

Spectrophotometric determination of ampicillin and amoxicillin using tetracyanoethylene reagent in aqueous solution

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Abstract

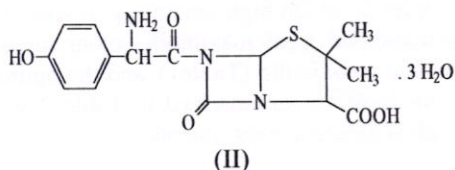
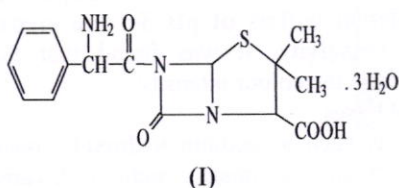
A simple, accurate and sensitive spectrophotometric method is described for the determination of ampicillin and amoxicillin in pure forms and in pharmaceutical formulations. The method is based on the reaction of these drugs in aqueous solution with tetracyanoethylene reagent in the presence of sodium hydroxide to produce a yellow coloured species measurable at $\lambda_{\max}$ 322 and 325nm for ampicillin and amoxicillin respectively. The method has been used for the determination of 1.0-40 $\mu\text{g/ml}$ for both drugs. The products have apparent molar absorptivities of 7.545×10^3 and $9.915 \times 10^3 \text{ L.mol}^{-1}.\text{cm}^{-1}$ and Sandell sensitivities of 0.0532 and 0.042 $\mu\text{g.cm}^{-2}$ for above drugs respectively. Also, it was found that these products were formed in ratio of 1:1 species with stability constants of 7.96×10^4 and $3.91 \times 10^4 \text{ L/mol}$ for ampicillin and amoxicillin respectively. The method was applied successfully to the assay of ampicillin and amoxicillin in their pharmaceutical formulations and was agreed well with its certified value and with the standard addition procedure.

Keywords: Spectrophotometry, ampicillin, amoxicillin, aqueous solution, tetracyanoethylene

Introduction

Ampicillin [6- [D - (-)- α -aminophenyl acetamido] penicillanic acid] trihydrate (I) and amoxicillin [6R - 6 - (α - p - hydroxyphenyl - D - glycylamino) penicillanic acid] trihydrate (II) are classified as antimicrobial agents which are defined as substances that kill, suppress the growth, multiplication or prevent the action of microorganism[1].

Several analytical methods have been reported for the determination of ampicillin and amoxicillin in their pharmaceutical preparations including chromatography [2], flow injection analysis [3,4], chemiluminescence [5], polarography [6,7], fluorometry [8], and conductimetric titrimetry [9].



Various reagents have been used for the spectrophotometric determination of the above drugs, such as, sodium 1,2-naphthoquinone-4-sulfonic acid in pH 9.00 buffer solution [10], acetylacetone—formaldehyde reagent [11], 1,10-phenanthroline-copper (II) complex [12] and inorganic complex of Mo(V)—thiocyanate[13] were used for the determination of ampicillin. 3-Methyl benzothiazolin-2-one hydrazone [14], N-bromo and N- chlorosuccinamide[15], diphenylamine cinamaldehyde in the presence of 2-propanol and acetic acid [16] and diazotized p-nitroaniline [17] were used for the determination of amoxicillin. Folin-Ciocalteu reagent [18], copper(II)acetate [19], ascorbic acid after oxidation to dehydroascorbic acid [20], picric and picramic acids [21]

reagents were used for the determination of both drugs. Charge transfer complex formation reactions have been also used for the determination of ampicillin and amoxicillin as n-donor with different π -acceptors such as 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) [22], tetrachloro-p-benzoquinone (chloranil) [23], tetrafluoro-p-benzoquinone (fluoranil) [24], and tetrabromo-p-benzoquinone (bromanil) [25]. Some of these methods are time-consuming, required extraction step or indirect procedures and require strictly controlled reaction conditions and less sensitive. Tetracyanoethylene (TCNE) reagent is used widely for the determination of pure organic molecules and aromatic hydrocarbons [26], phenols and aryl-ethers [27] in addition to assay of pharmaceutical preparations [28-31]. But these methods are applied in organic medium and suffer from lack of sensitivity and selectivity. The goal of this study is developing a sensitive and selective spectro-photometric method for the determination of ampicillin and amoxicillin in pure form and in their pharmaceutical preparations with TCNE reagent in aqueous solution.

