Spectrophotometric Determination of Microgram Amounts of Aliphatic Amines with Quinalizarin

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الخلاصة

تم تطوير طريقة طيفية بسيطة وسريعة وحساسة لتقدير الأمينات الاليفاتية، تعتمد الطريقة على تفاعل انتقال البروتون مع كاشف كوين اليزارين في وسط متعادل لتكوين ناتج ذي لون بنفسجي ذائب في الماء يقاس أقصى امتصاص له عند طول موجي ٥٦٠ نانوميتر وبامتصاصية مولارية تراوحت بين ٣٦٠٠ لتر .مول⁻¹.سم⁻¹ لبيوتيل امين الثالثي و ٦٧٢٠ لتر.مول⁻¹.سم⁻¹ لبيوتيل أمين الثانوي. لقد بلغت دقة الطريقة (معدل الاسترجاعية) ١٠٠.١٥% وتوافق الطريقة (الانحراف القياسي النسبي) اقل من ٣.١%. تم دراسة تأثير مركبات مختلفة على تقدير الأمينات الاليفاتية كما تم إيجاد طبيعة وثابت استقرار النواتج المتكونة.

ABSTRACT

A simple, rapid and sensitive spectrophotometric method for the determination of aliphatic amines was developed. The method is based on the proton transfer reaction with quinalizarin reagent in aqueous neutral solution to form violet product which shows maximum absorbance at 560 nm with molar absorptivity ranged between 3600 $l.mol^{-1}.cm^{-1}$ for ter-butyl amine to 6720 $l.mol^{-1}.cm^{-1}$ for sec-butyl amine. The accuracy (average recovery) of the method was 100.15% and the precision (RSD) was less than 3.1%. The effect of various compounds on the determination of aliphatic amines has been studied. The nature and stability constant of the products have been estimated.

INTRODUCTION

Amines are organic derivatives of ammonia, containing a nitrogen atom with a lone pair of electrons, making amines basic and nucleophilic therefore most of the chemistry of amines depends on the presence of this lone pair of electrons[1,2]. Aliphatic amines find application in numerous and varied industries, including chemicals, paper, rubber, corrosion,

plastic and textile. Ethylamine may play important role in initiating the gastrin secretory response to a meal. Cyclohexylamine is a reactive primary amine, serving as an intermediate for a variety of useful amine derivatives[3,4].

Various π acceptors have been used for the determination of amines spectrophotometrically via n-π CT-complex formation reaction with 2,4,7-trinitrofluorenone[5], p-benzoquinone[6], 2,6-dichloro-p-benzoquinone[7], trinitrobenzene[8], 2,3,5,6-tetrachloro-p-benzoquinone (Chloranil)[9-12], 2,3,5,6-tetrafluoro-p-benzoquinone (Fluoranil)[13], 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ)[14,15] and 2,3,5,6-tetrabromo-p-benzoquinone (Bromanil)[16].

Benzylamine was determined spectrophotomertically by its reaction with Cu-NTA complex in aqueous solution leading to increase the absorbance at λ_{max} 832 nm with accuracy of 99% and RSD $< 4.6\%$ [17].

Primary and secondary aliphatic amines were determined spectrophotometrically by their reaction with 1,2,5,8-tetrahydroxy 9,10-anthraquinone (quinalizarin) forming violet adduct in aqueous solution[18,19]. Spectrophotometric method used for the determination of ethylamine using tetracyanoethylene (TCNE) reagent at λ_{max} 325 nm in aqueous solution with a molar absorptivity of 2831 l.mol^{-1}.cm^{-1} [20]. 2,4-Dinitro-1-fluorobenzene (DNFB) used for the spectrophotometric determination of cyclohexylamine at λ_{max} 353 nm in the organic medium with molar absorptivity of 13957 l.mol^{-1}.cm^{-1} [21].

Recently spectrophotometric method used for the determination of microgram amounts of aliphatic amines based on the proton transfer reaction using 3,5-dinitrosalicylic acid (DNS) reagent in basic medium forming coloured products with maximum absorption at 415 nm. The molar absorptivity ranged from 5100 l.mol^{-1}.cm^{-1} for tributylamine to 12000 l.mol^{-1}.cm^{-1} for dibutylamine[22].

In this work quinalizarin reagent was used for the spectrophotometric determination of aliphatic amines via proton transfer reaction in aqueous solution.

