Spectrophotometric determination of sulphite and thiamin hydrochloride using proton transfer reaction-application to water sample and pharmaceutical formation

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ABSTRACT

In this research, an easy, accurate and rapid spectrophotometric method for determination of sulphite and thiamin hydrochloride is described. This method used proton transfer reaction between sulphite ion with alizarine at (pH 6.73) to develop red colour adduct that bears maximum absorption at 524 nm. This method was developed to determination of thiamin hydrochloride by bleaching the red colour (alizarine-sulphite product) at the optimum conditions established for the determination of sulphite. Beer’s law is obeyed in the concentration range (10-1000) μg/25ml (0.4-40) of sulphite and 10-1500, μg/25ml (0.4-60) for thiamin hydrochloride at the selected wavelength, the molar absorptivity 2.6×10⁴ l.mol⁻¹.cm⁻¹ for SO₃²⁻ and 3.37×10⁴ l.mol⁻¹.cm⁻¹ for thiamine hydrochloride, Sandell's index for sensitivity 0.00307 μg.cm⁻² for sulphite and 0.0100 μg.cm⁻² for B₁, the precision (RSD) of the method is ±0.23- ±0.51% sulphite and ±0.15- ±0.25 thiamine hydrochloride. Good recoveries of sulphite and thiamine hydrochloride from water samples and pharmaceutical formation are achieved using the proposed method.

Introduction

Sulphites are compounds that contain the sulphite ion. Sulphites are naturally occurring matter in human body as well as in some foods and all wines to some extent. Sulphites are often used as a food preservative or enhancer in dried foods, shrimp are treated with sulphites on fishing vessels, in all food products containing at least 10 ppm of sulphite, sulphites are one of the food allergens causing difficult breathing in minutes after eating a food containing sulphites, people with aspirin sensitivity and asthmatics are at high risk for sulphites, the potential symptoms include swelling of the throat, sneezing, and migraine [1]. Thiamine hydrochloride (B₁ or aneurine) [2], named as the "thio-vitamine" is a sulfur-containing vitamin. B₁ was isolated and characterized in 1920, therefore it is named B₁, because it was the first organic compounds recognized and discovered as a vitamin. B₁ is soluble in water and some organic solvents. All living organisms use thiamine in their biochemistry, but it is only synthesized in bacteria, fungi, and plants. People need it to form adenosine triphosphate (ATP), which every cell of the body uses for energy[3] (Scheme 1).
B1 help for strengthen the immune system also help the human body's ability to withstand stressful conditions so it was called an anti-stress vitamin. Beriberi as a severe chronic disease results from vitamin B1 deficiency. Beriberi has a serious complications on the nervous system, and gastrointestinal system [4]. Thiamine in vitamin supplements can be destroyed and cleaved the thiamine molecule by sulphites. Sulphite cleaving the pyrimidine ring from the thiazole ring at the methylene bridge in the thiamine hydrochloride structure, the rate of this reaction was increased by acidic solution. Eating food containing sulphate can have thiamine-antagonistic activities by rumen bacteria which reduced sulphate to sulphite [5,6]. Alizarine is an organic compound known as Mordant red 11. Alizarine or 1,2-dihydroxyanthraquinone. Alizarine was the first natural pigment synthesised in 1869, throughout history alizarine was derived from roots of madder genus' plants and had been used as a prominent red dye [7] (Scheme 2).

Several spectrophotometric methods are used for determination of sulphite such as quantification of sulphite ions in environmental samples [8], wine samples [9], other method based on a discoloring reaction between sulphite and flower anthocyanins in alkaline medium [10], another spectrophotometric methods used phosphate buffer at pH 7 with malachite green [11], or based on the bleaching of brilliant green dye by sulphite [12]. A flow injection analysis used for determination of sulphite in food and beverages [13], also a flow-injection determination of sulphite in white wines involving gas diffusion through a concentric tubular membrane [14].

