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Electrochemical determination of minocycline in pharmaceutical preparations

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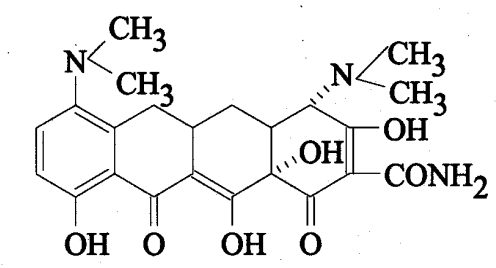
Abstract. The possibility of electrochemical determination of minocycline on mercury and solid electrodes by various voltammetric and polarographic techniques has been undertaken. The influence of some chemical (ionic strength and pH) and instrumental parameters on the main reduction signal of minocycline was investigated. The alternating current polarographic (ACP) technique was applied to the minocycline determination either in pure form or in a commercial pharmaceutical preparation. The common excipients did not interfere. The antibiotic could be detected in the concentration range 1 10^{-3} − 5 10^{-6} mol L^{-1}. The detection limit for the quantitative determination by ACP was 1 10^{-6} mol L^{-1} minocycline in aqueous medium of 0.1 M NaCl. The mean recovery was 99.2% with a relative standard deviation (RSD) of 2.93% (n = 8).

Introduction

Minocycline is the commercial name of the semisynthetic compound 7-dimethylamino-6-dimethyl-desoxytetracycline hydrochloride, that is a yellow crystalline, slightly hygroscopic, photo-sensitive powder. It belongs to the first group of semisynthetic tetracyclines beside doxycycline and methacycline. Its chemical structure (I) includes a second dimethylamino group. Minocycline has a major therapeutic interest, being active against staphylococci resistant to other tetracyclines. Today, an accessible semisynthetic method is used for its preparation, starting from 6-dimethyl-desoxytetracycline [1].



(I)

Analytical techniques employed for minocycline determination include chromatography [2-5], molecular absorption spectrometry [6-8], electrophoresis [9] and differential pulse polarography [10,11].

This paper discusses the possibility of using polarographic and voltammetric techniques for minocycline analysis as simple and accurate methods for its determination from dosage forms. The procedure involving, usually, a simple dilution because the most excipients did not interfere in the subsequent determination steps [12,13].

Due to the presence, in the molecular structure, of the possible reducible carbonyl groups, like in other previously investigated tetracyclines [14-17], the polarographic and voltammetric behaviour of this compound was tackled employing cyclic (CV), linear sweep (LSV), differential pulse (DPV) and square wave voltammetry (SWV) and sinusoidal alternating current polarography (ACP). The possibility of quantitative determination of minocycline was established using LSV, SWV, DPV and ACP.

Experimental

Apparatus

The voltammetric measurements were carried out using a Galvanostat/Potentiostat system Model 273 A with a multifunctional mercury electrode 303 A (EG & G Princeton Applied Research) and a Ag/AgCl reference electrode.

ACP with sensitive phase measurements were performed with a polarographic instrument PRG 3 (Solea-Tacussel), with three electrodes. A dropping mercury electrode with the following characteristics: glass capillary type CMT 10/68 Tacussel with the inner diameter 60 − 80 μm, q = 5.53 mg s^{-1}, τ = 2.08 s, was used as working electrode at E = − 1.00 V vs. SCE in 1.00 M NaOH, at a mercury column height of h = 65 cm. A K401 Radiometer calomel electrode and a Pt 121 coiled electrode (Solea-Tacussel) with a great active area were used as reference and auxiliary electrode, respectively.

A thermostated polarographic cell was used to do all measurements at controlled temperature (25.0 ± 0.1 °C). The dissolved oxygen from the polarographically analysed solutions was eliminated by bubbling a pure argon stream.

Reagents

All used substances were of analytical or pharmaceutical grade. The water used for the vessel cleaning and for solution preparation was tridistilled.

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Results and discussions

The elaboration of a quantitative polarographic and voltammetric determination method of a chemical compound needs, first of all, a rigorous study of the reduction or oxidation properties of the species at the chosen working electrode in different supporting electrolytes. Thus, minocycline electroactivity was first investigated by CV at a solid glassy carbon electrode (GCE) – figure 1A and a hanging mercury drop electrode (HMDE) - figure 1B.

