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A Charge Transfer Method for the Determination of Thiamine Hydrochloride Using 2,3- dichloro-1,4-dicyano-5,6-benzoquinone

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Authors' contributions

This work was carried out in collaboration between both authors. Author PU designed the study, author GN performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author PU managed the analyses of the study. Author GN managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this research is to determine a method based on the formation of charge transfer complex between thiamine and 2,3- dichloro-1,4-dicyano-5,6-benzoquinone (DDQ) that is simple, fast, economical and less laborious.
Objectives: To establish the degree of charge transfer complex formation between thiamine and DDQ, to determine the stability of the charge transfer (CT) complex with respect to time, temperature and pH. To apply the charge transfer complex in spectrophotometric determination of the drug, to determine the average recoveries of the drug in pure and commercial forms, to validate the proposed method using International Conference on Harmonization Guideline.
Study Design: A simple and sensitive spectrophotometric method.
Place and Duration of Study: Department of Chemical Engineering, Faculty of Engineering and Technology, Madonna University Nigeria, Akpugo campus) Enugu state, Nigeria and Department of

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pure and Industrial Chemistry, Faculty of Physical Sciences, University of Nigeria, Nsukka, Nigeria between January 2013 and November 2015.

Methodology: Transfer serial volumes of 0.036, 0.04 …… to 0.32 ml in 0.004 step of the standard thiamine (0.001 g/ml) solution equivalent to 5 µg/ml-80 µg/ml to different test tubes. Added 0.2 ml of buffer 8 to each test tube. Finally, add calculated volumes of DDQ (0.001 g/ml) solution in methanol to the content, mix and allow standing for 25 min at 40℃ before analysis at 474 nm against a methanol blank using an ultraviolet, visible spectrophotometer. Two thiamine hydrochloride tablets were grinded on a mortar. An amount equivalent to 0.01 g was weighed and dissolved in some methanol. It was stirred to extract the active ingredient, filtered with What man no. 1 filter paper and made up to 10 ml to give a theoretical 0.001 g/ml concentration. Similar volumes were transferred to different test tubes and prepared as described in the general procedure before analysis at 474 nm.

Results: A simple and sensitive spectrophotometric method is described for the assay of thiamine. The method is based on charge transfer complexation reaction of thiamine as n-electron donor with 2, 3 dichloro-5,6-dicyano -1,4- benzoquinone (DDQ) as π-acceptor to give highly coloured species with 1:1 stoichiometric ratio. The coloured product was quantified spectrometrically at 474nm under the optimized experimental conditions. Beer's law is obeyed over the concentration ranges of 5-80µg/ml. Formation and stability of the complex of thiamine were optimum at pH 8. The Apparent molar absorptivity was calculated to be 1.08 x $10^3 Lmol^{-1}cm^{-1}$ with the corresponding Sandell sensitivity of 2.68. Limit of detection and quantification of the drug based on this method were thiamine 1.23 and 3.37 respectively.

Conclusion: The proposed method was applied successfully to the determination of thiamine hydrochloride in pure and commercial forms with an average recovery of 96.2% and SD = 0.016. Statistical comparison of the results was performed using student's t-test and f-test at P = .05 confidence level.

Keywords: Thiamine hydrochloride; spectrophotometry; charge transfer complex; DDQ.

1. INTRODUCTION

Acceptors are aromatic systems containing electron withdrawing substituent such as nitro, cyano and halogen groups [1]. Electron donors are systems that are electron rich. The interaction between electron donor and acceptor results in the formation of charge transfer complex [2]. The term charge transfer denotes a certain type of complex which results from the interaction of an electron acceptor and an electron donor with the formation of weak bonds [3].