EXPERIMENTAL

Apparatus

A Shimadzu UV-210 A digital double beam spectrophotometer with 1-cm matched quartz cells was used for all spectral and absorbance measurements.

Reagents

All chemicals used were of the highest purity available.
Quinalizarin (2 x 10^{-4} M) solution:

This solution was prepared by dissolving 0.0136 g of quinalizarin in absolute ethanol in 250 ml volumetric flask. This solution was kept in brown bottle and it was stable for at least one month.

Aliphatic amines standard solutions

Ethylamine, sec-butylamine, tert-butylamine, benzylamine, dibenzylamine and cyclohexylamine were used in this work. Each compound of 100 ppm was prepared in aqueous solution.

Procedure for calibration

To a series of 10 ml calibrated flasks, transfer increasing volumes (mls) of 100 ppm of each amine solution to cover the concentration ranges as cited in (Table 3), followed by addition of optimum amounts of quinalizarin solution (Table 2). The solutions were then diluted to the mark with distilled water. Under the optimum conditions mentioned in Table (2), the absorbances were measured at 560 nm against reagent blank.

RESULTS AND DISCUSSION

Preliminary investigation

When dilute aqueous solution of ethylamine as a typical aliphatic amine and quinalizarin reagent were mixed in a aqueous solution a violet solution was observed with maximum absorption at 560 nm in contrast to the reagent blank which shows a maximum absorption at 490 nm (Fig. 1).
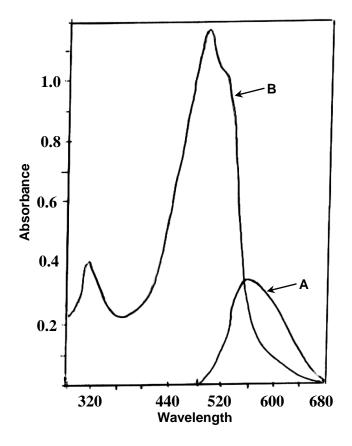

Figure (1): Absorption spectra of:
A: Ethylamine-Quinalizarin product versus reagent blank
B: Reagent blank versus distilled water

Optimization of conditions

The pH of ethylamine, a typical aliphatic amine was examined, it was 8. After the addition of 6 ml of 2 x 10^{-4} M quinalizarin the value of pH decreased to 7.2 therefore different buffers of pH 7.2 were prepared such as borate, carbonate, acetate and phosphate. It was found that these buffers decrease the absorbance (Table 1).

Table (1): Effect of buffer on the absorption of product

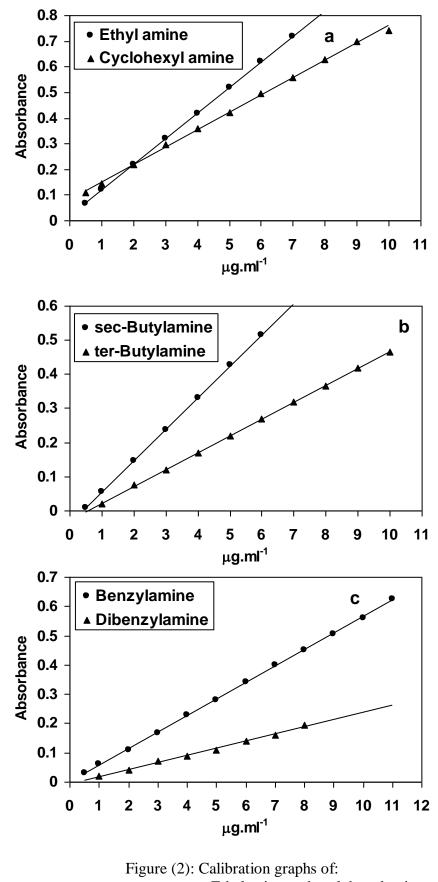| Buffer solutions (pH 7.2)	Borate	Carbonate	Acetate	Phosphate	Without
Absorbance	0.045	0.027	0.076	0.068	0.418

The effect of various parameters on the absorption of the coloured product has been investigated and the reaction conditions were optimized for each aliphatic amine. Table (2) shows summary of the optimum conditions for the determination of the studied aliphatic amines.

