Arabian Journal of Chemistry (2015) 8, 465–473

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Selective spectrophotometric determination of some primary amines using 2,4-dinitrofluorobenzene reagent

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Received 13 November 2010; accepted 21 January 2011
Available online 28 January 2011

KEYWORDS

DNFB;
Primary aromatic amines;
Spectrophotometry;
Aqueous solution

Abstract A spectrophotometric method is developed for the quantitative determination of some primary aliphatic and aromatic amines, i.e., allyl amine, 1,5-diaminopentane, 1,6-diaminohexane, cyclohexylamine, *m*-aminophenol, benzidine and *p*-phenylenediamine. The method is based on the interaction between these amines and 2,4-dinitrofluorobenzene (DNFB) reagent. The spectra of the products show maximum absorption that ranged from 355–357 nm and 366–377 nm with molar absorptivities that ranged from 1.086×10^4–6.398×10^4 and 7.566×10^3–1.581×10^4 l/mol cm for aliphatic and aromatic primary amines, respectively. Beer's law is obeyed in the concentration range of 0.25–8.0, 1.0–10, 0.25–2.50, 1.0–8.0, 2.0–20, 1.0–12.0 and 1.0–10.0 μg/ml for the above mentioned amines, respectively, and the mean percent recoveries ranged between 97.8% and 103.3% with precision (RSD) better than 5.5% for all the amines under study. In addition, the stability constant has been determined and the mechanism is proposed for the DNFB-amine products.

1. Introduction

A great number of aromatic amines are of considerable importance in industrial, toxicological and pharmaceutical aspects (Kirk-Othmer, 1979). Short-chain aliphatic amines are presented widely in the aquatic environment due to their wide spread use in several industrial, chemical and manufacturing applications (Lloret et al., 2004; Eller et al., 2007). 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 stinky smell, these compounds may be hazardous to human health as they are sensitizers and irritants to skin, eyes, mucus membranes and respiratory tract. In addition; they can react with certain nitrogen-containing compounds to form nitrosamines, which are potentially carcinogenic substances (Namiesnik et al., 2003).

Several spectrophotometric methods using various reagents have been described for the determination of aliphatic and aromatic amines. The interaction of *n*-alkylamine with chloranil has been investigated (Dwivedi and Banga, 1981), which in-

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.01.024

volves the participation of electron donor–acceptor complex between the primary aliphatic amines and chloranil resulting in mono and di-substituted products. Primary, secondary and tertiary amines, both aliphatic and aromatic amines, are shown to react with *p*-chloranil and form blue to purple colour in dioxane/2-propanol (1:4 v/v) (Smith and Davis, 1984) or used as a selective reagent for the determination of primary amines in aqueous solution (Al-Sabha, 1984). Also, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Al-Sabha, 1997) and *p*-fluoranil (Hanna, 1986) have been used as π-acceptors for the determination of primary aliphatic and aromatic amines as *n*-donors in aqueous medium. Some new 9-substituted 10-methyl acridinium trifluoromethanesulfonate salts have been synthesized and shown to react in methanol with aniline and *n*-butylamine to form derivatives which are absorbed at 445 and 439 nm, respectively (Dunning and Stewart, 1991). Sodium 1,2-naphthoquinone-4-sulphonate in the presence of a non-ionic surfactant and carbonate buffer solution is used for the determination of primary and secondary amines at 45 °C (Cabeza et al., 1994), also it was used for the determination of some drugs containing the primary amino group (Iskender and Sagirli, 2000). Terephthaloylacetylene forms an yellow or orange coloured product with primary and secondary amines and may be used for their detection (Tiwari et al., 2002). Quinoline-2-(carboxaldehyde) has been used as a reagent for the spectrophotometric detection of primary amines (You-Zung et al., 1990). *N*-Hydroxysuccinimidyl-naphthapyrone acetate has been utilized for the determination of primary and secondary amines at pH 8.5 which give the corresponding derivatives within 5 and 15 min (Liu et al., 2001). 9-Chloroacridine reagent (Steward et al., 1969; Steward and Lotti, 1970) has also been used for the determination of primary aromatic amines. This method needs heating the analytical solution and requires an excess of acridine reagent in order to achieve the reproducible results. *m*-Dinitrobenzene as π-acceptor has been used for the determination of several aliphatic amines as *n*-donors in acetone medium and the formation of charge transfer complex has been proposed to explain the spectroscopic behaviour of the mixtures (Siddiqi and Pathania, 2003). Most of the above methods suffer from several disadvantages that (a) they cannot be applied for all types of primary aliphatic and aromatic amines, (b) there is a lack of sensitivity and selectivity and (c) the estimation of these compounds is applied in the organic medium.