Vitamin B_1 (Thiamine) has its chemical name as 2-[3-[(4-Amino-2-methyl- pyrimidin-5-yl) methyl] -4-methyl – thiazol – 5 – yl] ethanol is a water-soluble vitamin which plays an important biological role in the metabolic process of carbohydrate in the human body [4].Thiamine or Thiovitamine (sulfur-containing vitamin) is the first vitamin of the water-soluble B complex category of vitamins [5]. It is heat labile, and considerable amounts are destroyed during cooking. It is unstable in acidic solutions. It acts like a weak base and can be absorbed on basic ion – exchange materials such as fuller's earth, a property used to concentrate it so that it can be detected at the levels needed for analysis. Thiamine is found in many foods such as pork, liver and whole grains [6]. The accurate estimation of the level of vitamin B_1 in the clinical setting as well as in food is very important [7]. Thiamine undergoes extensive phosphorylation in the liver to form thiamine pyrophosphate (TPP). All living organism use thiamine in their biochemistry, but it is synthesized in bacteria, fungi and plants. Animals must obtain it from their diet [8]. Previous studies have utilized different techniques for the estimation of thiamine hydrochloride which includes: electrochemical analysis method [9], high-performance liquid chromatography [10], spectrofluorimetry [11]. Also, the direct spectrophotometric method has been described for the determination of thiamine hydrochloride in the presence of its degradation products [12].Some of the methods mentioned above, lack sensitivity, they are laborious and involves liquid to liquid extraction procedures.

2. MATERIALS AND METHODS

All absorption measurements were made on 752W UV-Visible Grating Spectrophotometer and UV-1800 Shimadzu with 1 cm glass cell. All chemicals and reagents were of analytical grade

and were used as such. All laboratory reagents were freshly prepared. Pure Thiamine hydrochloride was supplied by Juhel Pharmaceuticals Nig. Ltd. Thiamine hydrochloride tablet was supplied by Genesis pharmaceutical Rumelfield, New York. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone(DDQ,98%) was supplied by Sigma- Aldrich Chemie, Germany.

2.1 General Procedure

Transfer serial volumes of 0.036, 0.04 …… to 0.32 ml in 0.004 step of the standard thiamine (0.001 g/ml) solution equivalent to 5 µg/ml-80 µg/ml to different test tube. Add 0.2 ml of buffer 8 to each test tube. Finally, add calculated volumes of DDQ (0.001 g/ml) solution in methanol to the content mix and leave to stand for 25 mins at 40℃ before analysis at 474 nm against a methanol blank.

2.2 Assay Determination of Thiamine

Two thiamine hydrochloride tablets were grounded on a mortar. An amount equivalent to 0.01 g was weighed and dissolved in some methanol. It was stirred to extract the active ingredient, filtered with what man no. 1 filter paper and made up to 10ml to give a theoretical 0.001 g/ml concentration. Similar volumes were transferred to the different test tube and prepared as described in the general procedure before analysis at 474 nm.

3. RESULTS AND DISCUSSION

3.1 Absorption Spectra

In this study 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol medium was used for the direct determination of thiamine hydrochloride. Charge transfer (CT) reactions have been widely studied [13,14]. A 2,3 dichloro-5-6-dicyano -1,4-benzoquinone solution in methanol displayed absorption peak at 350nm (Fig. 1).

This is similar to the result reported earlier in a preliminary study involving thrombin with DDQ in acetonitrile [15]. Thiamine hydrochloride showed absorption peaks at 229 nm, 254 nm, 269 nm (Fig. 2).

Mixing 2 ml solution of DDQ with the 2 ml solution of thiamine hydrochloride gave a reddish brown colouration upon reaction of yellow DDQ solution and a colourless solution of thiamine hydrochloride. This was suggestive of a charge transfer complex formation which showed a redshift at 474 nm (Fig. 3).

