The Use of 2,3-dichloro-1,4-naphthaquinone for the Spectrophotometric Determination of Some Primary Aliphatic Amines in Aqueous Solution

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الخلاصة

تم تطوير طريقة طيفية سهلة ودقيقة للتقدير الكمي لبعض الأمينات الأليفاتية الأولية (ايثانول أمين و ن- بيوتيل أمين و ن- هكسيل أمين). تعتمد الطريقة على التفاعل بين تلك الأمينات في الوسط المائي والكاشف ٣،٢ – ثنائي كلورو- ٤،١ – نفثاكوينون (DClNQ) بوجود محلو ل البيكاربونات المنظم لتكوين ناتج برتقالي اللون يمتلك أقصى امتصاص عند الطول ألموجي ٤٧٥ نانوميتر. لقد أمكن تطبيق قانون بير ضمن مدى التراكيز ١-٣٠ ، ٥-٢٠ و ٣-٣٠ مايكروغرام /مللتر لكل من ايثانول أمين و ن- بيوتيل أمين و ن- هكسيل أمين على التوالي . لقد وجد أن تلك الأمينات تكون نواتج مع الكاشف DClNQ بنسبة ١:١ ، وأن ثوابت استقرارها تراوح ت بين ١.٩٢×١٠^{٥} و ٤.١٤×١٠^{٥} لتر .مول$^{-١}$. لقد تم تطبيق الطريقة بنجاح في تقدير ايثانول أمين في مستحضره الصيدلاني.

Abstract

A simple and accurate spectrophotometric method was developed for the quantitative determination of some primary aliphatic amines in aqueous solution, i.e. ethanolamine, n-butylamine and n-hexylamine. The method is based on the interaction of these amines in aqueous medium with 2,3-dichloro-1,4-naphthaquinone (DClNQ) reagent in the presence of bicarbonate buffer solution to form an orange coloured products measurable at maximum wavelength of 475 nm. Beer's law is obeyed over the concentration range of 1-30, 5-20 and 3-30μg/ml for

ethanolamine, n-butylamine and n-hexylamine respectively. The DClNQ products were formed in the ratio of 1:1 amine: DClNQ, and their stability constants were ranged between 1.92×10^5 and 4.14×10^5 $L.mol^{-1}$. The method was successfully applied for the determination of ethanolamine in its pharmaceutical preparation.

Keywords: DClNQ; primary aliphatic amines; aqueous medium; spectrophotometry

Introduction

Short-chain aliphatic amines are presented widely in the aquatic environment due to their wide spread use in several industrial, chemical and manufacturing applications[1,2], Also; these amines are common components of biological systems as degradation products of organic materials such as amino acids and proteins. In addition to hygienic problems due to stinging smell, these compounds may be hazardous to human health as they are sensitizers and irritants to skin, eyes, mucus membranes and respiratory tract. Also; they can react with certain nitrogen-containing compounds to form nitrosamines, which are potentially carcinogenic substances[3].

The charge transfer (CT) complex formation reactions have been used in the spectrophotometric determination of amines as n-donor using various π-acceptors such as p-chloranil[4-6], p-fluoranil[7], p-bromanil[8] and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone(DDQ)[9]. The reaction between 1-fluoro-2,4-dinitrobenzene[10] or 1-chloro-2,4-dinitrobenzene[11] and aliphatic amines have also been studied. However; the above methods suffer from the selectivity for determination of primary aliphatic amines in the presence of other amines.

The goal of this study is to develop of selective spectrophotometric method for the determination of some primary aliphatic amines in the presence of aromatic primary amines, secondary and tertiary amines and other excipients with a DClNQ reagent which does not require any derivatization or catalyst but simply and rapidly reacts and forms stable colored products in addition to application of the proposed method for the determination of ethanolamine in its pharmaceutical formulation.

Experimental

Apparatus

All absorption measurements were made on a Shimadzu UV-210A double-beam spectro-photometer supplied with a digital printer DP80Z and matched 1-cm optical silica cells.

Reagents

All reagents used were of analytical grade and obtained from Fluka and BDH companies.

***DClNQ solution* (*2x10^{-3}M*)** is prepared freshly by dissolving 0.0454g of 2,3-dichloro-1,4-naphthaquinone in propanol (99.8%) and diluted to the mark in 100ml-volumetric flask with the same solvent.

