



SPECTROPHOTOMETRIC DETERMINATION OF AMINOPHENOL ISOMERS USING 9-CHLOROACRIDINE REAGENT

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A simple, sensitive and accurate spectrophotometric method is developed for the quantitative determination of aminophenol isomers, i.e. *o*-aminophenol, *m*-aminophenol and *p*-aminophenol. The method is based on the interaction between these amines and 9-chloroacridine reagent (9-CA). The spectra of the products show maximum absorption at 436 nm. Beer's law is obeyed in the concentration range of 0.25-12, 0.2-10 and 0.0-10 $\mu\text{g mL}^{-1}$ with molar absorptivity values 7.20×10^3 , 7.67×10^3 and $5.93 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ for the above mentioned isomers respectively. The mean percent recoveries are ranged between 97.3 % and 101.07 % with relative standard deviation (RSD) \leq 4.7 % for all the isomers. In addition, the stability constant has been determined and the mechanism is proposed for the 9-CA-aminophenol products.

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sensitive spectrophotometric method for analyses of aminophenol isomers. The method is based on the formation of coloured products from the reaction of these isomers with 9-chloroacridine reagent in ethanolic medium.

Introduction

Aminophenol isomers are primarily used as intermediates in the manufacture of dyes and pigments. Also used as a vulcanization accelerator and as an antioxidant in rubber compounds. These are crystalline solids of low volatility and cause contact dermatitis, which appears to be the greatest hazard arising from their use in industry.¹ These compounds are also the main metabolites of aniline both in vivo and in vitro.²

Several spectrophotometric methods using various reagents such as 4-aminoantipyrine,³ 2-methyl-5-vinylpyridine⁴ and salicylaldehyde⁵ are used for determination of *o*-aminophenol. Potassium iodate,^{6,7} crowned 2,4-dinitrophenolazophenol-barium (II) complex⁸ and 2,4-dinitrofluorobenzene⁹ are used for determination of *m*-aminophenol. Resorcinol,¹⁰ sodium sulphide,¹¹ 4-nitro- or 2,4-dinitrobenzaldehyde¹² and 5,7-dichloro-4,6-dinitrobenzofuroxan¹³ have been described for the determination of *p*-aminophenol. Charge transfer complex formation reactions have been widely used for determination of aminophenol isomers as n -donors with different π -acceptors such as chloranil,¹⁴ bromanil,¹⁵ fluoranil¹⁶ and DDQ.¹⁷ Also, 1,2-naphthoquinone-4-sulphonate (NQS) reagent has been used for determination of aminophenol isomers.¹⁸ However; most of these methods are either insufficiently sensitive or tedious and required an extraction step. Various analytical techniques have been reported for determination of aminophenol isomers such as thin layer chromatography,¹⁹ adsorptive stripping voltammetry,^{20,21} liquid chromatography,²²⁻²⁵ potentiometry²⁶ and fluorimetry.²⁷ These methods needed of highly sophisticated instruments. The present work describes a simple and

Experimental

Apparatus

Spectra and absorbance measurements were made with UV-Visible double beam spectrophotometers (Perkin-Elmer, lambda 25). with 1-cm matched silica cells. The pH measurements were made by using both Cyber Scan 510 pc. pH meter with a combined glass electrode. Heating of solutions is carried out on a water bath of frost instruments, LTD). Weighing is carried out on a sensitive balance type of Mettler H 54AR. All calculations in the computing process were done in Microsoft Excel for Windows.

Reagents

All chemicals used are of the highest purity available which are provided by BDH, Fluka and Molekula companies. 9-Chloroacridine (Eastman chemical Co.) was used as the chromogenic reagent. Absolute ethanol is used (ROTH Co.). Sodium hydroxide (1×10^{-2} M) and hydrochloric acid (1×10^{-2} M) solution are prepared by appropriate dilution of the concentrated NaOH (1 M) or HCl (1 M) solutions with distilled water.

9-chloroacridine reagent (1×10^{-3} M) (9-CA). The 25 ml solution is prepared by dissolving 0.0053 g of 9-chloroacridine in ethanol absolute and then the volume is completed to 25 ml in a volumetric flask. The solution is prepared daily and used immediately²⁸.