B1 can be determined with spectrophotometric methods in pharmaceutical preparation based on reaction of B1 with 7-chloro-4-nitrobenzoxadiazole to produce brown color complex measured at 434 nm [15], another used 1,2-naphthoquinone-4-sulphonate measured at 487 nm [16], others spectrophotometric methods using coupling reaction with diazotized sulfanilic acid [17], or diazotization reaction of B1 with procaine hydrochloride which reacted with sodium nitrate and hydrochloric acid to form a red water-soluble azo dye in basic medium [18], or using prussian blue reaction [19], also a flow injection method used for determination of B1 with diazetized metoclopramide [20], and a fluorimetric method can be used for the determination of B1 [21].

In this research sulphite and thiamine hydrochloride was determined with alizarine using proton transfer reaction, then applied the proposed method to various water samples and pharmaceutical formation respectively.

**Experimental**

**Apparatus**

Spectral and absorbance measurements are carried out using shimadzu UV-160, UV-Visible computerized double-beam spectrophotometer with 1-cm matched cells. The measurements of pH carried out by HANA pH meter.

**Chemicals**

The chemicals used in this research are of analytical grade reagent.

**Standard sodium sulphite solution:** this solution is prepared by dissolving a known amount of sodium sulphite anhydrous (Fluka) in distilled water containing 2 ml of 0.01% D(-) fructose as a stabilizer [22]. This solution is standardized by iodimetry. Working standard sulphite solution are prepared by suitable dilutions of the standard with distilled water. A standard sulphite solution is stable for at least 3 days.

**Thiamine hydrochloride (1000 μg/ml) solution.** It was prepared by dissolving 0.1000 g of B1 in distilled water and the final volume bring to 100 ml using a volumetric flask, this solution was stable for at least 2 days. Working solution of 100 μg / ml B1 solution was prepared by appropriate dilution of the stock solution with distilled water.

**Alizarine solution 3×10⁻³ M:** This solution was prepared by dissolving 0.0720 gm of alizarine (BDH) in distilled water and the volume was completed to

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**Scheme 1: Sulphite and thiamine hydrochloride structure**

**Scheme 2: Alizarine structure**
100 ml in a volumetric flask with distilled water, this solution was stable for at least 3 days. 

**Procedure for dosage forms (100 μg/ml) solution.**  
For B_{1} tablet, a ten tablets (200 mg B_{1}-HCl/tablet) were weighed, crushed and mixed well. A portion equivalent to 0.01 g was weighed and dissolved in 50 ml of distilled water, stirring and shaken well then filtered using filter paper, the final volume bring to 100 ml in a volumetric flask with distilled water. This solution was then treated as done in a recommended procedure.  

For capsule. At least ten capsules (5 mg B_{1}-HCl/capsule) of B_{1} drug were weighed. A portion equivalent to 0.01 g was weighed and dissolved in distilled water, mixed and shaken well, filtered using filter paper and diluted with distilled water to 100 ml in a volumetric flask, then it was proceeded as described under recommended procedure.

**Results and discussion**

To study the optimum conditions for the determination of sulphite and thiamine hydrochloride, the effect of many parameters on the intensity of the colored system have been studied then the optimum conditions have been selected.  

**Effect of buffer**

The pH of the solution (sulphite ion and alizarine solution) was measured before this study which found to be (6.73) then the effect of pH was studied by adding different amounts (0.1-3.0) ml of 0.1M of sodium hydroxide and 0.1 M of hydrochloric acid solutions to an aliquot containing 20 μg of sulphite. The intensity of absorption were measured against the reagent blank (Fig.1), while effect of adding of acid or base are omitted because of giving no useful results.

![Fig. 1: Effect of pH](image)

**Table 1: Effect of buffer solutions**

| ml of buffer solution | sodium acetate-acetic acid | phosphate buffer | KH_{2}PO_{4}-NaOH | Imidazole-HCl | KH_{2}PO_{4}-NaOH |
|----------------------|---------------------------|------------------|------------------|--------------|------------------|
|                      |                           |                  |                  |              |                  |
| 1                    | 0.071                     | 0.093            | 0.091            | 0.126        | 0.095            |
| 2                    | 0.062                     | 0.071            | 0.081            | 0.104        | 0.071            |
| 3                    | 0.049                     | 0.053            | 0.063            | 0.063        | 0.062            |
| 4                    | 0.030                     | 0.041            | 0.044            | 0.044        | 0.048            |
| 5                    | 0.018                     | 0.026            | 0.017            | 0.017        | 0.031            |

**Reaction time and temperature**

The effect of reaction time studied when the colour development was followed at room temperature and 0, 10, 20, 30,40 and 50°C in thermostatically controlled water-bath so the intervals against reagent blank treated similarly. The experimental results indicate that the absorbance reached maximum after 2 minutes at room temperature and remains constant more than 2 hours therefore, room temperature and 2 minutes reaction time were chosen.

