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Voltammetric determination of josamycin (a macrolide antibiotic) in dosage forms and spiked human urine

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Abstract

The voltammetric behaviour of josamycin (a macrolide antibiotic) has been studied using direct current (DC_t) alternating current (AC_t) and differential pulse polarography (DPP). In Britton–Robinson buffers, josamycin developed cathodic waves over the pH range 7–12. At pH 10, a well-defined cathodic wave with diffusion current constant of 1.06 ± 0.19 ($n = 5$) was obtained. The wave was characterized as being diffusion-controlled; and partially affected by adsorption phenomenon. The current-concentrations plots are rectilinear over the range 10–60 and 6–50 μg/ml using DC_t mode and DPP mode, respectively. The minimum detectability limit was 1.2 μg/ml (1.9×10^{-6} M) adopting the DPP mode. A method was proposed for the determination of josamycin in its tablets adopting both DC_t and DPP modes. The results obtained were in good agreement with those given by the manufacturer. The method was extended to the in-vitro determination of the drug in spiked human urine; the % recovery was $98.06 \pm 1.76\%$ ($n = 5$). The number of electrons involved in the reduction process was accomplished and a proposal of the electrode reaction was presented.

Keywords: Josamycin; Tablets; Urine; Voltammetry; Polarography

1. Introduction

Josamycin is a macrolide antibiotic that is particularly indicated for the treatment of infections of the skin, respiratory tract, ear, nose and throat. Its important pharmacokinetic properties include accumulation in certain cells and an increase in blood plasma levels after repeated ingestion [1]. Josamycin is also used in veterinary medicine to treat respiratory diseases or as feed additives to promote growth. Like other macrolide antibiotics, josamycin is a lipophilic molecule with a central lactone ring bearing 16 atoms to which several amino and sugars moieties are bound [2,3].

Most of the methods reported for the analysis of josamycin in biological fluids, tissues, eggs and milk rely on the use of liquid chromatography [4–18] in addition to capillary-electrophoresis [19]. The electrochemical behaviour of lanthanu-

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m(III) migration across a water/nitrobenzene interface, was used for the determination of josamycin [20]. No reports are available yet on the determination of josamycin in dosage forms. Although, chromatographic methods offer a high degree of specificity, yet the sample-clean up and the instrument limitations preclude their use for routine clinical analysis. This led us to study the electrochemical behaviour of josamycin at the Dropping Mercury Electrode (DME) with the aim of developing a simple and reliable method for its determination. The presence of the electroreducible conjugated diene groups initiated the present study. The results obtained were promising, and the method can be considered as an alternative substitute for the chromatographic methods. The most striking feature of the method as applied for urine is that, no prior treatment of the samples is necessary before measurement.

2. Experimental

2.1. Apparatus

The polarographic study and the DPP measurements were carried out using the Polarecord Model E506 Metrohm (Herisau, Switzerland). The electronically controlled droptime of 1 s was adjusted using a 663 VA Stand from the same company. The polarograms were recorded using a potential scan rate of 10 mV/s. A three-electrode system composed of the DME as the working electrode, an Ag/AgCl reference electrode and a platinum wire as the auxiliary electrode, was used. The solutions were purged with pure nitrogen gas for 5 min before being polarographed at room temperature. The phase selective alternating current (AC_t) behaviour of josamycin was studied using the same instrument. The superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

2.2. Materials and regents

- Josamycin was kindly provided by SAJA Pharmaceutical Company, Jeddah, Saudi Arabia. The potency as determined by the cylinder plate method was 1000 μg/ml. Tablets containing josamycin, 500 mg each (Batch No. 00L014) were obtained from the same company.
- Individual urine samples (3) was obtained from healthy volunteers (men, around 40 years old) and kept frozen until use after gentle thawing.
- Methanol, AR grade (Aldrich, USA).
- Britton–Robinson buffers (0.08 M) covering the pH range 7–12 [21]
- Standard solutions: a stock solution containing 1.0 mg/ml of josamycin was prepared in methanol then serially diluted with the same solvent as appropriate. The solutions are stable for 1 week if kept in the refrigerator.

