Spectrophotometric Determination of Some Phenolic Compounds Using N,N- diethyl –p-phenylenediamine (DE – PPD) and Benzoyl peroxide

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الخلاصة

تم تطوير طريقة طيفية سريعة وحساسة لتقدير بعض المركبات الفينولية (الفينول وأورثو– امينو فينول والفانفثول) وتطبيقاتها على دواء الاموكسيسلين تعتمد على تفاعل الاقتران التأكسدي للمركبات اعلاه مع N, N – ثنائي أثيل– بارافينلين ثنائي امين بوجود بيروكسيد البنزويل كعامل مؤكسد حيث تكونت صبغة الاندوفينول الزرقاء الذائبة لها اعلى امتصاص عند الاطوال الموجية ٦٢٧ و٦١٢.٥ و٦١١.٥ ٦٢٨.٥ نـانوميتر لكـل مـن الفينـول وأرثـو–أمينـو فينـول والفـانفثول وأموكسيسلين على التوالي. وقد بلغت الامتصاصية المولارية ١.٧٢ × ١٠^{4} و ١.٩٢ × ١٠^{4} و ١.٦٥×١٠^{4} و ١.٦٨ × ١٠^{4} لتر مول$^{-١}$ سم$^{-١}$ لتراكيز أتبعت قانون بير بحدود ٠.٢– ٤.٠ و ٠.٥– ١١.٠ و ٠.٦ – ١٦ و ٠.٨ – ٢٥ مايكروغرام مللتر$^{-١}$ للمركبات أعلاه على التوالي. تراوح معدل نسبة الاسترجاع بين ٩٨.٩٩% و١٠٠.٤٥% في حين كان الانحراف النسبي ≤ ١.٠٤ لجميع المركبات المدروسة. طبقت الطريقة بنجاح لتقدير الاموكسيسلين.

Keyword: Spectrophotometry: phenolic compounds; N,N-diethyl -p- phenylene diamine.

ABSTRACT

A rapid and sensitive spectrophotometric method has been developed for the determination of some phenolic compounds (phenol, o-aminophenol and α-naphthol) and was applied for determination of amoxicillin drug. The method is based on oxidative coupling reaction of these compounds with N,N-diethyl-p-phenylenediamine in the presence of benzoyl peroxide as oxidizing agent. The formed blue indophenol dyes have maximum absorptions at 627, 612.5, 611.5 and 628.5 nm. for phenol, o-aminophenol, α-naphthol and amoxicillin respectively. The

molar absorptivities are 1.72×10^4, 1.92×10^4, 1.65×10^4. l. mol^{-1}. cm^{-1} for concentrations obeyed Beer's law in the ranges 0.2 -4.0, 0.5 -11.0, 0.6 – 16.0 and 0.8 – 25.0 µg ml^{-1} for the above compounds respectively. The average recovery % was ranged between 98.99% 100.45 with relative standard deviation ≤ 1.04 for all the studied compounds. The method is applied successfully to the assay of amoxicillin.

INTRODUCTION

Phenolic compounds have great importance in the nutritional organoleptic and commercial properties of plant-derived food and beverages. Furthermore their consumption has been associated with positive health benefits such as antioxidant, antiviral, antiallergenic, cardioprotective, and anticarcinogenic effect. [1]

Several spectrophotometric methods have been proposed for the determination of present phenolic compounds using several reagents such as hydroxylamine and cerium (IV) [2], hydroxylamine and sodium nitroprusside [3], p-phenylazoaniline [4],4-aminoantipyrine and potassium ferricyanide [5], nitrous acid and resorcinol [6], 3-methyl –2-benzothiazoline hydrazone [7] 4-amino N, N-dimethyl aniline and dichromate [8] p-phenylene diamine [9] Anisidine [10] N,N-diethyl–p–phenylene diamine and N-bromosuccinimide [11], copper tetramine and Triiodite [12] Folin ciocalteu reagent [13].

The present paper describe a simple, sensitive and accurate spectrophotometric method for the determination of some phenolic compounds using N,N–diethyl–p-phenylene diamine (DE-PPD) in the presence of benzoyl proxide in alkaline medium.

EXPERIMENTAL

Apparatus

All spectral absorbance measurements were carried out on single beam spectrophotometer CECIL (UV – VIS) using 1-cm silica cell.

Reagents

All chemicals used were of analytical reagent grade.

N,N-diethyl–p-phenylenediamine hydrochloride (DE-PPD) solution ($1x10^{-2}$ M).

Prepared by dissolving 0.3995 g of DE - PPD in 200 ml ethanol. Working solution was prepared by further dilution of stock solution

Standard solutions of phenolic compounds (100 µg/ ml).