Aminophenol isomers. Ethanolic solution of 25 $\mu\text{g mL}^{-1}$ prepared for each of *o*-aminophenol (*oA*), *m*-aminophenol (*mA*) and *p*-aminophenol (*pA*).

Recommended procedure

Appropriate volumes containing 0.25-12, 0.20-10 and 0.00-10 $\mu\text{g mL}^{-1}$ of *oA*, *mA* and *pA* standard solutions followed by addition of 2.0, 2.5 and 2.0 ml of 9-CA reagent solution into separated 5-ml volumetric flasks; respectively. The solutions were diluted to the mark with absolute ethanol, and were left for 30 min at 50 °C for *oA* and for 30 and 15 min at 40 °C for each of *mA* and *pA* respectively. A portion of the solution was transferred into a 1cm silica cell to measure the absorbance at 436 nm against their respective reagent blank.

Results and Discussion

The reagent 9-CA was reacted quantitatively with aminophenol isomers in alcoholic medium and produce yellowish-green colored solutions immediately. The intensity of this colour increased when the reaction mixtures were heated in contrast to the reagent blank and show a maximum absorption at 436 nm, which was used in all subsequent experiments, where as blank reagent shows no absorption at this wavelength (Figure 1).

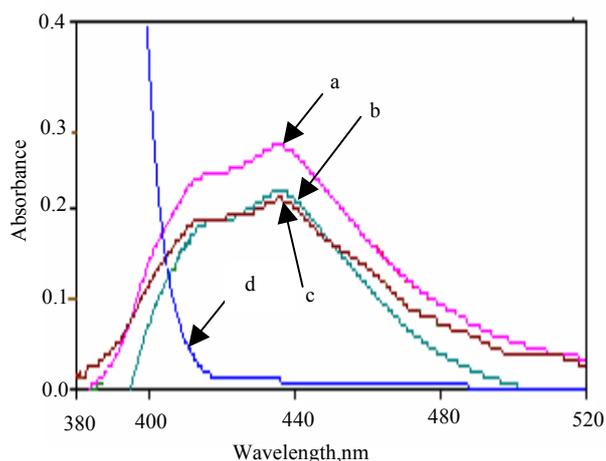


Figure 1. Absorption spectra of 2.5 $\mu\text{g mL}^{-1}$ of a) *pA*, b) *oA*, c) *mA* and d) Reagent blank against ethanol at optimum conditions

Selecting optimum reaction conditions

Preliminary experiments were carried out to choose the best solvent from water, methanol, ethanol, acetone and acetonitrile, optimum pH of the reaction mixtures, concentration of 9-CA, temperature of the reaction mixture and time allowed to the reaction mixture to develop highest colour intensity of the final product at 436 nm.

It was observed that a reaction mixture prepared from mixing 1 ml solution containing 2.5 $\mu\text{g mL}^{-1}$ of *pA*, as typical sample, with 1 ml of 1×10^{-3} M 9-CA in 5 ml of the reaction mixture prepared in ethanol absolute as solvent gave maximum absorbance compared to reaction mixtures prepared with other solvents (Table 1).

Table 1. Effect of solvent on colour intensity of *pA*-9-CA product.

<i>pA</i> , 5 $\mu\text{g mL}^{-1}$ dissolved in	9-CA 1 ml of 1×10^{-3} M dissolved in	Final dilution with	Absorbance
Water	Water	Water	-----
Water	Ethanol	Water	-----
Water	Ethanol	Ethanol	0.189
Ethanol	Ethanol	Ethanol	0.349
Water	Methanol	Water	-----
Water	Methanol	Methanol	0.326
Methanol	Methanol	Methanol	0.143
Water	Acetone	Water	0.274
Water	Acetone	Acetone	0.025
Acetone	Acetone	Acetone	-----
Water	Acetonitrile	Water	0.027
Water	Acetonitrile	Acetonitrile	0.276
Acetonitrile	Acetonitrile	Acetonitrile	-----

The pH of the reaction mixture was varied between pH 2.5 and 11.5 by adding 0.01 M of HCl or NaOH. It was found that the sensitivity of products were decreased. However; the pH of final solutions were measured in the absence of HCl and NaOH and found 9.50, 9.48 and 9.60 for *oA*, *mA* and *pA* respectively. Different buffer solutions (bicarbonate, borate and phosphate of pH 9.6) were also examined. These showed a negative effect on the absorbance of the products.