**Alizarine amount**

The effect of alizarine amount was studied by adding different amount (0.5, 1.0, 1.5, 2.0, 2.5, 3.0) ml of 3×10^{-3} M alizarine to solution containing (10, 25, 50, 75, 100, 150, 200, 300) μg.ml^{-1} sulphite, it was obtained that the absorbance reached maximum on using 2 ml of 3×10^{-3} M alizarine with r^{2} =0.9999204. Therefore, 2 ml of 3×10^{-3} M alizarine was used in all subsequent work.

**Effect of surfactants**

Various kind of surfactants were studied such as Triton X-100 (non-ionic surfactant), sodium dodecyl sulphate (anionic surfactant), cetyltrimethylammonium bromide and cetylpyridinium chloride (cationic surfactants). It was obtained from the results in (Table 2) that the addition of all kinds of surfactants gave no useful effect. Therefore, omitted in this study.

**Table 2: Effect of surfactant**

| Surfactant                      | Absorbance / ml of surfactant added |
|---------------------------------|------------------------------------|
|                                 | 1.0          | 3.0          | 5.0          | λ_{max}     |
| Cetyltrimethylammonium bromide  | 0.092        | 0.021        | 0.003        | 529         |
| Sodium dodecysulphate           | 0.133        | 0.102        | 0.093        | 533         |
| Cetyl pyridinium chloride       | 0.081        | 0.051        | 0.010        | 526         |
| Triton X-100                    | 0.112        | 0.097        | 0.054        | 518         |
| Without surfactulant            | 0.203        |              |              | 524         |

**Effect of time on the colour development**

The effect of time on the absorbance of the produced complex at the wavelength of maximum absorption at 524 nm, was studied under the optimal conditions.
Calibration curve
A procedure of calibration curve carried out by using a series of calibrated flasks (25 ml) containing an increasing volume of (0.1-10) ml of 100 µg .ml⁻¹ sulphite solution and 2 ml of 3.0×10⁻³ M alizarine reagent solution then standing for 3 minutes and dilution with distilled water to the mark, the absorbance are measured at 524 nm against the reagent blank. The range of Beer’s law was 10-1000 µg /25ml sulphite i.e (0.4-40) ppm (Fig.2). The molar absorptivity was 2.6 ×10⁴ l.mol⁻¹.cm⁻¹, and the Sandall sensitivity was 0.00307µg.cm⁻².

Final absorption spectrum: When sulphite and thiamine hydrochloride in an aqueous solution was treated according to the recommended procedure, the absorption spectrum showed a maximum absorption at 524 nm. The reagent blank shows low absorption at above wavelength (Fig.4 and 5).

Accuracy and precision
Under the experimental conditions which included in the recommended procedure, the precision and recovery of the method have been checked for 20,100, and 300 µg of sulphite, also the method have been checked for 100, 300 and 500 of thiamine hydrochloride, the recovery and relative standard deviation are shown in table 3, indicating the method has good accuracy and precision.

Table 3: Accuracy and precision

| Amount of SO₃²⁻ taken, µg/25 ml | Recovery*, % | Relative standard deviation*, % |
|-------------------------------|--------------|---------------------------------|
| 20                            | 99.50        | ± 0.49                          |
| 100                           | 100.29       | ± 0.23                          |
| 300                           | 100.46       | ± 0.51                          |
| Amount of B₁, µg              | Recovery(%) of B₁* | Relative standard deviation*, % |
| (200 mg /tablet) NDI-Iraq     |              |                                 |
| 100                           | 99.91        | 100.13 ± 0.15                   |
| 300                           | 100.03       | 100.24 ± 0.17                   |
| 500                           | 100.12       | 99.93 ± 0.25                    |

* Average of five determinations.

