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Electrochemical study of natamycin – analytical application to pharmaceutical dosage forms by differential pulse voltammetry

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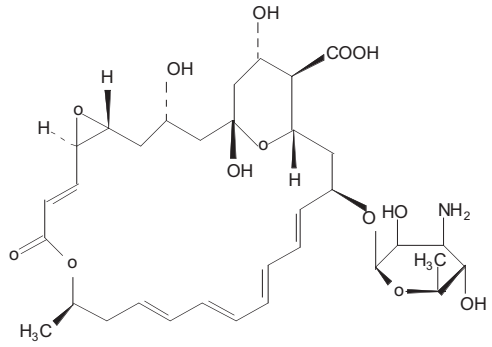
The electrochemical oxidation and determination of natamycin has been carried out at a carbon paste electrode in aqueous solutions in the pH range of 2.5–10.30 by cyclic and differential pulse voltammetry. Best results were obtained with the differential pulse voltametric technique in 0.5 M sulfuric acid at pH 1.82. The diffusion controlled nature of the waves was established. A differential pulse voltammetric technique for the determination of natamycin 0.5 M sulfuric acid which allows quantitation over the range of 2×10^{-6}–8×10^{-5} M range method is proposed. Limit of detection and limit of quantitatification were 1.5×10^{-6} and 5×10^{-6} M, respectively. Based on this study a simple, rapid, selective and sensitive voltammetric method was developed for the determination of natamycin in capsules. In order to validate the proposed method, UV spectroscopy was applied.

1. Introduction

Natamycin (pimaricin) [(8*E,* 14*E,* 16*E,* 18*E,* 20*E*)-(1*R*, 3*S*, 5*R*, 7*R*, 12*R*, 22*R*, 24*S*, 25*R*, 26*S*)-22-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octa-cosa-8, 14, 16, 18, 20-pentaene-25-carboxylic acid] is the only antifungal drug approved for the treatment of fungal keratitis. Natamycin has been determined by UV-spectrophotometry (Brik 1981), but to the best of our knowledge, the electrochemical behaviour and voltametric determination of this drug have not yet been reported.

The purpose of this work is to develop a simple, rapid and sensitive voltammetric method for the determination of natamycin and applying it to pharmaceuticals.

The investigation of the electrooxidation of natamycin yielded information on clinical activities, because the explanation of the electrode reaction may provide information on drug receptor interaction (Wang 1988; Kauffmann and Vire 1993).



Natamycin

2. Investigations, results and discussion

The voltammetric oxidation at the carbon paste electrode was investigated in the pH range 1.82–10.30. As seen from Fig. 1 (differential pulse voltammogram), one well defined oxidation peak is present at pH 1.82.

Fig. 2a shows the relationship between peak potential and pH. The effect of pH on the peak current (Fig. 2b) shows a maximum at pH 1.82 (0.5 M sulphuric acid), thus this pH value and this supporting electrolyte were chosen to carry out the electroanalytical study.

The effect of various electrolytics such as sulphuric acid, phosphate, acetate and Britton-Robinson buffers was examined (Table 1). For analytical purposes the best response was obtained with 0.5 M sulphuric acid.

Cyclic voltammograms were recorded at different potential scan rates between 10 and 1500 mVs^{-1}. Cyclic voltammetric measurements performed on 8×10^{-5} M natamycin solution in the presence of 20% methanol showed a one

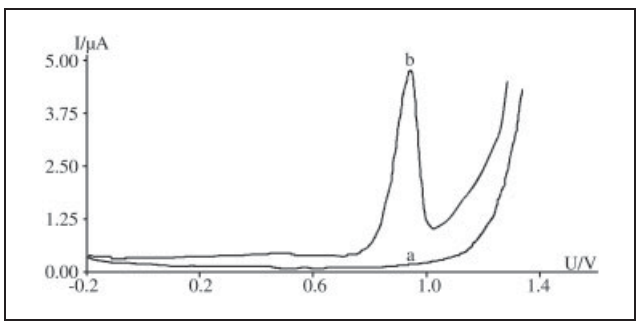


Fig. 1: Differential puls voltammogram of natamycin in 0.5 M sulphuric acid (20% methanol) at pH: 1.82, a) blank b) 8×10^{-5} M natamycin

