Study of Vitamin-B_1 Interaction with Dihydroxyanthraquinone Dye and its Thermodynamic Elucidation

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Abstract: Interaction of Alizarin Red-S dye with thiamin (vitaminB1) was studied using spectrophotometric method at room temperature and neutral pH. The yellowish color appeared due to the Alizarin Red-S interaction with vitamin B1. According to the Benesi-Hildebrand equation, the related equilibrium constant, K_b, for the interaction has been determined and the Gibbs free energy of interaction has been calculated. Also the Benesi-Hildebrand equation has been used for assay of vitamin B1 in tablets. Results show that the method has good sensitivity for vitamin B1 and is in good agreement with HPLC method.

Keywords: Thermodynamic study, Binding constant, Thiamin, Gibbs energy.

1. INTRODUCTION

Thiamin (2-[3-[(4-amino-2-methyl-pyrimidin-5-yl) methyl]-4-methyl-thiazol-5-yl] ethanol), is a water soluble vitamin also called vitamin B1 (Figure **1**). It is a natural essential nutrient which is found in rice bran, nuts, banana, soybean, green peas and etc. Also it is added to some kind of foods and drinks to produce enriched products for special purposes. Thiamin was first discovered in 1910 by Umetaro Suzuki in Japan when researching how rice bran cured patients of beriberi [1]. It was first crystallized by Jansen and Donath in 1926 and its chemical composition and synthesis was finally reported by Robert R. Williams in 1935 [2, 3].

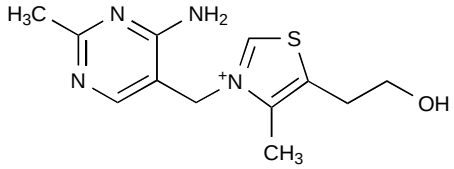

Figure 1: Chemical structure of thiamin.

Phosphate derivatives of thiamin play great roles as co-factor in enzymatic processes inside human body including carbohydrate metabolism and nerve transduction [4]. It is also used in the metabolism of branched chain amino acids and may have non co-enzyme roles in excitable cells [5]. Thiamin level in blood is an important diagnostic factor of sudden infant death syndrome [6]. Also, beriberi is a chronic deficiency disease that results from inadequate dietary intake or impaired absorption of thiamin, as in chronic alcoholism, so quantification of thiamin has vital importance and due to its use in pharmaceutical formulations such as tablets, syrups and ampoules, accurate analytical methods for quality control of thiamin in dosage forms are also needed.

There are various methods for analysis of thiamin in the literature. Liquid chromatography [7-9], spectrophotometry [10], spectroflurimetry [11], voltammetry [12], flow injection analysis [13] were reported. Biosensors found applications in thiamin analysis too [14].

Study of the interaction of small molecules (such as drugs) with dyes, polymers, surfactants, biological macromolecules and etc., is a good way in understanding of the molecules structure and functions [15-17]. In this study, we used spectrophotometric method for the thermodynamic study of interaction between thiamin and Alizarin Red-S (Figure **2**) and its application in assay of thiamin in tablets.

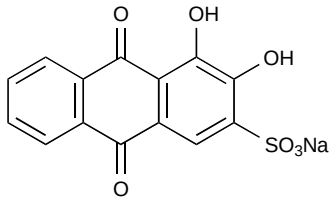

Figure 2: Chemical structure of Alizarin Red-S.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

Thiamin (vitamin B1), Alizarin Red-S and all the salts were purchased from E. Merck (Darmstadt,

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Germany). The chemicals are analytical grade and all of them used without any purification. All solutions were prepared with double distilled water (conductivity≈3μS). Stock solutions of 1 × 10^{-4} M Alizarin Red-S and 1 × 10^{-2} M of thiamin was prepared by dissolving appropriate amounts of them in distilled water.

2.2. Sample Solutions Preparation

An accurately weighed amount of 10 powdered Vitamin B1 tablets was dissolved in water. The excipients were separated by filtration and filter paper was washed three times with water. The filtrate and washing solutions of the tablet samples were transferred into 100 ml calibrated flask and diluted to the mark with water

2.3. Procedure

All pH measurements were made at 25˚C, using Metrohm 744 pH meter (Metrohm, Switzerland). Absorption spectra were recorded on a Perkin-Elmer Lambda 25, double-beam UV–Vis spectrophotometer with 1.0 cm matched quartz cells and thermostat cell holder for adjusting the temperature. An 1100 series Agilent HPLC apparatus (Agilent technologies, USA) equipped with quaternary pump, degasser and diode array detector was used. Separations carried out on a ZORBAX- ODS column (150×4.6 mm I.D., 5μm particle size). Chromatographic method used as below: solvent A: methanol, solvent B: Water, gradient elution: 0-1 min 5% A and 95% B, 1-2 min A reaches to 13% with a linear gradient, 2-5 reaches to 35%, flow rate =1 $ml.min^{-1}$, λ=288nm.