Nature of the reaction between SO₃²⁻ and alizarine.
the reaction ratio of sulphite with alizarine reagent was studied by using Job's method of the continuous variations and mole ratio method. The obtained results (Fig.6 & 7) showed that the molar ratio of sulphite to alizarine was found to be 1:1.
Hence the coloured product may have the following suggested mechanism (Scheme 3):

![Scheme 3: Suggeste mechanism of sulphite-alizarine](image)

**Effect of foreign ions**

The effect of many foreign ions on the determination of 20 μg sulphite per 25 ml of solution is examined under the experimental conditions used. Also the effect of interferences is examined on the determination of thiamin hydrochloride (100 μg/25ml). The results obtained are summarized in table 4 & 5.

**Table 4: Effect of foreign ions**

| Foreign ion | Form added | Relative error (%) |
|-------------|------------|--------------------|
|              |            | 50                 | 150               | 300               | 500               |
| I           | KI         | -1.82              | -2.56             | -6.15             | -9.33             |
| Cl-         | NaCl       | +2.33              | +20.54            | +40.21            | +73.33            |
| F-          | NaF        | +25.51             | +37.33            | +61.66            | +95.76            |
| Br-         | KBr        | -1.78              | -2.55             | -4.15             | -5.25             |
| CO$_3^{2-}$ | Na$_2$CO$_3$ | +5.67          | +8.43             | +20.32            | +33.17            |
| SO$_4^{2-}$ | Na$_2$SO$_4$ | -0.29            | -0.43             | -0.59             | -0.69             |
| S$_2$O$_3^{2-}$ | Na$_2$S$_2$O$_3$ | -4.82          | -2.55             | -0.03             | -0.82             |
| PO$_4^{3-}$ | NaH$_2$PO$_4$  | -0.47           | -0.58             | -0.27             | -0.63             |
| C$_2$O$_4^{2-}$ | Na$_2$C$_2$O$_4$ | -1.08         | -1.15             | -1.38             | -1.47             |
| CH$_3$COO$^-$ | CH$_3$COONa | -0.87           | -0.91             | -0.86             | -0.89             |
| La$^{3+}$   | La$_2$(CO$_3$)$_3$ | -0.32         | -0.21             | -0.10             | -0.42             |
| Bi$^{3+}$   | Bi(NO$_3$)$_3$ | -0.09         | -0.27             | -0.31             | -0.90             |
| Ba$^{2+}$   | BaCl$_2$, 2H$_2$O | -0.66        | -0.62             | -0.52             | -0.78             |
| NH$_4$$^+$  | NH$_4$Cl    | -0.49             | -0.91             | -0.52             | -0.56             |
| Fe$^{3+}$   | Fe(NO$_3$)$_3$, 5H$_2$O | +0.10       | +3.82             | +11.29            | +23.20            |
| Fe$^{2+}$   | FeSO$_4$.7H$_2$O | +0.91        | +2.82             | +5.29             | +7.20             |
| Mg$^{2+}$   | MgSO$_4$.7H$_2$O | -0.26        | -0.27             | -0.63             | -0.34             |
| Hg$^{2+}$   | Hg(NO$_3$)$_2$.H$_2$O | -0.98      | -0.79             | -0.41             | -1.22             |
| Zr$^{4+}$   | ZrOCl$_2$.5H$_2$O | -1.19      | -0.79             | -0.52             | -1.23             |

The results were shown in (Table 4) indicated that the method seemed to be selective except towards Cl$, F^-$ and CO$_3^{2-}$ which interfere seriously, also ferric ion in high concentrations was interfered.

**Table 5: Effect of interferences on the determination of 100 μg B$_1$**

| Interferences | Recovery (%) of 100 μg B$_1$/μg of interference added |
|---------------|-------------------------------------------------------|
|               | 100 | 500 | 1000 |
| Acacia        | 100.21 | 100.09 | 100.12 |
| Glucose       | 100.12 | 99.97 | 100.31 |
| Lactose       | 100.33 | 100.13 | 100.40 |
| Menthol       | 100.42 | 100.36 | 100.17 |
| Starch        | 100.11 | 99.98 | 99.98 |