Standard solutions of primary aliphatic amines (250µg/ml) were prepared individually by dissolving 0.025g of pure amine (ethanolamine, n-butylamine and n-hexylamine in a minimum amount of ethanol and diluted to the mark with distilled water in 100 ml-volumetric flask. These solutions were further diluted with water as needed.

Ethanolamine oleate injection(5%) is prepared by dissolving 4.23 g oleic acid in 50 ml injection water followed by addition of 0.91 g ethanolamine and 2 ml benzyl alcohol with stirring until homogeneous solution was obtained.

Bicarbonate buffer solution of pH value 8.6 was prepared by dissolving 1.86 g of sodium hydrogen carbonate and 2.12 g of sodium carbonate in sufficient distilled water to produce 100 ml and adjusted to pH 8.6, using pH meter, by addition few drops of 0.01 M hydrochloric acid.

Recommended procedure

Aliquots of standard primary amine solutions of ethanolamine, n-butylamine and n-hexylamine were transferred separately into a series of 25ml calibrated flasks. To each of these were added 1.0 ml of 2×10^{-3}M DClNQ (1.5 ml in the case of ethanolamine) followed by addition 0.5 ml of bicarbonate buffer solution (0.6 ml in the case of ethanolamine) and the solutions were heated at 60°C for 20 min. for n-butylamine and n-hexylamine and 30 min for ethanolamine, then the solutions were cooled to room temperature and diluted to the mark with water. The absorbances of the products were measured at 475 nm against corresponding reagent blank.

Results and discussion

Absorption spectrum

Primary aliphatic amines are reacted with DClNQ in the presence of bicarbonate buffer solution to give an orange coloured complexes with maximum absorption spectra at 475 nm, and their reagent blanks gave maximum absorption at 340 nm (Figure 1).

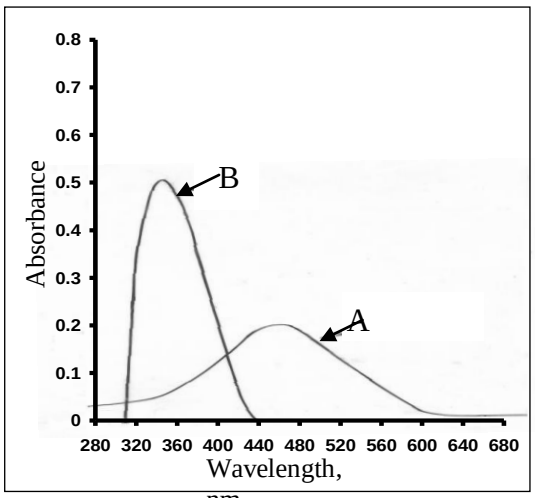


Figure 1: Absorption spectra for (A) the complex of 1.5 ml of 2×10^{-3}M DClNQ with 10µg/ml ethanolamine versus blank reagent and (B) blank versus distilled water under optimum conditions.

Effect of solvent

Various solvents such as methanol, ethanol, propanol, acetonitrile, acetone and water as medium for the reaction were examined in order to obtain high sensitivity and selectivity for determination of primary aliphatic amines, It was found that using propanol as solvent for DClNQ and water for primary aliphatic amines with dilution by water gave maximum colour intensity and high selectivity for the determination of these amines in the presence of aromatic amines, organic nitrogen compounds and other organic compounds. Therefore; this system of solvents is recommended in this method.

Effect of pH and buffer solutions

The effect of pH on the absorption of the products produced by the reaction of DClNQ with primary aliphatic amines was studied using different pHs of HCl and NaOH ranged from 2 to12. It was found that these products are formed in the final pH of 8.63 by addition of NaOH solution but the absorbance decreases after addition of HCl (Figure 2). Therefore different buffers of the same pH value were prepared by using bicarbonate, borate, phosphate and ammonia buffers to investigate the sensitivity of the amine–DCLNQ products. The results revealed that bicarbonate buffer solution (Na_2CO_3+$NaHCO_3$) gave high absorbance in comparison with other buffers,. However; the amount of bicarbonate buffer solution of pH 8.63 was studied and 0.5 ml was found to be optimum for n-butylamine and n-hexylamine and 0.6 ml for ethanolamine which are recommended in the subsequent experiments.

