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The non-aqueous titrimetric assay of selected antibiotics using tetra-N-butylammonium hydroxide as titrant

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Abstract: This study was carried out to develop a potentiometric titration method in non-aqueous media for the determination of some commonly used antibiotics. For this purpose, five antibiotics, namely ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride, were potentiometrically titrated using pyridine as solvent and tetrabutylammonium hydroxide as titrant, at 25°C and under a nitrogen atmosphere. The method was found to be highly accurate and precise having a relative standard deviation of less than 1.0%. Also it was shown that the method could be successfully applied to assay of commercial pharmaceuticals containing the above-mentioned antibiotics. The results of recovery studies for standard additions in pharmaceutical preparations were satisfactory. The proposed method is simple, rapid and sufficiently precise for quality control purposes.

Keywords: *Ampicillin; amoxycillin trihydrate; rifampin; netilmicin sulphate; ciproflaxacin hydrochloride; potentiometric titration; pyridine solvent; tetrabutylammonium hydroxide; titrimetric assay.*

Introduction

Many of the active components of pharmaceutical preparations are of organic origin and contain acidic and/or basic groups. Some of these substances are not soluble in water or decompose in aqueous media. As a continuing part of our studies on the titration of compounds in non-aqueous media, this study was carried out to investigate the possibility of titrating commonly used antibiotics such as ampicillin (I), amoxycillin trihydrate (II), rifampin (III), netilmicin sulphate (IV), and ciproflaxacin hydrochloride (V), in pyridine solution using a potentiometric method [1, 2]. The structural formulae of the antibiotics are given in Scheme 1.

When the literature on the determination of these compounds is examined, it can be seen that potentiometry in pyridine solution has not been investigated whilst the procedures employed previously are highly time-consuming and tedious. The USP recommends non-aqueous titrations for ampicillin and amoxycillin trihydrate [3–5]. Other methods for these antibiotics include iodometric and colorimetric determination of hydrolysis products [6–8], mercurimetric titration [9] and spectrophotometric methods [10, 11]. For rifampin in dosage form, the USP prescribes a microbiological assay [3]; however, the British Pharmacopoeia recommends a spectrophotometric assay for bulk drugs [12]. Among the other methods used for rifampin, either as a pure drug substance or in pharmaceuticals, are spectrophotometry, chromatography and amperometry [13–16]. Routine determinations of netilmicin sulphate in pharmaceuticals are usually performed by microbiological assays [4]. Other methods for netilmicin sulphate include GLC [17], TLC [18], HPLC [19]. There is no method given in USP (XXII) for the relatively new antibiotic ciproflaxacin hydrochloride. However, spectrophotometric and chromatographic methods are described in the literature [20–23].

It is obvious that a quick and reliable procedure is required for the determination of antibiotics, especially rifampin, netilmicin sulphate and ciproflaxacin hydrochloride. The purpose of the present investigation was to develop a simple non-aqueous titrimetric assay for the antibiotics mentioned above and to apply the procedure to pharmaceutical dosage forms.

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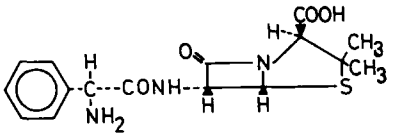
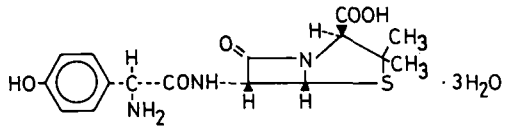


(I)



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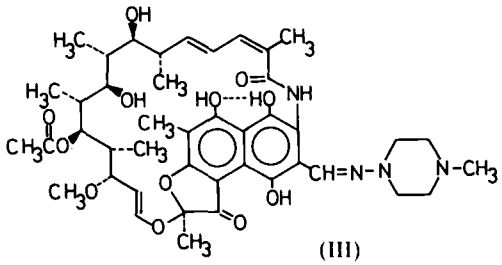