Prepared by dissolving 0.01 g of each phenol, o-aminophenol, α- naphthol and amoxicillin in 100 ml ethanol.

Benzoyl peroxide solution ($1x10^{-2}$M)

Prepared by dissolving 0.4845 g of benzoyl peroxide in 200 ml ethanol.

Sodium hydroxide solution 0.05 M

Prepared by dissolving 0.5gram in 250 ml. distilled water.

Recommended procedure for calibration:

Into a series of 25-ml calibrated flasks 3ml of $1x10^{-2}$ M DE-PPD solution was transferred. Add increasing volumes of phenolic working solutions (100 µg /ml), followed by 1.5 ml of $1x10^{-2}$ M benzoyl peroxide and 1.0 ml of 0.05 M sodium hydroxide. Dilute the solutions to the mark with ethanol and allow the reaction mixture to stand for 5 minutes. Measure the absorbance at 627, 612.5, 611.5, and 628.5nm for phenol, o-aminophenol α-naphthol and amoxicillin respectively against the reagent blank.

Procedure for the assay of amoxicillin in capsules:

Ten capsules were weighed and the amount of the powder containing 500 mg of amoxicillin was dissolved in distilled water and made up to 1000 ml with distilled water. The solution was filtered and then 100 µg ml^{-1} of amoxicillin solution was prepared, An aliquot of this solution was treated as described above under recommended procedure.

Result and discussion

A blue colour oxidative coupling products with an absorption maximum at 627, 612.5, 611.5 and 628.5 nm are formed when phenol, o-aminophenol, α-naphthol and amoxicillin were allowed to react with DE – PPD in the presence of benzoyl peroxide.

The absorption spectra of the resulting products are shown in Fig [2].

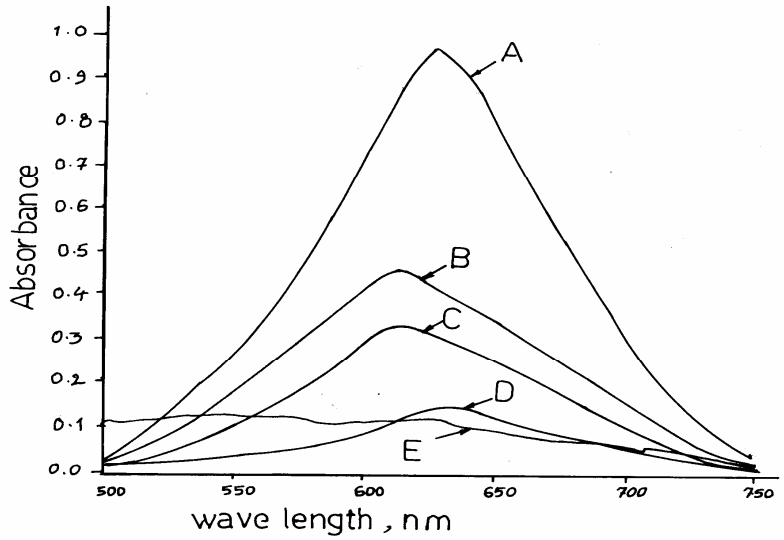

Fig2; Absorption spectra of the products from the reaction of DE-PPD and benzoyl peroxide with 3µg.ml^{-1} of
A- phenol, B- o-aminophenol, C- α-naphthol, D- amoxicillin,
E- Absorption spectraum of blank vs distilled water

Optimum reaction condition

The influence of various reaction variables were tested to establish the optimum conditions for the proposed procedure and the most suitable values of the variables tested based on absorbance obtained in each case. The phenol was used in all reaction.

Effect of diluents' agents on the absorbance and stability of the product:

The effect of distilled water and ethanol absolute as diluants on the colour intensity of the reaction product were studied. The results obtained in (Table 1) indicated that ethanol absolute which gave an stable product at room temperature is a suitable diluents agent.

It was found that the product was developed immediately and remained stable for at least 90 min; there after a slight decrease was observed.

Table 1: Effect of diluents on the absorbance and stability of the Product.

Time (min)	Diluent			
	Distilled Water		Ethanol absolute	
	Absorbance	λmax	Absorbance	λmax
0	1.119	670	0.817	627
5	0.838	669	0.811	627
15	0.633	668	0.813	627
30	0.483	665	0.814	627
45	0.417	663	0.814	627
60	0.342	659	0.813	627
75	0.294	654	0.814	627
90	0.231	636	0.812	627
120	0.196	621	0.809	627

Effect of DE – PPD concentration

The effect of changing the DE–PPD concentration on the absorbance was studied. Table 2 show that the absorbance increased with increasing DE–PPD concentration and reached maximum when using 3 ml of 1x 10^{-2} M DE – PPD. Therefore, this concentration was using in all subsequent experiments.