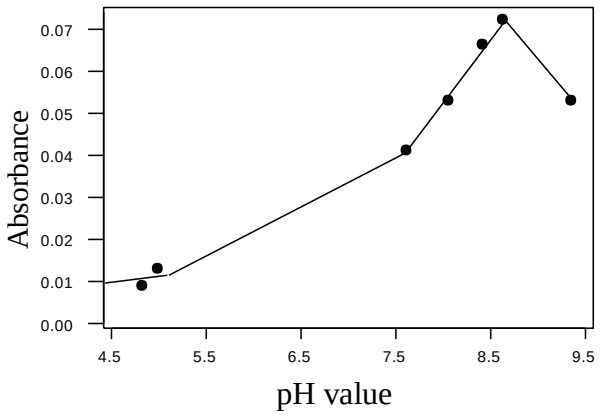


Figure 2: Effect of pH on the absorption of 10µg/ml ethanolamine with DClNQ reagent.

Effect of reaction time and temperature

The reaction time was determined by following the colour development at room temperature and in thermostatically controlled water-bath at different temperatures. The absorbance was measured against reagent blank treated similarly at 475 nm. It was observed that the absorbance reached maximum after addition of the reagent solutions after 10min for ethanolamine and 20 min for n-butylamine and n-hexylamine at 60°C, and remain constant more than 30 min (40min for n-butylamine). These temperatures and reaction time were chosen for colour development.

Effect of DClNQ concentration

The effect of different concentrations of DClNQ (0.002, 0.01, 0.1 and 1.0 M) dissolved in propanol on the absorbance of solution containing a fixed amount of the ethanolamine in the presence of bicarbonate buffer solution and dilution with water was studied. The results revealed the fact that 0.002 M DClNQ gave clear solution and other concentrations cause turbidity. However; it was found that absorbance reached maximum on using 1.5 ml for ethanolamine (Figure 3) and 1.0 ml of 0.002 M DClNQ for n-butylamine and n-hexylamine. Therefore, these volumes were used in subsequent experiments.

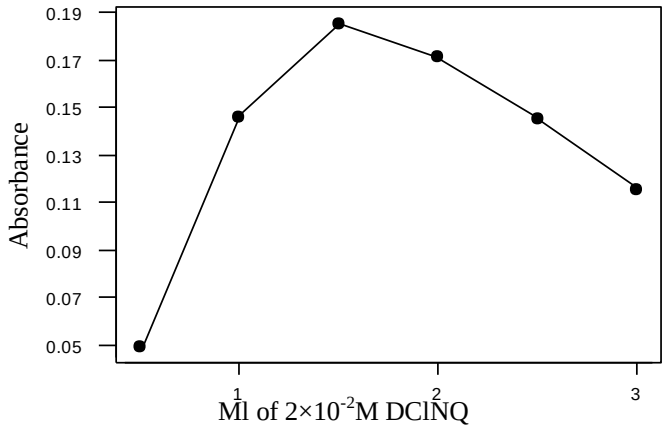


Figure 3: Effect of DClNQ amount on the absorption intensity of the reaction product of 10µg ml^{-1} ethanolamine

Effect of surfactant

Effect of various surfactants including cetavlon, cetyl perydinum chloride (CPC) and sodium dodecyl sulphate (SDS) were tested. It was found that no effect of these surfactants on the absorption of the studied amines.

Effect of order of addition

To obtain optimum results, the order of addition of reagents should be followed as given under the recommended procedure, otherwise a loss in colour intensity was observed.

However; the optimum reaction conditions for developing the colour intensity of DClNQ-aliphatic primary amine products are summarized in Table 1.

Table 1: Optimum reaction conditions of DClNQ reagent with aliphatic primary amines

Primary aliphatic amine	λ_{max} (nm)	**Temp.** (°C)	**Development time** (min.)	**Stability period** (min.)	**Bicarbonate buffer** (ml)	**DClNQ** 2×10^{-3}M(ml)
Ethanolamine	475	60	10	30	0.6	1.5
n-Butylamine	475	60	20	40	0.5	1.0
n-Hexylamine	475	60	20	30	0.5	1.0

Quantification

Under the experimental conditions described in Table 1, standard calibration graphs for aliphatic primary amines were constructed by plotting the absorbance versus concentration (Figure 4). The linearity ranges obeyed Beer's law and the molar absorptivities are cited in Table 2. The linearity was represented by the regression equation and the corresponding correlation coefficients for the amines determined by the proposed method, as shown in Table 2, represents excellent linearity.