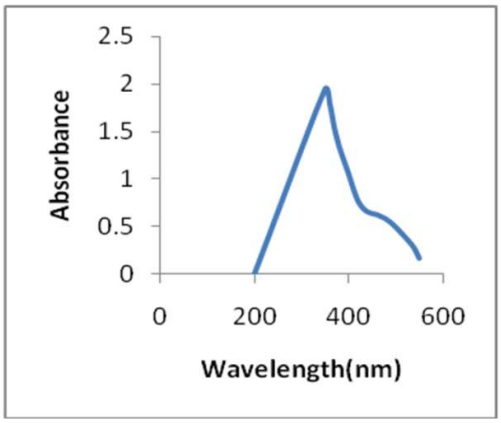


Fig. 1. Absorption spectra of DDQ

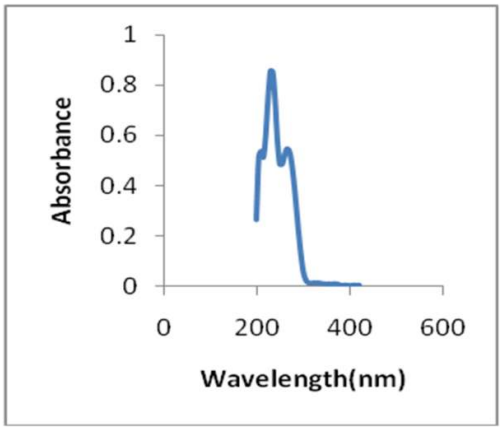


Fig. 2. Absorption spectra of thiamine

3.2 Stoichiometric Ratio

The molar ratio of the reactants, thiamine hydrochloride and DDQ in the charge transfer complex was determined by the mole ratio method and was found to be 1:1 as seen from (Table 1).

This suggests that one mole of nitrogen atom in the thiamine hydrochloride reacted with one mole of DDQ as represented in Scheme 1.

3.3 Optimal Conditions for the Formation of Thiamine – DDQ Complex

(Fig. 4) shows a plot of absorbance versus time. Although the reaction was immediate, maximum complexation was attained by 25 mins at room temperature.

The change in absorbance of thiamine hydrochloride – DDQ complex with temperature is shown in (Fig. 5). The result indicates that the maximum stability occurred at 40℃.

The plot of pH against absorbance for thiamine hydrochloride-DDQ complex is represented in (Fig. 6). The result shows that the maximum peak was seen at pH 8 which shows that the best medium for the formation of this complex is buffer 8.

Table 1. Optimum conditions, statistical data and regression equation of thiamine complex

Parameters	Values
Molar Absorptivity/Lmol^{-1}cm^{-1}	1.08 x 10^3
Stoichiometric ratio	1:1
Sandell's sensitivity/µgcm^{-2}/0.001A	2.68
Regression equation Y*	
Slope(b)	0.006
Intercept (c)	0.061
LOD	1.23
LOQ	3.73
Correlation coefficient	0.952
Average percentage recovery/%	96.2

**Average of five independent analyses*

3.4 Validation Using Beers Law

3.4.1 Linearity

The proposed method was validated using ICH guideline [16]. Fig. 7 represents the least square fit of absorbance versus concentration for the thiamine – DDQ complex at 474 nm from the linear equation:

$$A_{474nm} = 006\,[D] + 061 \quad (1)$$

Where [D] is the concentration of thiamine in µg/ml. R^2 has the value of 0.952.

Conformity with Beers law was evident between the concentration ranges of 5 – 80 µg/ml. The correlation coefficient was 0.952 indicating good linearity over the working concentration range. This is slightly similar to a reported work involving thiamine using flow injection method [17].