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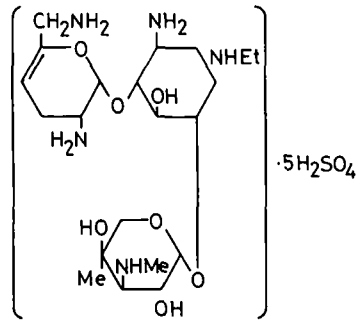
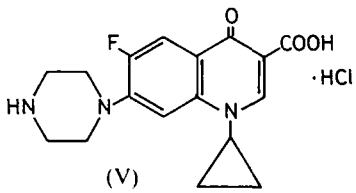


(IV)



(V)

Scheme 1

Experimental

Apparatus

An Orion 720A digital pH-ionmeter equipped with a combined pH-electrode (Ingold) was used for throughout the study. The electrode was modified by replacing the aqueous saturated KCl solution with a saturated anhydrous methanolic solution of KCl. All titrations were performed manually in under a nitrogen atmosphere, at 25 ± 1°C in a jacketed glass reaction cell as described previously [1].

Materials

Ampicillin, amoxycillin trihydrate and ciproflaxacin hydrochloride, obtained from Fako Drug Company (Turkey), were of chemically pure laboratory working standards having purities of 99.5, 99.0 and 99.7%, respectively.

Rifampin, obtained from Refik Saydam Hygiene Centre (Turkey), was of a chemically pure laboratory working standard, with a purity of 99.9%.

Netilmicin sulphate, received from Eczacibaşi Drug Company (Turkey), was of a chemically pure laboratory working standard, having a purity of 98.9%.

All the antibiotics were dried in a vacuum oven before being used, except for amoxycillin trihydrate.

Alfasilin: Fako Co.; labelled to contain ampicillin trihydrate equivalent to 500 mg ampicillin per capsule.

Alfoxil: Fako Co.; labelled to contain amoxycillin trihydrate equivalent to 500 mg amoxycillin per tablet.

Rifadin: Sifar Co.; labelled to contain 150 mg rifampin per capsule.

Netromycin: Eczacibaşi Co.; labelled to contain netilmicin sulphate equivalent to 150 mg netilmicin base per 1 ml of an ampoule.

Proxacin: Fako Co.; labelled to contain ciproflaxacin hydrochloride equivalent to 250 mg ciproflaxacin per tablet.

Tetrabutylammonium hydroxide was purchased from Merck (Darmstadt, Germany) as a 0.100 M solution in 2-propanol–methanol and was diluted with 2-propanol to give an approximately 0.040 M solution. This solution was standardized against sublimed benzoic acid (Merck) and kept in a dark-coloured ground glass-stoppered flask in a refrigerator.

Pyridine and 2-propanol were purchased from Merck and used after purification according to [24].

Antibiotic solutions. Four antibiotics (15–50 mg; ampicillin, amoxycillin trihydrate, netilmicin sulphate and ciproflaxacin hydrochloride) were dissolved in minimum amounts of water, depending upon their molecular weights, and diluted to 25 ml with pyridine. Rifampin solution was prepared by dissolving directly in pyridine. Aliquots of 20 ml were taken from the test solutions and titrated with standard tetrabutylammonium hydroxide with stirring, at 25°C, under nitrogen atmosphere. All the assay solutions were prepared immediately before titration.

Application of the proposed methods to pharmaceutical preparations

Capsules. Fifteen alfasilin capsules were weighed and their average contents calculated. The contents were pooled and finely powdered and the required amount of powder weighed accurately, dissolved in the minimum amount of water and diluted to 25 ml with pyridine. The same procedure was applied to rifadin capsules but the sample was dissolved directly in pyridine.

Tablets. Fifteen alfoxil or proxacin tablets were weighed and their average weight calculated. All the tablets were finely powdered, the required amount of this powder dissolved in the minimum amount of water and diluted to 25 ml with pyridine.

Ampoule. An ampoule of netromycin was transferred into a 100-ml standard flask and dissolved in pyridine. This was used as a stock solution. The required volume of this stock solution was diluted with pyridine to 25 ml. The 20 ml aliquots taken from the solutions of the pharmaceutical preparations also were titrated with tetrabutylammonium hydroxide solution under the same conditions as standard antibiotics.

