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Spectrophotometric determination of 4-aminobenzoic acid using charge transfer complexation

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Abstract : A simple and sensitive spectrophotometric method is described for the quantitative determination of 4-aminobenzoic acid (PABA). The method is based on charge transfer complexation reaction of PABA 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 474nm under the optimized experimental conditions. Beer's law is obeyed over the concentration ranges of 5-90µg/ml. The apparent molar absorptivity and corresponding Sandell sensitivity were calculated and are reported. The limit of detection and quantification were 0.55 and 1.67 respectively. The proposed methods were applied successfully to the determination of PABA in pure and commercial forms with good average recovery of 102.4 %. 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, PABA, Charge transfer complex, DDQ.

Introduction

PABA [4-aminobenzoic acid] is an aminobenzoic acid isomer that combines with pteridine and glutamic acid to folic acid. The fact that 4-amino benzoic acid absorbs light throughout the UVB range has also resulted in its use as an ingredient in sunscreens[1]. Also it is used as a component of some medicines e.g analgesic or anesthetic preparations sunscreen agents and bentiromide[2-4] .Various methods used for the analysis of PABA include HPLC [5] GC [6]. Spectrophotometric methods have been used for the determination of PABA; most of the methods are based on diazotization of PABA and coupling the corresponding agent such as Braton Marshall reagent and phyloroglucinol[7], N-(I-napthyl) ethylediamine dihydrochloride[8]. Indirect spectrophotometric [9] and oxidation [10] methods have been reported for the determination of PABA.

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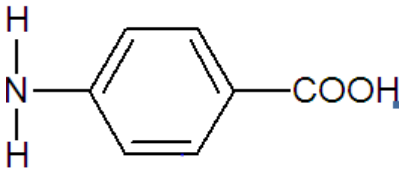


Fig 1: Structure of 4-aminobenzoic acid

However many of these methods lack sensitivity, require costly equipments and are laborious. Therefore, a method that is simple, sensitive and less laborious is required for the determination of 4-aminobenzoic acid.

Experimental

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 and Methods

Pure 4-aminobenzoic acid was supplied by Mallinckrodt USA. The commercial tablet of 4-aminobenzoic acid was sourced from the local market (Puritan's Pride, USA), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone(98% purity) was supplied by Sigma- Aldrich Chemie, Germany.

All chemicals used were of analytical grade and were used as such. All laboratory reagents were freshly prepared.

Preparation of reagents and standard solutions

Preparation of standard solution of 2, 3-dichloro-5, 6- dicyano 1, 4- benzoquinone

Exactly 0.027 g of DDQ was weighed. The weighed amount was dissolved in small amount of methanol and made up to 10 ml mark of a volumetric flask to give a 1.0 $x10^{-2}moldm^{-3}$ solution. Further dilutions to lower the concentration were carried out.

Preparation of standard solutions of 4-aminobenzoic acid (PABA)

Exactly 0.014 g of 4-aminobenzoic acid was weighed. The weighed amount was dissolved in small amount of methanol and made up to 10 ml mark in a volumetric flask to give a 1.0 $x10^{-2}moldm^{-3}$ solution. Further dilutions were carried out.

Absorption spectra

Exactly 4 ml solution of PABA ($7.29x10^{-5}$M) in methanol was scanned against a blank of methanol in the wavelength range of 199 nm-819 nm using a UV-Vis spectrophotometer. 2ml of 2,3-dichloro -5,6 – dicyano-1,4 - benzoquinone ($1.0x10^{-2}$ M) solution in methanol was mixed with 2ml of PABA ($1.0x10^{-2}$ M) solution in methanol, the resultant colour was scanned in the wavelength of 350-600 nm against a methanol blank

Proposed general procedure

Transfer serial volumes of 0.02ml to 0.36ml of standard PABA solution (0.00lg/ml) in a 0.04 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 474 nm.