The cyclic voltammogram obtained at the HMDE shows two reduction peaks at waves during the direct scan, anodic-cathodic and a well-defined oxidation peak at the inverse scan: cathodic-anodic, whereas the cyclic voltammogram obtained at the GCE presents only one reduction and one oxidation peak at the direct and inverse scan, respectively. Cyclic voltammograms demonstrated that minocycline is electroactive at both mercury and solid working electrodes. In both cases, the form of the cyclic voltammogram and the heights of maximum peak currents are dependent on the scan rate, optimum values being among 200 − 600 mV s^{-1}. Differences between the potential of the cathodic reduction peak E_{pc} and that of the anodic oxidation peak E_{pa} were ΔE_{Hg} = 135 mV at the HMDE (considering the reduction peak with E_{pc}= − 0.850 V) and ΔE_{GC} = 137 mV at the GCE. Though the two differences ΔE_p have close values, both are different from the values corresponding to reversible electrode processes (0.059/*z* V), indicating that the reversibility of these processes is low.

The minocycline electroactivity and the possibilities of its quantitative determination were also investigated by LSV, SWV and DPV. All these voltammetric techniques demonstrated the minocycline reducibility at a mercury electrode. The reduction process gives a main reduction peak at potentials among −0.800 and −1.200 V vs. Ag/AgCl and also some other peaks in the range −0.350 − 0.450 and/or −1.200 − 1.400 V vs. Ag/AgCl. By applying these three techniques the main signal was well defined and sensitive to the variation of the experimental parameters further presented.

Subsequently minocycline reduction was also studied at the DME in aqueous acid, neutral and alkaline media, using phase sensitive ACP. The sinusoidal alternating current polarogram of minocycline presents more peaks, their number and shape being influenced by the chemical parameters of the system in which the reduction process occurs (ionic strength, nature and concentration of supporting electrolyte, pH). Due to these reasons ACP was used to evidence the shape modifications of the current-potential curves during the variation of those parameters or of the instrumental parameters. Thus, to evaluate the ionic strength and the pH influence on the polarograms, aqueous NaOH and NaCl solutions of various concentrations and Britton-Robinson buffers, respectively, were used.

The sinusoidal alternating current polarograms obtained for minocycline reduction in solutions of different NaCl concentrations (see Fig. 2) indicated that the ionic strength influences the polarogram shape, the peaks number and the maximum peak currents. Thus, depending on the ionic strength, the polarograms showed 3 or 4 peaks: one very tight and sensitive to ionic strength at cathodic potentials among − 0.400 and − 0.600 V, a larger one (at high ionic strength) or two peaks (at low ionic strength) in the range −

