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Voltammetric and spectrophotometric techniques for the determination of the antihypertensive drug Prazosin in urine and formulations

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Abstract

A sensitive method was developed to determine Prazosin using a nafion modified carbon paste electrode (NMCPE). Prazosin was accumulated at a potential of 750 mV in Britton–Robinson buffer (pH 6.0) and then a negative sweep was made obtaining a cathodic peak close to 0 V. Cyclic voltammetric studies indicated that the process was quasi-reversible, and fundamentally controlled by adsorption. To obtain a good sensitivity, the instrumental and accumulation variables were studied using differential pulse voltammetry (DPV). Adsorptive voltammetric peak currents showed a linear response for Prazosin concentrations in the range between 4.0×10^{-11} and 4.0×10^{-8} M with two different slopes, and a detection limit (LOD) of 3.1×10^{-11} M was obtained. The variation coefficient (CV) for a 8.0×10^{-10} M solution ($n = 10$) was 4.08%. A spectrophotometric study of Prazosin was also carried out and two absorption bands were obtained at 246 and 329 nm (pH 1.8). The band at 329 nm was pH-dependent and its height and position changed with the pH values, so this allowed the pK'_a determination (7.14 ± 0.20) using different methods. The detection limit reached by means of UV-spectrophotometry was 0.9×10^{-7} M, and the variation coefficient for 1.5×10^{-5} M Prazosin solutions was 1.14% ($n = 10$). Although the sensitivity of the UV-spectrophotometric method was lower than that obtained using adsorptive stripping-differential pulse voltammetry (AdS-DPV), it could be applied to the determination of Prazosin in Minipres tablets. The voltammetric method was used for the determination of the drug in human urine samples at trace levels with good recoveries.

Keywords: Prazosin; Differential pulse voltammetry; Nafion modified carbon paste electrodes; UV spectrophotometry

1. Introduction

Prazosin hydrochloride, 1-4 (amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)

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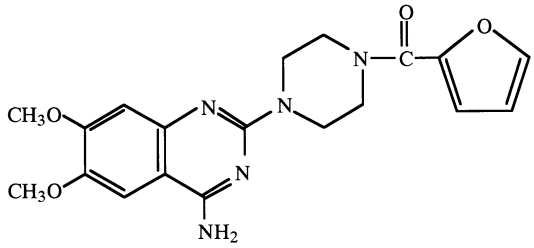
piperazine HCl, (M.W. 419.9) is a drug commonly used in the treatment of hypertension and congestive heart failure [1–4] because it has selective α1-adrenoceptor blocking properties [5–7].

This drug is a quinazoline derivative and its formulations are official in the British [8] and United States Pharmacopoeia [9].



Prazosin has been determined by different methods, for example: titrimetry [10], UV-spectrophotometry in methanolic-acid medium [11,12] and visible spectrophotometry with derivatization [13], fluorimetry [12], TLC [14,15], capillary electrophoresis [16] and HPLC with different detectors: UV [17–19], fluorescence [20–22] and with amperometric detection at a glassy carbon electrode [23]. A method based on a radioreceptor assay has also been developed, measuring the radioactivity by means of liquid scintillation counting after a lengthy and complicated incubation–separation process, reaching detection levels of 0.05 pmol [24].

With the arrival of the new voltammetric techniques, especially the impulse techniques [25], the analysis range of the organic molecules is considerably enlarged [26]. Using differential pulse polarography (DPP), Prazosin has been determined in methanolic-acetic acid [27] and in methanolic-sulphuric acid [28]. Reduction peaks at 126 and −1020 mV respectively were obtained. Among these modern techniques, the stripping techniques are the most sensitive. They have been combined with different redissolution techniques [29] and have been applied to all kinds of organic samples [30]. Working with these techniques, very different electrodes can be used: metallic like Au, Pt and Hg [31], vitreous and paste carbon, and especially the modified ones. This last group of electrodes has brought new endless possibilities because, on the base of any kind of electrode and through different procedures, its surface can be modified conferring new chemical properties which affect its selectivity [32] and sensitivity [33].

Nafion is a perfluorosulfonate cation exchange resin with some characteristics that make it a very adequate modifier for carbon paste electrodes. It is chemically and electrochemically inert, hydrophilic, insoluble and stable in water [34].

Nafion has been used to modify different types of electrodes, among these electrodes, carbon paste ones [35–37] can be prepared mixing the nafion with graphite and nujol, obtaining a homogeneous paste [38], or pipetting a small volume of nafion solution onto the surface of a previously prepared carbon paste electrode [39].