2.3. Procedures

2.3.1. Procedure for tablets

Weigh and pulverize 20 tablets. Transfer an accurately weighed quantity of the powder equivalent to 40 mg of josamycin into a small flask. Shake for 30 min with 80 ml of aqueous (50%) methanol and filter into a 100 ml measuring flask. Wash the conical flask and the filter then transfer the washing into the same measuring flask. Complete to the mark with aqueous methanol. Transfer aliquot volumes containing suitable amounts of the drug into a series of 25 ml measuring flasks, so that the final concentration of the drug is within the working range (Table 2). Complete to the mark with BRb of pH 10. Transfer the whole contents of the flask into the polarographic cell. Pass nitrogen gas for 5 min. Record the current in both the DC_t and DPP mode within the range -1.2– -1.6 V. Determine the nominal content of the tablets using either the calibration graphs or the corresponding regression equation.

2.3.2. Procedure for urine

Into a series of 25 ml measuring flasks, transfer 1 ml of urine. Add increasing volumes of a stock solution of josamycin containing 250 μg/ml, so that the final drug concentration is within the range 6–36 μg/ml. Mix well using a vortex mixer then complete to the mark with BRb of pH 10. Pour the whole contents of the flask into the

polarographic cell and pass nitrogen gas for 5 min. Record the current within the range −1.2– −1.6 V adopting the DPP mode. Determine the concentration of the drug in urine using the corresponding regression equation.

3. Results and discussion

Fig. 1 shows typical DC_t and DPP polarograms of josamycin (40 μg/ml) in BRb of pH 10. The DC_t wave is well-defined and the DPP peak is very steep. The reduction of josamycin at the DME was found to be pH-dependent. The wave began to develop at pH 7 and improved upon increasing the pH up to pH 12. The $E_{1/2}$ values showed positive shift upon increasing pH as shown in Fig. 2 and Table 1. The values of $E_{1/2}$ (mV) were linear function of pH over the range 7–12, the relation between them can be expressed by the equation:

$$E_{1/2}\ (\text{mV}) = 41.4\ \text{pH} - 1838.5 \quad (R = 0.9915).$$

A plot of $E_{1/2}$ versus pH values gave two straight lines with intersection at pH 7.3 (Fig. 3). This value (7.3) corresponds to the pK_a of josamycin; and is in agreement with the reported value (7.1 in 50% aqueous ethanol) previously mentioned in the literature [22]. Logarithmic analysis of the reduction waves of josamycin in BRb of different pH values resulted in straight lines. The values of αn_a were calculated according to the treatment of Meites and Israel [23] and are listed in Table 1. Assuming that the rate-determining step involves the transfer of two electrons, the values of αn_a point out to the completely irreversible nature of the reduction waves. The number of protons $Zn(H^+)$ consumed in the rate-determining step can be obtained from the following formula [24]:

$$\Delta E_{1/2}/\Delta\text{pH} = 0.059 Z_{(H^+)}/\alpha n_a.$$

The value of αn_a are obtained from the equation: $E = E_{1/2} - ((0.059/\alpha n_a)\log(i/\text{id} - i))$ where α is the transfer coefficient and n_a is the number of electrons transferred in the rate-determining step.

The values of protons consumed at different pHs are abridged in Table 1, the small figures obtained confirm the irreversible nature of the reduction waves.

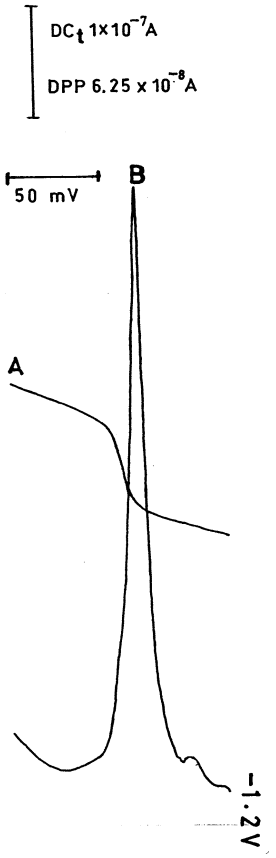

Fig. 1. Typical polarograms of josamycin (40 μg/ml) in BRb of pH 10. (A) DC_t mode; (B) DPP mode.

