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Polarographic determination of some penicillins through nitrosation

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Abstract

Direct current and differential pulse polarography DPP were used for the determination of three penicillins, namely, ampicillin, benzylpenicillin and carbenicillin, in pure form and in their dosage forms. The method is based upon treatment of penicillins with nitrous acid followed by polarographic measurement of the produced derivatives polarographically. The nitroso derivatives formed exhibited reduction waves over the whole pH range in Britton–Robinson buffers. The waves were characterized as being diffusion-controlled and free from adsorption phenomena. The current-concentration plots were rectilinear over the concentration range 8–200 and 2–160 μg ml^{-1} for DC_t and DPP, respectively, for all the studied compounds. The proposed method was further applied to determine penicillins in pharmaceutical preparations, and the results obtained were in good agreement with those given by the companies.

Keywords: Penicillins; Benzylpenicillin; Ampicillin; Carbenicillin; Polarography; Pharmaceutical analysis

1. Introduction

Penicillins are among the oldest and most frequently prescribed natural antimicrobials. Since 1940s, much effort was expanded on the isolation, purification, structure elucidation and evaluation of these compounds. Evaluation of penicillins by physical or chemical means had been thoroughly investigated, in order to substitute the tedious and lengthy microbiological methods. The physicochemical methods have the advantages of speed, precision and economy. In 1976, Hughes et al. [1] had presented an excellent review including a critical and exhaustive survey of the literature dealing with the physical and chemical methods reported for penicillins. Untermann and Weissbuch [2] published another review emphasizing on the electrochemical methods described for penicillins. Later on Fairbrother [3] reviewed the chemical methods reported for antibiotics. The British Pharmacopoeia 1993 [4] recommends titrimetric methods, acidimetric or mercurimetric for penicillins. The United States Pharmacopoeia 1990 [5], similarly recommends a titrimetric iodometric method. Regarding the electrochemical methods, polarography was applied to study the degradation of ampicillin [6] and benzylpenicillanic acid [7]. Benzylpenicillin was polarographi-

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cally determined in citrate buffer based on the activity of the thiol group [8,9].

Functionalization polarography, i.e. the conversion of an electroinactive compound into an active one is achieved by the introduction of an electroactive group through chemical reactions. These reactions should occur selectively, quickly and with a yield of about 100%. Work from our laboratory proved that several compounds could be assayed through this approach [10–13].

This paper describes the polarographic determination of some penicillins through treatment with nitrous acid. The method is simple, rapid and can be readily adopted for routine analysis in control laboratories

2. Experimental

2.1. Materials and reagents

1. Benzylpenicillin (CID Pharm. Co., Giza, Egypt)
2. Ampicillin (Misr Pharm. Co., Cairo, Egypt)
3. Carbenicillin (Pfeizer Italiana S.P.A., Italy)
4. Pharmaceutical preparations containing these compounds were obtained from commercial sources in the Egyptian market.
5. Britton–Robinson buffers (14) pH range 2–10 (0.08 M)
6. Sodium nitrite (Merck, FRG): 3% aqueous solution
7. Ammonium sulphamate (Fluka): 5% aqueous solution

2.2. Apparatus

The polarographic study [14] and the differential pulse measurements (DPP) were carried out using the polarecord E 506 Metrohm (Herisau, Switzerland). The electronically controlled drop time was adjusted using a 663 VA Stand from the same company to be 1 s. The polarograms were recorded with a potential scan rate of 10 mV s^{-1}. A three-electrode system was used consisting of a dropping mercury electrode (DME) as the working electrode, Ag/AgCl reference electrode and a graphite rod as an auxiliary electrode, was used.

2.3. Solutions

Stock solutions: 0.1% aqueous solutions of the studied penicillins were prepared in distilled water and diluted as appropriate.