UV-spectrophotometry is widely used for the determination of organic substances [40,41] because this technique allows their rapid and precise determination. The absence of specific UV-spectrophotometric data concerning Prazosin in aqueous medium encouraged us to study its behaviour in Britton–Robinson buffer. Although the sensitivity of this method is lower than for voltammetric techniques, it is useful to determine drugs in pharmaceutical formulations [42–44].

In this work, AdS-DPV was used to determine Prazosin in pharmaceutical formulations and in human urine samples based on adsorption properties of this drug at the NMCPE. UV-spectrophotometry was also used to analyze Prazosin in pharmaceutical formulations. For this reason, all factors that may influence both the UV-spectrophotometry and the AdS-DPV were studied to find out the most sensitive instrumental conditions.

2. Experimental

2.1. Reagents

Prazosin hydrochloride was obtained from Sigma and it was dissolved in de-ionized water in the range of concentrations between 1.0×10^{-8} and 1.0×10^{-3} M. Graphite was ultra F purity (Ultracarbon, Bay City, MI) with a grain size of < 33 mm. The paste agglutinant was Nujol

(Aldrich, Milkwaukee, WI) ($d = 0.838$ g cm^{-3}). Nafion, dissolved in aliphatic alcohols (5% v/v) was provided by Aldrich. Dissolutions of the nafion stock solution were prepared using methanol. The measurements were made in Britton–Robinson buffer (0.04 M in acetic, boric and phosphoric acids) and the pH was adjusted with 2 M NaOH solution. The supporting electrolyte, $NaClO_4$ was added to Britton–Robinson to obtain a concentration of 0.01 M in the cell. The cartridges used to carry out the solid-phase extraction were LC-18 Supelco (Bellefonte, PA). All other chemicals were of analytical reagent grade and the water used was de-ionized, obtained from a Milli-Q system (Millipore, Bedford, MA).

2.2. Instrumentation

An Eco Chemie Autolab pGStat 10 voltammetric analyzer coupled with a Metrohm 663 VA stand was used to carry out voltammetric and adsorptive stripping experiments. An EXP8551 (Pentium 120) personal computer was used with the electrochemical software package GPES 4.4 (Eco Chemie).

A three-electrode system was used: a platinum counter, an Ag/AgCl reference and a home made nafion modified carbon paste electrode (NMCPE) as working electrode. A Radiometer PHM 92 laboratory pHmeter was used. Spectral and absorbance measurements were made with a double beam UVICON 992 spectrophotometer (Kontron Instruments, Milan, Italy). Matched quartz cells (1 cm) were used to carry out the spectrophotometric study over the spectral range 200–900 nm at a scan rate of 200 nm min^{-1}, against a blank.

2.3. Modification of the working electrode

The paste was prepared by mixing graphite and nujol in the ratio 75:25 and then was put into a polyethylene supporting tube (4.6 mm i.d.) and smoothed onto paper to obtain a shiny appearance. Then 0.01 ml of a 0.05% nafion–methanol solution was put on the electrode surface and dried carefully with a hair dryer. To obtain reproducible results, it was necessary to dry the nafion drop by putting the hair dryer at a distance of 50 cm from the nafion drop. The electrical contact was assured by inserting a copper wire into the NMCPE and connecting it with a voltammetric analyzer through a banana plug. The working electrode could only be used once because Prazosin was strongly adsorbed on the electrode; therefore fresh electrode surfaces were easily generated by extruding a small amount of paste from the tip of the electrode, scraping off the excess and smoothing on paper. Before each measurement, a cathodic sweep between -1.2 and 1.2 V at pH 6.0 (Britton–Robinson buffer 0.04 M) was made to activate the electrode. To achieve a good cleaning of the counter and auxiliary electrodes and the cell, these were immersed in an alkaline solution (0.1 M NaOH) and successive anodic scans between -1.2 and 1.2 V were carried out.

2.4. Voltammetric procedure

A volume of 25 ml of Britton–Robinson buffer at pH 6.0 and 0.01 M in $NaClO_4$ and appropriate volumes of Prazosin solution were added to the cell. Then, a stream of oxygen-free nitrogen was bubbled through the solution for 10 min with the working electrode out of the cell, and then the electrode was placed in the cell and the Prazosin was deposited on the electrode at 0.75 V for 150 s with a stirring speed of 2000 rev./min. The stirring was stopped, and after a 20 s rest period, a cathodic potential was carried out between -1.2 and 1.2 V, using DPV as redissolution technique. For optimum operational parameters see Table 1. All measurements were carried out at room temperature.