Table (2): Summary of optimum conditions for the determination of aliphatic amines

Aliphatic amines	λ_{max} (nm)	Temp. (^{o}C)	Development time (min)	Stability period (min)	Quinalizarin $2x10^{-4}$ M (ml)
Ethylamine	560	50	35	25	6
sec-Butylamine	560	40	50	40	6.5
tert-Butylamine	560	40	30	15	6.0
Benzylamine	560	40	30	30	5
Dibenzylamine	560	RT	20	25	5
Cyclohexylamine	560	RT	10	50	6

Analytical data

Under these optimum conditions, linear correlation were obtained for the aliphatic amines over the ranges shown in Table (3). A negative deviation from Beer's law was observed at higher concentrations of aliphatic amines. The molar absorptivities for aliphatic amines ranged between 3600 l.mol^{-1}.cm^{-1} for tert-butylamine and 6720 l.mol^{-1}.cm^{-1} for sec-butylamine indicates the method is sensitive.

Figure (2): Calibration graphs of:
        a: Ethylamine and cyclohexylamine
        b: sec-Butylamine and tert-butylamine
        c: Benzylamine and dibenzylamine

Table (3): Analytical parameters

Aliphatic amines	Linearity range (μg.ml^{-1})	Molar absorptivity (l.mol^{-1}.cm^{-1})	Slope	Intercept	Correlation coefficient
Ethylamine	0.5-7	4490	0.0999	0.0192	1.0000
sec-Butylamine	0.5-6	6720	0.0921	-0.037	0.9999
tert-Butylamine	1-10	3600	0.0493	-0.0256	0.9998
Benzylamine	0.5-11	6030	0.0563	0.0015	0.9998
Dibenzylamine	1-8	4830	0.0245	-0.0067	0.996
Cyclohexylamine	0.5-10	6690	0.0676	0.0832	0.9985

Accuracy and precision

The recovery and the relative standard deviation (RSD) were determined at three different concentrations. The results shown in Table (4) indicate very good accuracy (average recovery %) 100.15% while the RSD is < 3.1%.

Table (4): The accuracy and precision of the method

Aliphatic amines	Amount added (μg.ml^{-1})	Recovery* (%)	Average recovery (%)	RSD* (%)
Ethylamine	2	98.17		1.75
	4	100.96	99.49	0.87
	6	99.35		0.55
sec-Butylamine	3	97.90		1.30
	4	101.21	99.31	0.91
	5	98.83		0.77
tert-Butylamine	4	102.34		1.73
	6	98.51	100.56	1.14
	8	100.82		1.15
Benzylamine	5	101.42		1.27
	7	101.00	100.07	0.72
	9	100.79		0.63
Dibenzylamine	5	99.257		3.01
	6	102.86	101.32	2.11
	7	101.85		1.95
Cyclohexylamine	4	100.84		0.94
	6	98.99	100.16	0.86
	8	100.64		0.71

Effect of intereferences

To check the selectivity of the method, 100 $\mu g.ml^{-1}$ of ethylamine as a typical aliphatic amine was determined using the recommended procedure in the presence of various foreign organic and inorganic compounds. The results shown in Table (5) indicate that the method was selective. Arginine interferes seriously due to the presence of basic amino group in its side chain.

Table (5): Effect of interferences

Foreign compounds	Fold excess added	Relative error (%)
N-Methyl aniline	10	0.0
	15	2.2
	25	5.6
Acrylamine	10	2.0
	15	3.3
	20	6.0
N,N-Dimethyl aniline	20	0.0
	25	2.8
	30	5.5
Aniline	10	2.7
	20	5.33
	40	12.6
Benzamide	10	-1.1
	20	-4.4
	30	-5.5
Acetanilide	10	2.0
	15	4.0
	20	6.0
EDTA	10	0.0
	15	3.3
	20	5.3
Glucose	10	0.0
	25	3.6
	50	5.3
Glycine	10	1.56
	20	3.9
	50	6.8
Proline	10	0.0
	20	2.2
	60	6.1
4-Hydroxy proline	10	2.6
	20	4.4
	30	8.3
Alanine	10	2.7
	20	5.3
	40	12.6