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.

2.2. Reagents

All reagents used were of analytical grade and obtained from Fluka and BDH companies.

2.2.1. TCNE solution ($1 \times 10^{-2} \text{ M}$) is prepared freshly by dissolving 0.129g of tetracyanoethylene (recrystallized by chlorobenzene) in absolute ethanol and diluted to the mark in 100ml-volumetric flask with absolute ethanol.

2.2.2. Standard solutions of ampicillin and amoxicillin (500 $\mu\text{g/ml}$) are prepared by dissolving 0.05g pure form of ampicillin and amoxicillin in a minimum amount of ethanol and diluted to the mark with distilled water in 100 ml-volumetric flask. These solutions were further diluted with water to required concentrations for working solutions.

2.2.3. Sodium hydroxide solution ($\sim 5 \times 10^{-3} \text{ M}$) is prepared by dissolving 0.02g of sodium hydroxide with distilled

7. Interference

The extent of interference by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing 20 µg/ml of amoxicillin and various amounts

of diverse species in final volume of 25 ml. It was found that the studied excipients do not interfere in the present method, even when present in large excess. An error of 5.0% in the absorbance readings was considered tolerable. Typical results are given in Table 5.

Table 5: Effect of excipients for assay of amoxicillin

Foreign compound	Recovery % of 20µg/ml of amoxicillin per mg foreign compound added							
	1.25	2.5	5.0	7.5	10	15	20	25
Glucose	-	101.02	101.92	102.06	102.66	103.73	104.52	106.31
Lactose	00.12	00.98	01.62	02.43	04.02	04.98	06.70	-
Starch	01.21	07.19	02.88	03.56	04.22	04.92	05.98	-
Urea	-	01.23	02.33	04.01	04.53	04.91	05.65	-
Albumin	00.22	01.33	02.67	03.92	04.73	05.42	-	-
Sodium chloride	00.15	01.29	02.99	04.38	05.63	-	-	-
Sodium bicarbonate	09.31	07.31	06.01	05.21	05.03	04.21	-	-

8. Application

Applications of the proposed method to the assay of various pharmaceutical samples of ampicillin and amoxicillin (capsule, injection and tablet) gave a reproducible and accurate results. Further, the proposed method is very economical when compared to

chromatographic British pharmacopoeia method [32]. However; the validity of the method was confirmed by applying the standard addition procedure, (Fig.3) and the results suggested that there is no interference from any excipients, which are present in commercial dosage forms, Table 6.

Table 6: Assay of ampicillin and amoxicillin drugs in some pharmaceutical formulations by the proposed method and standard addition procedure.

Drug determined	Pharmaceutical preparation ^a	Drug content (mg)	Drug content (mg) found			
			Direct method	Recovery ^b %	Standard addition	Recovery %
Ampicillin	Capsule	250	249.31	99.73	252.50	101.00
	Suspension	250	250.86	100.34	254.16	101.66
	Injection	500	494.66	98.93	498.33	99.60
Amoxicillin	Capsule	250	248.73	99.26	246.66	98.66
	Suspension	250	242.61	97.04	253.33	101.33

^a Akai Co.

^b Average of four determinations.