Quantitative determination of PABA

Two 4-aminobenzoic tablets were finely powdered in a crucible. An amount equivalent to 0.0l g was weighed and dissolved into a beaker with some quantity of methanol. The solution was stirred to extract the active ingredient, filtered and made up to 10ml to produce a theoretical concentration of 0.001g/ml. Different volumes similar to the one prepared in the general procedure were taken and treated similarly as was done with the proposed procedure before analysis at 474nm against a methanol blank.

Results and discussion

DDQ as π-electron acceptors often forms highly coloured electron-donor, electron-acceptor or CT complexes with various donors which provide the possibility of determination of drugs by spectrophotometric methods. Also DDQ is an electron deficient molecule due to the electron withdrawing effect of the two cyano and the two chloro groups [11].

In this study 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol medium was used for direct determination of 4-aminobenzoic (PABA).

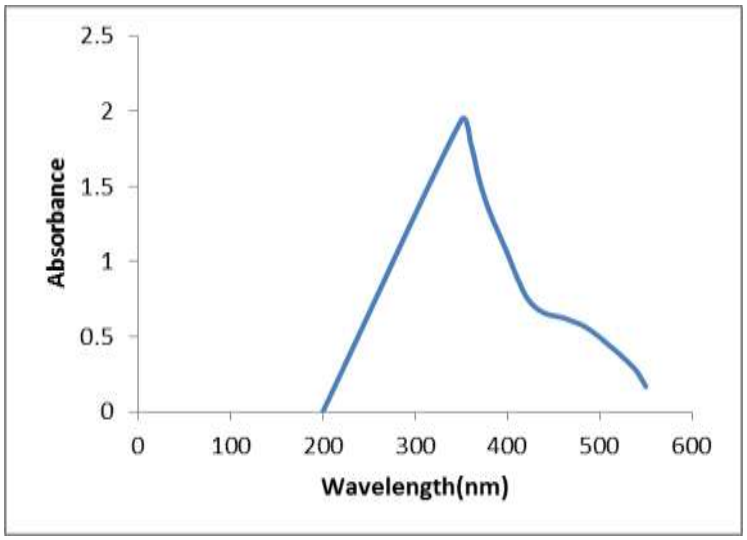


Fig. 1 Absorption spectra of DDQ in methanol

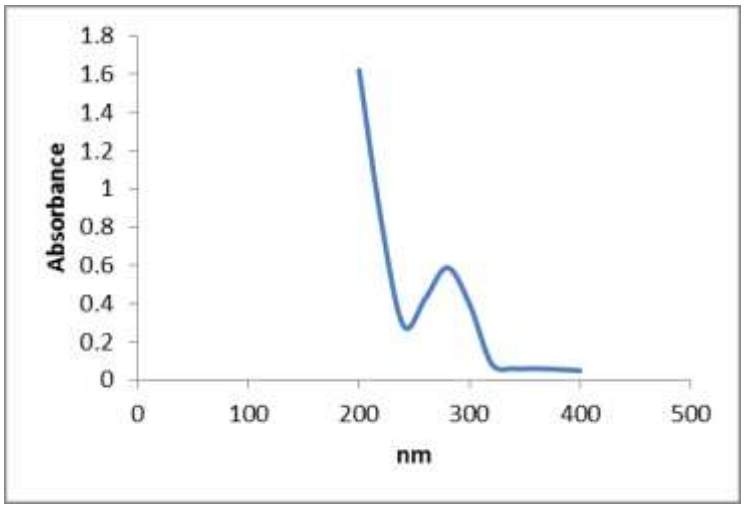


Fig. 2 Absorption spectra of PABA in methanol

A 2,3 dichloro-5-6-dicyano -1,4-benzoquinone solution in methanol displayed absorption peak at 350 nm (Fig 1). This is similar to an earlier preliminary study[12]. PABA showed absorption peak at 280 nm (Fig 2).

