

Estimation of thiamine hydrochloride and sulphite using charge transfer complex reaction

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Abstract

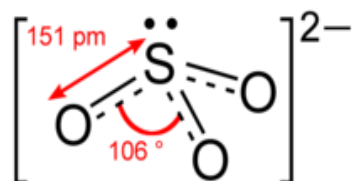
A new spectrophotometric method is used for estimating Thiamine hydrochloride in its pure form and pharmaceutical formulations, the present method depends on the reaction between Thiamine hydrochloride and chrome azurol S to form a red complex which exhibit maximum absorption at 510 nm, the addition of Sulphite to the resulted red complex is bleaching it and this step was used for the determination of Sulphite which is considered an attacker of Thiamine hydrochloride, therefore, this method was developed for determination of Thiamine hydrochloride in presence of Sulphite. The linearity of the proposed method was obeyed Beer's law from 2 to 48 and 0.04 to 2.4 ppm for Thiamine hydrochloride and Sulphite respectively. Also, the method sensitivity was measured by molar absorptivity values $5.9 \times 10^4 \text{ l.mol}^{-1} \cdot \text{cm}^{-1}$ and $1.68 \times 10^4 \text{ l.mol}^{-1} \cdot \text{cm}^{-1}$ for Thiamine hydrochloride and Sulphite respectively. The present method accuracy (average recovery) 100.06% and 100.04% the precision (RSD) of the method is ± 0.98 - $\pm 1.27\%$ and ± 0.76 - ± 1.47 of Thiamine hydrochloride and Sulphite respectively. This method was applied for the estimation of B1 and SO_3^{2-} in pharmaceutical preparations and various water samples respectively.

Introduction

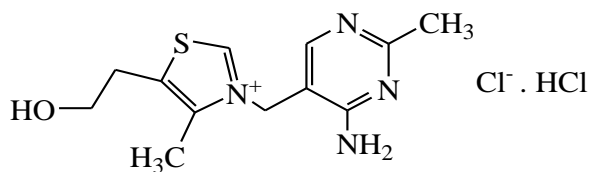
Thiamine hydrochloride was discovered in 1897, also it is known in chemistry as Vitamin B₁ (Scheme 1) [1]. Food is the important source of B₁. The disorders resulted from the deficiency of B₁ called beriberi, Korsakoff's syndrome, and Korsakoff's psychosis, which were treated with using B₁ as food supplement [2]. B₁ is an organosulfur compound, very soluble in water, glycerol and methanol, structure of B₁ consists of an amino pyrimidine and thiazole ring linked together by methylen bridge. B₁ is stable in cold storage conditions and acidic medium, while heat and alkaline medium makes it unstable [3].

Sulphite (Scheme 1) naturally occurs in human body, fermented foods, also Sulphite is a additives to all kind of meats, canned goods, wine, juices, dried fruit and seafood, Sulphite has been effected on lactic acid through the process of producing prevalent bacteria during wine production, that fact has been examined by many studies which have been illustrated the inhibitory properties of Sulphite on it. Sulphite additives have been intended primarily for controlling the growth of microbial, and preventing the spoilage of food or browning [4]. Sulphite is counted among food allergens. People were exposed to an elevated risk when they

were eating food with Sulphite additives especially those having asthmatics and salicylate sensitivity (aspirin sensitivity). The potential symptoms were: difficult breathing, migraine, sneezing, throat swelling, the anaphylaxis as well as life-threatening reactions were considered to be rare [5].



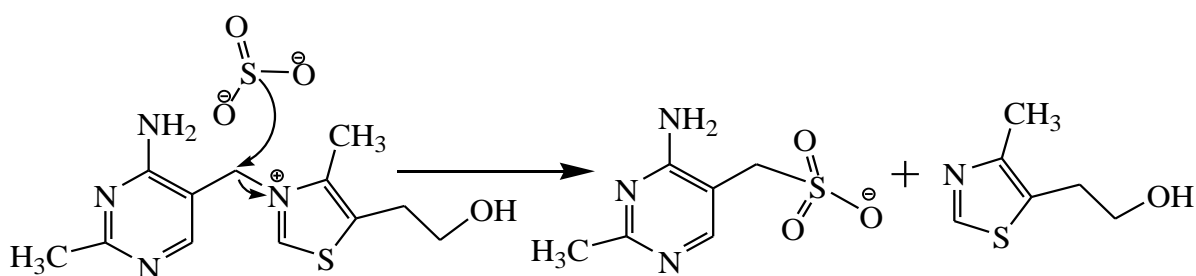
Sulphite ion
 SO_3^{2-}
 80 g/mol



Thiamine hydrochloride
 $\text{C}_{12}\text{H}_{17}\text{ON}_4\text{S}\cdot\text{Cl}\cdot\text{HCl}$
 Mw = 337.27 g / mol