2.5. Spectrophotometric procedure

Prazosin was dissolved in Britton–Robinson at pH 1.8, in a concentration range between 1.0×10^{-7} and 5.0×10^{-5} M and this solution was added to the quartz cell to perform the measurements at $\lambda = 246$ nm, against a blank prepared with Britton–Robinson buffer at pH 1.8. The determination of pK'_a was made by studying the variation of the absorbance on the pH at 317, 329 and 341 nm. The pH of the solution was varied in the range between 1.8 and 12.5, and absorbance

measurements were performed at each 0.5 pH unit except for the pK'_a zone, where measurements were carried out at each 0.25 pH unit. The temperature was kept constant at 25°C and the Prazosin concentration was 5.0×10^{-5} M for all the pH range considered.

2.6. Prazosin assay in formulations

The determination of the drug in formulations was performed by using voltammetric and spectrophotometric techniques and for voltammetric assay the standard addition method was also used, adding aliquots of 125 μl of a 1.0×10^{-4} M Prazosin solution. Five Minipres tablets, each containing 2 mg of Prazosin were triturated in an agate mortar, pounded and finally dissolved in de-ionized water for 30 min. The insoluble excipient was filtrated and washed with water. The solution and washing water were transferred to a calibrated flask and diluted to 100 ml with water. An aliquot of 100 μl of this solution was added to 25 ml of Britton–Robinson buffer (pH 6.0) and the voltammograms were recorded following the voltammetric procedure described above. For the UV-spectrophotometric determination of Prazosin, an aliquot of 700 μl of the above mentioned solution was added to 25 ml of Britton–Robinson buffer (pH 1.8). Then the quartz cell was filled with this solution and the measurement was made.

Table 1
Optimum operational parameters selected for the determination of Prazosin solutions by AdS-DPV and AdS-SWV

Parameter	Variation interval	DPV	SWV
Step (mV)	1–10	9	8
Pulse amplitude (mV)	10–100	90	50
Modulation time (ms)	2.5–50	2.5	–
Interval time (s)	0.2–1.0	0.40	–
Frequency (Hz)	25–200	–	150
pH	1.0–12.0	6.0	6.0
Ionic strength (M of $NaClO_4$)	0–0.3	0.01	0.01
Deposition time (s)	0–240	180/150	180/150
Deposition potential (V)	0.5–1.0	0.75	0.75
Stirring speed (rev./min)	0–3000	3000	3000

2.7. Prazosin assay in urine

The determination of Prazosin in urine required a solid–liquid extraction with LC-18 cartridges. These were conditioned with 2 ml of 0.5% acetic acid. Then, 1 ml of spiked urine was added, and after that, two sequential washings were made (0.4 ml of 40% methanol in water followed by 0.4 ml of 20% acetone in water). The elution was carried out by the addition of 2 ml of 75% methanol in water. Appropriate volumes of this solution were added to Britton–Robinson buffer (pH 6.0) placed in the cell, and the voltammograms were recorded following the voltammetric procedure described above. The determination of Prazosin was made following the standard addition method.

3. Results and discussion

3.1. Voltammetric study

3.1.1. Cyclic voltammetry

Prazosin was deposited on the NMCPE electrode for 150 s at a potential of 750 mV, with a stirring speed of 2000 rev./min. Then cathodic–anodic cyclic voltammograms, of a 4.0×10^{-6} M Prazosin solution in Britton–Robinson buffer (pH 6.0), were performed between 1000 and -500 mV at a scan rate of 50 mV s^{-1} (Fig. 1).

In the cyclic voltammogram three peaks appeared, at -78 mV in the cathodic branch and at 18 and 919 mV in the anodic one. The cathodic peak only appeared in the first scan if a previous anodic deposition potential was applied, or if the scan was begun at 1000 mV or higher oxidation potentials; so this peak was due to the reduction of a product of Prazosin oxidation. The anodic peak at 18 mV was formed as a consequence of the oxidation of the cathodic event in a quasi-reversible process, with $\Delta E = 96$ mV and $i_c/i_a =$

