Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(2):59-66
(http://derpharmachemica.com/archive.html)

Spectrophotometric determination of erythromycin using charge transfer complexation

Ukoha O. Pius[1] and Nwanisobi C. Gloria[2*]

[1]*Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria*
[2]*Department of Chemical Engineering, Madonna University Nigeria, Akpugo campus, Nigeria*

ABSTRACT

A simple and sensitive spectrophotometric method is described for the quantitative determination of erythromycin. The method is based on charge transfer complexation reaction of, erythromycin as n-electron donor with 2, 3 dichloro-5,6-dicyano-1,4- benzoquinone (DDQ) as π-acceptor to give highly coloured complex with 1:1 stoichiometric ratio. The coloured products were quantified at 464nm under the optimized experimental conditions. Beer's law is obeyed over the concentration ranges of 5-150µg/ml. The apparent molar absorptivity was calculated to be 1.27 x 10^3Lmol^{-1}cm^{-1} and corresponding Sandell sensitivity of 1.56. The limit of detection and quantification were 2.1and 6.4 respectively. The proposed methods were applied successfully to the determination of erythromycin in pure and commercial forms with good average recovery of 105.4 %. Statistical comparison of the result was performed with regards to accuracy and precision using student's t-test and f-test at 95% confidence level.

Keywords: Spectrophotometry, Assay, Erythromycin, Charge transfer complex, DDQ.

INTRODUCTION

Charge transfer complex was first introduced by Mulliken as a certain type of complex resulting from interactions of donor and acceptor with the formation of weak bonds [1] and discussed widely by Foster [2]. Donor acceptor properties are prerequisites for the formation of charge transfer complexes. Most drugs have –NH or –NH_2 groups which behave as bases (electron donors) and could form complexes with acids (electron acceptor).Various cases have been reported [3].

Erythromycin,(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethyl amino)-3-hydroxy-6-methyl oxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione [4]is a macrolide antibiotic used for the treatment of urinary tract infection. Literature revealed different techniques for the analysis of erythromycin which includes spectrofluorimetry [5] capillary electrophoresis [6], HPLC [7], microbiological method [8] spectrophotometry [9]. However many of these methods require costly equipments and tedious experimental procedure. Therefore, a method that is simple, sensitive and less laborious is required for the determination of erythromycin.

MATERIALS AND METHODS

Instruments
All spectrophotometric measurements were carried out using a UV-1800 Shidmazu and 752w UV - Vis grating with a silica glass cell of l cm thickness.

Materials

All chemicals used were of analytical grade and were used as such. Erythromycin powder was supplied by A C and drug pharmaceutical limited, Enugu, Nigeria. The commercial erythromycin tablet (500mg per tablet) was purchased from the local market (Syncom formulations limited, India) 2,3 dichloro – 5, 6- dicyano-1,4-benzoquinone DDQ (98% purity) was supplied by Sigma Aldrich, Germany.

Method

Preparation of solutions

Exactly 0.027 g of DDQ was weighed. The weighed amount was dissolved in small amount of methanol and made up to mark to 10 ml in a volumetric flask to give a 1.0 $x10^{-2}moldm^{-3}$ solution. Further dilution to lower the concentration (5 x 10^{-5} M) was prepared. Also, 0.073 g of erythromycin was weighed. The weighed amount was dissolved in small amount of methanol and made up to mark to 10 ml in a volumetric flask to give a 1.0 $x10^{-2}moldm^{-3}$ solution. Further dilution to lower the concentration (1.4 x 10^{-5} M) was prepared.

Absorption spectra of erythromycin

Exactly 4 ml solution of erythromycin (1.4 x 10^{-5} M) in methanol was scanned against the blank of methanol in wavelength range of 199 nm-814 nm using a UV-Vis spectrophotometer

Absorption spectra of erythromycin-DDQ complex

Exactly 2 ml of 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone ($1.0x10^{-2}$ M) solution in methanol was mixed with 2 ml 0f erythromycin ($1.0x10^{-2}$ M). The resultant colour was developed and scanned between 350-600 nm against a methanol blank.