The titrations were repeated for different amounts of each antibiotic and pharmaceutical preparation.

Results and Discussion

Determination of standard active components

Ampicillin, amoxyicillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride were titrated potentiometrically with tetrabutylammonium hydroxide as titrant in pyridine. The titration curve of the antibiotics, except for ciproflaxacin hydrochloride, showed one well-defined S-shaped stoichiometric end-point (see Figs 1–4). On the other hand, the titration curve for ciproflaxacin hydrochloride gave two well-defined S-shaped stoichiometric end-points (Fig. 5).

The determination of the equivalence points from the potentiometric data was carried using the Gran's method [25]. The end-points for both ampicillin (I) and amoxycillin trihydrate (II) corresponded to one equivalent of base and related to the neutralization of the −COOH group.

The end-point corresponding to one equivalent of base in the titration curve of rifampin (III) can be attributed to one of the −OH protons located in position 1 or 8 of the naphthalene ring. The value of half-neutralization potential and the significant jump in the titration curve of this compound indicate that the titrated −OH proton is much more acidic than expected [26]. This may be a result of the formation of a hydrogen bond between two −OH groups, which would cause an increase in the acidity of one of the hydrogens.

When the stoichiometry of the single end-point in the titration curve of netilmicin

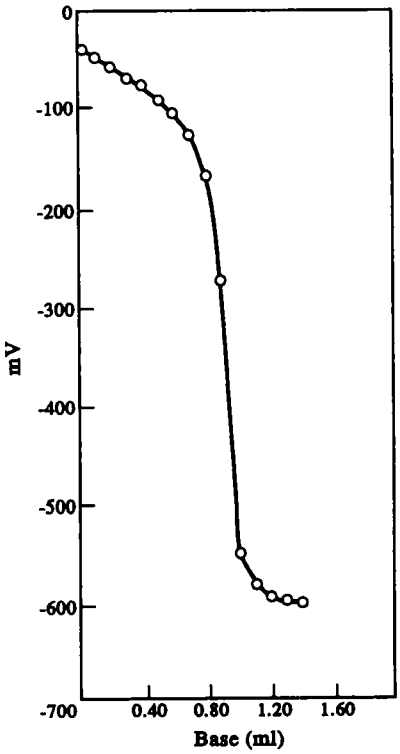


Figure 1
Potentiometric titration curve for ampicillin titrated with tetrabutylammonium hydroxide in pyridine solution.

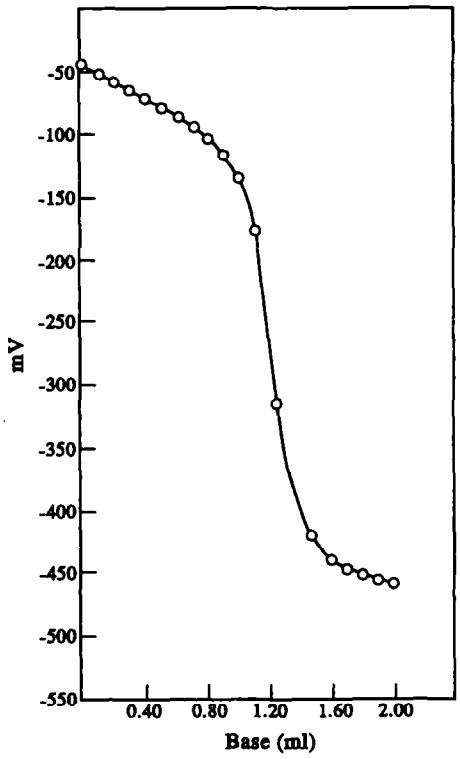


Figure 2
Potentiometric titration curve for amoxycillin trihydrate titrated with tetrabutylammonium hydroxide in pyridine solution.