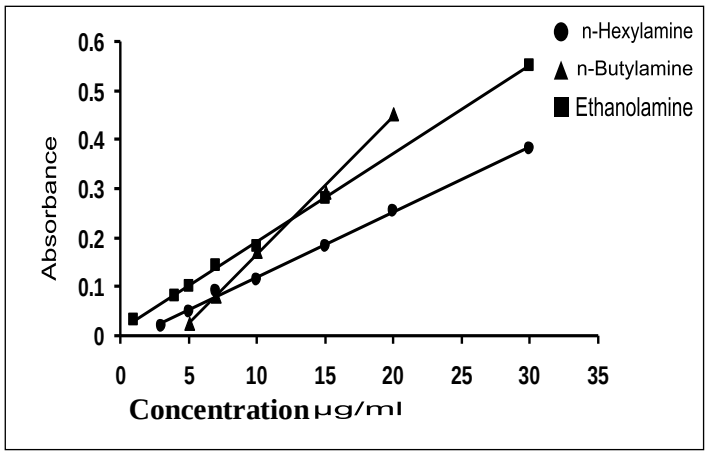


Figure 4: Calibration graphs for aliphatic primary amines

Table 2: Summary of optical characteristics and statistics for the proposed method.

Parameters	Product of DClNQ with		
	Ethanolamine	n-Butylamine	n-Hexylamine
Linearity range(µg/ml)	1-30	5-20	3-30
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.091×10^3	2.051×10^3	1.343×10^3
Slope	0.0179	0.0281	0.0133
Correlation coefficient	0.9992	0.9981	0.9978
Intercept	0.0123	-0.1145	-0.0154

Precision and accuracy

Six replicate measurements are performed at three different concentrations of each amine. The relative standard deviation and recovery results indicated the high precision and accuracy of the proposed method (Table 3).

Table 3: Precision and accuracy of the proposed method

Amine	Amount Added (µg/ml)	Recovery[#] (%)	Average recovery(%)	RSD[#] (%)
n-Hexylamine	5	102.65	100.54	2.74
	10	100.24		1.11
	20	98.70		0.86
n-Butylamine	7	101.23	100.09	2.63
	10	99.41		0.82
	15	99.65		0.47
Ethanolamine	5	101.81	100.22	2.52
	10	99.30		1.51
	20	99.55		0.76

[#]Average for six determinations

Interferences

The interference of various organic nitrogen compounds including secondary, tertiary aliphatic and aromatic amines, amides, other organic and excipients compounds on the determination of 10µg/ml of n-butylamine (as example for primary aliphatic amines) were examined. It was found that these compounds did not affect the accuracy of the determination of n-butylamine, even when these compounds were present in large excess amounts compared with that of n-butylamine. The results are summarized in Table 4.

Analytical application

The proposed method has been applied for the determination of ethanolamine in synthetic ethanolamine oleate injection as pharmaceutical preparation and gave good accuracy and precision, the

results obtained were compared favorably with the official method[12] (Table 5). Statistical analyses of the results using the t-test at 95% confidence level, for four freedom degrees, showed that the calculated value (1.11) did not exceed the theoretical value (2.78).

Table 4: Effect of foreign compounds on the recovery of 10μg/ml n-butylamine

Foreign compound	Fold excess added	Recovery* (%)
Dibutylamine	7	103.31
	9	104.82
	10	106.11
Triethylamine	7	102.96
	9	104.01
	12	106.33
Aniline	7	103.71
	10	104.43
	12	112.57
p-Anisidine	7	102.92
	9	104.01
	12	106.32
o-Tolidine	7	103.22
	10	104.25
	11	107.43
Diethanolamine	5	103.41
	7	104.82
	8	108.22
p-Aminobenzoic acid	8	100.01
	9	104.26
	10	107.34
N,N-Dimethylaniline	7	103.37
	9	104.41
	13	112.21
Acacia	20	100.21
	25	100.61
	30	109.53
Glucose	15	100.24
	25	103.61
	30	108.62
Glycerol	10	100.18
	20	101.29
	30	106.26
Oleic acid	5	102.21
	6	104.36
	7	108.41
Starch	7	101.23
	8	103.17
	9	110.03
Xylose	10	102.01
	20	103.14
	30	107.66

*Average of three determinations.