It was found that 2.0, 2.5 and 2.0 ml of 1×10^{-3} M solution of 9-CA in 5 ml of the reaction mixtures, containing 2.5 $\mu\text{g mL}^{-1}$ of each isomer in ethanol medium separately, showed maximum absorbance at 436 nm after 10 min at room temperature for *oA*, *mA*, and *pA* respectively.

The effect of temperature on the maximum absorbance of the reaction products for aminophenol isomers was studied at temperatures 24 °C, 40 °C and 50 °C at the above stated optimum conditions. It was found that the maximum absorbance was obtained at 50 °C after 30 min for *oA*, 40 °C after 30 min for *mA* and 40 °C after 15 min for *pA*. The absorbance remained constant over an appreciable time period ranging from 40-55 mins but decreased thereafter indicating dissociation of the product.

Quantitation

Under the experimental conditions described above, standard calibration graphs of products for aminophenol isomers with 9-CA reagent were constructed by plotting absorbance versus concentration as shown in Figure 2. The linear regression coefficient of the plot were 0.999, 0.999 and 0.999 with molar absorptivity values 7.202×10^3 , 7.671×10^3 and 5.936×10^3 for *oA*, *mA* and *pA* respectively indicating the high sensitivity of the method

Limit of detection (*LOD*), which is the smallest concentration that can be measured, and limit of quantitation (*LOQ*), which is the level above which quantitative results may be obtained with a specified degree of confidence, are determined by taking the ratio of standard deviation of the blank with respect to water and the slope of calibration curve by applying the following equations:

$$LOD = \frac{3.3 \sigma B}{S}$$

$$LOQ = \frac{10 \sigma B}{S}$$

whereas:

σB = standard deviation for six determinations of blank

S = slope of calibration graph for each isomer

LOQ is approximately 3.3 times LOD . Naturally, the LOQ slightly crosses the lower limit of Beer's law range, (Table 2). Sandell's sensitivity was determined by 0.001/slope.

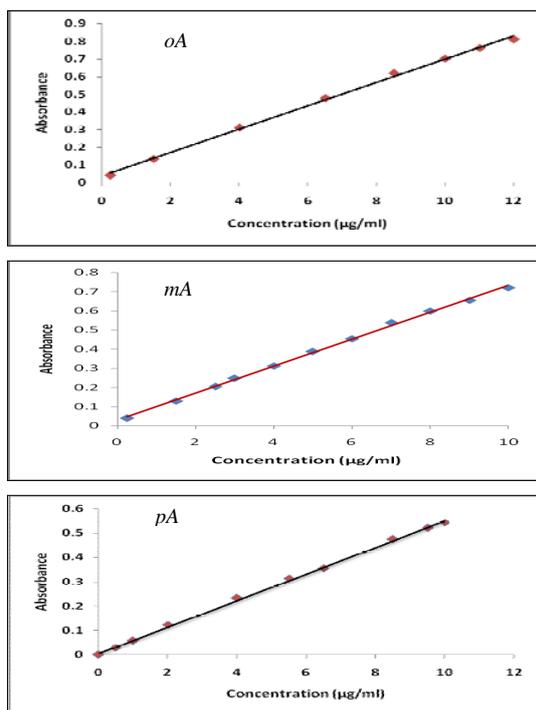


Figure 2. Calibration graphs of 9-CA-aminophenol isomers products

Table 2. Summary of optical characteristics and statistic data for the proposed method

Parameter	<i>oA</i>	<i>mA</i>	<i>pA</i>
Beer's law limits, $\mu\text{g mL}^{-1}$	0.25 -12	0.20 -10	0.00 -10
Molar absorptivity $\text{L mol}^{-1} \text{cm}^{-1}$	7.20×10^3	7.67×10^3	5.93×10^3
LOD, $\mu\text{g mL}^{-1}$	0.233	0.133	0.177
LOQ, $\mu\text{g mL}^{-1}$	0.706	0.404	0.539
Sandell's sensitivity $\mu\text{g cm}^{-2}$	0.0151	0.0142	0.0183
Average recovery, %*	98.83	97.33	101.07
Correlation coefficient	0.998	0.998	0.999
Regression equation, Y^{**}			
Slope, a	0.066	0.070	0.054
Intercept, b	0.037	0.030	0.007
RSD**	≤ 4.6	≤ 4.7	≤ 1.36

*Average of six determinations.