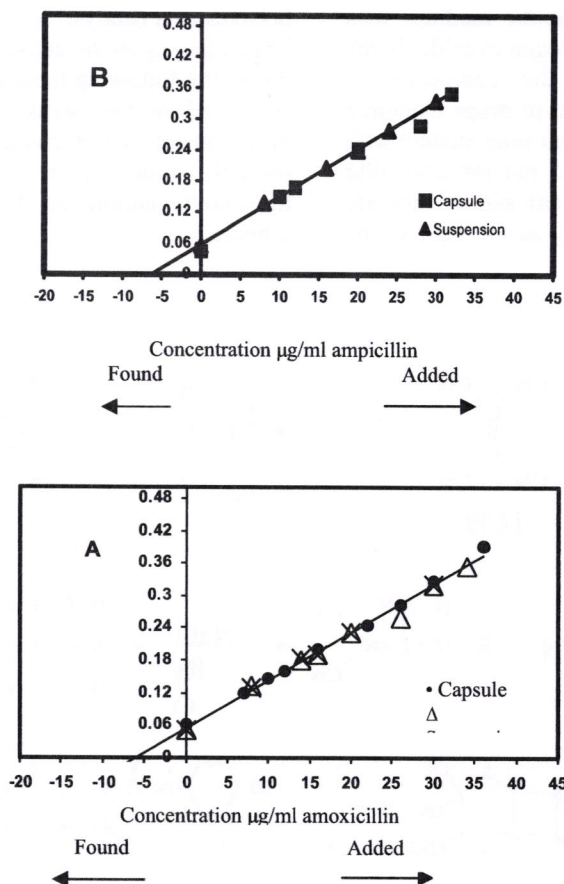


Fig.3 Standard addition plots for the recovery of 6µg/ml of ampicillin (A) and amoxicillin (B) in their dosage forms.

9. Stoichiometric Relationship

The mole ratio of the complexes formed between the drugs and the reagent used was investigated applying the continuous variation (Job's) method [33] using equimolar solutions ($2 \times 10^{-3} \text{M}$) of the drugs and TCNE reagent. The

results showed in Fig. 4 indicated that the complexes are formed in the ratio of 1:1. This may attributed that the amino group present in drug structures is responsible for the formation of the complex.

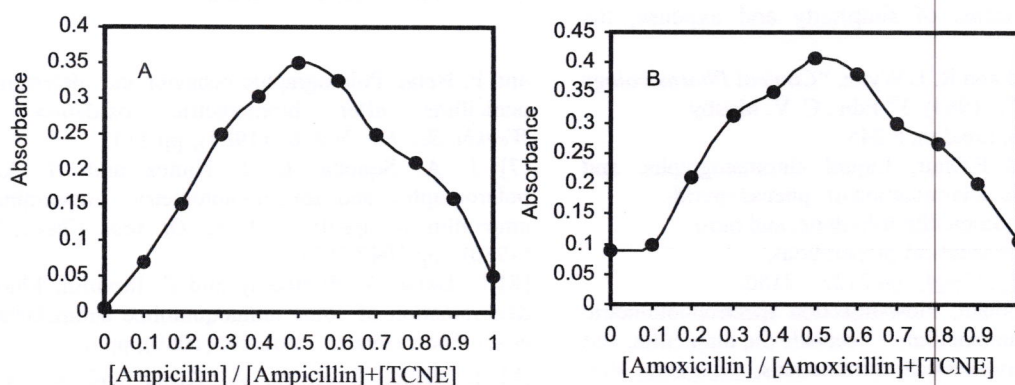


Fig.4 Continuous variation plots of TCNE with ampicillin (A) and amoxicillin (B).