2.4. Procedure

2.4.1. The derivatization process

Transfer aliquots covering the working concentration range into separate 25 ml volumetric flasks. Add to each flask 0.5 ± 0.1 ml of 1 M HCl

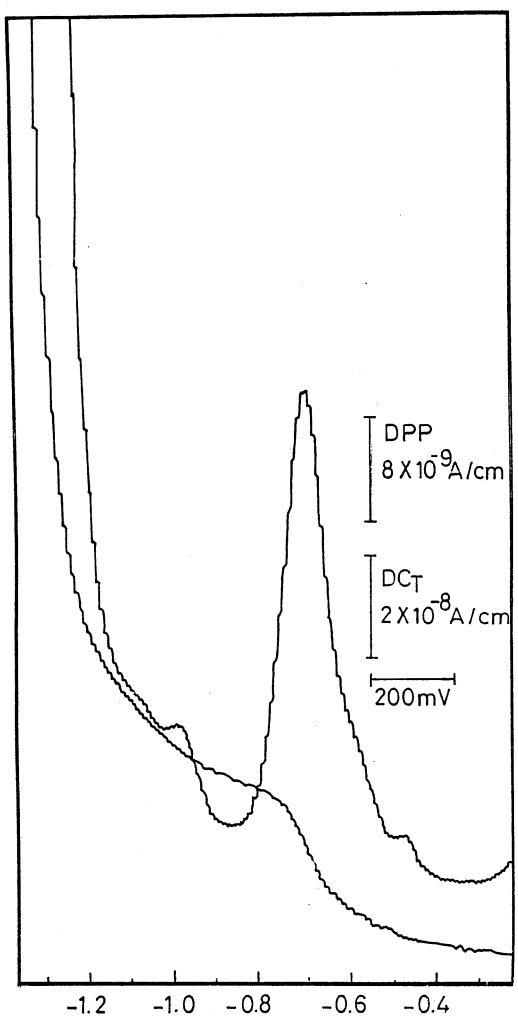

Fig. 1. Typical polarogram of ampicillin (corresponding to 40 μg ml^{-1} ampicillin) treated with nitrous acid in BRb pH 3.0.

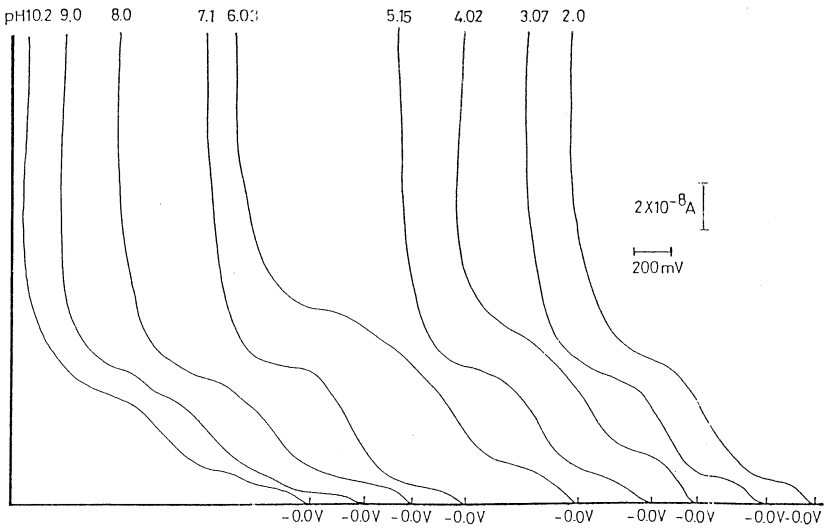

Fig. 2. Effect of pH on the polarographic behaviour of carbenicillin (corresponding to 40μg ml^{-1} carbenicillin) after treatment with nitrous acid.

followed by 2 ± 0.1 ml of 3% sodium nitrite solution. Heat in a boiling water bath for 10 ± 1 min, then cool. Add 2 ml of 5% ammonium sulphamate solution, then shake well until no more nitrogen evolves. Neutralize with 1 M NaOH then fill up to the mark with Britton–Robinson (BRb) buffer of pH 3.