1.5 mL of dye solution (1×10^{-4} M) placed in the cell and different volumes of thiamin stock solution were added. We give the concentration of the stock solution of thiamin higher to avoiding increasing in sample solution volume and consequent dilution in dye solution (thiamin adding solution is less than 20 micro liter) also we add the similar volume of water to the reference cell for considering any dye concentration changes constant. According to the fact that Alizarin Red-S is a pH indicator and due to effect of pH on absorption spectra of dye, pH of solutions adjusted at the neutral pH range (6.5-7.5) using the dilute solutions of sodium hydroxide and hydrochloric acid.

15 minutes after every addition, absorption changes in 422 nm were recorded at 25˚C. Concentration of thiamin in sample solution determined by the Benesi-Hildebrand equation ($1/\Delta A = f\,(1/C_T)$) [19]. It should be noted that, concentration of dye were kept constant by adding very low volumes of thiamin stock solution.

3. RESULTS AND DISCUSSIONS

3.1. Study of Interaction Between Thiamin and Alizarin Red-S

The electronic spectrum of Alizarin Red-S was recorded and variation in maximum absorbance of dye during addition of thiamin was followed spectrophotometrically (Figure **3**). The binding constant was determined from the effect observed in the absorbance of Alizarin Red-S at 422 nm upon addition of the thiamin according with the Benesi-Hildebrand treatment [20]. As shown in Figure **4**, there is a linear relation between 1/ΔA and 1/C, which indicates a certain interaction between dye and thiamin.

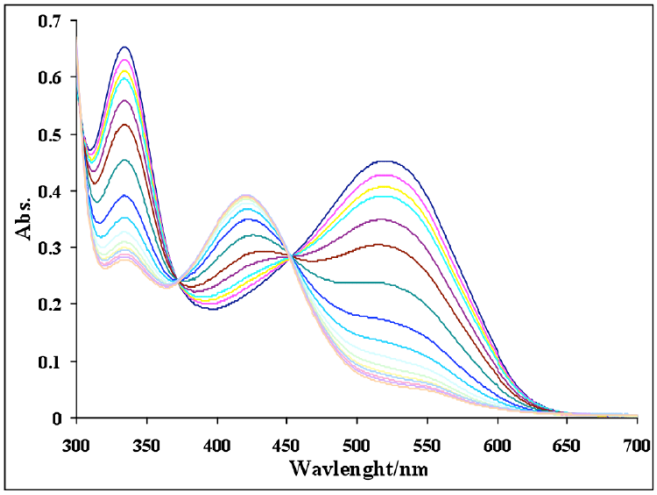

Figure 3: UV-Vis spectra of dye during thiamin addition (thiamin concentration was increased as titration method and it is changed from 10^{-7} up to10^{-3} molar).

3.2. Determination of Binding Constant

The value of the binding constant, K_b, was obtained according to Benesi-Hildebrand equation and the method described previously [18-23]. By assuming that there is only one type of interaction between thiamin and dye in aqueous solution, so the Eqs, (1) and (2) can be established:

$$\text{Alizarin Red-S (ARS) + Thiamin (T)} \rightleftharpoons \text{ARS-T} \quad (1)$$

$$K_b = \frac{[ARS-T]}{[ARS][T]} \quad (2)$$

Where K_b is binding constant, by assuming [ARS-T] $=C_b$:

$$K_b = \frac{C_b}{(C_{ARS} - C_b)(C_T - C_b)} \quad (3)$$

Where C_{ARS} and C_T are the analytical concentrations of Alizarin Red-S and Thiamin in solution, respectively. According to the Beer's law:

$$C_{ARS} = \frac{A_0}{\varepsilon_{ARS} l} \quad (4)$$

and

$$C_b = \frac{A - A_0}{\varepsilon_b l} \quad (5)$$

Where A_0 and A are the absorbance of Alizarin Red-S in the absence and presence of Thiamin, respectively. E_{ARS} and ε_b are the molar extinction coefficients of dye and the complex, respectively. *l* is the light path of the cell (1 cm).