** $Y = aX + b$, where X is the concentration of isomer in $\mu\text{g mL}^{-1}$.

Interferences

The interference from various organic nitrogenous compounds including primary, secondary, tertiary aliphatic amines, amides, phenols and n-hexane on the determination of $5 \mu\text{g mL}^{-1}$ of *pA* (as example for aminophenol isomers) was examined by using recommended procedure. It was found that these compounds did not affect the accuracy of the determination of the above compound indicating the method is selective for the determination of aminophenol isomers which are containing primary aromatic amino group. An error of $\pm 5.0\%$ in the absorbance readings was considered tolerable. The results are summarized in Table 3.

Table 3. Effect of foreign compounds on the determination of $5 \mu\text{g mL}^{-1}$ *pA*

Foreign compound	Amount added, $\mu\text{g mL}^{-1}$	Recovery, %
Hexylamine	40	98.90
	100	98.18
	300	93.80
Di-n-propylamine	40	97.82
	100	96.73
	300	93.09
Tri-butylamine	40	97.46
	100	97.09
	300	93.46
Phenol	250	100.00
	500	96.36
	800	94.91
Acrylamide	250	101.46
	500	103.64
	1000	105.09
Benzamide	250	98.91
	500	95.30
	1000	94.20
Toluene	250	102.50
	500	96.40
	1100	94.50
n-Hexane	250	103.63
	500	97.10
	1100	94.57

Stoichiometry and Stability constant

The stoichiometry of the reaction of aminophenol isomers with 9-CA reagent was studied by the molar ratio and Job methods,²⁹ using solutions of $1 \times 10^{-4} \text{M}$ for each isomer and 9-CA reagent. As shown in Figure 3, the results indicate that 1:1 aminophenol to reagent was formed using both above methods. This indicates that amino group present in the isomer is responsible for the formation of the products.

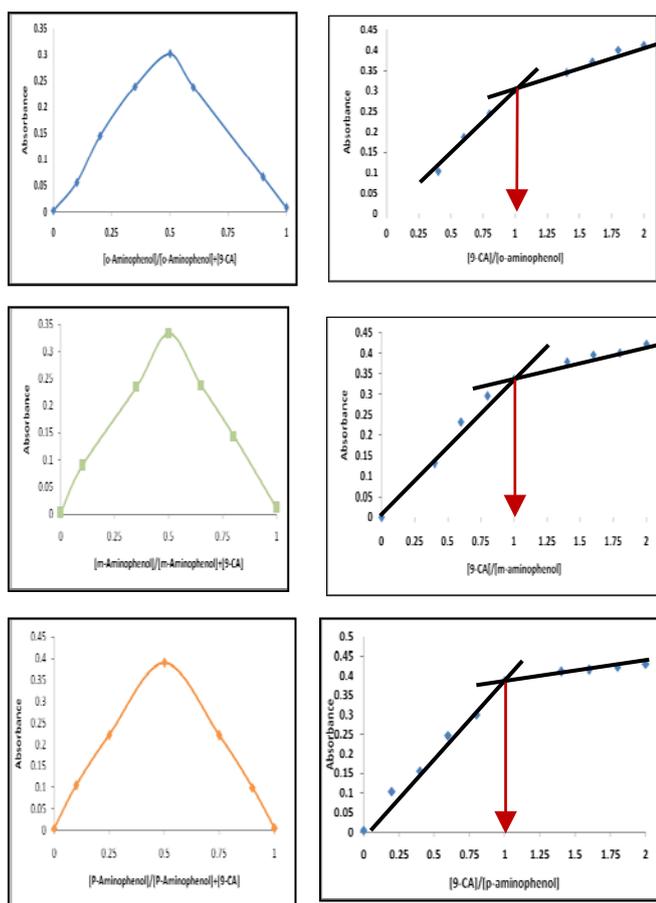


Figure 3. Job and mole ratio plots for aminophenol isomer–9-CA products.