The present work describes a simple and sensitive spectrophotometric method for the analyses of some primary aliphatic and aromatic amines. The method is based on the formation of DNFB-amine derivative from the amine and DNFB.

2. Experimental

2.1. Apparatus

All absorption measurements were made on a Shimadzu UV-210A double-beam spectrophotometer supplied with a digital printer DP80Z and matched 1-cm optical silica cells. Heating of solutions was carried out on a water bath of frost instruments, LTD. The reading of pH was made on a PW 9420 pH meter supplied with an electrode type CE 10–12 pH. Weighing was carried out on a balance type of Mettler H 54 AR.

2.2. Reagents

Analytical grade chemicals, acetonitrile and distilled water were used. Standard solutions of primary aliphatic amines (250 μg/ml) in acetonitrile were prepared and aromatic amines (100 μg/ml) were dissolved in a small amount of absolute ethanol and diluted with distilled water. These amines are supplied by FLUKA & BDH companies. 0.01 M of DNFB (Sigma Co.) was prepared in acetonitrile for the determination of aliphatic amines or by dissolving in a small amount of absolute ethanol and diluting with distilled water for the determina-

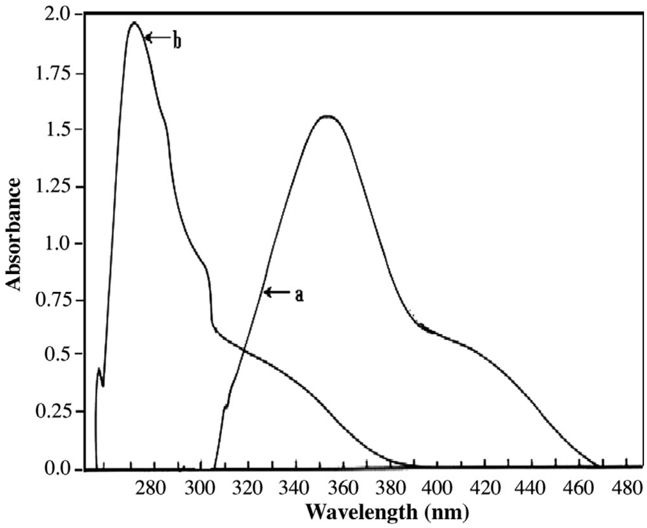


Figure 1 Absorption spectra of 15 μg/5 ml 1,6-diaminohexane with DNFB against reagent blank (a) and blank against acetonitrile (b) plotted under the general procedure conditions.

Table 1 Wavelengths, bases and the optimum amounts of base and DNFB for the studied amines.

Compounds	λ_{max} (nm)	Base	Base amount 10^{-2} M (ml)	DNFB 10^{-2} M (ml)
1,5-Diaminopentane	355	–	–	0.2
Allylamine	353	–	–	0.2
1,6-Diaminohexane	353	NaOH	0.5	0.6
Cyclohexylamine	357	–	–	0.4
Benzidine	377	$NaHCO_3$	0.4	3.0
m-Aminophenol	366	CH_3COONa	1.4	1.5
p-Phenylenediamine	375	$NaHCO_3$	1.0	1.0

Table 2 Effect of solvent on the absorption of amine-DNFB product.

Amine dissolved in	Reagent dissolved in	Dilution by	Absorbance of	
			1,6-Diaminohexane (2 μg/ml)	Benzidine (10 μg/ml)
Water	Water	Water	0.048	0.049
Water	Ethanol	Water	0.259	−0.030
Ethanol	Ethanol	Ethanol	0.675	0.019
Water	Methanol	Water	0.173	0.039
Methanol	Methanol	Methanol	0.364	0.011
Water	Acetone	Water	0.648	0.032
Acetone	Acetone	Acetone	0.165	0.024
Water	Acetonitrile	Water	0.248	0.021
Acetonitrile	Acetonitrile	Acetonitrile	1.399	0.027

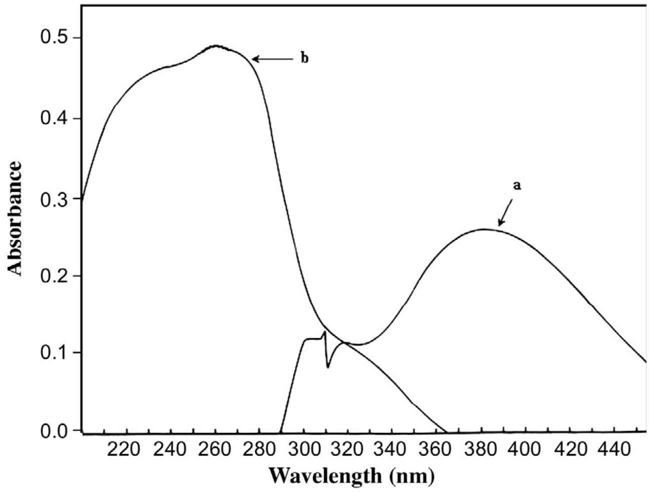


Figure 2 Absorption spectra of 400 μg/25 ml benzidine with DNFB against reagent blank (a) and blank against distilled water (b) plotted under the general procedure conditions.