**Application of the method:**

The proposed method was successfully applied for the determination of sulphite and thiamine hydrochloride in various water samples and pharmaceutical preparations respectively. A good recovery was obtained from the results showed in Table (6 & 7).
The proposed method is simple, sensitive, inexpensive and does not require temperature control or solvent extraction step. Sulphite in different water samples has been carried out by the rapid reaction of sulphite with alizarine at pH 6.73 to form a red water soluble and stable product, which exhibit maximum absorption at 524 nm. Beer's law were obeyed in the concentration range of (0.4–40) ppm of sulphite with a molar absorptivity of $2.6 \times 10^4$ L.mol$^{-1}$ .cm$^{-1}$, Sandell sensitivity index of 0.00307 μg.cm$^{-2}$ respectively, and relative standard deviation of $±0.23 – ±0.51$, the proposed method is developed for determination of B$_1$ by bleaching red colour of sulphite-alizarine product, Beer's law were obeyed in the concentration range of (0.4–60) ppm of sulphite with a molar absorptivity of $3.37 \times 10^4$ L.mol$^{-1}$ .cm$^{-1}$, Sandell sensitivity index of 0.0100μg.cm$^{-2}$ respectively, and relative standard deviation of $±0.15 – ±0.25$ depending on the concentration level. Good recoveries of sulphite and B$_1$ from various water samples and pharmaceutical preparation are achieved using the proposed method.

### Table 6: Determination of sulphite in water samples

| ml of water sample | Sulphite added, μg | Tap water | Well water from village in Mosul | Natural spring water | Sea water from white middle sea in Turkey |
|-------------------|-------------------|-----------|---------------------------------|---------------------|------------------------------------------|
|                   |                   | Recovery (%) |
| 1                 | 5                 | 101.23    | 101.06                          | 99.97               | 100.22                                   |
| 3                 | 5                 | 101.33    | 101.14                          | 100.03              | 101.46                                   |
| 5                 | 5                 | 100.94    | 101.22                          | 100.12              | 101.91                                   |
| 7                 | 5                 | 101.53    | 101.43                          | 100.06              | 100.87                                   |
| 1                 | 50                | 102.42    | 101.53                          | 99.98               | 100.43                                   |
| 3                 | 50                | 102.13    | 101.28                          | 99.96               | 100.22                                   |
| 5                 | 50                | 102.55    | 101.62                          | 99.98               | 102.51                                   |
| 7                 | 50                | 101.43    | 101.33                          | 100.08              | 102.63                                   |
| 1                 | 100               | 100.93    | 100.94                          | 100.12              | 101.31                                   |
| 3                 | 100               | 100.94    | 100.53                          | 100.11              | 101.83                                   |
| 5                 | 100               | 102.53    | 100.21                          | 100.23              | 102.44                                   |
| 7                 | 100               | 102.41    | 101.63                          | 100.28              | 102.58                                   |

### Table 7: Determination of thiamine hydrochlorid

| Amount of B$_1$, μg | Recovery (%) of B$_1^{+}$ (200 mg/tablet) | Recovery (%) of B$_1^{+}$ (5 mg capsul) |
|---------------------|------------------------------------------|----------------------------------------|
| 50                  | 101.33                                  | 100.94                                 |
| 100                 | 100.62                                  | 100.72                                 |
| 200                 | 100.85                                  | 101.07                                 |

The value of t-test was calculated [24]. It did not exceed the theoretical values for five degrees of freedom at the 95% confidence level when the proposed method has been compared with literature method [25] as shown in Table 8 & 9.

### Table 8: The result of t-test analysis.

| Drug                  | Pharmaceutical preparation | t-test |
|-----------------------|----------------------------|--------|
| B$_1$ (NDI-Iraq)      | Tablet                     | 0.4284 |

### Table 9: The result of t-test analysis.

| Sample                | Tap water | Natural spring water |
|-----------------------|-----------|----------------------|
| The value of t-test   | 1.0137    | 0.7213               |

Comparison of the methods

Table 10, shows a comparison between the proposed method and the other literature spectrophotometric methods through some measured analytical parameters.