Stoichiometry of erythromycin –DDQ reaction

Job's method of continuous variation was employed [10]. A ($3.0x10^{-3}$ M) solution of erythromycin in methanol and ($3.0x10^{-3}$ M) solution of 2, 3- dichloro -5, 6- dicyano -1, 4- benzoquinone in methanol were used in the experiment. A 10 ml volume of mixtures of solution comprising complementary proportions of the solution (0:10, 1:9 , 2:8 , 3:7 , 4:6 , 5:5 , 6:4 , 7:3 , 8:2,9:1,10:0) were transferred to different tubes and the complex formed for each reaction mixture was allowed to stand for 15 min at room temperature, before analysis at 464 nm against a blank containing methanol.

Optimization of reaction conditions

Effect of time on the formation of erythromycin – DDQ complex

The absorbance of mixtures of 2 ml of erythromycin ($3.0x10^{-3}$ M) solution in methanol and 2 ml of DDQ ($3.0x10^{-3}$ M) solution in methanol were determined at various time interval from 0 min to 40 min at 464 nm at room temperature against a methanol blank.

Effect of temperature on erythromycin-DDQ complex

The absorbance of mixtures of 2 ml of erythromycin ($1.0x10^{-3}$ M) solution in methanol and 2 ml of DDQ($1.0x10^{-3}$ M) solution in methanol were determined at various temperatures from 0 ℃ – 60 ℃ at 464 nm for 15min against a blank containing methanol. The temperature was maintained using a thermostated water bath.

pH study on erythromycin – DDQ complex

A 0.5 ml of (1.0 x 10^{-3} M) erythromycin was placed in 13 sample containers, 0.5 ml of DDQ (1.0 X 10^{-3} M) was added. The content was shaken and 4ml of buffer 1-13 were added to each mixture. The mixtures were left to stand for 15 min at 60 °C before analysis at 464 nm against a methanol blank.

Proposed general procedure

Transfer serial volumes of 0.02ml to 0.6ml of standard erythromycin solution (0.00lg/ml) in a 0.02 step into different test tubes. Add 0.2ml of buffer 8 into each set up before making up with methanol solvent. Allow each set up to stand for 15min at 60 ^{0}C before analysis against a methanol blank at 464 nm.

Assay determination of erythromycin

One tablet containing 500mg of erythromycin was powdered. An amount equivalent to 0.01g was weighed and dissolved with some methanol solvent to extract the active ingredient, the solution was filtered and made up to 10ml to produce a theoretical 0.001g/ml. Different volumes similar to the one prepared in general procedure were taken and treated before analysis at 464nm against a blank.

RESULTS AND DISCUSSION

Absorption spectra

2, 3-dichloro-5, 6- dicyano 1, 4- benzoquinone reacts instantaneously with basic nitrogenous compounds to form charge - transfer complexes of n - π type. It is known for its interaction with drugs having donor sites in their structures and form ion-pair charge transfer complexes which offers a basis for quantification of drugs[11],[12] .In this experiment 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol was used for analysis of erythromycin . The spectrum of DDQ displayed absorption band at 350 nm (Fig.1). Erythromycin in methanol displayed absorption bands at 214 nm (Fig. 2). Mixing the solution of erythromycin in methanol to a solution of DDQ in methanol causes a red shift in the absorption maxima with a new absorption peaking at 464 nm (Fig. 3).Interaction of erythromycin in methanol with DDQ in methanol solution was found to yield a reddish brown colouration.