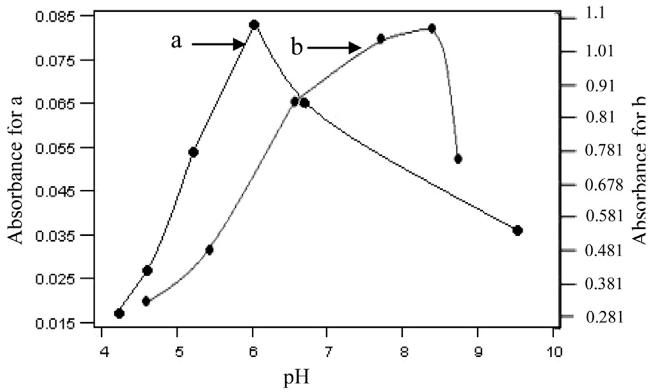


Figure 3 Effect of pH on the absorbance of DNFB product with (a) 10 μg/ml benzidine, (b) 2 μg/ml 1,6-diaminohexane.

tion of aromatic amines. 0.1% of sodium dodecyl sulphate (SDS) and Triton-x 100, 0.001 M of sodium hydroxide, 0.01 M of sodium bicarbonate and sodium acetate solutions were prepared in distilled water.

Table 3 Order of addition.

Order of addition	Order No.
DNFB + 1,5-diaminopentane or cyclohexylamine or allylamine	I
DNFB + 1,6-diaminohexane + NaOH	II
Benzidine + DNFB + Tween-80 + $NaHCO_3$	III
DNFB + *m*-aminophenol + CH_3COONa	IV
p-Phenylenediamine + DNFB + $NaHCO_3$	V

2.3. General procedures

2.3.1. Determination of aliphatic amines

Increasing volumes (ml) containing 1.25–50, 5–50, 1.25–12.5 and 5–40 μg of allyl amine, 1,5-diaminopentane, 1,6-diaminohexane and cyclohexylamine were added to separated sets of 5 ml volumetric flasks and mixed with the optimum amounts of DNFB as cited in Table 1 followed by the addition of 0.5 ml of NaOH in the case of 1,6-diaminohexane. Then the solutions were diluted to the mark with acetonitrile solvent and left for 30 min at room temperature (60 min at 40 °C for cyclohexylamine). The absorbances were measured at the appropriate λ_{max} against the respective reagent blank.

2.3.2. Determination of aromatic amines

According to the order of addition for each amine cited in Table 3, increasing volumes (ml) containing 25–300, 50–500 and 25–250 μg of benzidine, *m*-aminophenol and *p*-phenylenediamine are mixed into separated sets of 25 ml volumetric flasks with the optimum amounts of DNFB and base as cited in Table 1, in addition to 1.8 ml of tween-80 in the case of benzidine. The solutions were left for 60, 100 and 40 min at 50 °C, 70 °C and 60 °C for benzidine, *m*-aminophenol and *p*-phenylenediamine, respectively. After cooling to room temperature the solutions were diluted to the mark with distilled water and the absorbances were measured at the appropriate λ_{max} against the respective reagent blank.

3. Results and discussion

The effect of various parameters on the absorption intensity of the formed dye was studied and the reaction conditions are optimized.

Table 4 Optimum conditions for the determination of primary amines with DNFB reagent.

Compound	Temp. (°C)	Development time (min)	Base 10^{-2} M (ml)	DNFB 1×10^{-2} M (ml)	Order of addition[b]	Surfactant 0.1%	Stability period (min)	Final pH
1,5-Diaminopentane	RT[a]	30	–	0.2	I	–	30	8.88
Allylamine	RT	20	–	0.2	I	–	40	8.77
1,6-Diaminohexane	RT	30	NaOH (0.5 ml, 10^{-3} M)	0.6	II	–	60	8.40
Cyclohexylamine	40	60	–	0.4	I	–	30	8.50
Benzidine	50	60	$NaHCO_3$ (0.4)	3.0	III	Tween-80 (1.8 ml)	60	6.02
m-Aminophenol	70	100	CH_3CO_2Na (1.4)	1.5	IV	–	45	6.60
p-Phenylenediamine	60	40	$NaHCO_3$ (1.0)	1.0	V	–	80	6.60

[a] Room temperature = 28 °C.
[b] As cited in Table 3.