2.4.2. The polarographic determination of penicillins

Transfer quantitatively the contents of the measuring flask into the polarographic cell. Pass pure nitrogen gas for 5 min. Record the polarogramms over the potential range 0–1 V versus Ag/AgCl electrode. Plot the measured currents (μA) in both the DC_t and DPP modes to get the calibration graphs. Alternatively, derive the corresponding regression equations for both modes.

2.4.3. Application to dosage forms

2.4.3.1. Procedure for capsules. Evacuate the contents of 20 capsules. Extract a known weight of the powder equivalent to 1.0 g of the drug by shaking with three successive 25 ml portions of water. Filter the combined extracts into a 100 ml calibrated flask. Wash the filter and dilute to the mark with the same solvent. Transfer an accurately measured volume of the filtrate into 25 ml volumetric flask. Prepare the corresponding derivative, then proceed as described in Section 2.4.2.

2.4.3.2. Procedure for syrups (dried powder to prepare syrups). Mix the contents of ten bottles. Weigh accurately an amount of the powder equivalent to 1.0 g of the drug. Transfer into 100 ml volumetric flask. Dissolve the drug in water and fill up to the mark with the same solvent. Proceed as described in Section 2.4.2.

2.4.3.3. Procedure for vials (dried powder to prepare injections). Mix the contents of five vials. Weigh accurately an amount of the powder equivalent to 1.0 g of the drug. Transfer into 100 ml volumetric flask. Dissolve the drug in water and fill up the mark with the same solvent. Proceed as described in Section 2.4.1. then Section 2.4.2.

3. Discussion

Treatment of penicillins with nitrous acid was found to yield polarographically active deriva-

tives. Fig. 1 shows a typical polarogram of ampicillin, as a model example, after the derivatization. Fig. 2 shows the effect of pH on the development of the polarographic waves of carbenicillin as a model example. For all the studied penicillins, the $E_{1/2}$ values were shifted to more negative potentials upon increasing pH. Linear regression analysis of $E_{1/2}$ values versus pH resulted in the following equations:

$E_{1/2}$ (V) = $-0.570 - 0.025$ pH

($R = 0.9956$)

$E_{1/2}$ (V) = $-0.452 - 0.075$ pH

($R = 0.9785$)

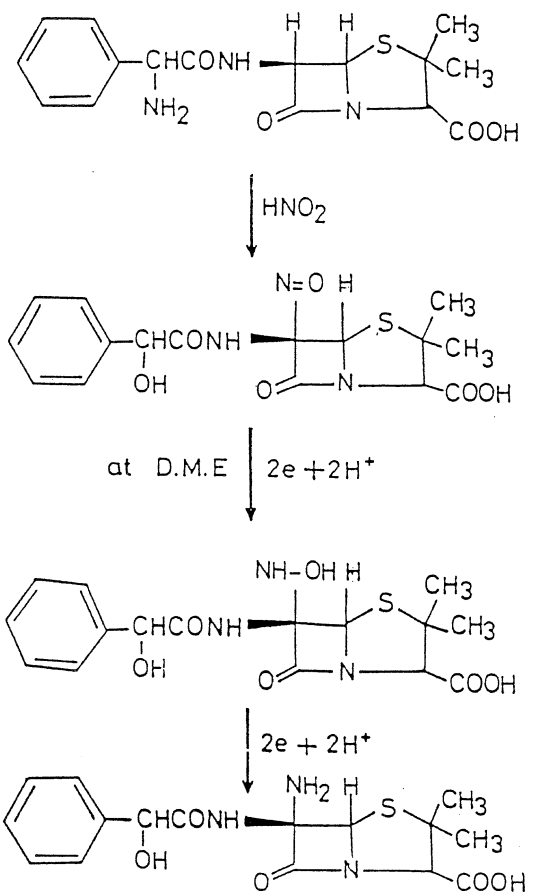

Fig. 3. Proposed mechanism of reduction of ampicillin treated with nitrous acid.