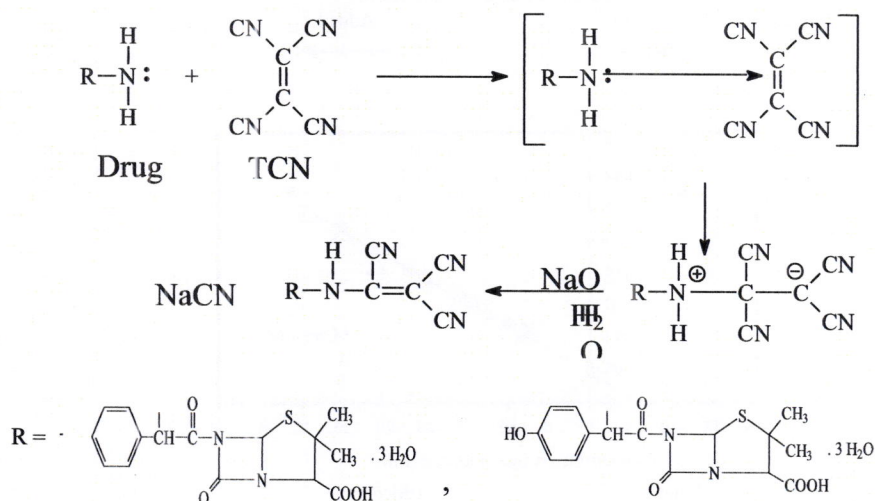
The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the drug and TCNE to one containing an excessive amount of TCNE reagent. The average conditional stability constants of the complexes are 7.96×10^4 and $3.91 \times 10^4 \text{ L.mol}^{-1}$ for ampicillin and amoxicillin respectively. This indicates that the complex is relatively stable.

10. Mechanism

The nature of the reaction between primary amines in aqueous solution and TCNE reagent is not clearly understood. Many methods have been described the formation of π - π and n - π charge-transfer complexes between TCNE and organic molecules including amines [34-36], Durgn [37] indicated that tetracyanoethylene reacts with primary and secondary aliphatic and aromatic amines in boiling tetrahydrofuran to give N-tricyanovinylamine and Middleton et al [38] observed that the pentacyanopropenide ion is formed in the

reaction of TCNE with aqueous pyridine in the presence of sodium bicarbonate in a weak acidic medium with liberation of carbon dioxide and hydrogen cyanide. In the present work it was observed that the complexes is formed in ratio of 1:1 after addition of drugs including primary amino group to TCNE reagent immediately with maximum absorption at 322 and 325 nm for ampicillin and amoxicillin respectively, and their sensitivities are increased within 5 min after cooling in ice bath in the

presence of sodium hydroxide, the base may be behaves as a catalyst for the final product formation. Depending upon the above experimental observation and others the following mechanism explained in scheme1 is suggested in two steps: (1) the initial rapid colour development is $n-\pi$ charge-transfer complex formation instantly followed by (2) conversion to the N-tricyanovinylamine as final product as proposed in scheme 1.



Scheme 1 The proposed chemical mechanism for the drug-TCNE product

Conclusion

The proposed method is simple, rapid, sensitive and economical compared to already reported methods and do not require any pretreatment of the drugs or extraction procedure and has a good accuracy and precision. On the other hand, in terms of simplicity and expense, the

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method could be considered superior in comparison with the chromatographic British Pharmacopoeia method and the previously reported methods, especially with those based on non-aqueous medium.

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الملخص

تم وصف طريقة طيفية بسيطة وحساسة لتقدير الأمبسلين والأموكسيسيلين بأشكالهم النقية وفي مستحضراتهما الصيدلانية. تعتمد الطريقة على تفاعل تلك الأدوية في المحلول المائي مع كاشف رباعي سيانوأثيلين بوجود هيدروكسيد الصوديوم لتكوين نواتج صفراء اللون تمتلك أقصى امتصاص عند الأطوال الموجية 322 و 325 نانومتر لكل من الأمبسلين والأموكسيسيلين على التوالي. أمكن تطبيق قانون بير بحدود تركيز 1.0-40.0 مايكروغرام/مللتر لكل الدوائين وبامتصاصية مولارية 7.545×10^3 و 9.915×10^3 لتر/مول.سم في حين كانت حساسية ساندل 0.0532 و 0.042 مايكروغرام/سم² لكل من الأمبسلين والأموكسيسيلين على التوالي. كما وجد أن تلك النواتج تتكون بنسبة 1:1 ، وأن ثابت استقرارها 7.96 و 3.91 لتر/مول للأدوية أعلاه على التوالي. تم تطبيق الطريقة بنجاح لتقدير الأمبسلين والأموكسيسيلين في مستحضراتهما الصيدلانية وكانت النتائج متفقة مع المحتوى الأصلي وطريقة الإضافة القياسية