

The Spectroscopic Determination of Aqueous Sulfite Using Ellman's Reagent

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Abstract

At room temperature and in N_2 -purged pH 8 aqueous phosphate buffer, the sulfite ion (SO_3^{2-}) cleaves the disulfide bond in Ellman's reagent, 5-5'-Dithiobis-(2-nitrobenzoic acid), and displaces one of the chromophoric anions, producing a yellow hue. Absorption spectroscopy of the resulting chromophore, 5-mercapto-2-nitrobenzoate, permits a quantitative assay of the initial sulfite concentration using a linear relationship in accordance with the Beer-Lambert law. This colorimetric method was validated with several test reactions and was determined to be accurate to within a 2% average relative error. Its sensitivity has been demonstrated down to 0.8ppm sulfite ($10^{-5}M$). One drawback of Ellman's reagent is that it will react with other compounds containing thiol groups; yet, in some interesting cases, such as in the water boiler industry, other such compounds are absent and this technique would provide a reliable, inexpensive, and field-usable method for determining sulfite concentration.

Introduction

Sulfur-oxy anions play an important role in our nutrition and environment. One such anion, the trigonal pyramidal sulfite (SO_3^{2-}), has several fascinating and uncommon uses. Sulfites are currently used to control microbial growth, bleach certain food starches, and prevent spoilage of certain perishable foods, beverages, and pharmaceuticals. Several examples of where sulfites are used include shrimp, dried apricots, dried raisins, lettuce and other vegetables, and potato chips. Furthermore, their antioxidant and antimicrobial properties play an important role in wine-making. The sulfites either inhibit or kill bacteria and wild

yeast, thus encouraging rapid and clean fermentation of wine grapes. The paper and pulp industries use sulfites as additives to improve strength, increase brightness, and lower pulping energy. The photographic industry uses sulfite for the testing of fixing baths, stop baths, and developers. Sulfite is used in the water boiler industry as an oxygen scavenger, which binds well with excess oxygen to form sulfate. Yet sulfite concentrations in boiler and process waters must be monitored routinely to avoid overtreatment, which can lower the pH and cause rust. Waste treatment plants remove residual chlorine in wastewater by injection of either sulfur dioxide gas or a solution of sodium sulfite or sodium bisulfite (strong reducing agents). In practice, most plants subject to dechlorination requirements run relatively high-sulfite residuals to ensure complete chlorine removal at all times. However, excess sulfite in wastewater effluents may be harmful to marine life. Because sulfites are widely used in industries, a method for monitoring its levels is a necessity for environmental safety.

Sulfite may also be harmful to some humans with allergies. A 1985 study by the Federation of American Societies for Experimental Biology found that in some patients with asthma, ingesting sulfites might lead to an acute and sometimes life-threatening attack of asthma. This study prompted the U.S. Food and Drug Administration (FDA) to issue regulations prohibiting the use of sulfites on fresh fruits and vegetables that are usually eaten raw. In addition, the FDA now requires other foods, such as wine, to indicate the presence of sulfites on their labels. The FDA estimates that one in 100 people is sulfite-sensitive to some degree, but for the 10 percent of the population who are asthmatic, up to 5 percent are at risk of having an adverse reaction to the substance.

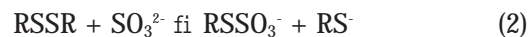
Current methods of analysis of sulfite include basic test strips, an iodide-iodate titration,¹ and sulfite acidification to sulfur dioxide gas. These kits vary in price and accuracy. However, a spectroscopic method would be cheaper because it would only require a spectrophotometer, which is already available in most scientific labs.

Further, it would be convenient, reliable, and accurate at low concentrations. The aim of the research was to determine a simple spectroscopic method to measure sulfite in solution using 5-5'-Dithiobis-(2-nitrobenzoic acid), or DTNB (also called Ellman's reagent). The method established in the research is also compared to previous studies on DTNB.

Since the first synthesis of 5-5'-Dithiobis-(2-nitrobenzoic acid), DTNB, by George L. Ellman in 1959 for the quantitative analysis of mercaptans, "Ellman's reagent" has been commonly used for the determination of thiols in biochemical samples.² An organic disulfide, DTNB reacts with aliphatic thiol compounds to produce equimolar concentrations of a mixed disulfide and a luminescent thiol, 5-mercapto-2-nitrobenzoate (MNB), according to Equation 1.



The use of DTNB has been extended to the reaction with sulfite ions (SO_3^{2-}), which displaces the thiol anion forming an organic thiosulfate, called a "Bunte" salt (Equation 2).^{3,4,5,6}



The reaction of DTNB with sulfite ions has led to studies for a spectrophotometric determination of sulfite.^{3,4} While useful in biochemical samples, the method for sulfite analysis proposed by Johnston et. al. may not be effective in aqueous solutions. Such a method may interest the boiler industry. Humphrey et. al. demonstrated the usefulness of a similar method in aqueous solutions stabilized with the disodium salt of ethylenediamine tetraacetate (EDTA). This compound was introduced with the intention of stabilizing the readily oxidizable sulfite in aerated solutions. The method presented here demonstrates an effective sulfite assay in aqueous solutions without the use of EDTA and outlines some of the parameters governing the reaction of DTNB with sulfite and with L-cysteine in the presence of oxygen and ultraviolet light. A detailed comparison of the present method with a commercially available titration kit demonstrates the enhanced accuracy (<2% avg.

relative error) and range of analysis (<1ppm).