Scheme 1 : Structure of sulphite and thiamine hydrochloride

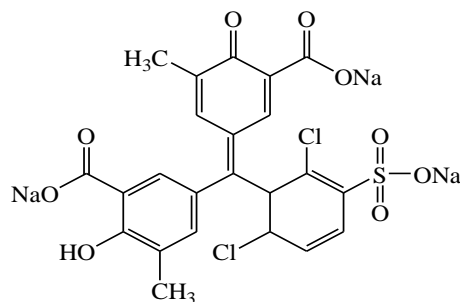
Sulphite was attacked Thiamine hydrochloride at the methylene bridge in the structure, that was lead to cleave ring of pyrimidine from thiazole, and this is the source of the important relationship between Thiamine hydrochloride and Sulphite (Scheme. 2). Acid solution was increased the attacking of Thiamine by Sulphite, this fact was studied from many researches on some kind of dogs in deferent ages, the dogs have been diagnosed with a deficiency of Thiamine resulted from feeding the dogs with meat treated by Sulphite. also B₁ is degraded in shell and raw fish via enzyme thermolabile thiaminases. [6 and 7].



Scheme 2: Sulphite attacked thiamine hydrochloride

A spectrophotometric methods were suggested for estimating B₁ and Sulphite such as determination of B₁ by precipitation of sulphur as barium sulphate [8], or produced a brown color which resulted from the reaction between B₁ and 7-chloro-4-nitrobenzoxadiazole [9] measured at 434 nm, also B₁ reacted with 1,2-naphthoquinone-4-sulphonate and measured at 487 nm[10]. Various technique have been used in the determination of B₁ using fourier transform infrared [11], other method based on coupling between multivariate calibration of partial least square and spectrophotometry[12], or determination B₁ and B₆ in Injection formulation [13]. Other spectrophotometric method estimating B₁ Spectiphotometrically in pharmaceutical forms, tablet and capsule dosage[14]. Tablets in Bangladesh [15], or using diazotized coupling reaction with sulfanilic acid [16], or Procaine HCl[17]. Moreover, using prussian blue dye to spectrophotometric assay of B₁[18], also, diazotized metoclopramide has been used for evaluating B₁ with a flow injection [19], as well as, others techniques was used for determining B₁ with a fluorimetric method [20], enzymatic catalytic method [21] or high performance liquid chromatography [22]. A spectrophotometric quantification of Sulphite was reported in literatures in various samples such as: environmental samples [23], wine

drink samples [24], dried fruits and vegetables [25]. Others method based on using malachite green dye with phosphate buffer at pH 7 [26], or depends on visible color changes [27], or using a flow injection analysis for estimating Sulphite in food and beverages [28], Finally, Sulphite has been evaluated in beer sample [29]. Chrome azurol S (CAS) as an organic reagent (Scheme 3) is also named as mordant blue, the properties of CAS is a red to brown powder, and dissolves easily in water. CAS was almost used for evaluation of several cations such as: iron, titanium, samarium(III), aluminium(III), and europium(III) [30,31]. The study aims to suggest an easy and affordable spectral method to study both vitamin B₁ as well as Sulphite and the possibility of applying the proposed method to pharmaceutical products of B₁ and Sulphite in various water samples.



Chrome azurol S or 5-[(3-carboxy-5-methyl-4-oxo-2,5-cyclohexadien-1-ylidene)-(2,6-dichloro-3-sulfophenyl)methyl]-2-hydroxy-3-methylbenzoic acid tri sodium salt
 $C_{23}H_{13}Cl_2Na_3O_9S$, Molecular Weight $605.28 \text{ g.mol}^{-1}$

Scheme 3 : Structure of CAS molecule

Chemicals and Equipment:

Instrumental

Spectrophotometric readings were reported with UV-Visible double-beam computerized recording spectrophotometer (shimadzu UV-160) and 1-cm cells. pH reading have been carried out using HANA pH meter.