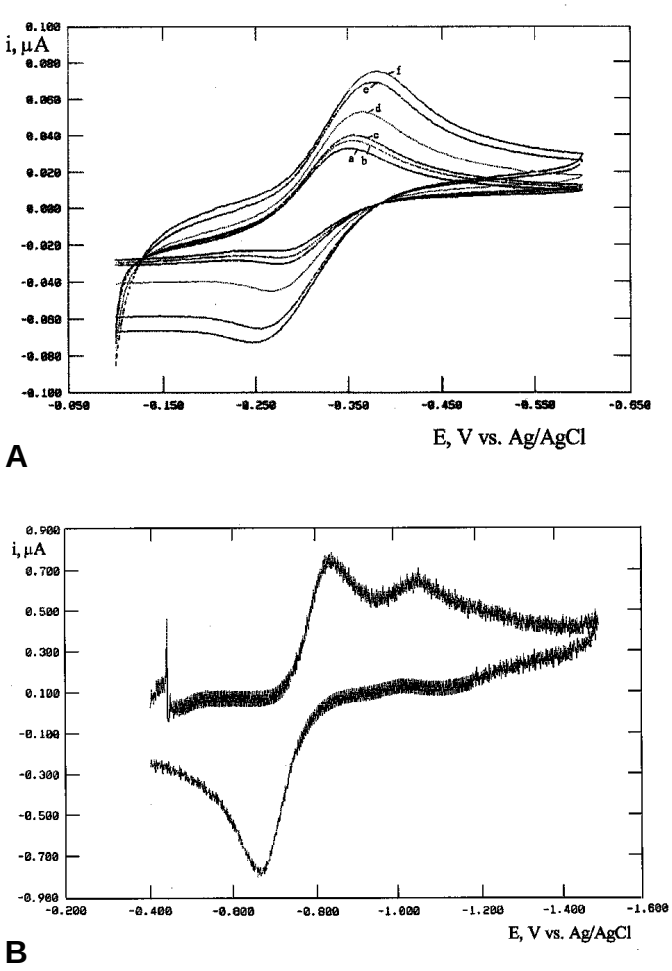

Fig. 1. Anodic - cathodic cyclic voltammograms of minocycline (C = 2 10^{-4} mol L^{-1}) in 0.1 M NaCl at **A)** GCE: a) 60 mV s^{-1}, b) 80 mV s^{-1}, c) 100 mV s^{-1}, d) 200 mV s, e) 400 mV s^{-1}, f) 500 mV s^{-1} and **B)** HMDE, v = 200 mVs^{-1}.

0.900 to −1.300 V and a strongly distorted peak, situated at potentials from −1.600 to −1.800 V vs. SCE.

The pH influence on the shape of the minocycline alternating current polarograms proved that only two peaks appear in weak acidic media at potentials among − 1.100 and − 1.400 V vs. SCE. In alkaline buffers, as in NaOH solutions, three or even four peaks were registered in the same potential range.

Comparing the sinusoidal alternating current polarograms with the cyclic voltammograms obtained for minocycline at the HMDE and at GCE (Fig. 1), it can be observed that the reduction process is complicated by other physico-chemical phenomena (adsorption) which are taking place at the electrode-solution. However, in 0.1 M NaCl solution the signal corresponding to the minocycline reduction is in the range −0.800 to –1.100 V vs. SCE both in CV and ACP (signals were strongly distorted by the ionic strength and the pH variation).

Subsequently, the influence of the characteristic instrumental parameters (the amplitude and the frequency of the superimposed alternating signal, the scan rate, the angle of

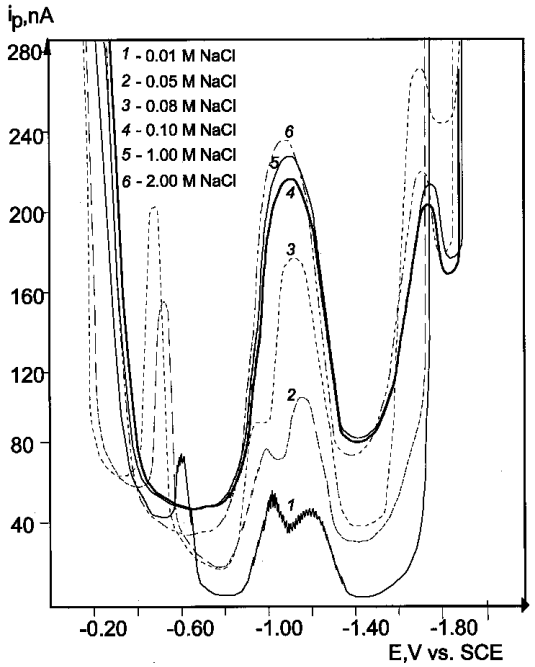

Fig. 2. The influence of ionic strength on the minocycline reduction using ACP in NaCl. C = 2 10^{-4} mol L^{-1} minocycline; ν = 80 Hz; ΔE = 10 mV; ϕ = 0°.

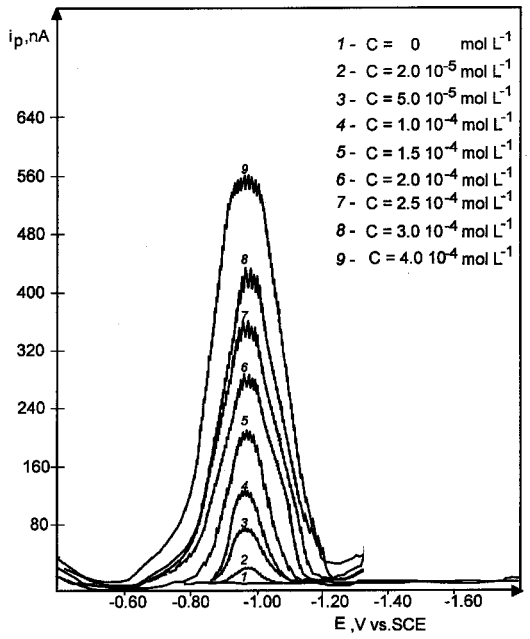

Fig. 3. Sinusoidal alternating current polarograms for minocycline determination in 0.1 M NaCl the concentration range 2 10^{-5} − 4 10^{-4} mol L^{-1}, ν = 100 Hz; ΔE = 10 mV; ϕ = 0°.