### Table 10: Comparison of the methods.

| Analytical parameters | Present method | Literature method |
|-----------------------|----------------|-------------------|
|                       |                | Sulphite ion [25] | B$_1$ [26] |
| Method                | Alizarine      | Fer(II)-1.10-PHENANTHROLINE | Chromate-DPC |
| pH                    | 6.73           | 3.00               | 1.41      |
| Buffer                | -------        | Glycine-HCl        | 2 N H$_2$SO$_4$ |
| $\lambda_{max}$ (nm)  | 524            | 510                | 543       |
| Reaction time (min)   | Immediately    | Immediately        | 5         |
| Stability period (minutes) | 120         | 120                | 120       |
| Beer’s law range (ppm)| $0.4-40 \text{SO}_2^\text{2}^-$ | $0.04-4.0$ | $0.4-40$ |
| Molar absorptivity (1.mol$^{-1}$.cm$^{-1}$) | $2.6 \times 10^4 \text{SO}_2^\text{2}^-$ | $4.56 \times 10^4$ | $1.5 \times 10^4$ |
| R.S.D. (%)            | $±0.23 – ±0.51$ | $±0.52 –±1.31$ | $±0.31±0.57$ |
| Colour of the product | Red            | Orange –red        | Pink-violet |
| Application of the method | Pharmaceutical preparations for B$_1$ and water samples for SO$_2^-$ | Water samples | Pharmaceutical preparations |

The results in (Table 10) are indicated that the method is sensitive and has an application part.

### Conclusion

The proposed method is simple, sensitive, inexpensive and does not require temperature control or solvent extraction step. Sulphite in different water samples has been carried out by the rapid reaction of sulphite with alizarine at pH 6.73 to form a red water soluble and stable product, which exhibit maximum absorption at 524 nm. Beer's law were obeyed in the concentration range of (0.4–40) ppm of sulphite with a molar absorptivity of $2.6 \times 10^4$ L.mol$^{-1}$.cm$^{-1}$, Sandell sensitivity index of 0.00307 μg.cm$^{-2}$ respectively, and relative standard deviation of $±0.23 – ±0.51$, the proposed method is developed for determination of B$_1$ by bleaching red colour of sulphite-alizarine product, Beer's law were obeyed in the concentration range of (0.4–60) ppm of sulphite with a molar absorptivity of $3.37 \times 10^4$ L.mol$^{-1}$.cm$^{-1}$, Sandell sensitivity index of 0.0100μg.cm$^{-2}$ respectively, and relative standard deviation of $±0.15 – ±0.25$ depending on the concentration level. Good recoveries of sulphite and B$_1$ from various water samples and pharmaceutical preparation are achieved using the proposed method.
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التقدير الطيفي للكبريتيت وهيدروكلوريد الثيامين باستخدام تفاعل انتقال البروتون - التطبيق على نماذج مائية ومستحضرات صيدلانية

باسمة أحمد عبدالهادي
قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق

ملخص

يتضمن البحث طريقة طيفية بسيطة وسريعة لتقدير الكبريتيت وهيدروكلوريد الثيامين. تعتمدت الطريقة المقترحة على تفاعل الكبريتيت كاشف الاليزارين عند دالة حامضية 6.73 لتكون ناجح أحمرون الناتج الأحمر (كبريتيت – اليزارين) في الظروف المثلى لتقدير الكبريتيت. وتنبع الطريقة قانون بير في نطاق التركيز (10-10000) ميكروغرام / 25 مل (0.4-40) من الكبريتيت و 10-15000 ميكروغرام / 25 مل (0.4-60) لهيدروكلوريد الثيامين عند الطول الموجي المختار ومعامل الامتصاص المولاري 2.6 × 10^{-4} لتر. مول^{-1} . سم^{-1} لإليكبريتيت و 3.37 × 10^{-4} لتر. مول^{-1} . سم^{-1} لهيدروكلوريد الثيامين أما دلالة ساندل للحساسية فقد كانت 0.00307 لكبريتيت و 0.0100 لهيدروكلوريد الثيامين والانحراف القياسي النسيم تراوح بين ±0.23 لكبريتيت و ±0.15 لهيدروكلوريد الثيامين وكانت استرجاعية الطريقة جيدة للكبريتيت وهيدروكلوريد الثيامين في النماذج المائية والمستحضرات الصيدلانية على التوالي.