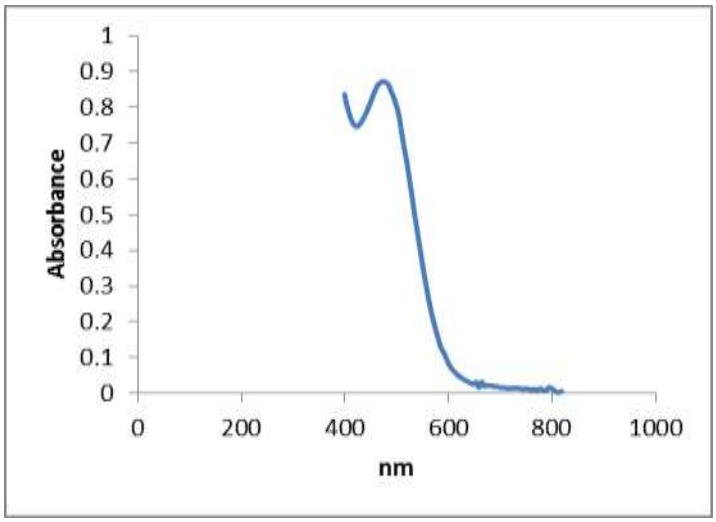
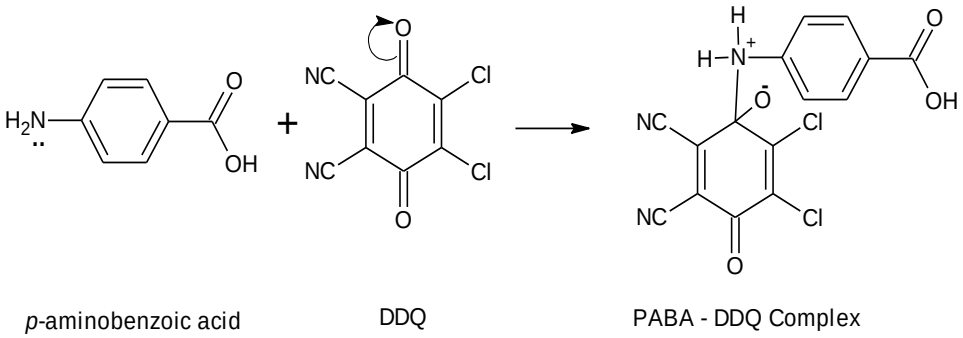


Fig. 3 Absorption spectra of erythromycin-DDQ complex

A reddish colouration was seen upon reaction of yellow DDQ solution and a colourless solution of PABA, this was suggestive of charge transfer formation which showed a bathochromic shift at 474nm (Fig 3). Scheme 1 shows the charge transfer complex formed between PABA and DDQ which reveals a free terminal basic amino groups[13].



p-aminobenzoic acid DDQ PABA - DDQ Complex

Scheme 1 Interaction of PABA with DDQ to form charge transfer complex

The donation process from PABA donor to acceptors can occur either from the lone pair of electron on the nitrogen atom of amino groups or benzene rings[14]. Fig 4 represents the mole ratio plot. Result showed a point of inflexion at 1.0 indicating a 1:1 mole ratio of PABA: DDQ. This result indicates that only one site in the PABA that participated in the formation of the CT complex and a univalent charged species is the possible sign of the CT process [15].