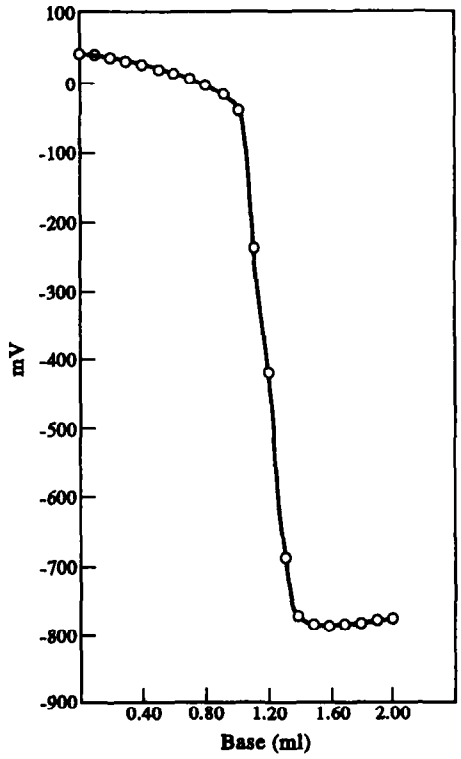


Figure 3
Potentiometric titration curve for rifampin titrated with tetrabutylammonium hydroxide in pyridine solution.

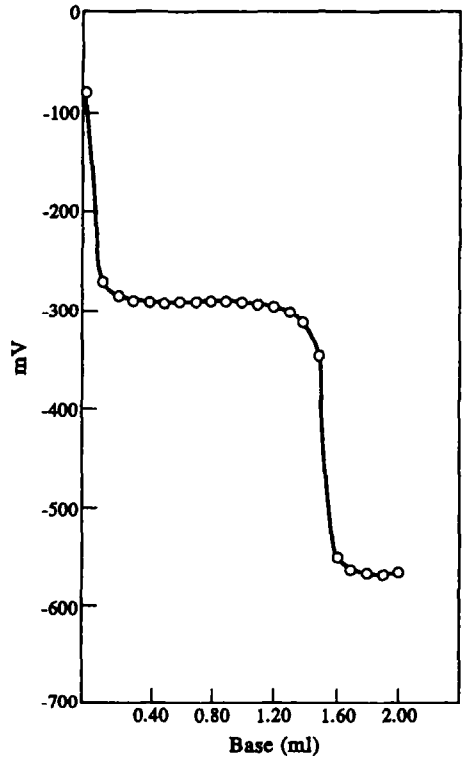


Figure 4
Potentiometric titration curve for netilmicin sulphate titrated with tetrabutylammonium hydroxide in pyridine solution.

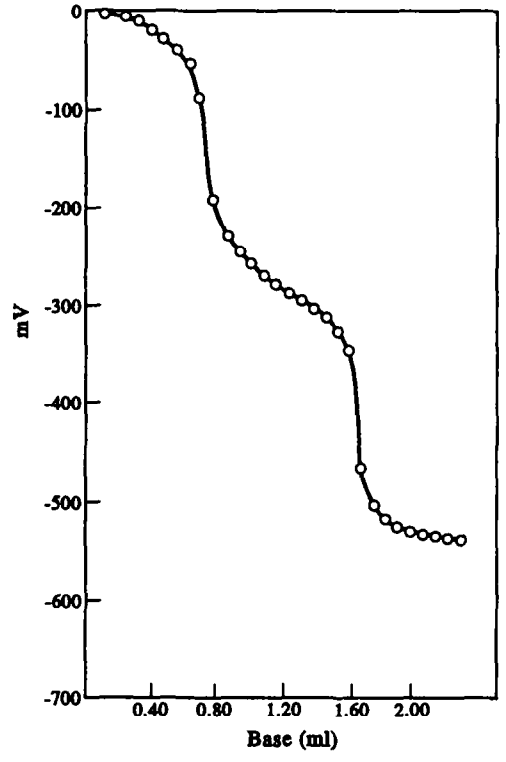


Figure 5
Potentiometric titration curve for ciproflaxacin hydrochloride titrated with tetrabutylammonium hydroxide in pyridine solution.

sulphate (IV) is examined, it can be seen that 1 mol netilmicin sulphate is equivalent to 10 moles of base. This shows that the protons of 5 moles H_2SO_4 in each mole of netilmicin sulphate are titrated together instead of being titrated individually. The double end-points observed in the titration curve of ciproflaxacin hydrochloride (V) correspond to one mole of base in each case. The first one corresponds to the proton of the hydrochloride and the second to the carboxyl proton.