Experimental

Reagents. Ellman's reagent, DTNB (99%), was obtained from Aldrich Chemical Co. in Milwaukee, Wis.; laboratory-grade sodium sulfite was obtained from Flinn Scientific, Inc., in Batavia, Ill.; L-cysteine (98+%) and Tris-[hydroxymethyl]-aminomethane (99.9+%) were obtained from Sigma Chemical Co. in St. Louis, Mo. These reagents were used without further purification.

Solutions. The DTNB solutions were prepared $1.0 \times 10^{-3}\text{M}$ by dissolving the DTNB with about 1mL 95% ethanol and diluting with N_2 -purged pH 8.0 0.1M Tris buffer. Further dilutions yielded $1.0 \times 10^{-4}\text{M}$ and $5.0 \times 10^{-5}\text{M}$ DTNB solutions. The sodium sulfite solutions were prepared in N_2 -purged water and diluted in volumetric flasks to obtain 10^{-4}M samples. Aqueous $2.5 \times 10^{-4}\text{M}$ L-cysteine solutions were similarly prepared.

Equipment. Absorption measurements were made with a Perkin Ellmer Model Lambda 3B UV-visible spectrophotometer, using quartz cuvettes with 1cm path lengths. Comparison testing was done with a Hach Inc. titration analysis 1-200mg/L sulfite detection kit (Model SU-5, Cat. #1480-02).

Procedure. For the reaction of DTNB and cysteine, the cysteine was added in fivefold excess relative to DTNB to ensure complete dissociation of MNB. The cysteine was directly introduced into the solutions. The concentration of DTNB was made in fivefold excess relative to the concentration of the sulfite solutions. The DTNB and sulfite were reacted in equal volumes of 5mL, and all absorbance measurements were taken from about 2mL aliquots in triplicate. Reference solutions included a 50:50 mixture of DTNB in pH 8 buffer solution and deionized water.

Results and Discussion

DTNB-Cysteine reaction. In order to determine the em of MNB, a standard reaction was followed with DTNB and L-cysteine. It was observed that the cysteine completely cleaved the DTNB and formed double the molarity of the chromophoric thiol (MNB). Note that this is contrary to Equation

1; the cysteine is oxidized and forms cystine, while the DTNB is reduced and cleaved at the disulfide bond. Using a spectrophotometer, the peak absorbance at 410nm of 10^{-4}M MNB was determined to be 1.660 with a molar extinction coefficient of $16,600\text{cm}^{-1}\text{M}^{-1}$. This value does not equal the em previously reported.^{1,4,7} Humphrey et. al. documented $15,500\text{cm}^{-1}\text{M}^{-1}$ as the em value of MNB. Discrepancies in the em values of MNB are also noted by Riddles et. al.⁸

DTNB-Sulfite reaction. It was hypothesized that a solution of $1.0 \times 10^{-4}\text{M}$ sulfite would react similarly with DTNB to form $1.0 \times 10^{-4}\text{M}$ MNB (see Figure 1). This is in accordance with Equation 2; the sulfite displaces the chromophoric thiol, MNB. The absorbance measurements of this solution at 410nm were near 1.600. This value is within 4% of the absorbance from the DTNB-cysteine reaction, justifying the reaction of DTNB with sulfite as seen in Equation 2. The absorbance of MNB was then measured as a function of the initial sulfite concentration for various samples. The data demonstrated a linear relationship between the absorbance of MNB and initial concentration of sulfite (see Tables 1 and 2). The sensitivity of this method has been demonstrated as low as 10^{-5}M sulfite.

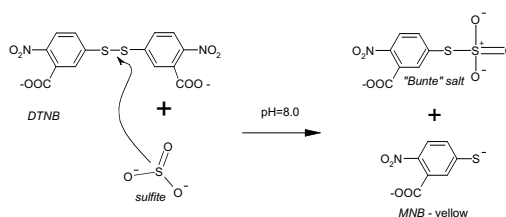


Figure 1. MNB (5-mercapto-2-nitrobenzoate) is displaced by sulfite.

| mL MNB | mL SO_3^{2-} | mL Tris Buffer | $[\text{SO}_3^{2-}]$ (mol/L) | Avg. Abs. |
|--------|-----------------------|----------------|------------------------------|-----------|
| 5 | 5 | 0 | 1.0×10^{-4} | 1.610 |
| 5 | 4 | 1 | 8.0×10^{-5} | 1.300 |
| 5 | 3 | 2 | 6.0×10^{-5} | 1.008 |
| 5 | 2 | 3 | 4.0×10^{-5} | 0.718 |
| 5 | 1 | 4 | 2.0×10^{-5} | 0.434 |

Table 1. Comparison of the concentration of sulfite with the absorbance of MNB produced in the DTNB-sulfite reaction.