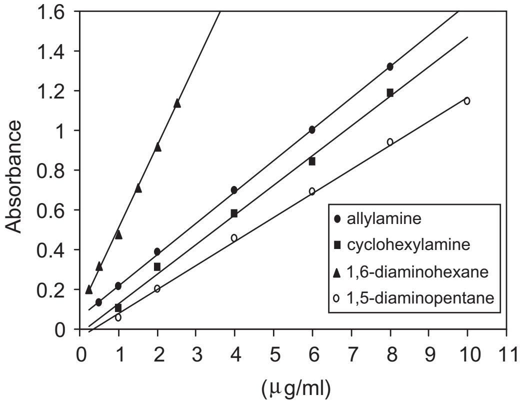


Figure 4a Calibration graphs for aliphatic amines.

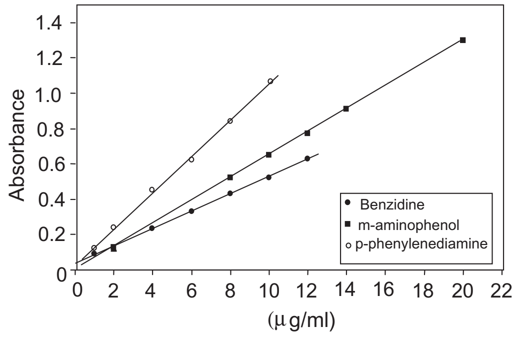


Figure 4b Calibration graphs for aromatic amines.

3.1. Effect of solvents

In the preliminary investigation work, it was found that DNFB reagent reacted with primary, secondary, tertiary aliphatic and aromatic amines and amides in the alcoholic medium of methanol, acetonitrile, acetone, ethanol, in addition to water. It was observed that most of these amines reacted with DNFB to produce various colours except the acetonitrile when used as a solvent for aliphatic amines and DNFB with diluting the same solvent lead to the production of yellow coloured products with aliphatic amines (in the presence of a base in the case of 1,6-diaminohexane) and gave maximum absorption at wavelengths that ranged between 355 and 357 nm for given amines (Fig. 1 and Table 2 for 1,6-diaminohexane as example), whereas other amines were either unreacted or gave low response. On the other hand, it was found that DNFB reacted with primary aromatic amines and produce yellow coloured products when water is used as the solvent for both DNFB and aromatic amine in the presence of a base and diluting with water gave a maximum absorption at wavelengths that ranged between 355 and 377 nm for given amines (Fig. 2 and Table 2 for benzidine as example). Therefore; these systems of solvents are recommended for the determination of aliphatic and aromatic amines.

3.2. Effect of pH and buffer solutions

The effect of pH on the absorption of the products was studied using different pH values of HCl or NaOH that ranged from 2 to 12. As shown in Fig. 3, it was found that the products formed with maximum absorption when the final pH of the solutions

Table 5 Summary of optical characteristics and statistics for the proposed method.

DNFB product with	Parameters						
	λ_{max}	Linearity range (µg/ml)	Limit of detection (µg/ml)	Molar absorptivity (L mol^{-1} cm^{-1})	Intercept	Slope	Correlation coefficient
Allylamine	353	0.25–8.0	0.0270	1.09×10^4	0.0590	0.1579	0.9998
1,5-Diaminopentane	355	1.0–10.0	0.0371	1.13×10^4	−0.0214	0.1488	0.9980
1,6-Diaminohexane	353	0.25–2.5	0.0120	6.40×10^4	0.0902	0.4150	0.9989
Cyclohexylamine	357	1.0–8.0	0.0706	1.40×10^4	−0.0457	0.1213	0.9992
Benzidine	377	1.0–12.0	0.0240	1.58×10^4	0.0396	0.0487	0.9997
m-Aminophenol	366	2.0–20.0	0.0988	7.56×10^3	−0.0007	0.9999	0.9999
p-Phenylenediamine	375	1.0–10.0	0.0497	1.20×10^4	0.0254	0.1023	0.9993

was 8.40 for 1,6-diaminohexane and 6.02 for benzidine as examples from the studied amines after the addition of NaOH, and a decrease in absorbances was observed through the addition of HCl, which may be attributed to the liberation of hydrogen fluoride. Therefore, different buffers with the above optimum pH were prepared to examine the sensitivity. It was found that they have no effect on the colour intensity.

Table 6 Precision and accuracy data for primary aliphatic and aromatic amines determination obtained by the proposed method.