$E_{1/2}$ (V) = $-0.479 - 0.027$ pH

($R = 0.9938$)

for benzylpenicillin, ampicillin and carbenicillin, respectively.

A plot of the $E_{1/2}$ values of nitrosoampicillin versus pH gave three segments with two breaks at 4 and 7.5 (Fig. 4) corresponding to the pK_a of ampicillin 2.66 and 7.2, respectively [15].

Logarithmic analysis of the waves of the three compounds gave straight lines. If the rate determining step involves the transfer of two electrons (a free radical one electron transfer is not likely to occur) the slopes of the plots point out to the complete irreversibility of the reduction process. The αn_a values were calculated according to the treatment of Meites [16] and were found to change irregularly with pH.

3.1. Characteristics of the wave

The limiting current for the studied compounds was found to be a linear function of the square root of the mercury height (h). A plot of $\log h$ versus $\log i$ gave a straight line with a slope of about 0.5 for all the studied penicillins. The wave heights were independent of the buffer concentration. All these facts indicate a typical diffusion-controlled reduction process.

3.2. Stability

The produced derivatives were found to be stable for about 6 h at room temperature. In BRb pH 3.0 (the analytical pH), the studied penicillins gave their corresponding wave heights for more than 2 h, then they began to decrease slowly.

3.3. The diffusion-current constant at 25°C (id)

The diffusion current constants (Id) were calculated according to Ilkovic equation for varying concentrations; the results are abridged in Table 1. The calibration graphs for the derivatives were rectilinear over the ranges 8–200 μg and 2–160 μg ml^{-1} for all the studied penicillins as shown in Tables 2 and 3. The correlation coefficients are close to unity in all cases.

Table 1
Polarographic data for the derivatives of penicillins using DC_t mode

Compound	id/C	$Id = id/C\, m^{2/3} t^{1/6}$	Regression equation	R
Benzylpenicillin	2.56 ± 0.03	2.27 ± 0.02	$C = 0.00026 + 3.5\ id$	0.99995
Ampicillin	3.47 ± 0.03	2.64 ± 0.02	$C = 0.0043 + 3.5\ id$	0.99996
Carbenicillin	4.18 ± 0.02	3.18 ± 0.01	$C = 0.00057 + 4.1\ id$	0.99996

Table 2
Polarographic determination of pure samples of pencillins after derivatization using DC_t mode

Compound	μg Taken	μg Found	% Found	
			Proposed	Official [4,5]
Benzylpenicillin	8	8.1	101.3	
	16	16.0	100.0	
	32	31.8	99.4	
	80	79.0	98.8	
	120	121.0	100.8	
	160	160.0	100.0	
	200	199.0	99.5	
Mean ± S.D.			100.0 ± 0.8	99.8 ± 1.04
Ampicillin	8	7.9	98.8	
	16	15.7	98.1	
	32	31.5	98.4	
	80	80.1	100.1	
	120	119.0	99.2	
	160	159.0	99.4	
	200	201.0	100.5	
Mean ± S.D.			99.1 ± 0.95	100.6 ± 1.39
Carbenicillin	8	7.9	98.8	
	16	15.8	98.8	
	32	31.5	98.4	
	80	79.5	98.4	
	120	118.5	98.8	
	160	159.5	99.7	
	200	197.0	98.5	
Mean ± S.D.			98.8 ± 0.40	99.5 ± 1.08

3.4. Mechanism of electrode reaction

Upon treatment with nitrous acid, the polarographically inactive penicillins were converted into active derivatives. Position 6 is reported to be the active site in pencillins [17], therefore, it is proposed that the corresponding nitroso derivatives will be obtained as shown in Fig. 3. The formation of the nitroso derivative was confirmed through its reaction with cyanoacetamide where a yellow product with λ maximum at 327 nm was formed [18]. The nitroso derivatives were reduced at the DME on two steps [19]. The first step was the formation of hydroxylamine with the consumption of two electrons. Only in acidic medium, further reduction to the primary amine occurs. The last step involves two more electrons. It is worthy mentioning that in ampicillin in particular the α-amino group is converted by nitrous acid to the corresponding alcoholic group. However, this reaction has no effect on the polarographic behavior of derivatized ampicillin.

