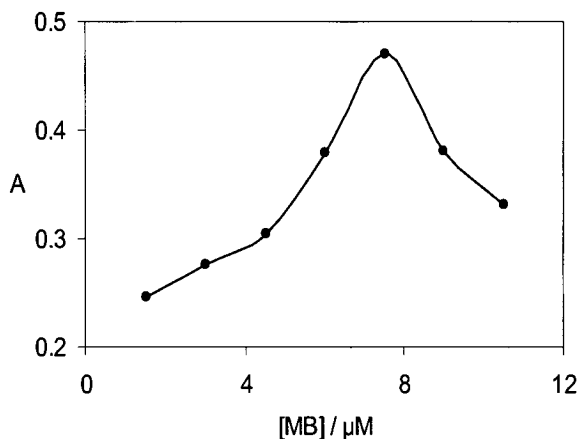
In order to establish experimental condition under which the inhibitory effect of sulphite and therefore the sensitivity of its determination to be at maximum, various acids were tested and sulphuric acid was found to be the best one. Then, dependence of reaction rate to temperature, concentration of  $H_2SO_4$ , MB, bromate and the effect of ionic strength were studied. The absorbance change after fixed time as a measure of initial rate, were used to plot the graph for each variable and the optimum conditions were taken from the graphs for the subsequent study of the variables.

The effect of sulfuric acid on the reactions was studied in the concentration range of 0.04-0.72 mol  $L^{-1}$  of  $H_2SO_4$ . The reaction rate increases with increasing concentration of sulfuric acid up to 0.40 mol  $L^{-1}$ . At higher concentrations, the rate decreases. This decrease in rate at higher acidic concentration may be attributed to protonation of MB, which might stop oxidation or making it quite difficult to occur. So for further works the concentration of 0.40 mol  $L^{-1}$  was selected.

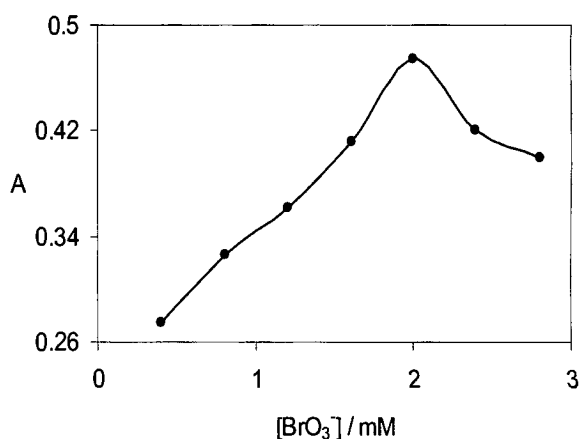
Effect of MB concentration in the range of  $1.5-10.5 \times 10^{-6}$  mol  $L^{-1}$  on the results was investigated. As is shown in Fig. 1, it was indicated that the absorbance difference increases almost linearly up to the concentration of MB up to  $7.5 \times 10^{-6}$  mol  $L^{-1}$ .

The dependence of bromate concentration on the rate of retarded oxidation of MB was studied in the range of  $4.0-28 \times 10^{-3}$  mol  $L^{-1}$ . As is shown

in Fig. 2 the rate of reaction increased up to  $2.0 \times 10^{-3}$  mol  $L^{-1}$  of bromate and this concentration was selected as optimum.