3.1. Study of wave characteristics

The waveheight (W) of the reduction waves increased with increasing the mercury height (h) over the range 40–75 cm; a plot of W versus $\sqrt{h}$ resulted in a straight line. A plot of log W versus log h gave a straight, the slope of which was 0.56. Changing the buffer concentration over the range 0.012–0.076 M (final concentration) resulted in a negligible effect on the waveheight. These two characteristics point out to a diffusion-controlled wave, partially affected by adsorption phenomenon. This fact was further confirmed by studying the alternating current behaviour of josamycin. At pH 10 (the analytical pH) and

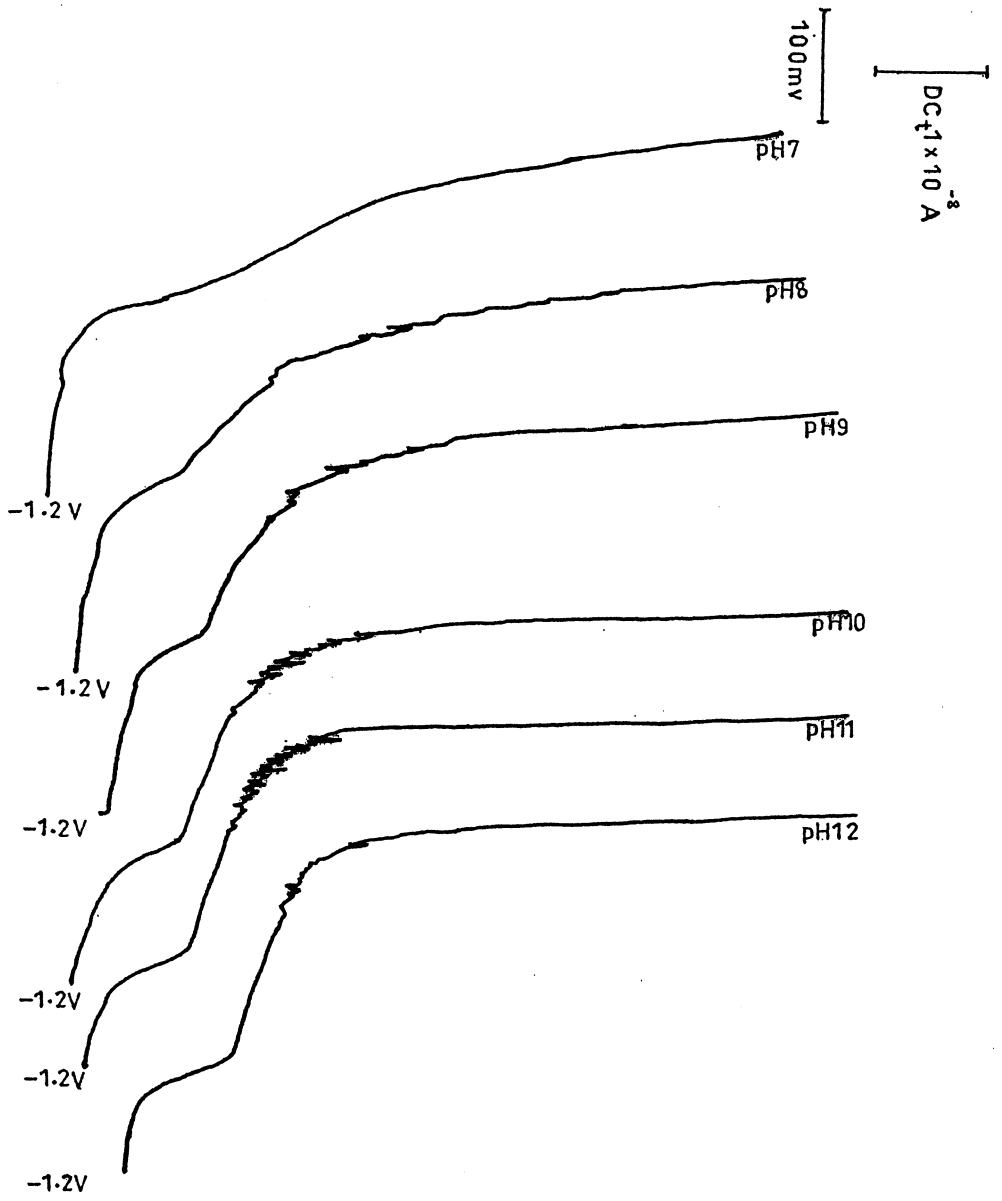

Fig. 2. Effect of pH on the development of the polarographic waves of josamycin (40 μg/ml) in BRb.

using a phase-selective angle of 90°, Fig. 4 shows that only the depolarizer—but not its reduction product—is adsorbed to the mercury surface. The figure also shows that, the summit potential (E_s) has been shifted 20 mV more negative than the corresponding $E_{1/2}$ value.

The diffusion current constant ($Id = id/Cm^{2/3}t^{1/}$ 6) was calculated according to Ilkovic equation [25] using five different concentrations of josamycin and was found to be 1.06. Similarly, the diffusion coefficient was calculated [25] and was found to be 7.67×10^{-7} cm^2/s. This small value may be attributed to the bulky nature of the molecular structure of josamycin.