Table 3
Polarographic determination of pure samples of pencillins after derivatization using DPP mode

Compound	μg Taken	μg Foundt	% Found	
			Proposed	Official [4,5]
Benzylpenicillin	2.0	2.0	100.0	
	8	8.0	100.0	
	16	15.9	99.4	
	32	32.2	100.6	
	80	81.0	101.3	
	120	119.0	99.2	
	160	162.0	101.3	
Mean ± S.D.			100.24 ± 0.84	99.8 ± 1.04
Ampicillin	2	2.0	100.0	
	8	8.0	100.0	
	16	15.8	98.8	
	32	32.0	100.0	
	80	80.5	100.6	
	120	120.5	100.4	
	160	158.0	98.8	
Mean ± S.D.			99.58 ± 0.78	100.6 ± 1.39
Carbenicillin	2	2.0	100.0	
	8	8.1	101.3	
	16	16.2	101.3	
	32	32.0	100.0	
	80	80.0	100.0	
	120	119.0	99.2	
	160	161.0	100.6	
Mean ± S.D.			100.3 ± 0.71	99.5 ± 1.08

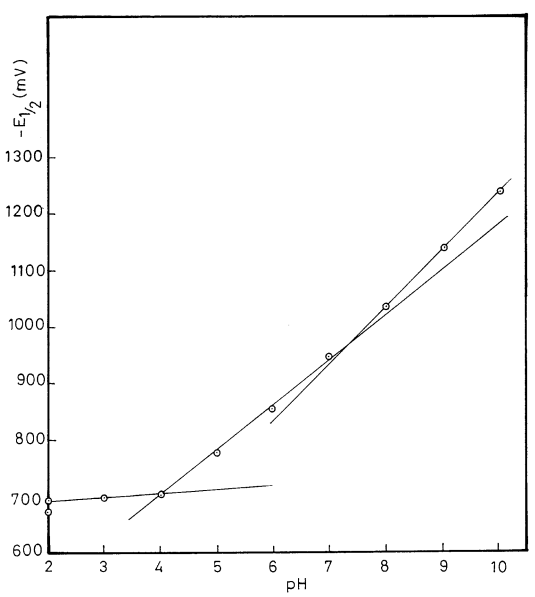

Fig. 4. Plot of the $E_{1/2}$ of nitrosoampicillin versus pH.

3.5. Analytical application

The suggested method was further applied to dosage forms (syrups, capsules and vials), the results in Table 4 are in good agreement with those obtained with the official methods [4,5]. Statistical analysis of the results obtained by both methods using the Student's *t*-test and variance-ratio *F*-test shows no significant difference regarding accuracy and precision [20].

Excepients frequently added to dosage forms, such as talc, starch, magnesium stearate, lactose and gelatine did not interfere with the assay. The most susceptible degradation pathway of penicillins is hydrolysis, i.e. introduction of H_2O or OH^- group resulting in the formation of penicilloic acid [21] in which the active site for electrophilic substitution is not available, i.e. the degradation products of penicillins do not inter-

Table 4
Polarographic determination of pencillins after derivatization in dosage forms using DC_t mode