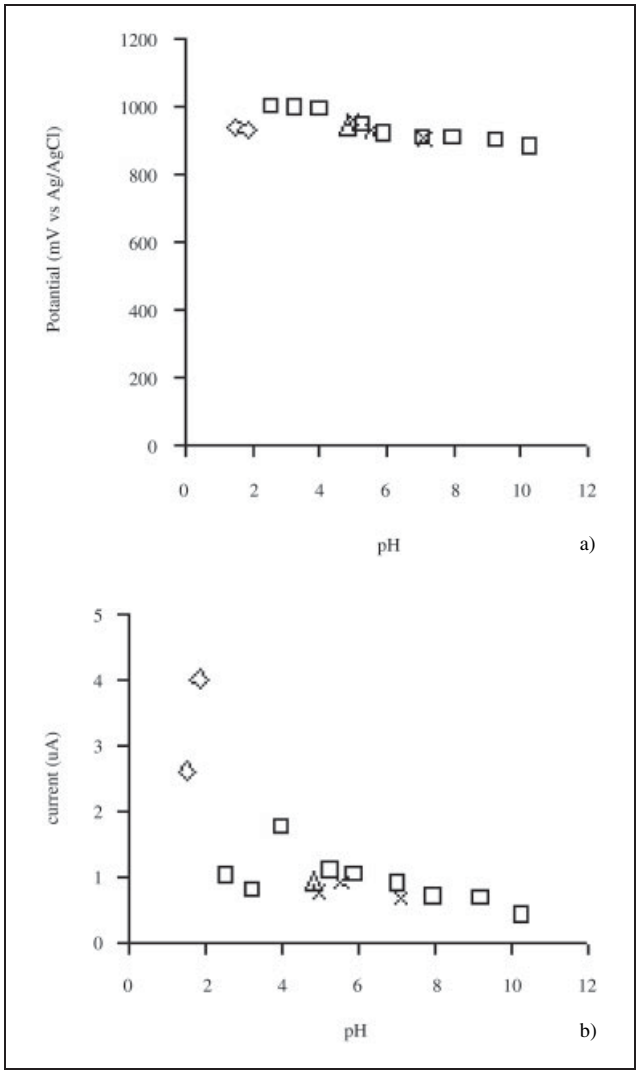


Fig. 2: The effect of pH on a) natamycin peak potential and b) peak current natamycin concentration, 8×10^{-5} M; (quadrangle): sulphuric acid; (square): Britton-Robinson buffer; (triangle): acetate buffer; (multiplication): phosphate buffer

nonreversible nature of the peak in the range of scan rates between 10 and 1500 mVs^{-1} (Fig. 3).

The linear relationship existing between peak current and square root of the scan rate (correlation coefficient 0.987) with a slope of 0.93, showed that the oxidation process is predominantly diffusion-controlled over the whole scan rate range studied. A plot of logarithm of peak current versus logarithm of scan rate gave a straight line (correlation coefficient 0.986) with a slope of 0.66 (close to 0.5), which is the expected value for an ideal reaction of solution species (Laviron 1980).