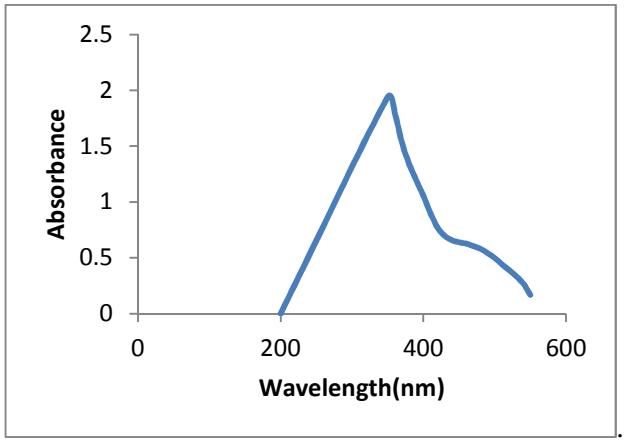


Fig. 1 Absorption spectra of DDQ in methanol solvent

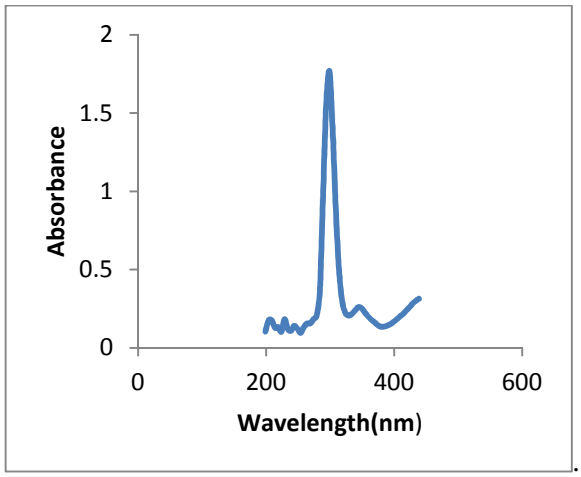


Fig. 2 Absorption spectra of erythromycin

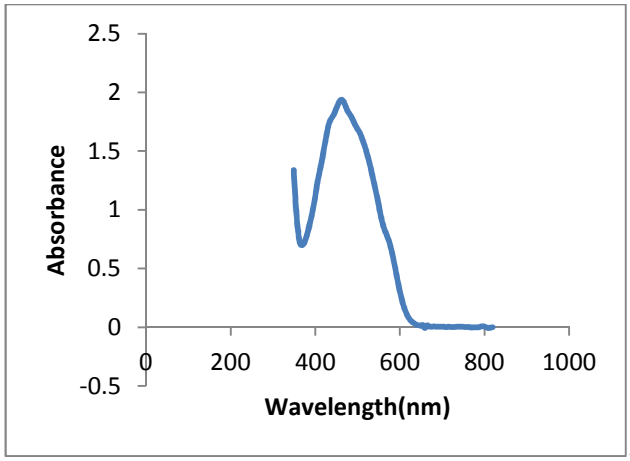


Fig. 3 Absorption spectra of erythromycin-DDQ complex in methanol solvent

Stoichiometry of erythromycin-DDQ reaction

The stoichiometric ratio of the reactants was determined using the Job's continuous variation method. It showed a point of inflexion at 1.0 indicating a 1:1 mole ratio of erythromycin: DDQ (Fig. 4).