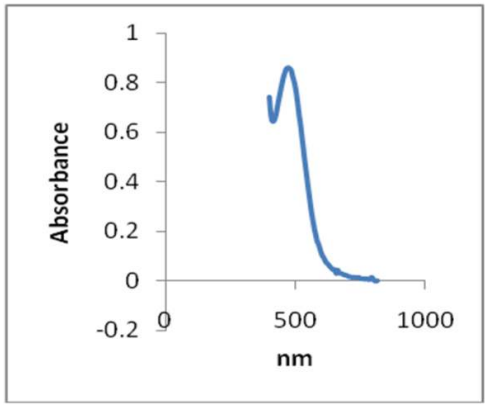


Fig. 3. Absorption spectra of thiamine-DDQ complex

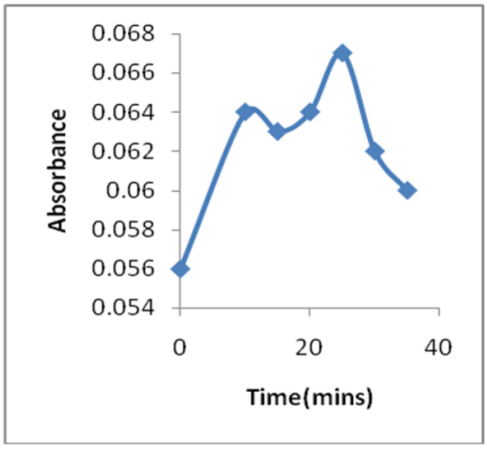


Fig. 4. Effect of time on complex formation

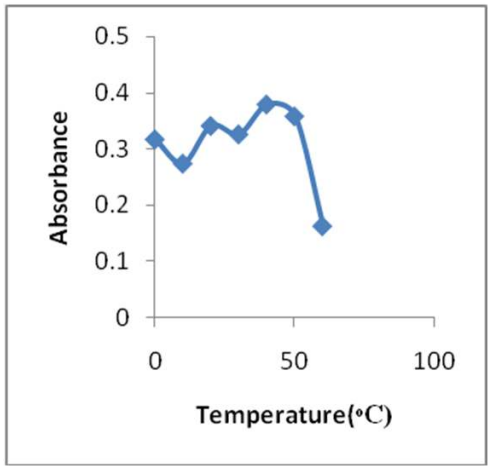
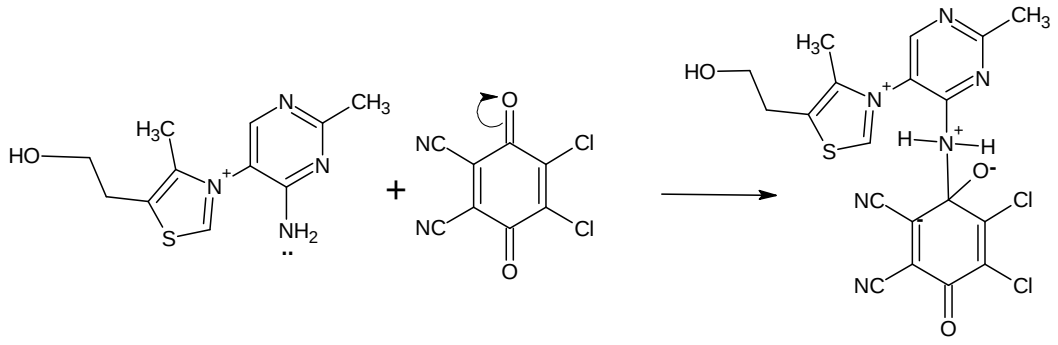


Fig. 5. Effect of temperature on complex formation



Scheme 1. Proposed reaction mechanism of thiamine with the formation charge transfer complex

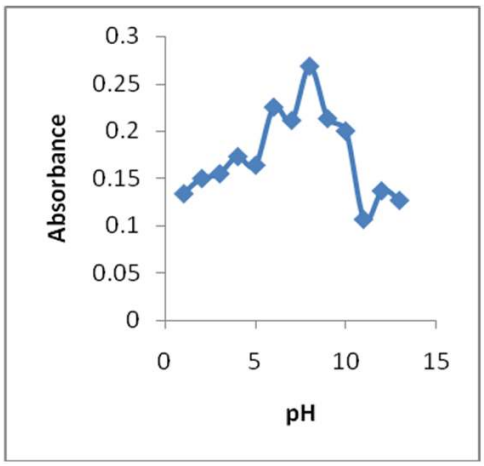


Fig. 6. Effect of pH on complex formation

3.4.2 Sensitivity

The values of limit of quantification and detection were determined using the following equations (2)

$$LOD = 3.3\ \delta/S,\ LOQ = 10\delta/S \quad (2)$$

The limit of detection, quantification, Sandell sensitivity and molar absorptivity are all presented in Table 2. These values confirm the sensitivity of the method.