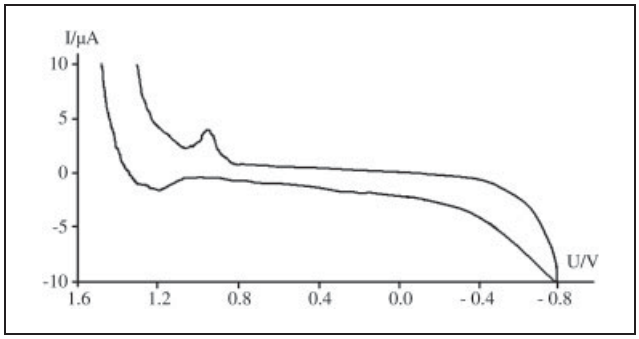


Fig. 3: Cyclic voltammogram obtained from 8×10^{-5} M natamycin in 0.5 M sulphuric acid (20% methanol) at pH: 1.82. Scan rate: 100 mVs^{-1}

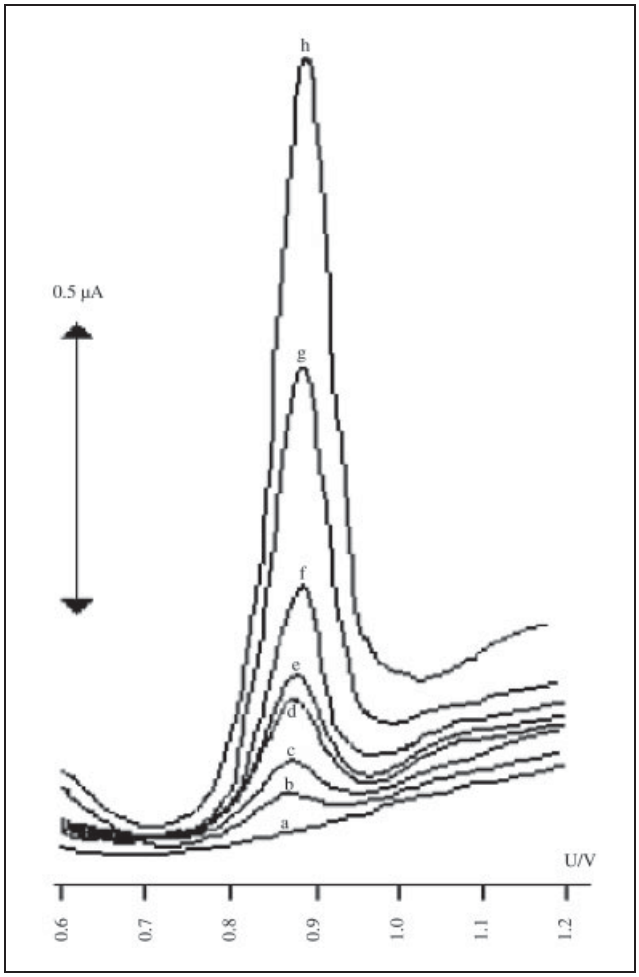


Fig. 4: Differential pulse voltammograms of natamycin in 0.5 M sulphuric acid 20% methanol) at pH: 1.82. a) blank; b) 2×10^{-6} M; c) 6×10^{-6} M d) 8×10^{-6} M; e) 2×10^{-5} M; f) 4×10^{-5} M; g) 6×10^{-5} M; h) 8×10^{-5} M

In order to quantitative determination of natamycin cyclic and differential pulse voltametric techniques were applied. The differential pulse mode yielded a voltammogram in which the peak current was greater than that obtained by cyclic voltammetry.

The peak currents increased linearly with increasing amounts of natamycin by differential pulse voltammetry technique (Fig. 4). Under the experimental parameters described below linear calibration curves were obtained in the range of 2×10^{-6}–8×10^{-5} M. The regression equation of the calibration curve was calculated as

$$I(\mu A) = 1.46 \times 10^4\, C + 0.067)\ (r = 0.990) \qquad (1)$$

Table 1: Voltammetric characteristics of 8×10^{-5} M natamycin in various buffer systems (20% methanol) by differential puls voltammetry

Buffer	Concentration	Studied pH range	Selected pH	E_p/V	i_p/μA
Sulfuric acid	0.5 M	1.82	1.82	0.93	4.02
Britton-Robinson	0.04 M	2.5–10.30	2.55	0.99	1.01
Phosphate	0.1 M	4.97–7.12	5.55	0.93	1.40
Acetate	0.1 M	4.82	4.82	0.94	1.60