The percentage of each antibiotic (chemically pure laboratory working standard) was calculated from the potentiometric titration data. The accuracy and precision of the proposed method were tested by five successive determinations carried out on ampicillin, amoxycillin trihydrate, rifampin, netilmicine sulphate and ciproflaxacin hydrochloride. The results are tabulated in Table 1.

As seen from the data in Table 1, the mean values obtained by the proposed method are in good agreement with the nominal value given for each antibiotic and furthermore the relative standard deviations are less than ±1%. This indicates that the accuracy and precision of this method is quite satisfactory.

Determination of the active components in pharmaceuticals

In order to evaluate the applicability of the method to pharamceutical preparations, ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride were determined in alfasilin, alfoxil, rifadin, netromycin and procaxin, respectively, under the same conditions as employed for the pure antibiotics. The fact that the mV values before the end-points in the titration curves of pure antibiotics and their corresponding pharmaceuticals are almost identical, provides evidence that the titration curves are not due to other impurities which might be present in the pharmaceutical preparations.

Table 2 summarizes the results obtained for each antibiotic in the corresponding pharmaceuticals, expressed as percentages of the nominal contents. The recoveries are in good agreement with the nominal contents and the RSD values are less than 1%. Thus, the precision is very satisfactory for the analysis of pharmaceutical preparations as well as bulk drugs. These results indicate that the content of each antibiotic in the pharmaceuticals can be safely determined by using this method without interference from other substances in the preparations.

The recovery studies of standard additions to commercial pharmaceuticals also were carried out in order to provide further evidence of the validity of the method. The results related to these studies are presented in Table 3. It can be seen from this table that the mean recoveries and RSD values are in the range of 99.95–

Table 1
Titrimetric determinations on antibiotics which are chemically pure laboratory working standards

Antibiotics	Proposed method Mean (%)	RSD	Nominal value (%)
Ampicillin	98.9	0.78	99.5
Amoxycillin trihydrate	99.2	0.63	99.0
Rifampin	99.7	0.33	99.9
Netilmicin sulphate	98.6	0.51	98.9
Ciproflaxacin hydrochloride	99.8	0.47	99.7

*Mean and relative standard deviation for five determinations.

Table 2
Titrimetric determination of antibiotics in some pharmaceutical preparations

Pharmaceuticals	Antibiotics	Recovery (% ± RSD)*
Alfasilin	Ampicillin	103.20 ± 0.65
Alfoxil	Amoxycillin trihydrate	102.25 ± 0.70
Rifadin	Rifampin	101.80 ± 0.75
Netromycin	Netilmicin sulphate	100.40 ± 0.85
Proxacin	Ciproflaxacin hydrochloride	101.30 ± 0.20

*Mean and relative standard deviation of five determinations.
Recovery relative to nominal content.

Table 3
Recovery studies of standard additions to some pharmaceutical preparations

Pharmaceuticals	Antibiotics	Added (mg)	Found (mg)	Recovery (%)*
Alfasilin	Ampicillin	3.00	3.00	100.00
		4.00	3.97	99.25
		5.00	5.03	100.60
Alfoxil	Amoxycillin trihydrate	3.00	3.01	100.33
		4.00	4.09	102.25
		5.00	5.08	101.60
Rifadin	Rifampin	6.00	5.93	98.83
		8.00	8.08	101.00
		10.00	9.98	99.80
Netromycin	Netilmicin sulphate	2.00	2.02	101.00
		2.50	2.49	99.60
		3.00	3.02	100.67
Procaxin	Ciproflaxacin hydrochloride	2.00	2.02	101.00
		2.50	2.54	101.60
		3.00	3.06	102.00

* Ampicillin: mean ±RSD = 99.95% ± 0.68%; amoxycillin trihydrate: mean ±RSD = 101.39% ± 0.98%; rifampin: mean ±RSD = 99.88% ± 1.09%; netilmicin sulphate: mean ±RSD = 100.42% ± 0.73%; ciproflaxacin hydrochloride: mean ±RSD = 101.53% ± 0.50%.