Table I. The influence of the amplitude and frequency of the superimposed alternating voltage on the maximum peak current in ACP reduction of minocycline; C = 4 10^{-4} mol L^{-1}; 0.1 M NaCl; ϕ = 0°.

Δ*E (mV)*	ν *(Hz)*	i_p *(nA)*
2		125
4		185
6	80	240
8		310
10		350
10	36	
40	96	
10	65	245
80	350	
100	555	
140	600	

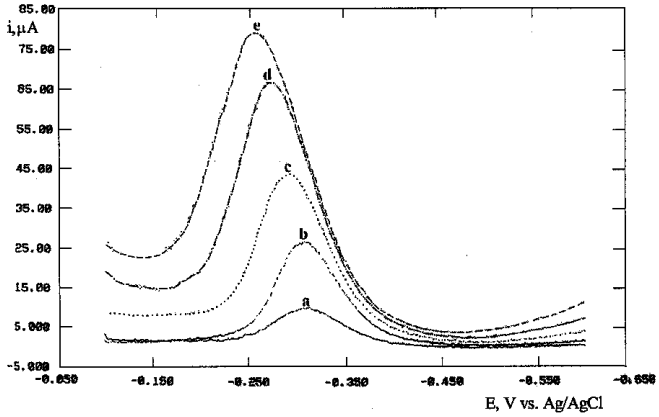

Fig. 4. The influence of the pulse height on the maximum peak current (i_p) for the minocycline reduction in 0.1 M NaCl by DPV on GCE (C = 2 10^{-4} mol L^{-1} minocycline); **a)** ΔE = 10 mV, **b)** 25 mV, **c)** 50 mV, **d)** 75 mV, **e)** 100 mV.

the detection phase) on the obtained signals was checked up and optimised. Some results using ACP are listed in table I. The dependence of the maximum peak currents on the magnitude of the tested parameters is obvious.

Further, the influence of minocycline concentration on the maximum peak current intensities in ACP was investigated in 0.1 M NaCl medium in the range 2 10^{-5} − 4 10^{-4} mol L^{-1} (see Fig. 3). The calibration graph at different amplitudes of the superimposed alternating potential (see the equations presented in Tab. II) proved an increased sensitivity of the minocycline determination at high amplitudes of the alternative superimposed signal.

The ACP technique was applied for the minocycline determination either in pure form or in a commercial pharmaceutical preparation (Mestacine tablets). The common excipients did not interfere. The mean recovery was 99.2% with a RSD = 2.93% for n = 8.

After the establishing of the optimal experimental conditions (chemical and instrumental parameters), figure 4, minocycline was also determined on GCE using DPV.

The possibility of minocycline determination at lower concentration levels was investigated by adsorptive stripping voltammetry at the HMDE in aqueous 0.1 M NaCl solutions. The obtained voltammograms presented two peaks (E_{p1} = −0.340 V and E_{p2} = 0.550 V vs. Ag/ AgCl), but only the first

Table II. Performances of quantitative minocycline determination employing different electrochemical techniques.