Compound	Amount added (μg/ml)	Recovery[a] (%)	Average recovery (%)	RSD[a]
Allylamine	2.0	100.0	103.0	0.822
	4.0	107.5		0.628
	6.0	101.6		3.836
1,5-Diaminopentane	2.0	100.0	101.3	0.680
	4.0	101.5		0.865
	8.0	102.5		0.250
1,6-Diaminohexane	0.5	102.0	97.8	1.225
	2.0	95.0		0.500
	2.5	96.5		1.808
Cyclohexylamine	2.0	100.5	100.1	0.580
	6.0	100.0		0.168
	8.0	100.0		0.227
Benzidine	1.0	102.5	101.8	1.431
	2.0	100.6		2.100
	3.0	102.5		0.572
m-Aminophenol	3.0	107.1	102.9	5.236
	12.0	102.5		2.550
	20.0	99.1		1.885
p-Phenylenediamine	2.0	102.5	103.3	4.168
	4.0	105.0		1.592
	8.0	102.5		1.305

[a] Average for six determinations.

3.3. Effect of base

Various bases, such as NaOH, KOH, Na_2CO_3, $NaHCO_3$, NH_4OH and CH_3CO_2Na were examined in order to obtain high sensitivity. It was found that these bases reduced the intensity of the aliphatic amine-DNFB products except of 1,6-diaminohexane which shows increasing colour intensity in the presence of NaOH. For the aromatic amines, it was found that $NaHCO_3$ gave high intensity with benzidine and *p*-phenylenediamine and CH_3CO_2Na for *m*-aminophenol. The optimum amounts of these bases are cited in Table 4.

3.4. 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 at 5 min intervals against the reagent blank treated similarly. It was observed that the absorbance reached a maximum after a time that ranged between 30 and 60 min at room temperature (cyclohexylamine at 40 °C) and remains constant between 30 and 60 min for aliphatic amines, and between 40 and 60 min at a temperature that ranged between 50 °C and 70 °C and remains constant between 45 and 80 min for aromatic amines (Table 4).

3.5. Effect of DNFB concentration

The effect of changing the DNFB concentration on the absorbance of a solution containing a fixed amount of the primary amine was studied. It is evident that the absorbance increases with increasing DNFB concentration and reached a maximum at optimum concentrations cited in Table 4.

3.6. Effect of surfactants

Effect of various surfactants including cetyltrimethylammonium bromide (CTAB), cetylperydinum chloride (CPC), sodium dodecyl sulphate (SDS), tween-80 and triton x-100 was tested. It was found that these surfactants caused a decrease

Table 7 Effect of foreign compounds on the determination of 1,5-diaminopentane and *m*-aminophenol.

Foreign compound	Amount of interference (mg) added to		Relative error (*E*%)	
	1,5-Diaminopentane	*m*-Aminophenol	1,5-Diaminopentane	*m*-Aminophenol
Dibutylamine	0.3	0.2	1.66	−2.47
	0.6	0.4	2.02	−1.48
	0.3	0.6	2.50	7.54
Diethanolamine	0.1	0.2	2.72	−4.05
	0.4	2.0	−1.16	−1.78
	0.5	5.0	−8.78	−10.26
Triethylamine	0.1	0.2	2.60	−2.65
	0.4	0.6	−0.36	0.88
	0.8	0.8	−22.29	7.08
Formamide	0.3	0.2	3.27	3.26
	1.0	1.0	−3.91	2.38
	4.0	10.0	−23.48	2.25