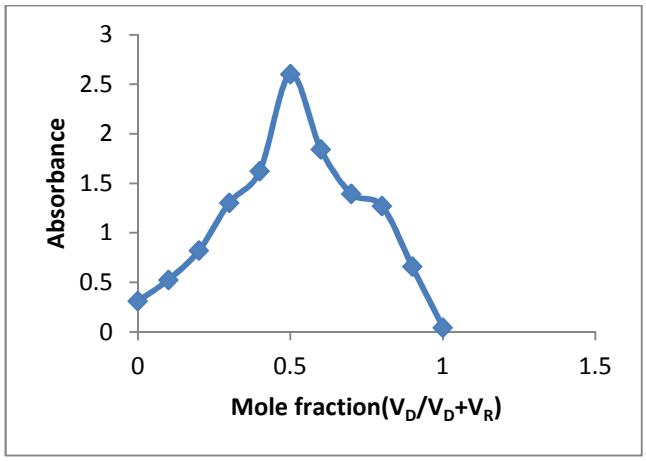
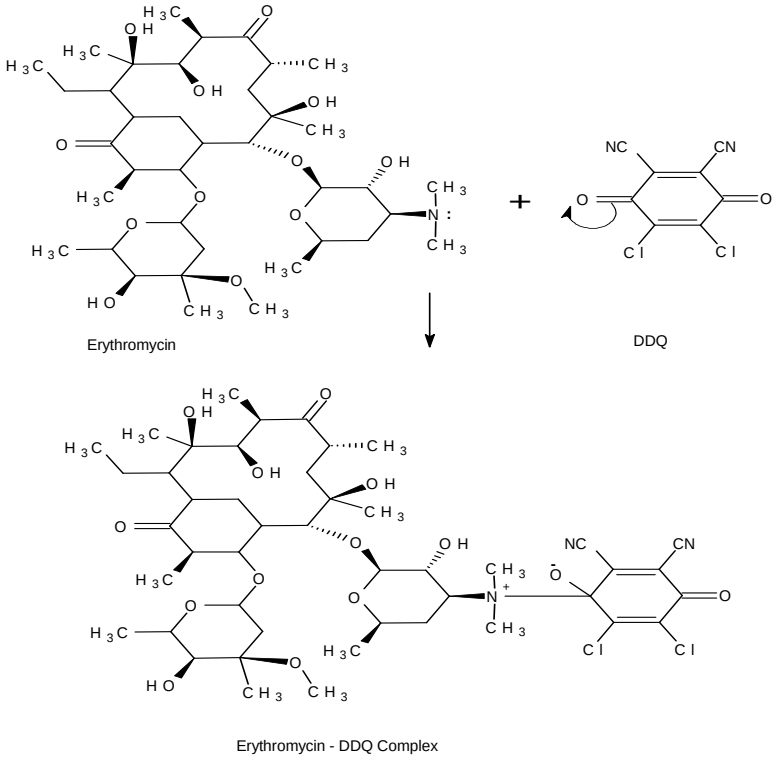


Fig. 4 Job's plot for erythromycin-DDQ complex

This indicates that one mole of erythromycin interacted with one mole of DDQ meaning that only one site on the erythromycin basic structure interacted with DDQ as shown in scheme 1



Scheme 1: Proposed interaction of erythromycin with DDQ to form charge transfer complex

Maximum time for the formation of erythromycin-DDQ complex

Formation of reddish brown colouration has been reported to be responsible for the charge transfer interaction [13]. Although the interaction of erythromycin and DDQ was instantaneous but maximum complexation was reached at 15min (Fig. 5).

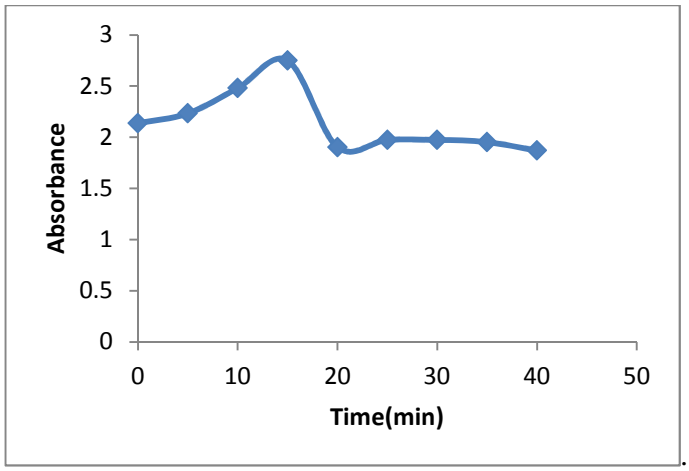


Fig. 5 Effect of time on erythromycin-DDQ complex

Effect of temperature on the formation of erythromycin-DDQ complex

Most reactions are faster when the operating temperature is increased. Increase in temperature increases both the collision of the reacting particles and hence the rate of reaction [14]. The change in absorbance of erythromycin–DDQ complex with temperature is shown in (Fig. 6). Result shows that maximum stability occurred at 60 ^{o}C. As absorbance is directly proportional to concentration, increase in absorbance is an indication of increase in complex formation [15]. The increase in complex formation increased as the temperature increased from 0 ^{o}C - 20 ^{o}C and decreased at 40 ^{o}C. There was an increase from 50 ^{o}C and this shows the unstable nature of the complex formation resulting from higher collision.