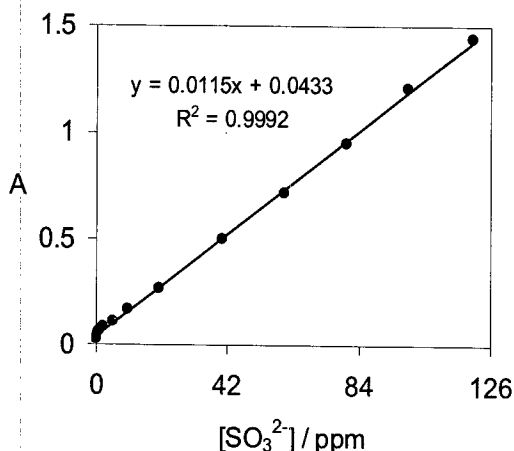
**Figure 1.** Optimization of Methylene blue concentration. Conditions: Sample:  $H_2SO_4$ , 1.0 mL ( $4.0 \text{ mol } L^{-1}$ );  $SO_3^{2-}$ , 400  $\mu g$  and  $BrO_3^-$ , 1.0 ml ( $2.0 \times 10^{-2} \text{ mol } L^{-1}$ ).



**Figure 2.** Optimization of  $BrO_3^-$  concentration. Conditions: Sample:  $H_2SO_4$ , 1.0 mL ( $4.0 \text{ mol } L^{-1}$ ); Methylene blue, 0.5 ml L ( $1.5 \times 10^{-4}$ ) and  $SO_3^{2-}$ , 400  $\mu g$ .

Under the optimum conditions, the effect of ionic strength was studied using a solution of 3.0 mol  $L^{-1}$   $KNO_3$  in the range of 0.0 to 0.9 M. It was observed the change decreased by increasing the ionic strength. Thus, we continued our study in the absence of  $KNO_3$ .

The influence of temperature between 10-40  $^{\circ}C$  was studied and it was observed that increasing the temperature up to 20  $^{\circ}C$ , increased the reaction rate. The decrease in the reaction



**Figure 3.** Calibration curve. Conditions: H<sub>2</sub>SO<sub>4</sub>, 1.0 mL (4.0 mol L<sup>-1</sup>); Methylene blue, 0.5 mL (1.5 × 10<sup>-4</sup>); BrO<sub>3</sub><sup>-</sup>, 1.0 mL (2.0 × 10<sup>-2</sup> mol L<sup>-1</sup>); temperature 25 °C and 210 s.

rate at higher temperature may be due to partial dissociation of MB at higher temperature. Thus, 20 °C was selected as optimum temperature.

Optimized time was found by measuring the absorbance and its change during 0.5-4.5 minutes. The reaction rate increase up to 210 seconds. At still longer time, no considerable effect on the rate of reaction was observed.

### Calibration Curve, Detection Limit and Reproducibility

Calibration graph was obtained by applying the fixed time method under the optimum conditions stated in the previous section. A plot of the absorbance changes after 210 seconds versus sulphite concentration in the reaction mixture are linear in the range of 0.05-120 mg L<sup>-1</sup> sulphite. The linear regression equation relating initial rate, is given in equation (2). It is in agreement with the proposed rate equation (1)

$$A = 0.0115 C + 0.0433 \quad (R^2 = 0.9992, n = 6) \quad (2)$$

Where C is concentration (mg L<sup>-1</sup>) of sulphite and A is the change in absorbance. The experimental limit of detection is 0.04 mg L<sup>-1</sup> (three times of noise). The relative standard deviation (R.S.D. %) of determination of 4.0 and 20.0 mg L<sup>-1</sup> (n = 6) of sulphite were 0.9 and 0.5 %, respectively. Table 1 summarizes the figures of merit of the method.

**Table 1.** Features of the method

Equation	$\Delta A = 0.0115 C + 0.0433$
Regression coefficient	$R^2 = 0.9992, n = 6$
Linear range <sup>a</sup>	0.05-120
Detection limit <sup>a</sup>	0.04
Quantitation limit <sup>a</sup>	0.05
R.S.D. % <sup>b</sup>	
Low level	0.9
High level	0.5

<sup>a</sup>mg L<sup>-1</sup>

<sup>b</sup>4.0, 20.0 mg L<sup>-1</sup> of sulphite

A: Change in absorbance, C: Concentration of sulphite in mg L<sup>-1</sup>

### Study of Interferences

The effect of various cations (as Na<sup>+</sup> or K<sup>+</sup> salts) and anions (as NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>) on the determination of 2.0 mg L<sup>-1</sup> of sulphite ion were studied. Interfering species were added to the samples at higher concentrations than those usually present in real samples and the obtained signal were compared with those containing no foreign species. The results are listed in Table 2.