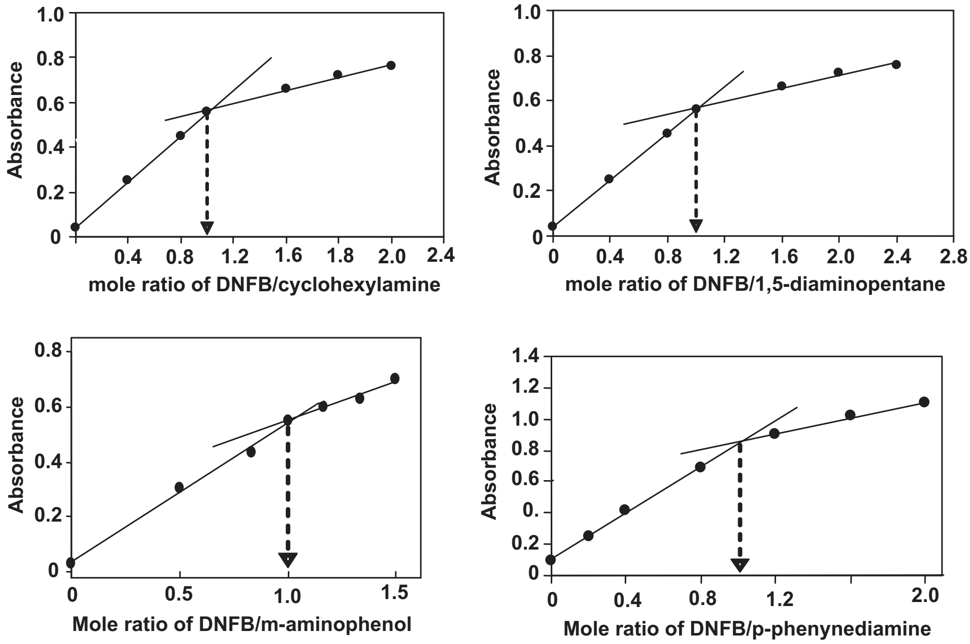


Figure 5 Mole ratio plots of DNFB products with samples of primary aliphatic and aromatic amines at their corresponding λ_{max} and under their optimum conditions.

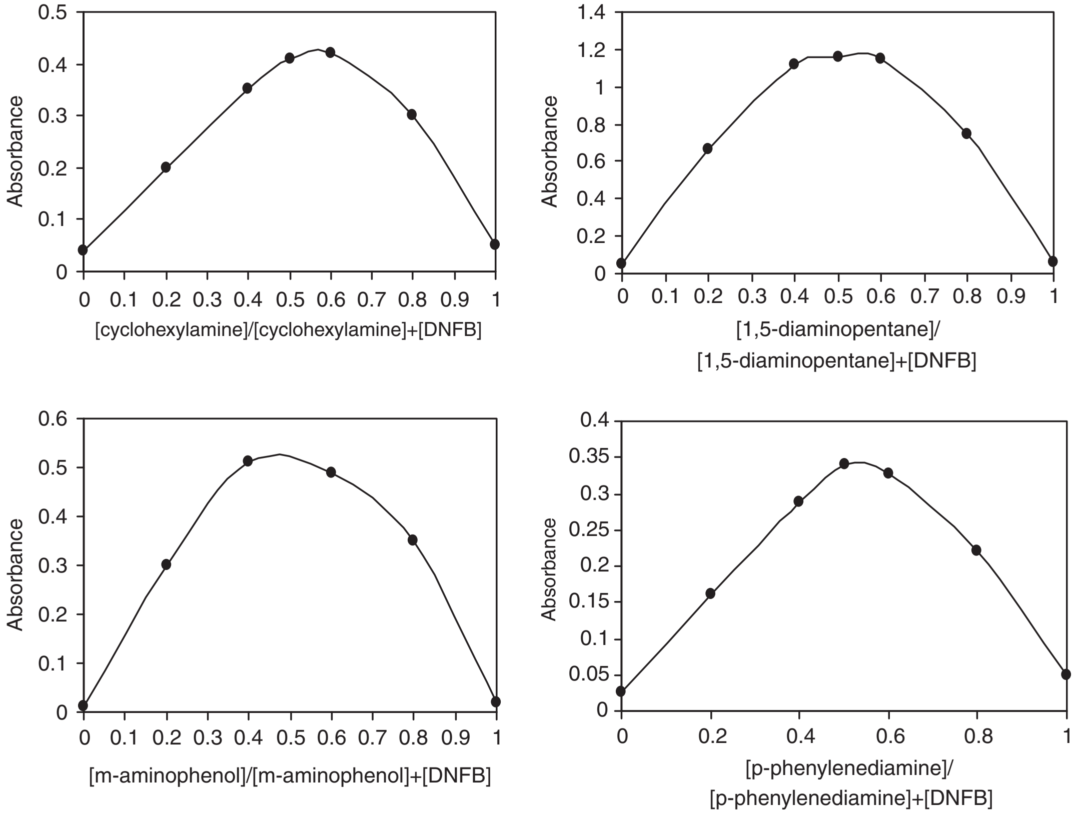


Figure 6 Job plots for the studied aliphatic and aromatic primary amines.

in the absorbances of both aliphatic and aromatic amines except for benzidine-DNFB product which shows increasing intensity in the presence of 1.8 mL of tween-80 which is recommended in the procedure (Table 4).