Table 2: Analytical parameters for the determination of natamycin by differentail pulse voltammetric and UV-spectrophotometric methods

Method	Linearity range (M)	Slope (μAM^{-1})	Intercept (μA)	Corr. coeff. r	RSD of slope	Limit of detection LOD (M)	Limit of qantification LOQ (M)
Differential pulse voltammetry	2×10^{-6}–8×10^{-5}	1.46×10^4	0.067	0.990	0.22	1.5×10^{-6}	5×10^{-6}
UV-spectro-photometry	10^{-6}–8×10^{-6}	6.54×10^4	0.019	0.995	0.24	1.88×10^{-7}	6.27×10^{-7}

Table 3: Content of natamycin capsules obtained by differential pulse voltammetric and UV-spectrophotometric methods

Method	Labelled natamycin (mg per capsule)	Amount found* (mg) (X ± SE)	RSD (%)	Recovery (%)	t-test of (p = 0.05)
Differential pulse voltammetry (DPV)	25.00	25.22 ± 0.26	1.03	100.88	0.484**
UV-spectro-photometry	25.00	25.40 ± 0.29	1.14	101.60	

* Each value is the mean of 10 experiments
X, mean; SE, standard error; RSD, relative standard deviation
** $t_{theoretical}$: 2.306 (95% confidence limit)

The characteristics of the calibration plots are listed in Table 2.
LOD: limit of detection and LOQ: limit of quantitation of the procedures were also shown in Table 2, which were calculated on the peak current using the following equations:

$$\mathrm{LOD} = 3\,\mathrm{s/m} \quad (2)$$

$$\mathrm{LOQ} = 10\,\mathrm{s/m} \quad (3)$$

where s, the noise estimate, is the standard deviation of the peak currents (five runs) of the sample, m is the slope of the calibration curve.
For comparison, the spectrophotometric behaviour of natamycin in methanol was studied in a range of wavelengths from 200 to 350 nm. Good results were obtained at 317 nm (Fig. 5).
Linear calibration curves were obtained for natamycin in the range of 10^{-6}–8×10^{-6} M (Table 2). The regression equation of the calibration curve for UV-spectrophotometry was calculated as:

$$A = 6.54 \times 10^4 C + 0.019 \; (r = 0.995) \quad (4)$$

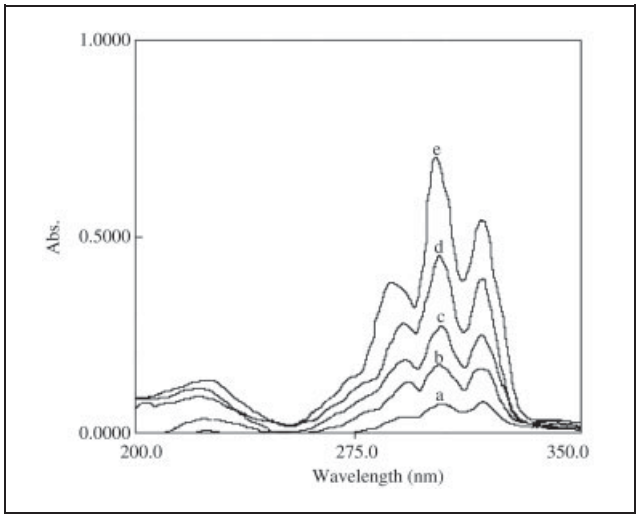


Fig. 5: UV spectrum of natamycin in methanol : a) 10^{-6} M , b) 2×10^{-6} M, c) 4×10^{-6} M, d) 6×10^{-6} M , e) 8×10^{-6} M