Foreign compounds	Fold excess added	Relative error (%)
Leucine	10	-1.9
	20	-3.7
	50	-8.5
Isoleucine	14	-2.1
	28	3.9
	42	-9.4
Ammonium chloride	10	1.1
	20	2.2
	50	6.1
Serine	10	2.4
	20	3.5
	50	8.0
Tryptophan	10	-1.8
	20	-3.5
	50	-7.3
Methionine	10	-2.1
	20	-4.5
	50	-9.0
Threonine	10	0.0
	20	1.1
	50	6.3
Cysteine	10	2.6
	20	4.0
	50	9.0
Asparagine	10	0.8
	20	1.5
	50	6.0
Glutamine	10	2.1
	20	4.0
	50	9.1
Arginine	1	92.25
	2	185.48

The nature and stability constant of the coloured product

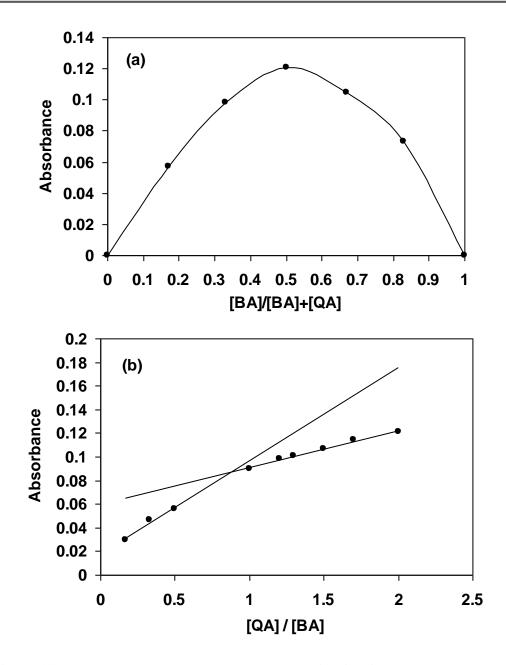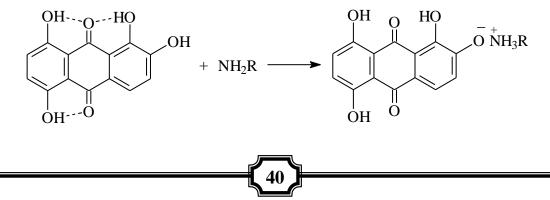The nature of the coloured products was studied by applying Job's and mole ratio methods[23] (Figure 3).

**Figure (3): a. Job's method for benzylamine-quinalizarin product
b. Mole ratio method for benzylamine-quinalizarin product**

Results show that the mole ratio of quinalizarin to aliphatic amines was 1:1 indicating that one amino group responsible for the formation of the product. Therefore, the formation of the product may occur as follows[24]:

The stability constants of these products were determined (Table 6), the results show that the stability constants ranged from 1.00×10^6 l.mol^{-1} for ethylamine to 3.75×10^6 l.mol^{-1} for sec-butylamine indicating high stabilities for these products.

Table (6): The stability constants of the products

Aliphatic amines	Stability constant* (l.mol^{-1})
Ethylamine	1.00×10^6
sec-Butylamine	3.75×10^6
tert-Butylamine	1.55×10^6
Benzylamine	1.74×10^6
Dibenzylamine	2.51×10^6
Cyclohexylamine	2.00×10^6

* Average of three values

Comparison of Methods

Table (7) gives the results obtained by application of the present method and literature methods to the determination of cyclohexylamine.

Table (7): Comparison of the present method with other methods

Analytical parameter	Present method	Alizarin red sulphonate (ARS) method[19]	2,4-Dinitro-1-fluorobenzene (DNFB) method[21]
λ_{max} (nm)	560	530	353
pH	Without	Without	8.4
Medium	Aqueous	Aqueous	Organic
Temp. (°C)	R.T	40	40
Development time (min)	10	30	60
Stability period (min)	50	60	30
Molar absorptivity l.mol^{-1}.cm^{-1}	6690	7790	13957
Linearity (μg.ml^{-1})	0.5-10	0.1-10	1-8
Recovery (%)	100.16	101.03	100.166
RSD (%)	< 1	< 1	< 1
Analytical application	-	Pharmaceutical preparations	-

Results shown in Table (7) confirm that the present method was rapid, simple, has low detection limit, wide range for determination and cheap since it does not require neither organic solvent nor solvent extraction.

CONCLUSION

A spectrophotometric method has been developed for the determination of aliphatic amines in aqueous solution, based on proton transfer reaction with quinalizarin reagent. The method was simple, sensitive, low cost and does not require solvent extraction.

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