3.2. Analytical applications

At pH 10 (the analytical pH) josamycin exhibited well-defined cathodic DC_t waves and steep DPP peaks. The current–concentration plots are rectilinear over convenient ranges in both modes. Table 2 presents the analytical performance data (working range, regression equations, correlation coefficient … etc.) of the proposed methods. Statistical evaluation of the regression lines, in both modes, with regard to the standard deviation of residuals ($S_{x/y}$), standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) gave the values abridged in the same table; these small values point out to the high precision of the proposed methods [26]. The minimum detectability limit ($S/N = 2$) was determined adopting the DPP mode and was found to be 1.2 μg/ml (1.9×10^{-6} M). Solutions of jasomycin in BRb of pH 10 were found to be stable for more than 100 min.

The validity of the method was assessed by applying the method to an authentic sample of josamycin. Both DC_t and DPP were successfully utilized. The results are shown in Table 3. The results are in agreement with those given by the Manufacturer adopting a microbiological assay method.

Both DC_t and DPP modes were further successfully applied to commercial tablets containing josamycin after simple extraction with aqueous (50%) methanol. The results are shown in Table 3. The results are in good agreement with those obtained by the manufacturer adopting a microbiological method.

Josamycin is given orally in a dose of 500 mg three times daily. This dose results in a urine level of concentration of about 10 μg/ml. This concentration lies well within the working range of the DPP mode. The method could be successfully applied to the analysis of spiked urine. No prior extraction step was necessary. The relation between the concentration of josamycin in urine and the current adopting DPP mode was rectilinear over the range 6–36 μg/ml (Fig. 5). Linear regression analysis of the data gave the following equation.

$$\text{ip } (\mu A) = 0.0057C - 0.0138 \quad (R = 0.9988),$$

when ip is the peak current in μA in the DPP mode. C is the concentration in μg/ml.

Analysis of samples of spiked human urine was conducted after dilution with BRb of pH 10. The results shown in Table 4 are satisfactorily accurate and precise.

3.3. Mechanism of the electrode reaction

The number of electrons involved in the electrode reaction could be accomplished through comparative study of the waveheight of josamycin

Table 1
Effect of pH on the development of the cathodic waves of josamycin

pH	$E_{1/2}$ (−mV)	$\Delta E_{1/2}/\Delta$pH	id/C (μA/mM)	$W_{1/2}$ (Mv)	αn_a	$Z(H^+)$
7.0	−1540					
		20				
8.0	−1520		1.1458	30	0.8148	0.28
		50				
9.0	−1470		1.0417	40	0.7037	0.60
		50				
10.0	−1420		1.1458	30	0.9630	0.82
		50				
11.0	−1370		1.1979	40	0.7222	0.61
		20				
12.0	−1350		1.3542	30	0.7222	0.24

Where α, is the transfer coefficient; n_a, is the number of electrons transferred in the rate-determining step; $Z(H^+)$, number of protons transferred in the rate-determining step; $W_{1/2}$, is the half-peak width in DPP mode.