By displacing C_{ARS} and C_b in Eq, (3) by Eqs, (4) and (5), Eq, (6) can be deduced:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_{ARS}}{\varepsilon_b} + \frac{\varepsilon_{ARS}}{\varepsilon_b . K} . \frac{1}{C_T} \quad (6)$$

Plot of (1/ ($A-A_0$)) versus ($1/C_T$) is linear and the binding constant (K_b) can be estimated from the ratio of the intercept to the slope [19, 20]. Figure **4** shows the plot of (1/ ($A-A_0$)) versus ($1/C_T$), at specified experimental conditions. The Gibbs free energy of interaction of Alizarin Red-S with Thiamin can be obtained by the following equation:

$$\Delta G_b^0 = -RT \ln K_b \quad (7)$$

The obtained amount for the Gibbs energy of the interaction between ARS-T is -19.81 kJ/mol.

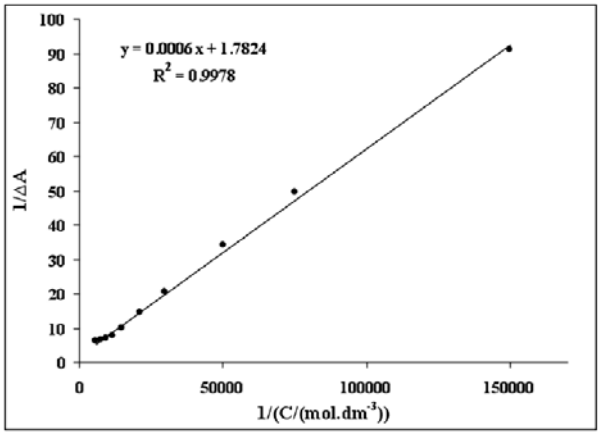

Figure 4: Variation of $1/\Delta A$ vs. $1/C_T$ at 1×10^{-4} M dye solution and pH=7.

3.2. Study of Dye Concentration

For optimization of dye concentration, differences of absorbance (ΔA) during addition of constant amount of thiamin, plotted versus concentration of dye. As shown in Figure **5** in concentrations above 1×10^{-4} M, ΔA reaches to a limiting value and remain constant. So, 1×10^{-4} M selected as optimum concentration of Alizarin Red-S.

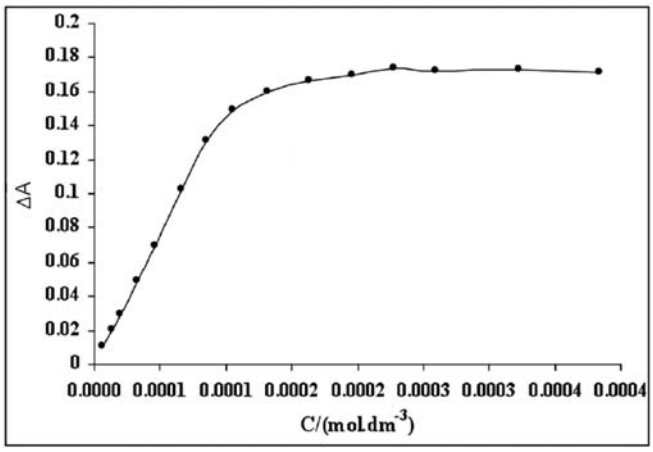

Figure 5: Plot of ΔA vs. C_T at the specified conditions.

3.3. Effect of Time

The absorbance of solution was read after 1, 5, 8, 12 and 20 min of thiamin addition. As shown in Figure **6**, in the range of 5 to 15 minutes no variation in absorbance was observed. So, 10 minutes selected as optimum time.

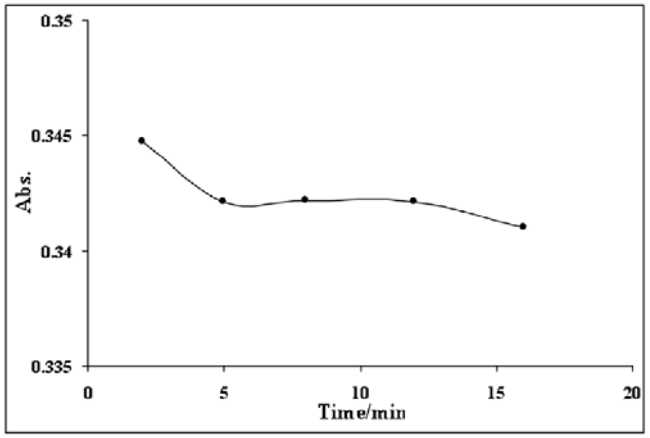

Figure 6: Effect of time on variation of ΔA at constant dye concentration.