Chemical Materials

All the chemical materials which were used in this work are of analytical grade reagents.

Thiamine hydrochloride (1000 µg / ml) solution. 0.1000 g of B₁ was dissolved in 100 ml of distilled water using a volumetric flask, this solution was stable for at least 3 days. 100 µg/ml B₁ working solution was prepared by appropriate dilution of the stock solution with distilled water.

Standard Sodium Sulphite solution: 0.1 N solution of sodium Sulphite was prepared approximately by dissolving a known amount of anhydrous sodium Sulphite (Fluka) in distilled water containing 2 ml of 0.01% D(-) fructose as a stabilizer [32]. Then standardized this solution with iodimetry. Working solution was prepared using a suitable dilutions of the standard solution with distilled water. This solution was stable more than three days.

Chrome azurol S ($1 \times 10^{-3} \text{ M}$) solution. 0.0605 g of chrome azurol S (BDH) was dissolved in distilled water, then complete the volume with distilled water in a 100 ml volumetric flask, this solution was stable more than 1 week.

Formaldehyde solution (0.1%): formaldehyde solution (Fluka) was prepared by diluted 0.1 ml of formaldehyde with distilled water in 100 ml volumetric flask.

Procedure for dosage forms

Ten capsules (5 mg B₁-HCl/capsule) of Thiamine hydrochloride drug were weighed. Then, an equivalent weight to 0.0100 g was dissolved with distilled water, mixed and filtered by Whatman No.1 filter paper, after that, this solution was diluted to 100 ml in a volumetric flask with distilled water. Also, a 10 tablets (200 mg B₁-HCl/tablet) have been weighted, then crushed, weighed an equivalent to 0.0100 g, dissolved in distilled water with stirring then filtered with filter paper and diluted to 100 ml in with distilled water using a volumetric flask.

Results and Discussion

Optimum Conditions

Many experimental conditions have been affected on the intensity of the colored system, for this reason, those various parameters were studied to choose or selected the optimum conditions.

Effect of Addition Acid and Base

Several kinds of acids 0.1 N of H₂SO₄, HCl, HNO₃ and CH₃COOH were added in different quantities 0.5-3.0 ml, as well as, the same additions of bases quantity were carried with 0.1 N such as NaOH, KOH and Na₂CO₃. The experimental work resulted that, there is no useful effect of the addition of any kind of acid or base, therefore this study was omitted.

Effect of Buffer

The measured pH was 4.7 after the addition of CAS, So that, various kinds of buffers at pH 4.7 were prepared as shown in Table 1. It was obtained from the tabulated results in Table 1 that all of these buffers decrease the intensity of absorbance, therefore, adding of buffer solutions was not recommended.

Table 1: Effect of buffer solutions

Buffer solution	Citric acid-sodium citrate	Tartaric acid-NaOH	Sodium acetate-acetic acid	Citric acid-NaOH	Succinic acid-NaOH	KH-Phthalate
Absorbance	0.0310	0.0430	0.0440	0.0520	0.0610	0.0290
Absorbance without buffer			0.2120			

Effect of Time and Temperature

The effect of time on colour evolution of the reaction mixture was studied. This study was carried out using thermostatically water bath at room temperature and 10, 20, 30,40 and 50°C. Figure 1 shows that the maximum absorbance was after 2 min. at room temperature, so this time at room temperature have been selected for colour development.