According to the results obtained from above stoichiometry, the apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the each isomer and 9-CA (A_s) to one containing an excessive amount of 9-CA reagent (A_m). The average conditional stability constants of the complexes are calculated by the following equation :

$$K_c = \frac{1-\alpha}{\alpha^2 C}$$

where

K_c is the association constant ($L mol^{-1}$)

α is the dissociation degree and

C is the concentration of the product which is equal to the concentration of aminophenol and

$$\alpha = \frac{A_m - A_s}{A_m}$$

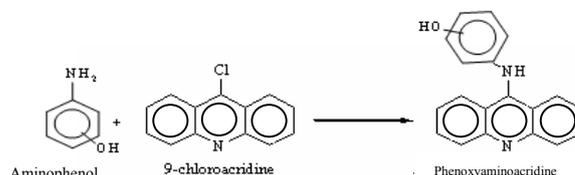
The results shown in Table 4 indicate that the products are relatively stable.

Table 4. Association constants of the 9-CA-aminophenol isomer products.

Iso-mer	Volume of 1×10^{-4} M, ml	Absorbance		α	Average K_{st} , $L mol^{-1}$
		A_s	A_m		
oA	0.5	0.213	0.409	0.4792	3.06×10^4
	1.0	0.307	0.557	0.4488	
	1.5	0.335	0.601	0.4426	
mA	0.5	0.283	0.305	0.07213	1.61×10^5
	1.0	0.358	0.430	0.1674	
	1.5	0.448	0.533	0.1595	
pA	0.5	0.260	0.400	0.3500	3.7×10^4
	1.0	0.380	0.511	0.2564	
	1.5	0.422	0.535	0.2112	

Mechanism

The colored products produced from the reaction of aminophenol isomers with 9-chloroacridine suggested that the amino functional group present in the molecule is necessary for the reaction. Aromatic nucleophilic substitution is enhanced in structures which contain an electronegative heteroatom.³⁰ This ease of nucleophilic substitution can be harnessed for the purposes of analytical chemistry when the 9-position of the acridine ring bears a facile leaving group and substituted by amino group in this reaction to form 9-aminoacridinium derivatives. However; the chemical reaction mechanism is proposed in Scheme 1.



Scheme 1. Proposed chemical reaction mechanism

Comparison of the proposed method with reported methods

The proposed method compared favourably with other reported spectrophotometric methods as shown in Table (5). However, the present method is simpler than other methods as no pH adjustment or use of buffer solutions and no need of surfactants, moreover the proposed method is more sensitive than other methods.

Conclusion

The proposed method is simple, rapid, selective, sensitive and economical compared to previously reported methods as it does not require any pretreatment or extraction procedure and has good accuracy and precision. The method, in terms of simplicity and expense, is considered superior to the previously reported methods.

Table 5. Comparison of spectrophotometric methods with the proposed method

Reagent	Amine	λ_{\max} , nm	T, °C	Developing time, min	Linearity range ($\mu\text{g}\cdot\text{ml}^{-1}$)	Molar absorptivity, $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$	Remark	Ref.
p-BA	oA	430	25	40	0.2 - 5.0	9.43×10^3	Need surfactant and buffer solution	21
	mA	420	55	40	0.4 - 6.0	6.45×10^3		
	pA	355	40	40	0.2 - 6.4	3.36×10^3		
NQS	oA	488	25	5	0.20-10.0	5.16×10^3	Need surfactant and buffer solution	18
	mA	480			0.08-7.2	6.61×10^3		
	pA	535			0.08-18.0	7.64×10^3		
9-CA	oA	436	50	30	0.25 -12.0	7.20×10^3	Need heating	Present method
	mA		40	30	0.20 -10.0	7.67×10^3		
	pA		40	15	0.00 -10.0	5.93×10^3		

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