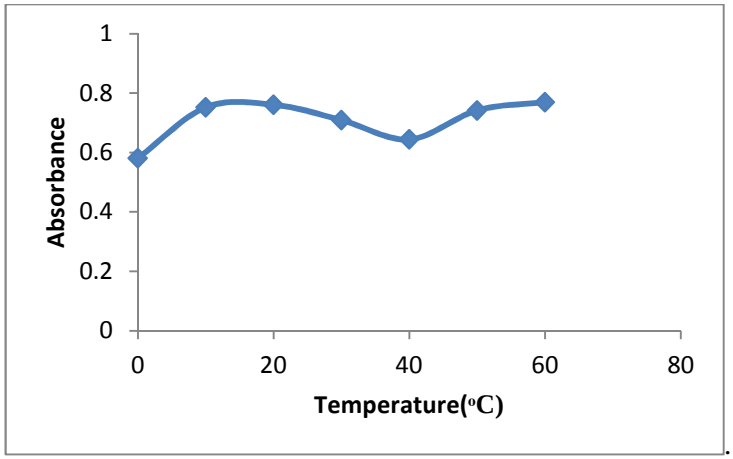


Fig. 6 Effect of temperature erythromycin-DDQ complex

Effect of pH medium on the formation of erythromycin-DDQ complex

Buffers 1-13 were used for the pH studies. A plot of pH against absorbance for the erythromycin - DDQ complex (Fig. 7) gave a maximum peak at pH 8 which means that the complex formation is favorable in alkaline medium.

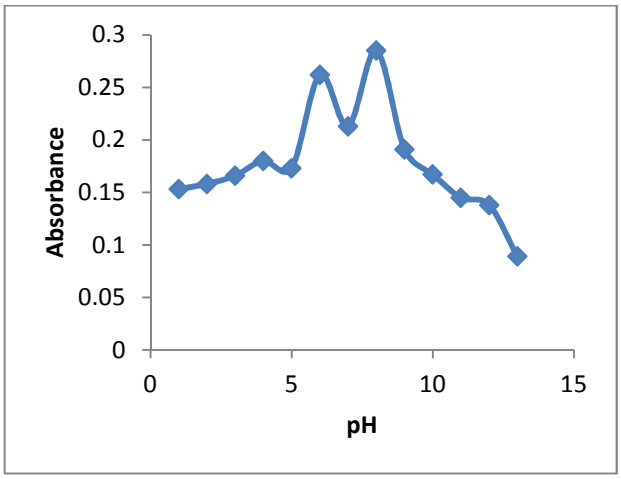


Fig. 7 Effect of pH formation on erythromycin-DDQ complex

Validity of Beers law

The proposed method was evaluated using the International conference on harmonization guideline (ICH) [16]. Beer's law was obeyed between the concentration ranges of 5-150ug/ml (Fig. 8) with R^2 value of 0.994.

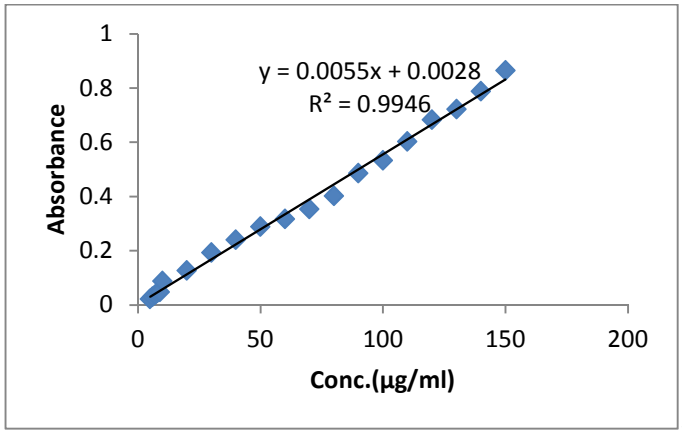


Fig.8 Beer's plot for the formation of erythromycin complex

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the following equation

LOD = $\frac{3.3 \times \sigma}{s}$, LOQ = $\frac{10 \times \sigma}{s}$ …… (1)