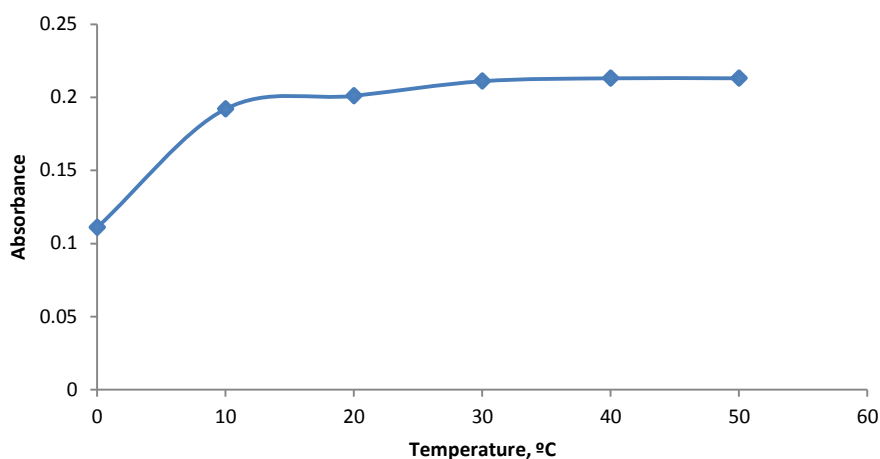


Fig.1: Effect of temperature on the colour intensity

Effect of Reagent Quantity

Various volumes 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml of 1×10^{-3} M CAS quantity have been studied. The experimental results showed that the intensity of absorbance reached maximum when 2 ml of 1×10^{-3} M CAS was used, also the value of r^2 was equal to 0.9992. Therefore, 2 ml of 1×10^{-3} M CAS was selected to be added for the subsequent experiments.

Effect of Surfactant Agents

Three kind of surfactants have been studied: Triton X-100 as a non-ionic surfactant, sodium dodecyl sulphate as a anionic surfactant, cetyltrimethylammonium bromide and cetylpyridinium chloride as a cationic surfactants. The results tabulated in Table 2 show that the addition of any kind of surfactants has no effect on the colour system. So that this study was omitted.

Table 2: Effect of surfactant

Surfactant	Absorbance / ml of added surfactant					
	0.5	1.0	2.0	3.0	4.0	5.0
Triton X-100	0.2080	0.1880	0.1350	0.0920	0.0240	0.0040
Sodium dodecylsulphate	0.2000	0.1890	0.1120	0.0530	0.0220	0.0070
Cetyl pyredinium chloride	0.2090	0.1780	0.1330	0.1110	0.0920	0.0060
Cetyltrimethylammonium bromide	0.2090	0.1980	0.1280	0.1210	0.0820	0.0060
Without addition of surfactant	0.2190					

Stability of The Colored System:

The effect of time on the absorbance of the colored product was studied with various quantities of Thiamine hydrochloride under the optimum conditions. Figure 2 shows that the color of product was formed immediately and become stable after three minutes, Moreover, the absorbance was remained constant more than one hour.

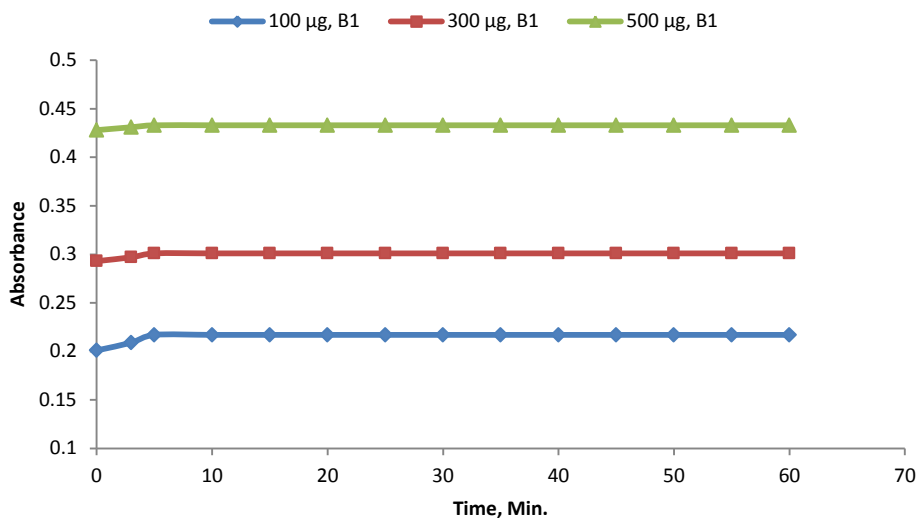


Fig. 2: Stability of the colored product.

Calibration graph and final absorption spectrum

A series of 25 ml volumetric flasks containing 0.5-12.0 ml of 100 µg /ml of B₁ solution, 2 ml of 1×10^{-3} M CAS solution was added for studying the calibration curve of B₁. After standing for two minutes, the volumes were diluted to the mark with distilled water, then, measured the absorbance of red complex at 510 nm against the reagent blank solution. The linearity of the proposed method was obeyed Beer's law between 2 - 48 ppm. The values of molar absorptivity and Sandell's sensitivity were 5.9×10^4 l.mol⁻¹.cm⁻¹ and 0.005716 µg.cm⁻² respectively (Fig.3).