| mL MNB | mL SO ₃ ²⁻ | mL Tris Buffer | [SO ₃ ²⁻] (mol/L) | Avg. Abs. |
|--------|----------------------------------|----------------|--|-----------|
| 5 | 5 | 0 | 1.0x10 ⁻⁴ | 1.520 |
| 5 | 4 | 1 | 8.0x10 ⁻⁵ | 1.230 |
| 5 | 3 | 2 | 6.0x10 ⁻⁵ | 0.940 |
| 5 | 2 | 3 | 4.0x10 ⁻⁵ | 0.637 |
| 5 | 1 | 4 | 2.0x10 ⁻⁵ | 0.335 |
| 10 | 1 | 9 | 1.0x10 ⁻⁵ | 0.186 |

Table 2. Comparison of the concentration of sulfite with the absorbance of MNB produced in the DTNB-sulfite reaction with adjusted spectroscopic reference solution.

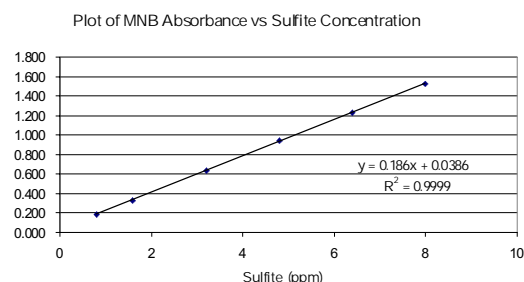


Figure 2. Adjusted sulfite determination graph.

| Sulfite sample | Actual Mass (g) | Calculated Mass (g) | Error |
|-----------------|-----------------|---------------------|-------|
| A | .1261 | .1298 | 2.90% |
| B | .0946 | .0966 | 0.06% |
| C | .0631 | .0656 | 4.00% |
| D | .0315 | .0318 | 0.83% |
| Average % error | — | — | 1.95% |

Table 3. Calculated percent error from the DTNB-sulfite method.

| Standard sample, Sulfite (ppm) | Hach Inc. kit (ppm) | Error |
|--------------------------------|---------------------|-------|
| 16 | 14.7 | 8.0% |
| 12.8 | 11.5 | 10.0% |
| 9.6 | 8.3 | 13.3% |
| 6.4 | 5.8 | 10.0% |
| 1.6 | 1.9 | 20.0% |
| Average % error | — | 12.3% |

Table 4. Calculated percent error for the Hach Inc. detector at low sulfite concentrations.

Validation. This method was validated with several standard sulfite samples and determined to be accurate to within a 2% average relative error (see Table 3) for dilute concentrations of sulfite (10⁻⁵ – 10⁻⁴ M). The method was then tested against the accuracy

of a commercial sulfite detection kit. The Hach Inc. titration analysis sulfite detection kit had an average relative error of 12% (see Table 4) for dilute sulfite solutions.

Stability of Sulfite Solutions. It was initially observed that the absorbance of MNB was less than expected and decreased over time after the DTNB-sulfite reaction. The presence of oxygen was found responsible for the rapid degradation of sulfite solutions, and all dilutions were then done with nitrogen-purged water. This differs from the EDTA in pH 7 buffer shown in the results of Humphrey et. al. Johnston et. al. similarly conducted their experimentation in pH 7 buffer, but neglected to indicate sulfite degradation. Nevertheless, there is still uncertainty over the time dependency of the nitrogen-purged DTNB-sulfite reaction. The absorbance of MNB still decreased significantly after a period of about 20 minutes. There is a possibility that UV light will degrade MNB or promote a side reaction. In a DTNB-sulfite reaction placed in separate cuvettes, one in the dark, and one in UV light, MNB in the cuvette in the presence of UV light had a significantly lower absorbance. Nevertheless, all reactions were conducted expeditiously.

Application. Other existing techniques for sulfite analysis are either very expensive (such as analyzing the SO₂ gas produced under acid treatment) or have limited accuracy and utility (such as iodine-iodate titration). The present technique, however, could be translated into a simple color chart comparison for field use. One drawback to the use of DTNB is that it will react with any other compounds present that contain thiol groups; yet, in some interesting cases such as in the water boiler industry, other such compounds are absent, and this technique would provide a reliable, inexpensive, and field-usable method for determining sulfite concentration.

Boiler water is normally basic (pH 8–11), and the added sulfite (20–80ppm) and phosphate (30–80ppm) prevent scaling or corrosion on the walls of the boiler tanks. The reducing agent, sulfite, acts as an oxygen scavenger, forming the sulfate ion and preventing electrochemical side reactions.

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Boiler water also does not contain any other compounds that may skew the results. The method was used to test the boiler water with sufficient accuracy. Methods employed were the filtration of the boiler water sediment and dilution (by a factor of 10) to bring the sulfite levels in the desired range of 0.8–8ppm. The results of the DTNB-sulfite method were corroborated with the Hach Inc. detector kit, which is accurate at higher sulfite concentrations. This showed that the DTNB-sulfite method is applicable and advantageous in the water boiler industry. The method is currently being researched for use in other industries, such as food and wine. **MURJ**

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