101.53% and 0.50–1.09%, respectively, which is good evidence of the validity of the method.

As a result of this work, ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride can now be determined potentiometrically in pyridine by the proposed method. This non-aqueous titration procedure was successfully applied to the determination of pure authentic samples and some of their pharmaceutical preparations. In conclusion, the proposed potentiometric method could be utilized readily for routine analysis of pharmaceuticals since it offers a simple system and with short analytical time coupled with good reproducibility and accuracy.

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References

[1] T. Gündüz, N. Gündüz, E. Kiliç, F. Köseoğlu and S.G. Öztaş, *Analyst* **113**, 715–719 (1988).
[2] T. Gündüz, E. Kiliç, F. Köseoğlu and S.G. Öztaş, *Analyst* **113**, 1313–1316 (1988).
[3] *The United States Pharmacopeia*, 21st edn, pp. 59 and 566. Mack, Easton, USA (1985).
[4] *The United States Pharmacopeia*, 22nd edn, pp. 80–85 and 942–943 (1990).
[5] C. Casalini, L. Montecchi, D. Boccali and G. Cesarano *Bull. Chim. Farm.* **114**, 651–658 (1975).
[6] Code of Federal Regulations, Title 21, Part 440, US Government Printing Office, Washington, DC, USA (1981).
[7] H. Bundgaard and K. Ilver, *J. Pharm. Pharmacol.* **24**, 790–794 (1972).
[8] E.A. Ibrahim, S.M. Rida, Y.A. Beltagy and M.M. Abdel Khalek, *Pharmazie* **29**, 143–144 (1974).
[9] B. Nowak and H. Wollmann, *Pharmazie* **42**, 862–863 (1987).
[10] F. Belal, *Anal. Lett.* **16**, 1555–1566 (1983).
[11] R.C. Hiremath and S.M. Mayanna, *Microchim. Acta.* **1**, 265–270 (1986).
[12] *The Pharmacopoeia*, Vol. I, p. 509, Vol. II, pp. 627 and 780. H.M. Stationery Office, London (1980).
[13] C.S.P. Sastry, T.E. Divakar and U.V. Prassad, *Indian Drugs* **22**, 604–606 (1985).
[14] G.R. Rao, S.S.N. Murty and E.V. Rao, *Indian Drugs* **22**, 484–488 (1985).
[15] P. Barza, *Farmacia, Bucharest* **21**, 121–126 (1973).
[16] *British Pharmacopeia*, p. 957. H.M. Stationery Office, London (1988).
[17] J.W. Mayhew and S.L. Gorbach, *J. Chromatogr.* **151**, 133–146 (1978).
[18] F.R. Kunz and H. Jork, *Fresenius Z. Anal. Chem.* **329**, 773–777 (1988).
[19] T. Kavamoto, M. Watanabe, Y. Kondo and K. Mashimo, *Shimadzu Hyanon* **40**, 177–179 (1983).
[20] S.C. Mathur, S. Lal, N. Murugesan, Y.K.S. Rathore and P.D. Sethi, *Indian Drugs* **27**, 398–399 (1990).
[21] S.G. Shanbag, P.P. Thampi and C.S. Thampi, *Indian Drugs* **28**, 279–280 (1991).
[22] G.R. Rao, A.B. Avadhanulu and D.K. Vatsa, *Indian Drugs* **27**, 532–535 (1990).
[23] J. Parasrampuria and V.D. Gupta, *Drug Dev. Ind. Pharm.* **16**, 1597–1604 (1990).
[24] D.D. Perrin, W.L.F. Armarego and D.R. Perrin, in *Purification of Laboratory Chemicals*. Pergamon Press, Oxford (1966).
[25] E.P. Serjeant, in *Potentiometry and Potentiometric Titrations*. John Wiley and Sons, New York (1984).
[26] T. Gündüz, E. Kiliç, V. Ertüzün and G. Çetinel, *Analyst* **111**, 1439–1442 (1986).

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