3.7. *Effect of order of addition*

In order to obtain the high colour intensity the order of addition of reagents for aliphatic and aromatic amines should be

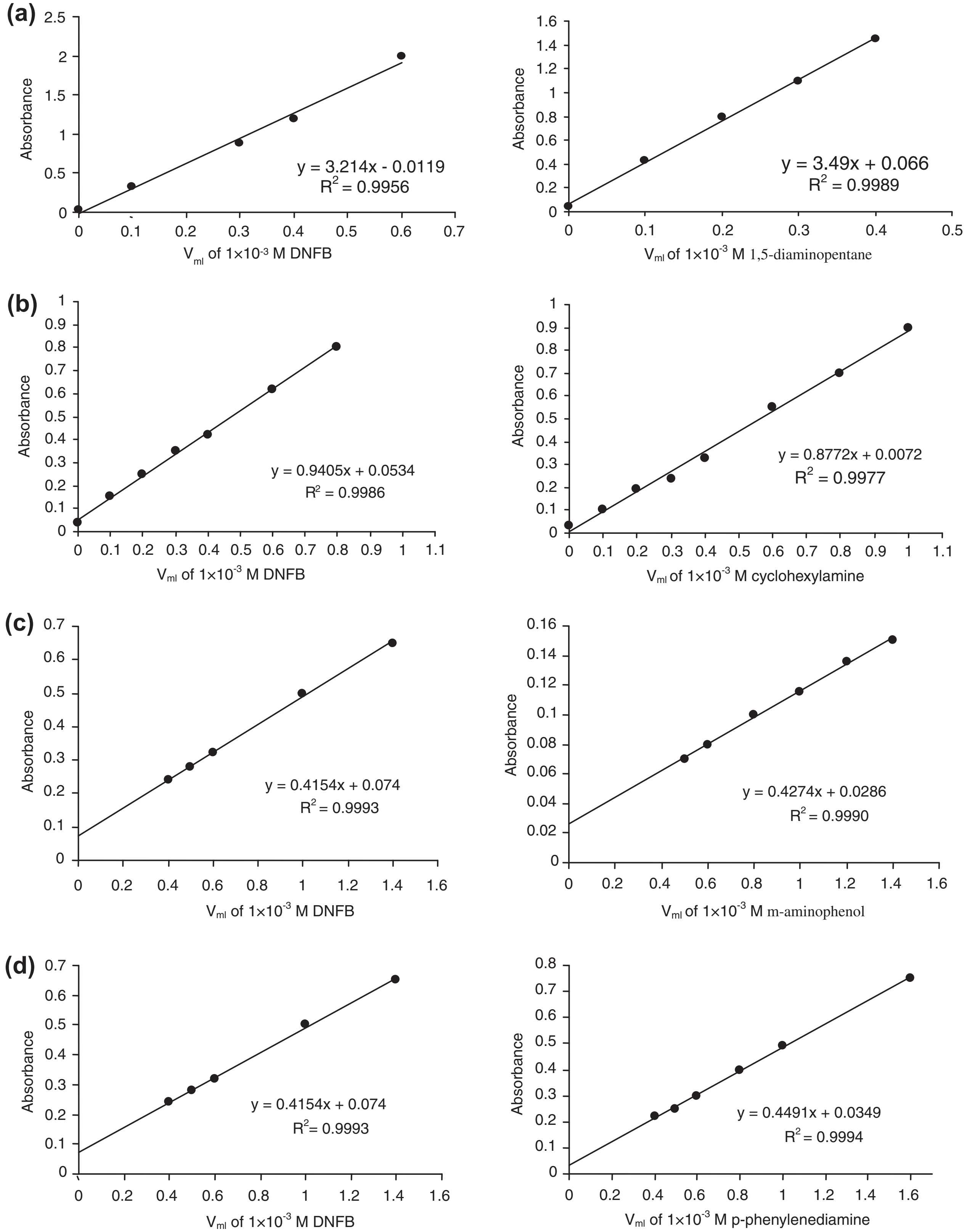


Figure 7 Slope ratio plots for DNFB product with (a) 1,5-diaminopentane, (b) cyclohexylamine, (c) *m*-aminophenol and (d) *p*-phenylenediamine.

carried out as shown in Table 3, otherwise a loss in colour intensity was observed.

However; the optimum reaction conditions for developing the colour intensity of the studied primary amine-DNFB products are summarized in Table 4.

3.8. Analytical parameters

Under the experimental conditions described in Table 4, standard calibration graphs for the studied primary aliphatic and aromatic amines with DNFB were constructed by plotting absorbance versus concentration (Fig. 4a and b). The correlation coefficients ranged from 0.9980 to 0.9999, indicating good linearity. Beer's law is obeyed in the ranges as cited in Table 5, and the molar absorptivity values indicate the high sensitivity of the proposed method.

3.9. Precision and accuracy

Six replicate measurements are performed at three different concentrations within the Beer's law limits of each amine. The relative standard deviation and recovery % results cited in Table 6 indicate the high precision and accuracy of the proposed method.

3.10. Interferences

The interference from various organic nitrogen compounds including secondary, tertiary amines and amides on the determination of 5 µg/ml of 1,5-diaminopentane (as example for primary aliphatic amines) and 12 µg/ml of *m*-aminophenol (as example for primary aromatic amines) was examined. It was found that these compounds did not affect the accuracy of the determination of the above compounds indicating the method is selective for the determination of compounds containing the primary amino group. An error of 5.0% in the absorbance readings was considered tolerable. The results are summarized in Table 7.