Table 2: Effect of DE – PPD concentration

ml.of DE-PPD $1X10^{-2}M$	1.0	2.0	2.5	3.0	3.5
Absorbance	0.323	0.579	0.707	0.811	0.813

Effect of benzoyl peroxide concentration.

The effect of changing the benzoyl proxide concentration on the absorbance was studied. Table 3 show that the absorbance reached maximum when using 1.5 ml of $1x10^{-2}$ M benzoyl peroxide.Therefore, this volume was using in all subsequent experiments.

Table 3: Effect of benzoyl peroxide concentration.

ml of $1x10^{-2}$ M benzoyl peroxide	0.1	0.5	1.0	1.5	2.0	2.5
Absorbance	0.286	0.609	0.730	0.813	0.746	0.748

Effect of alkaline medium

The effect of different alkaline was studied. The color product is formed in The use of 1 ml of 0.05 M sodium hydroxide solution gave a maximum intensity (Table 4).

Table 4: Effect of alkaline

ml of 0.05M base	**Absorbance**				
	0.2	0.5	1.0	1.5	2.0
Sodium hydroxide	0.383	0.539	0.813	0.704	0.538
Ammonium hydroxide	0.121	0.227	0.258	0.218	0.187
Sodium carbonate	0.062	0.105	0.129	0.121	0.218
Disodium tetraborate	0.221	0.364	0.466	0.473	0.461

Effect of temperature and reaction time.

The reaction time was studied by following the colour development at room temperature and different temperatures in thermostatically controlled water– bath and ice bath. The absorbance were measured and the reagent blank treated similarly. It was observed that formation of colored complex was achieved maximum immediately at room temperature and it remains stable for at least 90 min (Table 5).

Table 5: Effect of temperature on the absorbance and stability of the product.

Time/min	Absorbance		
	O ºC	R.T	70 ºC
5	0.305	0.807	0.213
15	0.311	0.812	0.210
30	0.304	0.811	0.193
45	0.301	0.814	0.196
60	0.298	0.810	0.184
75	0.292	0.811	0.178
90	0.283	0.813	0.180
120	0.292	0.806	0.172

Effect of order addition

To obtain optimum results the order of addition of reagents should be followed as given under the recommended procedure. Otherwise a loss in color intensity was observed (Table 6).

Table 6: Order of addition

Reaction compounds	Absorbance
DE-PPD+ phenol + benozyl peroxide+ sodium hydroxide	0.815
DE-PPD+ phenol+ sodium hydroxide + benzoyl peroxide	0.611
phenol+ sodium hydroxide + benzoyl peroxide + DE-PPD	0.148
DE-PPD+benzoyl peroxide + sodium gydroxide + phenol	0.132
phenol + benzoyl peroxide + sodium hydroxide + DE-PPD	0.133

However; the optimum reaction conditions for developing the color intensity of the products are summarized in Table 7.

Table 7: Optimum reaction conditions for the determination of phenolic compounds.

Phenolic componnd	λ_{max} (nm)	Temp (°C)	Development time (min)	Stability period (min)	DE-PPD 1×10^{-2}M (ml)	Benzoyl Peroxide 1×10^{-2} M (ml)	NaOH 0.05M (ml)
Phenol	627.0	R.T *	5	90	3	1.5	1.0
o-aminaphenol	612.5	R.T	5	90	3	1.5	1.0
α-naphthol	611.5	R.T	5	90	3	1.5	1.0
Amoxicillin	628.5	R.T	5	75	5	2.5	0.8

* Room temperature = 29 ± 3

Analytical Application:

Typical calibration data for the four phenolic compounds investigated from linear regression analysis of absorbance reading vs concentration of each compound gave the slopes, intercepts and correlation coefficients present in Table 8.

Table 8: Analytical data and spectral characteristics

Parameter	Phenol	o-aminophenol	α-naphthol	*Amoxicillin*
Beers law (μg. ml^{-1})	0.2-4.0	0.5-11	0.6-16	0.8-25
Molar absorptivity (l. mol^{-1} cm^{-1})	17225	19242	16592	16874
Slope	0.308	0.149	0.1061	0.0411
Intercept	+0.052	+0.038	+0.0121	+0.0309
Correlation coefficient	0.9990	0.9998	0.9997	0.9994

Accuracy and Precision:

To estimate the accuracy and precision of the method, six replicated determinations were made at three different concentration of

each phenolic compound. The results indicated that the present method is accurate and precise (Table 9).