Electrochemical technique	*Optimum parameters*	*Linear range (mol L^{-1})*	*Equation of the calibration graph*	*Detection limit (mol L^{-1})*	*Sensitivity (μA L/mol)*	*Correlation coefficient*
ACP	ΔE = 5 mV; ν = 100 Hz; ϕ = 0°	1 10^{-3} − 5 10^{-6}	i_p = − 0.0015+751 C	1 10^{-6}	750.99	0.992
	ΔE = 10 mV; ν = 100 Hz; ϕ = 0°		i_p = 0.00085 + 1400 C		1400.00	0.99992
	ΔE = 15 mV; ν = 100 Hz; ϕ = 0°		i_p = 0.0043 + 2097 C		2097.00	0.99995
LSV at HMDE	v = 400 mV s^{-1}	1 10^{-3} − 1 10^{-5}	i_p = 0.0039 + 678.97 C	8 10^{-6}	678.96	0.9997
DPV at HMDE	ΔE = 25 mV; v = 200 mV s^{-1}	1 10^{-3} − 1 10^{-6}	i_p = 2.37 + 48967 C	5 10^{-7}	48969.37	0.985
DPV at GCE	ΔE =50-100 mV; v = 200 mV s^{-1}	1 10^{-3} − 1 10^{-5}	i_p = 0.39 + 918.2 C	7.5 10^{-6}	918.59	0.994
SWV at HMDE	ΔE = 25 mV; ν = 150 Hz; v = 200 mV s^{-1}	1 10^{-3} − 1 10^{-6}	i_p = 3.7+38249 C	5 10^{-7}	38252.7	0.990
AdSV	E_{acc} = -0.950 V vs. Ag/AgCl; t_{acc} = 180 s; t_{eq} = 30 s; v = 200 mV s^{-1}	1 10^{-6} − 5 10^{-8}	i_p= −21 − 23897713C	1 10^{-9}	−23897734	0.996

one responded to the variation of minocycline concentration. In order to optimise the determination, the influence of some parameters (accumulation potential and time, anodic scan rate) on the current intensities of the two minocycline stripping peaks was studied (Fig. 5). At the established optimum conditions (E_{acc} = −0.95 V vs. Ag/ AgCl, t_{acc} = 180 s, $t_{eq.}$ = 30 s and v = 100 mV s^{-1}) the current intensity of the first peak responded linearly to minocycline concentration in the range 1 10^{-6} − 5 10^{-8} mol L^{-1}, according to the equation presented in table II.

The mean results of the quantitative minocycline determination by different electrochemical techniques are summarized and presented in table II.

Conclusions

Minocycline is polarographically and voltammetrically reducible at mercury (DME, HMDE) and solid (GCE) electrodes, presenting well-defined response signals in CV, LSV, SWV, DPV and AdSV at mercury electrodes and in DPV at GCE.

The ACP reduction curves are complex, the number and shape of the peaks depending on both the chemical parameters of the system and on the characteristic parameters of the employed technique.

ACP was used for the minocycline determination from dosage forms, this electrochemical method was rapid and did not need any sample pre-treatment other than the drug dissolution. The method enables the estimation of the drug concentration without interferences from the excipients contained in the formulation.

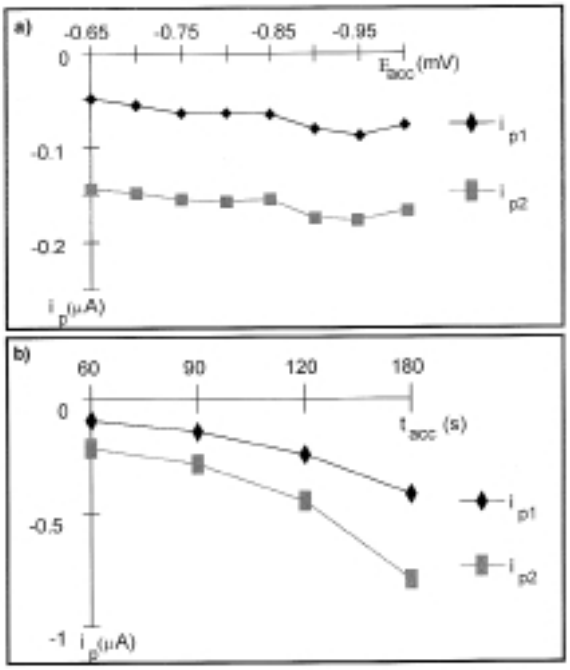

Fig. 5. The maximal peak currents variation in AdSV determination of minocycline. (C = 1 10^{-6} mol L^{-1}) with: **a**) accumulation potential E_{acc}, t_{acc} = 180 s, t_{eq} = 30 s, v = 100 mV s^{-1} and **b**) accumulation time t_{aac}, E_{acc} = − 0.950 V vs. Ag/AgCl, t_{eq} = 30 s, v = 100 mV s^{-1}.

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