3.11. Stoichiometric relationship

The molar ratio of the product formed between the studied amines and the reagent used was investigated applying the molar ratio method (Hargis, 1988) using equimolar solutions of the amine and reagent. The results indicated that the product was formed in the ratio of 1:1 (Fig. 5, typical examples have been plotted). This finding supports that the interaction of the studied amino compounds and the reagent used takes place at only one site which was the more sterically free terminal basic primary amino group. However; these results are proved by using Job (Fig. 6) and slope ratio methods (Fig. 7) (Hargis, 1988).

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the amine and DNFB (As) to one containing an excessive amount of DNFB reagent (Am). The average conditional stability constants of the products are calculated by the following equation:

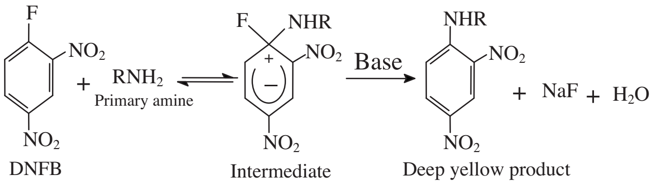
$$K_c = 1 - \alpha/\alpha^2\ C$$

$$\alpha = A_m - A_s/A_m$$

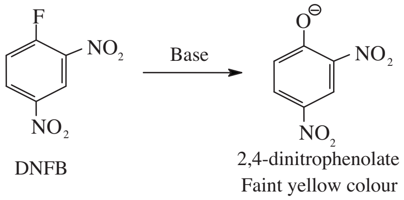
where K_c is the association constant (l mol^{-1}), α is the degree of dissociation and C the concentration of the product which is equal to the concentration of amine. The results shown in Table 8 indicate that the products are relatively stable.

Table 8 Association constants of the DNFB-primary amine products.

Amine	ml of 1×10^{-2} M amine/25 ml	Absorbance A_s	Absorbance A_m	α	Kst (l mol^{-1}) average
Allylamine	0.10	0.110	0.380	0.710	2.200×10^6
	0.30	0.463	0.780	0.406	
	0.50	0.942	0.980	0.038	
1,5-Diaminopentane	0.10	0.144	0.260	0.446	1.252×10^6
	0.20	0.438	0.500	0.124	
	0.30	0.724	0.790	0.083	
1,6-Diaminohexane	0.20	0.383	0.460	0.167	0.504×10^6
	0.30	0.588	0.700	0.160	
	0.40	0.725	0.920	0.211	
Cyclohexylamine	0.10	0.510	0.590	0.135	2.032×10^6
	0.15	0.671	0.780	0.139	
	0.20	0.927	1.040	0.108	
Benzidine	0.05	0.211	0.29	0.2724	1.95×10^5
	0.10	0.271	0.54	0.498	
	0.13	0.352	0.68	0.482	
m-Aminophenol	0.1	0.091	0.30	0.700	1.105×10^4
	0.2	0.214	0.59	0.637	
	0.25	0.231	0.74	0.687	
p-Phenylenediamine	0.06	0.103	0.27	0.6185	4.39×10^6
	0.1	0.184	0.47	0.6085	
	0.2	0.853	0.88	0.0306	



Scheme 1 Probable reaction mechanism of primary amines with DNFB reagent.



Scheme 2 Basic hydrolysis of DNFB.

3.12. Reaction mechanism

The reaction of DNFB with amines that own a free primary amine group results in the formation of coloured products in basic medium (Georgion et al., 1991; Correa et al., 2000). This reaction was first introduced by Sanger (Sanger, 1945) as means for the determination of the DNA sequence. Based on the mole ratio method and supported by Job and slope ratio methods, it was found that the primary amine interacted with the DNFB in the ratio of 1:1. This result indicates that the reaction between the amine and the reagent used takes place at only one site which was the more sterically free terminal amino group. The reaction is a typical nucleophilic substitution and proceeds through an intermediate product as cited in Scheme 1.

However; it was observed that blank gave a faint yellow colour with maximum absorption at 250 nm. This may be attributed to the formation of 2,4-dinitrophenolate ion through its basic hydrolysis (Correa et al., 2000) as cited in Scheme 2.

4. Conclusion

A simple, precise, selective and sensitive spectrophotometric method has been developed for the determination of trace amounts of some primary aliphatic and aromatic amines based on their reaction with DNFB to form coloured products. The method does not require any pretreatment of the amines or extraction procedure and has a good accuracy and precision.

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