The addition of Sulphite to the mixture of Thiamine hydrochloride and chrome azurol S, lead to bleach the red colour which was formed between B₁ and the reagent. The bleaching method was used to estimate Sulphite in various water samples. Therefore, a various amount 0.1-6 ml of 10 µg/ml of SO₃²⁻ solution have been added to the colored product (B₁-CAS), (500 µg B₁/25 ml +2 ml of 1×10^{-3} M CAS), all flasks were diluted with distilled water to the mark, then measured as above against reagent blank. Beer's law range was 0.04- 2.4 ppm of SO₃²⁻.The molar absorptivity and Sandell's sensitivity were 1.68×10^4 l.mol⁻¹.cm⁻¹, and 0.00476 µg.cm⁻² respectively (Fig. 4).

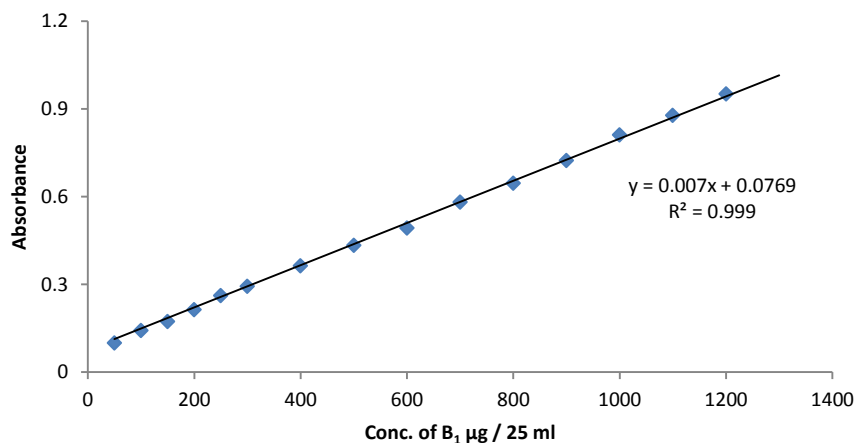


Fig. 3: Calibration graph of B₁

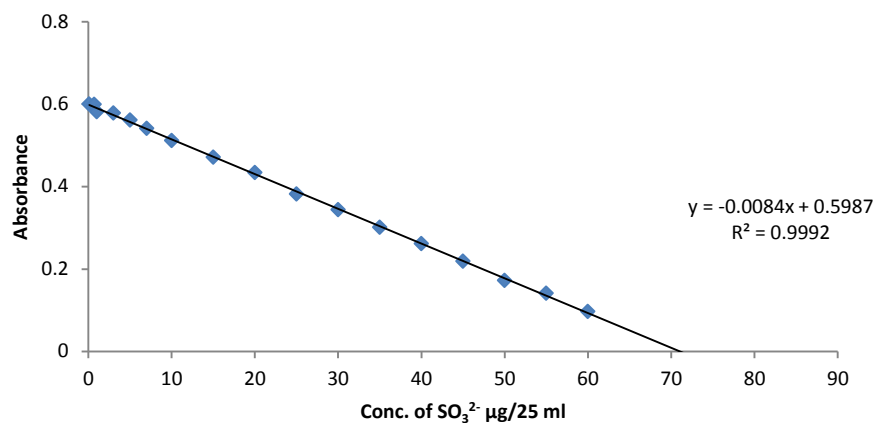


Fig. 4: Calibration graph of SO₃²⁻

When B₁ and SO₃²⁻ were treated according to the optimum conditions, the final absorption spectra at maximum wavelength 510 nm. was shown in Figure 5 .