The values of limit of detection, limit of quantification, apparent molar absorptivity and Sandell's sensitivity are all presented in table I

Table 1 Sensitivity and regression parameters

Parameters	Values
ʎ max	464 nm
Beer's law limits (µgml^{-1})	5-150
Molar absorptivity (Lmol^{-1}cm^{-1})	1.27 x10^{3}
Sandell's sensitivity (µgcm^{-2}/0.001A)	1.56
Limit of detection	2.1
Limit of quantification	6.4
Regression equation (Y^{a} = a +bC)	Y = 0.005x -0.002
Slope (b)	0.005
Intercept (a)	0.002
Regression coefficient	0.994

[a] *n=6*

[a] *Average of three determinations*

[a] *Six independent determinations*

Recovery studies on the formation of erythromycin-DDQ reaction

From the experiment done, table 2 shows that the percentage recoveries of erythromycin were found to be 105.4 % with a relative standard deviation of < 1.The average percentage recovery was satisfactorily high with low relative standard deviation which shows the reproducibility of this method.

Statistical analysis of the results obtained using student's t test and f test shows no significant difference to the reference [17].

Table 2 Application of the proposed method for the assay of erythromycin

Drug	Erythromycin taken (µg/ml)	Erythromycin found (µg/ml)	Recovery (%)	RSD (%)	Error (%)	t-test (2.03)	f-test (6.39)
Erythromycin	10	10.7	106.7	0.01	0.07	0.009	0.932
	50	51.4	102.8	0.01	0.03		
	90	101.5	112.7	0.002	0.13		
	110	116.4	105.8	0.002	0.06		
	130	132	101.5	0.01	0.02		
	150	154	102.7	0.002	0.03		

CONCLUSION

The proposed method for the determination erythromycin is simple, precise, sensitive and accurate. The proposed method has been validated and successfully applied for the quantitative determination of erythromycin with good accuracy and precision of 105.4 % and relative standard deviation of < 1.

REFERENCES

[1].J.El –Zaria, *Spectrochim. Acta Part A.* **2008,** 69**,** 216.
[2].R.Foster , Organic Charge – transfer complex, Academic press, London, **1967**,1-600.
[3].*Int.J.Electrochem.Sci.*2013 **8** 1274-1294
[4].R.Nawal, F.Hassan, Y.Pakiraz, N.Noha, *Journal of AOAC International*, **2006**, 89, 1276 – 1287.
[5].Y.Pakinaz, *Journal of Pharmaceutical and Biomedical Analysis*, **2002,** 27, 923 – 932.
[6].C.Flurer, Analysis of Macrolide Antibiotics by Capillary Electrophoresis, **1996,** 17, 359 – 366.
[7].M. Hedenmo, B. Eriksson, *Journal of chromatography .A.* **1995**, 692, 161 – 166.
[8].J.Bernabaeu, M.Camacho, M.Gilalegre, V.Ruz, *J. Pharm.Biomed Anal.* **1999**, 21, 347-353.
[9]. M.Walash, E.Sharaf, M.Metwallis and M.Redashabana, *Arch pharmacy Res.* **2004**, 27,720,729.
[10].P.Job , *Ann. Chimm. Phs.* **1928**, 9, 113
[11].T.Ghabsha, T.Sabha, S.Mtaiwti, *Journal of pure and Applied Sciences*, **2007**, 4, 13 – 28.
[12].D.Goweri, M.Rajenda, P.Latha, *Asian J. chemistry.* **2005**, 17, 1334-1333.
[13].Doaa F, Mossalamy E, Salih S,Spectrophotometric and kinetic Studies of Charge Transfer Compass of Pantoprazole with Chloranilic acid and DDQ as π - Acceptors, Dubai, **2013**.

[14].M.Chendo ,Comprehensive Practical Chemistry Hybrid Publishers Nigeria, **2002**, 130.
[15].U.Ajali, B.Ezema, P.Ukoha, *J. chem. soc. Nigeria* **2008**, 33, 23-28.
[16].International conferences on Harmonization of Technical Requirements for Human Use London, **2005**.
[17].BP Microbiological Assay **1998,** 38.