3.5 Accuracy and Precision

From the experiment done, Table 2 shows the percentage recoveries of thiamine which was found to be 96.2% with a relative standard deviation of < 1. Also, the t-test and f-test values were calculated and found to be 0.44 and 0.93 respectively at P = *.05* confidence level, this does not vary much from an earlier report [18]. The average percentage recovery was satisfactorily high with low relative standard deviation which shows the reproducibility of this method.

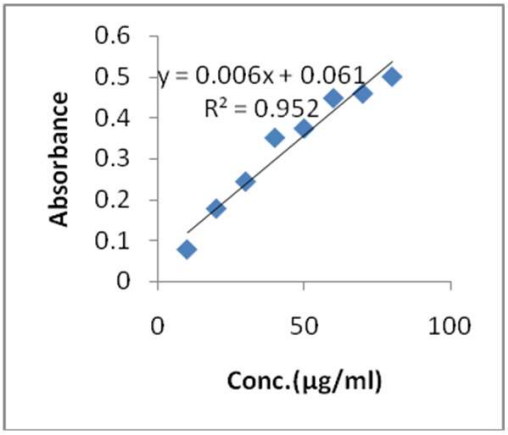


Fig. 7. Beers plot on thiamine complex

3.6 Interference Studies

Detailed studies on the interference of different excipients were made which includes magnesium stearate, corn starch, Talc (Table 3), no significant interference was observed in magnesium stearate (30 µg/ml), Talc (15-25 µg/ml, 35-40 µg/ml). However intolerable interferences were observed in magnesium stearate (10-25 µg/ml, 35- 40 µg/ml), Cornstarch (10-40 µg/ml), Talc (10 µg/ml and 30 µg/ml).

Table 2. Application of the proposed method for the assay of niacin

Taken (μgml^{-1})	Found (μgml^{-1})	[a]Recovery (%)	R.S.D (%)	T-test	F-test
10	7.11	71.79	0.09	0.44	0.93
30	28.53	95.20	0.004		
40	38.14	95.26	0.009		
60	58.30	121.7	0.01		
80	98.24	96.22	0.003		

[a]n Average of five independent analyses

Table 3. Pharmaceutical excipients used in the formulation of thiamine drug

Excipients (Added/μg/ml)	%Recovery ± S.D[a]						
	10	**15**	**20**	**25**	**30**	**35**	**40**
Maize starch	85±0.006	80±0.002	73±0.003	83±0.002	84± 0.002	72± 0.001	78±0.004
Talc powder	104±0.006	96±0.006	98±0.001	98±0.002	102±0.002	98±0.006	97±0.002
Magnesium Stearate	91±0.002	84±0.001	84±0.006	89±0.001	97±0.002	122±.001	116±0.003

20 μg/ml of thiamine was taken
[a]n Average of three determinations
three independent determinations

Application of the proposed method with combined excipient/drug ratio of 1.5:1 reveals tolerable interference with 96.2 % recovery value.

4. CONCLUSION

The proposed method is simple, sensitive, cost-effective and does not require liquid to liquid extraction procedures. Hence it was applied successfully to the determination of thiamine hydrochloride in pure and commercial forms. Application of the proposed method with combined excipient/drug ratio of 1.5:1 reveals tolerable interference with 96.2 % recovery value and low standard deviation of <1 which shows the reproducibility of the result.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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