4. ANALYTICAL APPLICATION

We used Benesi-Hildebrand equation for determination of thiamin in tablets. Wide linear concentration range of 1.31×10^{-4} – 6.60×10^{-6} M and

Table 1: Quantitative Characteristics of the Proposed Method

Compound	Scott equation	r	LOD*	LDR†
Thiamin	$1/\Delta A=0.0006\times1/C_T+1.176$	0.997	4.36×10^{-5}	1.31×10^{-4}-6.60×10^{-6}

*Limit of detection (mol/L).
†Linear dynamic range (mol/L).

Table 2: Study of Reproducibility and Comparative Study with HPLC Method (each Experiment Done 5 Times)

Labeled (mg)	Found (mg) proposed method	RSD%	Found (mg) HPLC method	RSD%
300	293.64±4.52	1.54	293.20±4.00	1.36

detection limit of 4.36×10^{-7} M made this method suitable for this purpose. Some of the analytical characteristics of this method were summarized in Table **1**.

Further experiments have been performed to assess the reproducibility of the method. Thus, five replicate determinations have been carried out and the relative standard deviation was calculated. RSD% of the method was 1.54 which indicates the proposed method is reproducible. In order to evaluation of the method we used a high performance liquid chromatographic method for determination of thiamin in tablets at 25°C, too. Typical chromatograms of standard thiamin and thiamin sample were shown in Figure **7**. Comparative results of two methods were presented in Table **2**. Results clearly show a good agreement between the methods.

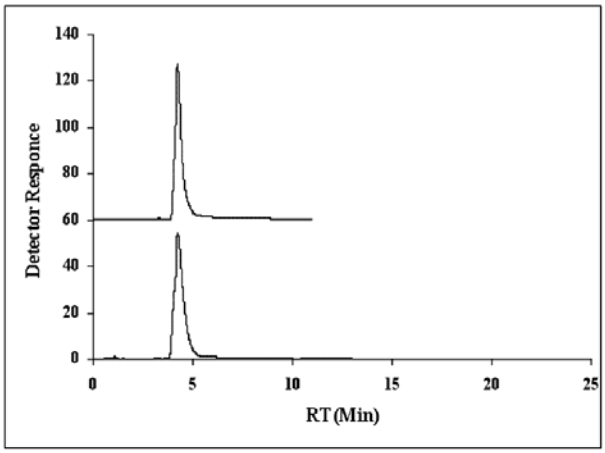

Figure 7: Typical chromatograms of (a) standard thiamin solution, (b) thiamin in tablet sample (solvent A: methanol, solvent B: Water, gradient elution: 0-1 min 5% A and 95% B, 1-2 min A reaches to 13% with a linear gradient, 2-5 reaches to 35%, flow rate =1 ml.min^{-1}, λ=288nm).

5. CONCLUSION

According to the obtained results for binding studies of interaction between Alizarin Red-S and vitamin B1 (Thiamin), it can be concluded that there is a strong interaction in this system. Benesi-Hildebrand equation was used for determination of thiamin analytical concentrations in the solution in the presence of Alizarin Red-S. The obtained results were in good agreement with the results obtained from HPLC method.

REFERENCES

[1] WWW. Supplement-information-library.com/thiamin.html.

[2] Jansen BCP, Donath WF. On the isolation of anti-beriberi vitamin. Proc K Ned Akad Wet 1926; 29: 1390-400.

[3] Williams RR, Ruehle AE. Studies of Crystalline Vitamin B1. XV. C-Methylated 6-Amino- and 6-Oxypyrimidines. J Am Chem Soc 1937; 59: 526-30. http://dx.doi.org/10.1021/ja01282a028

[4] Burgos S, Bohorquez DV, Burgos SA. Vitamin Deficiency-Induced Neurological Diseases of Poultry. Int J Poultry Sci 2006; 5(9): 804-807. http://dx.doi.org/10.3923/ijps.2006.804.807

[5] Bettendorff L. Thiamine in excitable tissues: Reflections on a non-cofactor role. Metabolic Brain Dis 1994; 9: 183-209. http://dx.doi.org/10.1007/BF01991194

[6] Chunag DT, Chuang JL, Wynn RM. Lessons from genetic disorders of branched-chain amino Acid metabolism. J Nutr 2006; 136: 243S-49S.

[7] Bohrer D, Nascimento PCD, Ramirez AG, Mendonca JKA, Carvalho LMD, Pomblum SCG. Determination of thiamine in blood serum and urine by high-performance liquid chromatography with direct injection and post-column derivatization. Microchem J 2004; 78: 71-76. http://dx.doi.org/10.1016/j.microc.2004.03.013

[8] Kimura M, Itokawa Y. Determination of thiamin and thiamin phosphate esters in blood by liquid chromatography with post-column derivatization. Clin Chem 1983; 29: 2073-75.