**Table 2.** Tolerance limit of diverse ions on the determination of 2.0 mg L<sup>-1</sup> sulphite ion

Foreign ions	Tolerated ratio of foreign ions to sulphite
Li <sup>+</sup> , Na <sup>+</sup> , K <sup>+</sup> , Ba <sup>2+</sup> , Mg <sup>2+</sup> , NH <sub>4</sub> <sup>+</sup> , Cu <sup>2+</sup> ,	100
Cl <sup>-</sup> , CO <sub>3</sub> <sup>2-</sup> , HCO <sub>3</sub> <sup>-</sup> , CH <sub>3</sub> CO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> , F <sup>-</sup>	100
Ni <sup>2+</sup>	80
Fe <sup>3+</sup>	60
NO <sub>2</sub> <sup>-</sup> *	25
Co <sup>2+</sup>	12
Hg <sup>2+</sup> , Ag <sup>+</sup>	10
Pb <sup>2+</sup> , Cd <sup>2+</sup>	5
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	0.5
VO <sub>3</sub> <sup>-</sup>	0.07
I <sub>3</sub> <sup>-</sup>	0.03

\*After masking with 5 mL of 0.25% of sulfamic acid.

### Practical Application

The proposed method was validated by applying it to the determination of sulphite in aqueous samples from different sources. After determination of sulphite concentration, the recovery was evaluated by adding different concentrations (5 and 10 mg L<sup>-1</sup>) of standard to each sample. The obtained results are listed in Table 3.

**Table 3.** Recovery of sulphite from water samples

Sample	Sulphite(mg L <sup>-1</sup> )	Recovery % <sup>b</sup>	
	Found <sup>a</sup>	1st addition	2nd addition
Well water 1	10.2 ± 0.2	97	99
Well water 2	8.2 ± 0.1	99	103
River water1	3.6 ± 0.07	99	98
River water2	4.1 ± 0.09	101	97
Tap water 1	5.3 ± 0.1	101	100
Tap water 2	4.7 ± 0.08	97	99

<sup>a</sup>Average of five determination ± standard deviation and after dilution 1:3.

<sup>b</sup>Addition of 5.0 and 10.0 mg L<sup>-1</sup> of sulphite.

### CONCLUSION

The possibility of determination of sulphite by preparing spectral absorbption of MB was investigated.

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Then reactants concentrations were optimized. After optimizing ionic strength, temperature and time, calibration curve was plotted.

Calibration curve gives us equation of curve ( $A = 0.0115 [\text{SO}_3^{2-}] + 0.0433$ ) and coefficient of correlation ( $R^2 = 0.9992$ ) that using it for evaluating of method and are criterion of precision. Finally, the effect of foreign ions that might be present in real samples was investigated and validity of method was examined by using it on the determination of sulphite in real samples. Recovery as a touchstone of applicability of method was determined.

The present method is rapid, accurate and precise. It can be attractive to analyze under ground water, river water and tap water because cheaper reagents are used, the analytical system is simple and needs only minimum maintenance. Most anions and cations do not interfere. The advantages of this method compared with the existing ones are summarized in Table 4.

**Table 4.** Comparative study

Features	Ref [19]	Ref [20]	Proposed method
Detection limit	0.4 mg L <sup>-1</sup>	0.08 mg L <sup>-1</sup>	0.04mg L <sup>-1</sup>
Linear range	1-20 mg L <sup>-1</sup>	0.08-40 mg L <sup>-1</sup>	16-80 mg L <sup>-1</sup>
R.S.D. % range	-	0.9-2.0 mg L <sup>-1</sup>	0.5-0.9 mg L <sup>-1</sup>

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