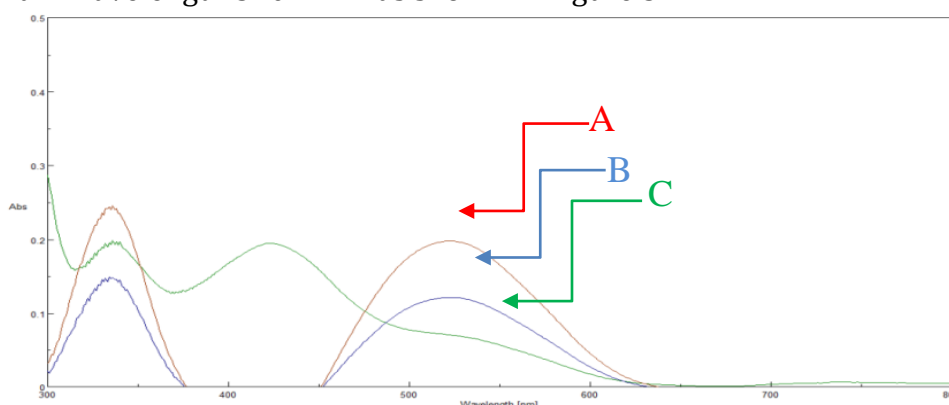


Fig 5 . : Final absorption spectra of 100 μg /25 ml B₁ treated according to the proposed method and measured against A: reagent blank, B: 50 μg of SO₃²⁻/25 ml measured against reagent blank, and C: blank measured against distilled water

Accuracy and Precision

The precision and recovery for the present method was examined for three concentration 50,100 and 300 μg B₁, also, the proposed method was checked for 5,10 and 25 μg SO₃²⁻, relative standard deviation (RSD%) and Recovery percentage (Rec%) were tabulated in table 3 which showed the good values of accuracy as well as precision for the proposed method.

Table 3: Accuracy and precision

Amount of B ₁ taken	Recovery(%) of B ₁ *		RSD*, %
	(200 mg /tablet) NDI-Iraq	(5 mg capsule) SDI-Iraq	
50	99.8700	100.0100	± 0.9800
100	100.2800	100.2300	± 1.0100
300	100.3100	100.4700	± 1.2700
Quantity of SO ₃ ²⁻ taken, μg/25 ml	R*, %	RSD*, %	
5	99.8800	± 0.7600	
10	100.3200	± 1.1100	
25	100.5000	± 1.4700	

* Average of 5 measurements.

Stoichiometric of B₁-CAS

To study the nature of the ratio for B₁ and CAS reaction, continuous variations method (Job's method) has been used. Figure 6 indicate that 1:1 is the molar ratio between B₁ and CAS.

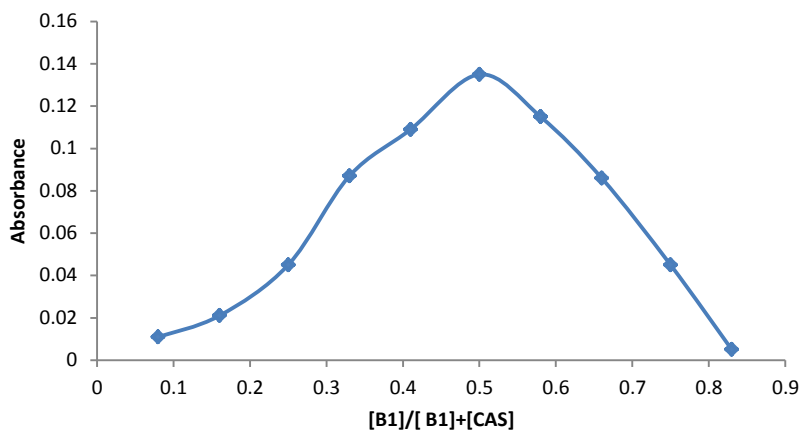


Fig 6.: Job's plot for B₁ – Chrome azurol S.

Hence, Figure 7 shows the mechanism of colored product formation :