Table 5: Determination of ethanolamine in its pharmaceutical preparation by the proposed method and comparison with the British pharmacopoeia method

Procedure applied	Pharmaceutical formulation	Drug amount present(µg/ml)	Recovery* (%)	Drug content found (mg)	Average (mg)
Proposed method	Injection (0.91 mg)**	5 10 20	102.51 103.34 101.62	0.932 0.940 0.924	0.932
British Pharmacopoeia method[12]	Injection (0.91 mg)**		101.67	0.925	

* Average of three determinations.
** Certified value

Stoichiometric Relationship

The mole ratio of the products formed between the aliphatic primary amines and the reagent used was investigated applying the continuous variation (Job's) method[13] using equimolar solutions (2×10^{-3}M) of the amine and DClNQ reagent. The results showed in Figure 5 (for ethanolamine as example) indicated that the product is formed in the ratio of 1:1 . This may attributed that the primary amino group present in the amines is responsible for the formation of the product.

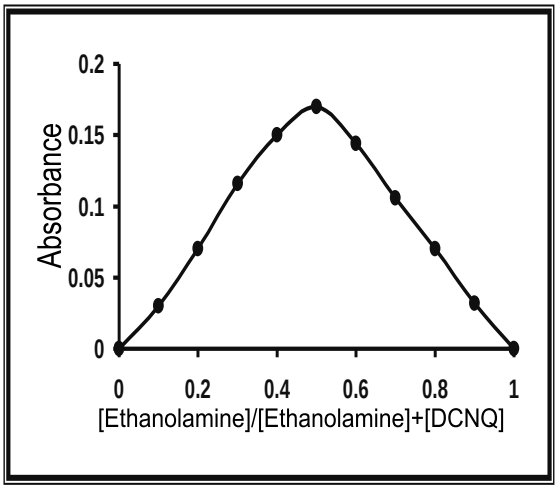
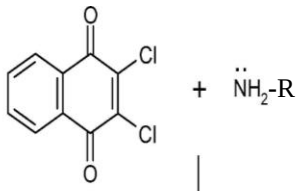


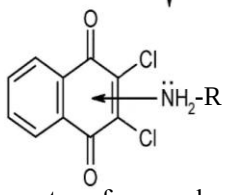
Figure 5: Continuous variation plot of ethanolamine with DClNQ reagent.

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the primary aliphatic amine and DClNQ reagent to one containing an excessive amount of DClNQ reagent. The average conditional stability constants of the products are 3.74×10^5 , 4.14×10^5 and 1.92×10^5 L.mol^{-1} for ethanolamine, n-butylamine and n-hexylamine respectively. This indicates that the products are stable.

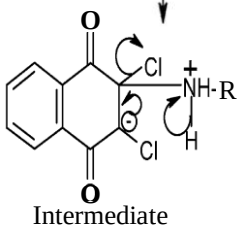
Reaction mechanism

On mixing the studied aliphatic primary amines with DClNQ reagent, there was an instantaneous formation of orange color with an absorption maximum centered around 475 nm. The intensity of this band goes on slow increasing with time at room temperature. The continuous increase in absorbance at 475 nm at 60°C is indicative of formation of the final reaction product, because at this wavelength neither the acceptor nor the donor absorbs. Hence, the reaction probably appears to proceed through the initial formation of the n-π charge transfer complex, which might be transformed into the final products[14-16]. The proposed mechanism may be shown as follows:



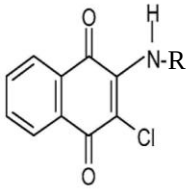


n-π charge transfer complex



Intermediate

-HCl



2N(alkylamino)-3-chloro-1,4-naphthoquinone

R= $-C_2H_4-OH$, $-C_4H_9$, $-C_6H_{13}$

* Average of six determinations.

Conclusion

The proposed method is simple, rapid, selective for the determination of aliphatic primary amines and economic compared with already reported methods and it does not require any pretreatment of the amines or extraction procedure and has a good accuracy and precision. On the other hand, in terms of sensitivity, the method could be considered superior in comparison with the titrimetric British Pharmacopoeia method and the previously reported methods, especially with those based on non-aqueous medium.

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