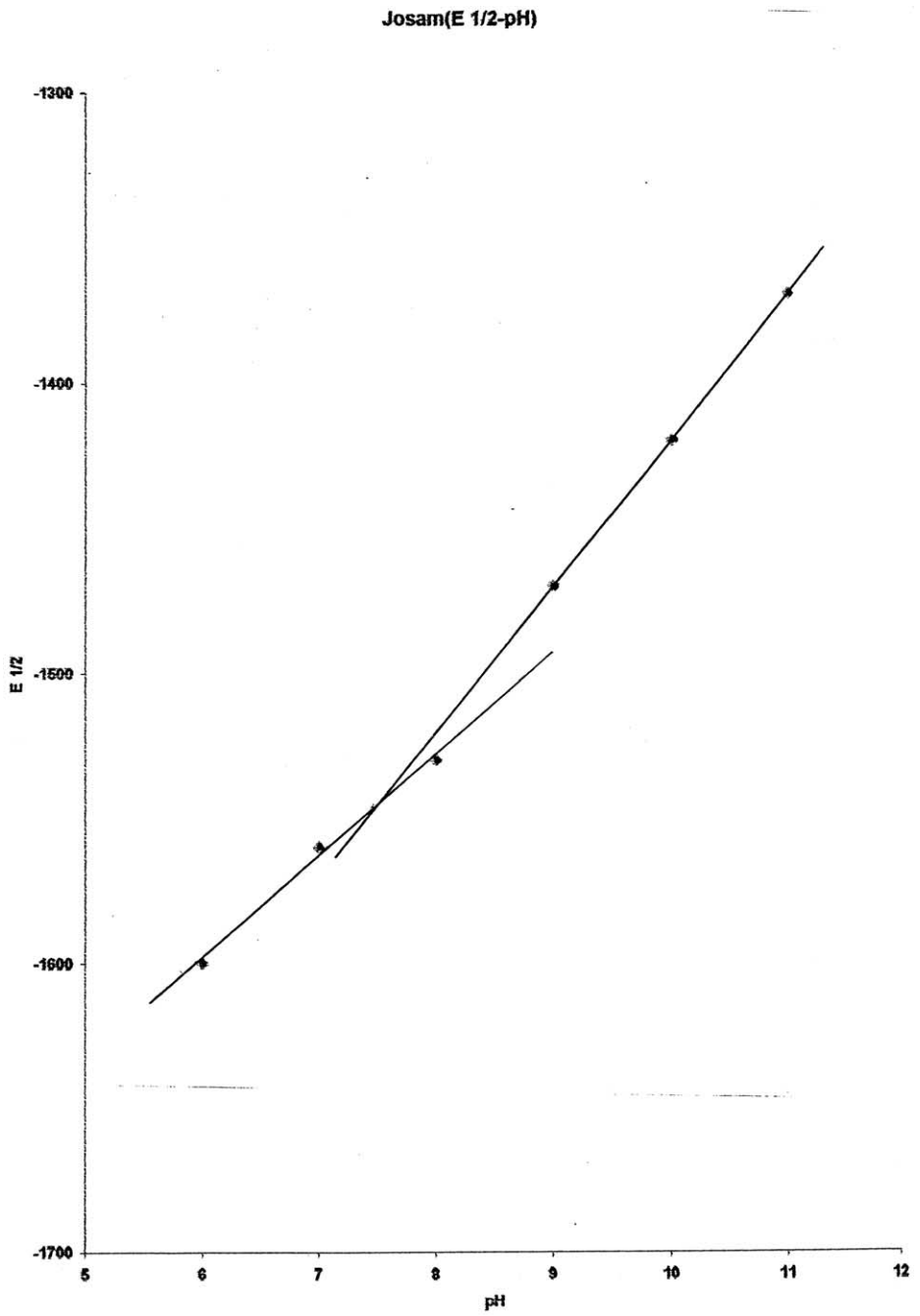

Fig. 3. Plot of half-wave potentials ($E_{1/2}$) of josamycin versus pH in BRb.

in BRb of pH 10 with that produced by an equimolar solution (3×10^{-5} M) of a previously studied compound having the same reducible function group and with nearly identical value of diffusion coefficient, that is acrivastine [27]. It was fund that both compounds gave two waves of

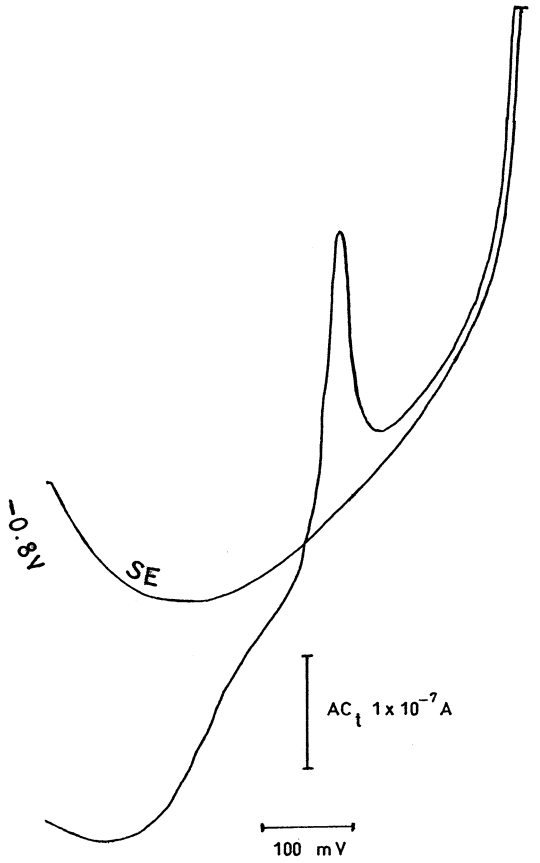

Fig. 4. Alternating current behaviour of josamycin (20 μg/ml) in BRb of pH 10. Superimposed alternating voltage 15 mV, selective phase angle 90° at a frequency of 75 Hz. SE, supporting electrolyte.