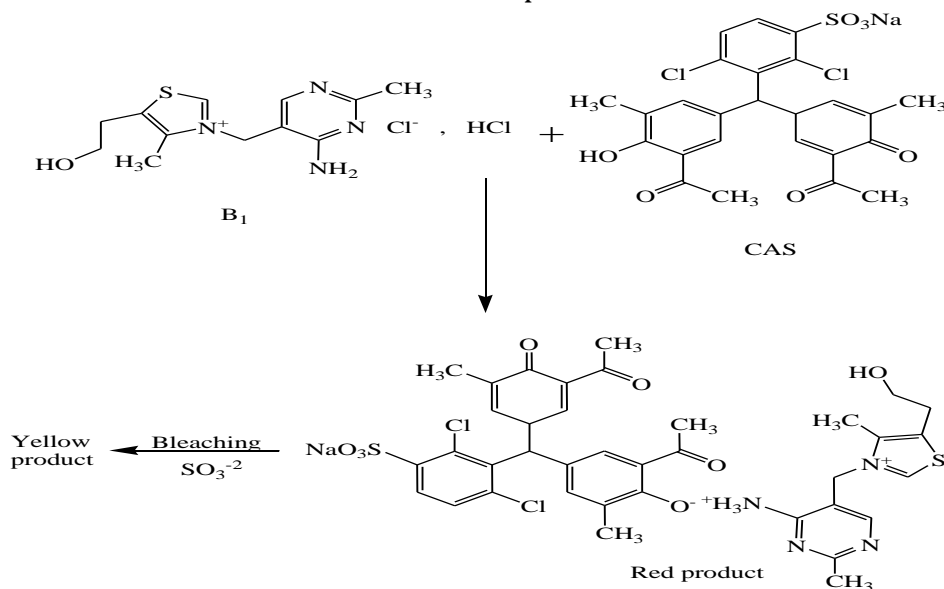


Fig 7 : Suggested mechanism for B₁ – CAS

Application of the present method:

Determination of B₁ and SO₃⁻² in pharmaceutical formulations and various water samples respectively have been applied successfully. Table 4 and 5 showing that the proposed method has a good recoveries when it was applied for tablets and capsule as well as water samples.

Table 4: Application of the present procedure for determination of B₁

Amount of B ₁ , µg	Recovery(%) of B ₁ *	
	(200 mg /tablet) NDI-Iraq	(5 mg capsul) SDI-Iraq
50	101.080	102.100
100	100.500	101.020
200	100.230	100.430

The bleaching method was applied for determination of Sulphite in various water samples, so that (1-7) mL were added to volumetric flasks containing different concentrations of Sulphite (5-100) $\mu\text{g}/25\text{ml}$, then measured the absorption at the chosen wavelength as shown in Table 5.

Table 5: Application of the present work for SO_3^{2-}

ml of water sample	Sulphite added, μg	Recovery (%)			
		Tap water	Well water*	Natural spring water	Sea water**
1	5	101.220	99.930	100.210	99.910
3	5	100.130	100.110	100.290	100.410
5	5	100.250	100.380	100.470	100.160
7	5	100.430	100.140	100.330	100.230
1	50	100.220	100.120	100.320	100.260
3	50	99.970	99.970	100.420	99.960
5	50	99.980	100.340	99.990	100.320
7	50	100.330	100.280	100.100	100.170
1	100	100.190	100.230	100.340	100.180
3	100	100.140	100.490	100.310	100.210
5	100	99.950	100.110	100.410	100.610
7	100	99.970	100.120	99.940	100.510

Water samples applied from * Al-Kaser village in Mosul, ** white middle sea in Turkey(Antalya).

t-test value has been calculated for B_1 tablet. T-test value[33] for 5 degrees of freedom at the confidence level (95%) did not exceed theoretical values when the present method was compared with the literature method [34]as it was tabulated in Table 6.

Table 6: The result of t-test analysis.

Medicine	t-test
B_1 (NDI-Iraq), Tablet	0.6912

Effect of interferences:

Many interferences which was effected on the determination of 100 μg $B_1/25\text{ ml}$ was examined under the optimum conditions. Table 7 was indicate that all kind of interferences was not effect or interfered except in Sulphite case which was interfered seriously.

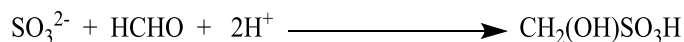
Table 7: Effect of interferences for 100 μg B_1 determination.

Interferences	Recovery(Rec%) of 100 μg B_1 / μg of interfere added		
	100	500	1000
Lactose	100.630	100.330	100.600
Glucose	100.320	99.980	100.410
Acacia	100.110	100.180	100.420
Menthol	100.610	100.320	100.120
Starch	100.630	99.970	99.990
Sulphite	78.520	55.210	32.710

Determination of B_1 in presence of Sulphite

When Sulphite was seriously interfered, so that, the present method was developed to evaluate B_1 in presence of SO_3^{2-} . SO_3^{2-} was removed from the reaction mixture using 0.1% of

formaldehyde solution, the formaldehyde–Sulphite was a very stable system against the oxidation or convert ions to another [35]:



A series of 25 ml calibrated flasks, containing 1 ml of 100 µg B₁ solution, 50-500µg of SO₃²⁻, then added to this mixture an increasing volume 0.5-1.5 ml of 0.1 % formaldehyde solution, shaken and standing for 5 min. 2 ml of 1×10⁻³ M CAS reagent solution was added. Then, the flasks have been diluted to the mark with distilled water, the absorbance were measured at 510 nm against reagent blank as shown in Table 8, which indicate that the removal of 50 -500 µg of Sulphite completely from B₁ solution has been achieved using 1 ml of 0.1% formaldehyde solution as it has been given the nearest values of absorbance when it compare to B₁ solution alone.