On the basis of these results, commercially available capsules containing 25 mg of natamycin were analysed by the proposed differential pulse voltammetric method and an UV-spectrophotometric method for comparison and were applied to the direct determination of natamycin in capsules, using the straight calibration line after adequate dilution (Table 3).
According to the student's t-test, the calculated t values did not exceed the theoretical value for significance level of 0.05. This result indicates that there is no significant difference between the proposed differential pulse voltametric method and the UV-spectrophotometric method as regards to accuracy and precision. Also, the proposed methods could successfully be applied to natamycin assay in capsules without any interferences.
Moreover, in order to know whether the excipients in the capsule show any interference with the analysis, the accuracy of the proposed method was evaluated by recovery studies after the addition of known amounts of pure drug to various pre-analysed formulations of natamycin. The procedure is specified in the Experimental part. As Table 3 shows, the results demonstrate the validity of the proposed method for the determination of natamycin in commercial dosage forms. The proposed methods were proved to have precision and accuracy adequate for the reliable analysis of natamycin.
The principle advantage of the proposed method over the UV-spectrophotometric method is that the excipients do not interfere and a separation procedure is not necessary.

3. Experimental

3.1. Apparatus

A model Metrohm 693 VA Trace Analyzer and 694 VA Stand was used for the voltammetric measurements, with a three-electrode system consisting of a carbon paste working electrode (φ = 3 mm, Metrohm), a platinum wire auxiliary electrode and an Ag/AgCl (NaCl 3 M, Metrohm) reference electrode. Before each measurement the carbon paste electrode was polished manually using weighing paper until a shiny surface and washed with ultrapure water.
Differential pulse voltammetry conditions were: pulse amplitude: 50 mV; pulse width: 50 ms; scan rate: 20 mVs^{-1}.
Spectrophotometric measurements were carried out with a Schimadzu UV 1605 spectrophotometer. Quartz cuvettes of 1 cm light path were used throughout.

3.2. Reagents

Natamycin was kindly provided by Santa Farma Inc. (Istanbul, Turkey). A stock solution of 1×10^{-3} M natamycin was prepared by dissolving an

accurate mass of the drug in an appropriate volume of methanol and kept in the dark in a refrigerator. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution by methanol (20%). Methanol (Riedel, 99.0%) was used for all of the solutions prepared. All solutions were protected from light and were used within 24 h to avoid decomposition. 0.5 M sulphuric acid, 0.1 M phosphate buffer (pH 4.97–7.12), 0.1 M acetate buffer (pH 4.82) and 0.04 M Britton-Robinson buffer (pH 2.5–10.30) were used as supporting electrolytes.

3.3. Calibration procedure for voltammetric determination

Natamycin was dissolved in methanol to obtain a 1×10^{-3} M stock solution. This solution was diluted with methanol to obtain a different natamycin concentration. Under optimum conditions linear calibration curves were obtained for natamycin in the range of $2 \times 10^{-6} - 8 \times 10^{-5}$ M with differential puls voltammetric technic.

3.4. Tablet assay procedure

Five capsules were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of a concentration of 1×10^{-3} M was weighed and transferred in to a 100 ml calibrated flask and completed to the volume with methanol. The contents of the flask were centrifuged for 15 min at 4000 rpm and then diluted to volume with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with methanol:selected supporting electrolyte solution in order to obtain a final solution of 20 : 80. Each solution was transferred in to the voltammetric cell and recorded as for the pure drug.

3.5. Recovery experiments in drugs

In order to study whether the excipients show any interference with the analysis, known amounts of the pure drug were added to the different preanalysed formulations of natamycin and mixtures were analysed by the method proposed. The recoveries obtained after five repeated experiments were calculated.

3.6. Spectrophotometric procedure

An appropriate amount of supernatant (obtained from capsule assay procedure) was added to the quartz spectrophotometric cuvettes. The sample solution absorptivity was measured at 317 nm. The concentration of natamycin was calculated from a prapared standard calibration curve.

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