Table 2
Analytical performance data of the proposed methods

Parameter	DC_t mode	DPP mode
Working range	10–60 μg/ml	6–50 μg/ml
Slope	0.00084	0.0041
Intercept	0.0245	0.0080
Correlation coefficient	0.9988	0.9987
Detection limit	–	1.2 μg/ml (1.9×10^{-6} M)
$S_{x/y}$	0.00083	0.0032
S_a	0.000773	0.0000363
S_b	0.000000474	0.0000696
Applications	Tablets	Tablets and urine

Table 3
Application of the proposed methods to the analysis of josamycin in pure form and commercial tablets

Material	DC_t	DPP	Reference method[b]
Authentic powder			
Number of experiments	4	5	100.0
% Recovery	100.45	100.03	
Standard deviation	1.23	1.91	
Variance	1.51	3.65	
Josaxin tablets[a] (*josamycin* 500 *mg/tablet*)			
Number of experiments	8	8	
% Recovery	102.62	102.14	99.60
Standard deviation	2.60	1.08	
Variance	5.29	1.1664	

[a] Product of Saja Pharmaceuticals, Jeddah, Saudi Arabia, batch no. 00L014.

[b] Results obtained by the manufacturer adopting a microbiological assay method.

equal heights. Hence, it is concluded that, two electrons are involved in the electrode reaction. Based on this fact, and by analogy to previous reports for compounds with the same function groups [27,28]. The following pathway for the electrode reaction is proposed:

$$R\text{–}CH{=}CH\text{–}CH{=}R' + 2e + 2H^+ \rightarrow R\text{–}CH_2\text{–}CH{=}CH\text{–}CH_2\text{–}R'$$

4. Conclusion

A simple and satisfactorily sensitive method has been developed for the determination of josamycin, in raw material, tablets and spiked human urine. The method is based upon measuring the cathodic polarographic current produced in BRb of pH 10. The method can be adopted for routine quality control of the drug and for its pharmacokinetic studies.

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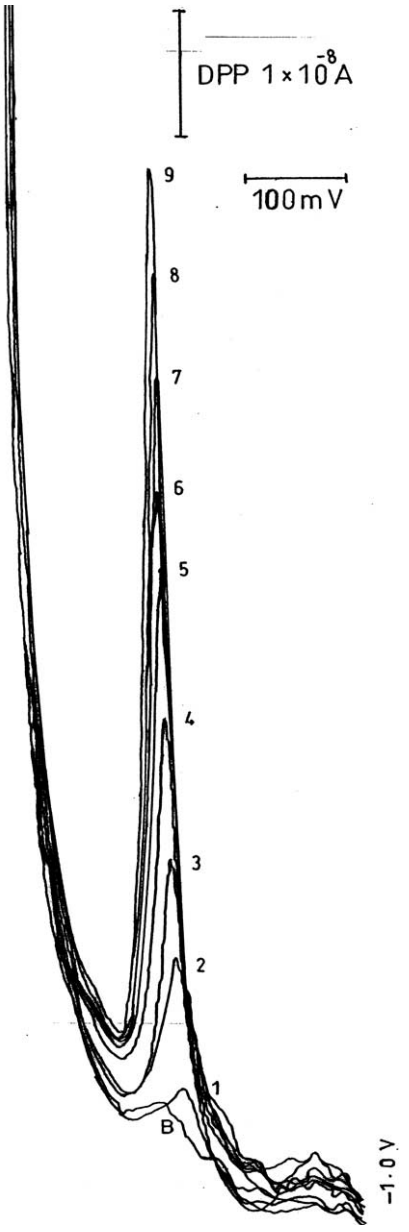

Fig. 5. DPP polarograms of josamycin in urine at pH 10. B, blank urine. (1) 6; (2) 8; (3) 12; (4) 16; (5) 20; (6) 24; (7) 28; (8) 32; and (9) 36 μg/ml.

Table 4
Analysis of josamycin in spiked human urine adopting the DPP mode

Added (μg/ml)	Found (μg/ml)	% Recovery
8.0	7.91	98.88
12.0	11.41	95.08
28.0	28.03	100.10
32.0	31.70	99.06
$\bar{X}$		98.28
SD		±1.9

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