Table 9: Accuracy and precision of the method

Phenolic compound	Mean recovery * (%)	RSD* (%)
Phenol	99.63	0.64
o- aminophenol	99.17	0.53
α- naphthol	98.99	0.72
Amoxicillin	100.45	1.04

*Average of six determinations

The nature of reaction product

DE-PPD oxidized in different oxidizing agent to p-benzoquinone diimine and then to p-benzoquinone monoimine which react with some phenolic compounds to produced indophenol dye in alkaline medium [9, 14].

A similar mechanism has been sngested by Al –Esawati. [11] for the reaction of DE-PPD with some phenolic compounds in the presence of N-bromosucinimide.

The stoichiometry of the reaction between DE-PPD and phenol in the presence of benzoylperoxide was investigated by mole – ratio method [15]. The obtained results showed the existent of 1:1 DE-PPD: phenol. Therefore the suggested mechanism for the formation of the dye can be written as follows:

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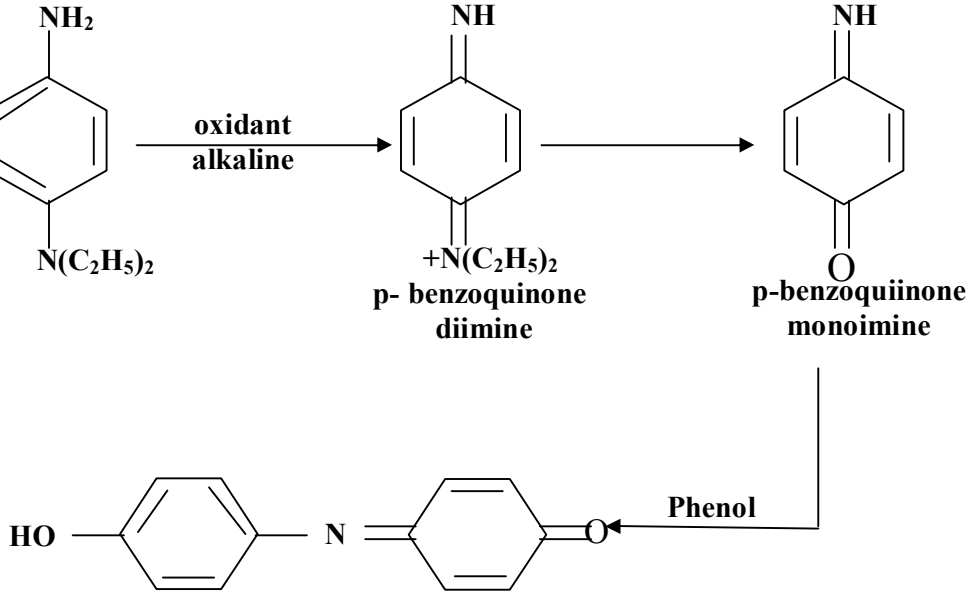

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of phenolic compound and DE-PPD to one containing an excessive of DE-PPD reagent. The average conditional stability constants of the dye are shown in Table 10.

Table 10: stability constant of the products obtained by the reaction of phenolic compounds with DE-PPD and benzoylperoxide

Phenolic compound	Stability constant (K) (l. mol^{-1})
Phenol	2.06×10^{4}
o-aminophenol	4.59×10^{4}
α- naphthol	3.77×10^{4}
Amoxicillin	1.48×10^{4}

Interferences

Amoxicillin is usually formulated in a capsule form therefore; the effect of some exceipients which often accompanied pharmaceutical preparation were studied. It was found that the exceipients do not interfer on the assay amoxicillin (Table 11).

The proposed method was applied by analyzing commerial formulation of amoxicillin (capsule) and comparing the results obtained with AL-Esawati method [11]. Satisfactory agreement between the results was obtained with an acceptable range of error (Table 12)

Table 11: Effect of interferences

Eceipient	Recovery (%)of 50 µg amoxicillin per µg foreign compound added		
	50	100	200
Starch	98.74	99.13	99.82
Glucose	99.16	100.31	99.79
Lactose	99.05	98.86	99.53
Acacia	100.13	99.74	100.35
Sodium citrate	98.42	99.11	100.09

Table 12: Assay of Amoxicillin in pharmaceutical preparation.

Pharmaceutical Formulation	Certified value (mg)	Present method (mg)	DE-PPD / N-bromosucinimide (mg)
Capsule*	250	248.66	250.93

* Mean of three determinations.

* Amoxicillin trihydrate, S.D.I-Iraq

Conclusion

A simple, rapid and sensitive spectrophotometric method for the determination of some phenolic compounds have been devised, based on the oxidative coupling reaction with DE –PPD and benzoylperoxide. The proposed method needs neither temperature control nor solvent extraction and it can be applied successfully for determination of amoxicillin in capsules.

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