Compound	μg Taken	μg Found	% Found	Official method (% found) [4,5]
Benzylpenicillin[a] capsules (250 mg ampicillin/capsule)	8	8.06	100.8	
	20	19.57	97.9	
	40	39.78	99.5	
	80	79.03	98.8	
	120	118.2	98.6	
Mean ± S.D.			99.1 ± 0.88	100.3 ± 1.2
Ampenicillin[a] syrup (250 mg ampicillin 5 ml^{-1})	8	7.95	99.5	
	20	20.0	100.0	
	40	39.78	99.5	
	80	80.64	100.8	
	120	120.43	100.4	
Mean ± S.D.			100.02 ± 0.58	100.8 ± 0.8
Penicillin G[b] vial (0.6 g penicillin/vial)	8	8.0	100.0	
	20	19.8	99.0	
	40	39.8	99.5	
	80	79.8	99.8	
	120	121.0	100.8	
Mean ± S.D.			99.82 ± 0.68	99.8 ± 1.04
Carbenicillin[c] vial (1 g carbenicillin/vial)	8	7.9	98.8	
	20	19.8	99.0	
	40	39.0	97.5	
	80	79.0	98.8	
	120	119.0	99.2	
Mean ± S.D.			98.7 ± 0.58	99.5 ± 1.08

[a] Product of Misr Pharm. Co. Egypt.
[b] Product of CID Pharm. Co., Giza, Egypt.
[c] Product of Pfier Italiana, S.P.A., Italy.

fere with the assay, therefore, the proposed method can be regarded as stability indicating one.

In conclusion, the polarographically inactive penicillins could be converted into active species through derivatization using nitrous acid. The suggested method has many advantages over the official methods [4,5], as it is less time-consuming, more specific and more sensitive.

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References

[1] D.W. Hughes, A. William, W.L. Wilson, Cand. J. Pharm. Sci. 11 (1976) 97.
[2] H.W. Unterman, S. Weissbuch, Pharmazie 29 (1974) 752.
[3] J.E. Fairbother, Pharm. J. 18 (1977) 509.
[4] British Pharmacopoeia, vol. I and II, Her Majesty's Stationary Office, London, 1993.
[5] The United States Pharmacopoeia XXII–National Formulary XVII, USP, Rockville, MD, 1990.
[6] G.O. El-Sayed, A.S. Amine, I.M. Issa, Anal. Lett. 27 (1994) 2515.
[7] M. Jamal, L.A. Stane, A.M. Knevel, J. Pharm. Sci. 67 (1978) 1917.
[8] M. Jamal, A.M. Kneval, Anal. Chem. 50 (1978) 1917.
[9] H. Oelschlager, in: D.P. Breimer, P. Speiser (Eds.), Topics in Pharmaceutical Sciences, Elsevier/North Holland, Amsterdam, 1981, p. 357.

[10] M.I. Walash, M. Rizk, F. Belal, A. El-Brashy, Microchem. J. 38 (1988) 300.
[11] F. Belal, F. Ibrahim, S.M. Hassan, F.A. Aly, Mikrochim. Acta 111 (1991) 61.
[12] F.A. Aly, F. Belal, A. El-Brashy, Pharm. World Sci. 15 (1993) 208.
[13] F.A. Aly, F. Belal, M.I. Walash, J. Pharm. Biomed. Anal. 13 (1995) 1127.
[14] J. Heyrovsky, P. Zuman, Practical Polarography, Academic Press, London, 1968, p. 179.
[15] J.P. Hou, J.W. Poole, J. Pharm. Sci. 58 (1969) 1510.
[16] L. Meites, Y. Israel, J. Am. Chem. Soc. 83 (1961) 4903.
[17] J. March, Advanced Organic Chemistry, Reaction Mechanisms and Structure, 2nd ed, McGraw Hill, New York, 1977, p. 542.
[18] N.A. Zakhari, Anal. Lett. 23 (1990) 1843.
[19] P. Kastening, in: P. Zuman, L. Meites, I.M. Kolthoff (Eds.), Progress in Polarography, vol. III, Wiley Interscience, New York, 1972, p. 259.
[20] J.D. Hinchen, Practical Statistics for Research, Methuen, London, 1969.
[21] Jaime. N. Delgudo, William A. Ramers (Eds.), Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott, Philadelphia, 1991, p. 234.