Table 8: Determination of B₁ in presence of SO₃²⁻

ml of 0.1% formaldehyde Solution	Recovery% */µg of Sulphite present in 25 ml								
	50	75	100	150	200	250	300	400	500
0.5	85.7798	85.7798	87.1559	89.9082	91.2844	47.7064	50.9174	54.5871	59.6330
1.0	100.000	99.0825	100.458	100.458	99.0825	98.6238	98.1651	99.0825	98.6238
1.5	92.6605	95.8715	96.7889	93.1192	92.6605	95.8715	92.2018	93.5779	92.6606

* Recovery without Sulphite = 100.009%

Comparison of the proposed methods

A comparison between the present method with the literatures methods throughout several analytical measured parameters was shown in Table 9, which were indicated that the present method was so sensitive with best application part.

Table 9: Comparison between the present method with the literature methods.

Analytical parameters	Present method	Literature methods	
		SO ₃ ²⁻ [25]	B ₁ [34]
Method	Chrome azurol S	Malachite green	Chromate-DPC
pH	4.7	7.0	1.41
Buffer solution	-----	Phosphate buffer	2N of H ₂ SO ₄
λ _{max} (nm)	510	615	543
Reaction time (min)	2	-----	5
Stability period (minutes)	2 days	-----	120
Beer's law range (ppm)	(0.04-2.4)SO ₃ ²⁻ (2-48) B ₁	0~ 0.6mg/L	0.4-40
ε (l.mol ⁻¹ .cm ⁻¹)	1.68×10 ⁴ SO ₃ ²⁻ 5.9×10 ⁴ B ₁	-----	1.5×10 ⁴
Recovery%	99.91-100.61 SO ₃ ²⁻ 100.23-102.10 B ₁	95.8%~103.1%	-----
RSD (%)	± 0.98± 1.27 B ₁ ± 0.76 ± 1.47 SO ₃ ²⁻	-----	±0.31±0.57
Product Color	Red	color-fading	Pink-violet
Application of the method	Pharmaceutical preparations and water samples	Food	Pharmaceutical preparations

Conclusion

The present method was considered to be a simple, sensitive, accurate, and not require solvent extraction or temperature control step. Also, the present method was successfully

applied to the estimation of B_1 and SO_3^{2-} in a pharmaceutical formulation and various water samples respectively.

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التقدير الطيفي للثيامين هيدروكلوريد والكبريتيت باستخدام تفاعل معقد انتقال الشحنة

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الخلاصة:

تم استخدام تفاعل نقل البروتون مع الكروم ازورول S في تحديد هيدروكلوريد الثيامين عن طريق تطوير طريقة طيفية بسيطة وسريعة وحساسة في المحلول المائي لتكوين ناتج أحمر يظهر أقصى امتصاص عند الطول الموجي 510 نانومتر ومعامل امتصاص مولاري 5.9×10^4 لتر.مول⁻¹.سم⁻¹ لثيامين هيدروكلوريد و 1.68×10^4 لتر.مول⁻¹.سم⁻¹ للكبريتيت. وتتبع الطريقة قانون بير على نطاق التركيز 2-48 جزء لكل مليون من الثيامين هيدروكلوريد و 0.04-2.4 جزء لكل مليون للكبريتيت. دقة الطريقة (معدل الاسترجاعية) 100.06% من B₁ و 100.04% من SO₃²⁻ والانحراف القياسي النسبي (RSD) للطريقة هي $0.98 \pm - 1.27 \pm$ B₁ و $0.76 \pm - 1.47 \pm$ SO₃²⁻. تم تطبيق هذه الطريقة بنجاح لتقدير ثيامين هيدروكلوريد وأيون الكبريتيت في المستحضرات الصيدلانية وعينات المياه المختلفة على التوالي، كما تم تطوير الطريقة المقترحة لتقدير الثيامين هيدروكلوريد بوجود الكبريتيت.