[9] Echols RE, Miller RH, Foster W. Analysis of Thiamine in Milk by Gas Chromatography and the Nitrogen-Phosphorus Detector. J Dairy Sci 1986; 69: 1246-49. http://dx.doi.org/10.3168/jds.S0022-0302(86)80530-6

[10] Aniceto C, Pereira AV, Costa-Neto CO, Fatibello-Filho O. Flow-injection spectrophotometric determination of vitamin B1 (thiamine) in multivitamin preparations. Lab Robotics Automation 1999; 11(1): 739-41. http://dx.doi.org/10.1002/(SICI)1098-2728(1999)11:1<45::AID-LRA6>3.0.CO;2-7

[11] Amjadi M, Manzoori JL, Orooji M. The Use of Crude Extract of Kohlrabi (brassica Oleracea Gongylodes) as a Source of

Peroxidase in the Spectrofluorimetric Determination of Thiamine. Bull Korean Chem Soc 2007; 28: 246-50. http://dx.doi.org/10.5012/bkcs.2007.28.2.246

[12] Aboul-kasim E. Anodic adsorptive voltammetric determination of the vitamin B_1 (thiamine). J Pharm Biomed Anal 2000; 22: 1047-54. http://dx.doi.org/10.1016/S0731-7085(99)00154-5

[13] Chen H, Zhu J, Cao X, Fang Q. Flow injection on-line photochemical reaction coupled to spectrofluorimetry for the determination of thiamine in pharmaceuticals and serum. Analyst 1998; 123: 1017-21. http://dx.doi.org/10.1039/a708762d

[14] Akyilmaz E, Yasa I, Dinckaya E. Whole cell immobilized amperometric biosensor based on Saccharomyces cerevisiae for selective determination of vitamin B_1 (thiamine). Anal Biochem 2006; 354: 78-84. http://dx.doi.org/10.1016/j.ab.2006.04.019

[15] Bordbar AK, Hosseinzadeh R, Omidiyan K. Potentiometric Study on Interaction of Dodecyltrimethylammonium Bromide with α-Amylase. Bull Chem Soc Jpn 2004; 77: 2027-32. http://dx.doi.org/10.1246/bcsj.77.2027

[16] Bordbar AK, Hosseinzadeh R. Binding of cetylpyridinum chloride to glucose oxidase. Coll Surf B 2006; 53: 288-95. http://dx.doi.org/10.1016/j.colsurfb.2006.09.019

[17] Bordbar AK, Hosseinzadeh R, Noroozi MH. Interaction of a homologous series of n-alkyl trimethyl ammonium bromides with egg white lysozyme; Microcalorimetric and spectroscopic study. J Thermal Anal Calorimet 2007; 87: 453-56. http://dx.doi.org/10.1007/s10973-005-6906-2

[18] Kanakis CD, Tarantilis PA, Polissiou MG, Diamantoglu S, Tajmir-Riahi HA. Antioxidant flavonoids bind human serum albumin. J Mol Struct 2006; 798: 69-74. http://dx.doi.org/10.1016/j.molstruc.2006.03.051

[19] Zhong W, Wang Y, Yu JS, Liang Y, Ni K, Tu S. The interaction of human serum albumin with a novel antidiabetic agent-SU-118. J Pharm Sci 2004; 93: 1039-46. http://dx.doi.org/10.1002/jps.20005

[20] Stephanos JJ. Drug-protein interactions: Two-site binding of heterocyclic ligands to a monomeric hemoglobin. J Inorg Biochem 1996; 62: 155-69. http://dx.doi.org/10.1016/0162-0134(95)00144-1

[21] Hosseinzadeh R, Geshlaghi M, Tahmasebi R, Hojjati F. Spectrophotometric study of interaction and solubilization of procaine hydrochloride in micellar systems. Cent Eur J Chem 2009; 7: 90-95. http://dx.doi.org/10.2478/s11532-008-0078-4

[22] Hosseinzadeh R, Geshlaghi M. Interaction and micellar solubilization of diclofenac with cetyltrimethyl Ammonium bromide. Collect Czech Chem Commun 2009; 74; 503-13. http://dx.doi.org/10.1135/cccc2008021

[23] Hosseinzadeh R, Maleki R, Matin AA, Nikkhahi Y. Spectrophotometric study of anionic azo-dye light yellow (X6G) interaction with surfactants and its micellar solubilization in cationic surfactant micelles. Spectrochimica Acta Part A 2008; 69; 1183-87. http://dx.doi.org/10.1016/j.saa.2007.06.022

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