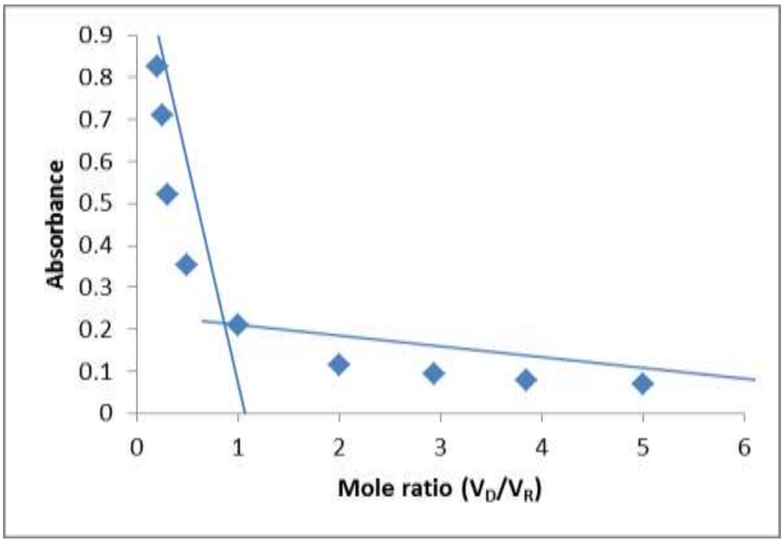


Fig 4 Mole ratio of PABA with DDQ

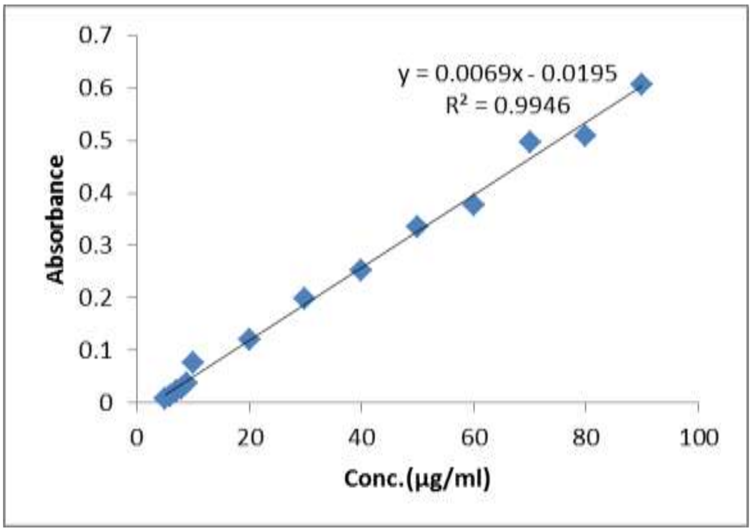
The optimum condition was determined by measuring the absorbance of the complex formed upon the addition of reagent solution to paraminobenzoic acid solution at room temperature. The reaction of PABA with DDQ was instantaneous while complete colour development was attained at 15min at room temperature (table 1). Also, table 1 reveals that maximum temperature was attained at 60°C. Between pH 1-13, effect of pH on the formation of PABA-DDQ complex was investigated. The result from (table 1) reveals that the maximum was seen at pH 8. This indicates that the best medium for the formation of this complex is pH 8.

Validity of Beers law

The proposed method was evaluated using the International conference on harmonization guideline (ICH) [16]. Fig 5 is the Beer's plot for the formation of PABA and DDQ complex, result indicates a linear range between 5-90ug/ml with R^2 value of 0.994. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the following equation

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

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



The limit of quantification and detection were found to be 1.67 and 0.55 with Sandell sensitivity of 6. Table 2 shows that the average mean percentage recovery was 102.4% with a low relative standard deviation. This shows that the proposed method is simple sensitive and reliable for the assay determination of PABA.

Table 1 Sensitivity and regression parameters

Parameters	**Values**
Molar absorptivity(L $mol^{-1}cm^{-1}$)	1.06 x 10^3
Maximum time of complex formation (min)	15
Maximum Temperature (°C)	60
Sandell sensitivity (µg/ml)	6
Regression equation [a] (Y = a + bx)	Y =0.006x-0.019
Slope	0.006
Intercept	0.019
Limit of quantification	1.67
Limit of Detection	0.55
Correlation (r^2)	0.994

[a] n =9

[a] Average of three determinations

[a] five independent determination

Table 2: Quantitative determination of PABA using the proposed method

Drug	**Taken (µg/ml)**	**Found µg/ml)**	**Recovery (%)**	**R.SD (%)**	**T-test**	**F-test**
PABA	8	9.9	124	0.17	0.51	0.74
	20	20.9	104.4	0.02		
	40	41.1	102.8	0.009		
	60	56.9	94.9	0.003		
	80	68.6	85.7	0.005		

Conclusion

The proposed method for the determination PABA is simple, precise and sensitive. The proposed method has been validated and successfully applied for the assay determination of erythromycin with good accuracy and precision of 102.4 %.

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