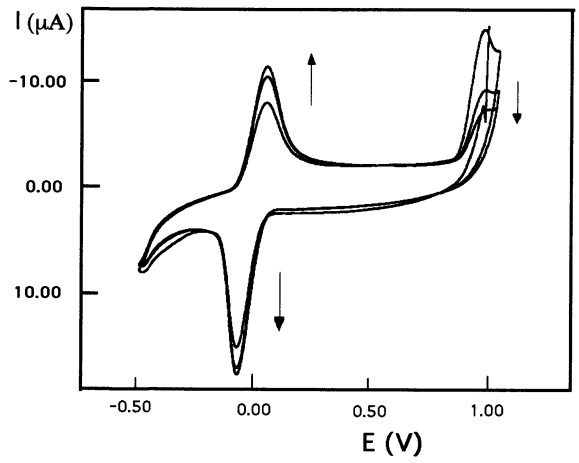


Fig. 1. Successive cathodic–anodic cyclic voltammograms of a 4.0×10^{-6} M Prazosin solution in B-R buffer (pH 6.0), after a deposition step (750 mV, 150 s and 2000 rev./min) at a scan rate of 50 mV s^{-1} using a NMCPE.

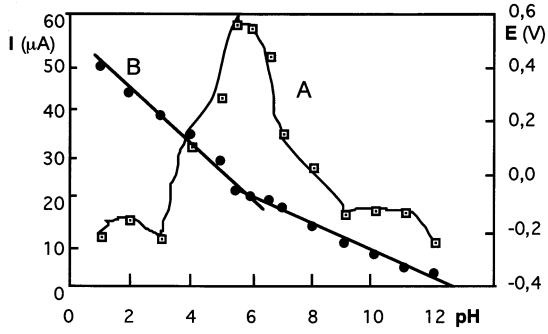


Fig. 2. Variation of peak current (A) and peak potential (B) with pH for 4.0×10^{-6} M Prazosin solutions by means of DPV on the NMCPE. For operating conditions, see Table 1.

1.66. The peak that appeared at 919 mV was due to the oxidation of the amino group in the Prazosin molecule with a chemical follow-up reaction occurring, giving rise to a product which exhibits a quasi-reversible couple at considerably lower positive potentials in a ECE process, similar to that described by Moane et al. [37] for the oxidation of the amino group of clembuterol. If successive cyclic scans are made, it can be observed (Fig. 1) that the peak at more positive potentials decreases as the number of scans increased, while the peaks of the quasi-reversible couple augmented, since this couple arises from the product of the follow-up reaction of the Prazosin oxidation.

Cyclic voltammograms were made at different rates between 25 and 1000 mV s^{-1}. Plotting $\log i$ vs $\log v$, a straight line was obtained, $\log i = -5.31 + 0.99 \log v$, with a slope very close to one, which is the expected value for an ideal reaction of surface species [45], so this process has an important adsorptive component, with strong adsorption and the relative contribution of adsorbed reactants increases at increasing scan rates [46].

The variation of scan rate brought out a slight change in the reduction peak potential according to equation: $E_p = 0.35 + 0.005 \log v$, which confirms the non-irreversibility of the process.

The variation of peak intensity was studied in the range of temperatures between 14 and 58°C using DPV for 2.0×10^{-8} M Prazosin solutions, and the temperature coefficients were calculated according to Meites [47], the obtained values being: 2.06% $°C^{-1}$ (14–20°C), 1.80% $°C^{-1}$ (20–26°C), 2.44% $°C^{-1}$ (26–30°C), 4.15% $°C^{-1}$ (30–34°C), −4.05% $°C^{-1}$ (34–40°C), 3.36% $°C^{-1}$ (40–46°C), −5.86% $°C^{-1}$ (46–50°C), 1.66% $°C^{-1}$ (50–58°C). For an adsorptive process, the temperature coefficients acquire random values, so in this case the process has an important adsorptive component.

3.1.2. Influence of pH on the reduction peak

The variation of peak intensity with pH, for a 4.0×10^{-6} M Prazosin solution was studied between pH 1 and 12, and the maximum peak intensity was found at pH 6.0 (Fig. 2), which was selected to carry out the quantitative determination. The E_p varied with pH obtaining two linear ranges, the first between pH 1.0 and 7.0, and the second between pH 7.0 and 12.0. The equations obtained were the following:

$$E_p\ (V) = 0.37 - 7.23 \times 10^{-2}\ pH; \qquad r = 0.9977$$

$$1 < pH < 7$$

$$E_p\ (V) = 0.13 - 3.67 \times 10^{-2}\ pH; \qquad r = 0.9938$$

$$7 < pH < 12$$

The pK'_a of the oxidized form of Prazosin, at which the electron transfer reduction mechanism changes, was obtained by the intersection of the

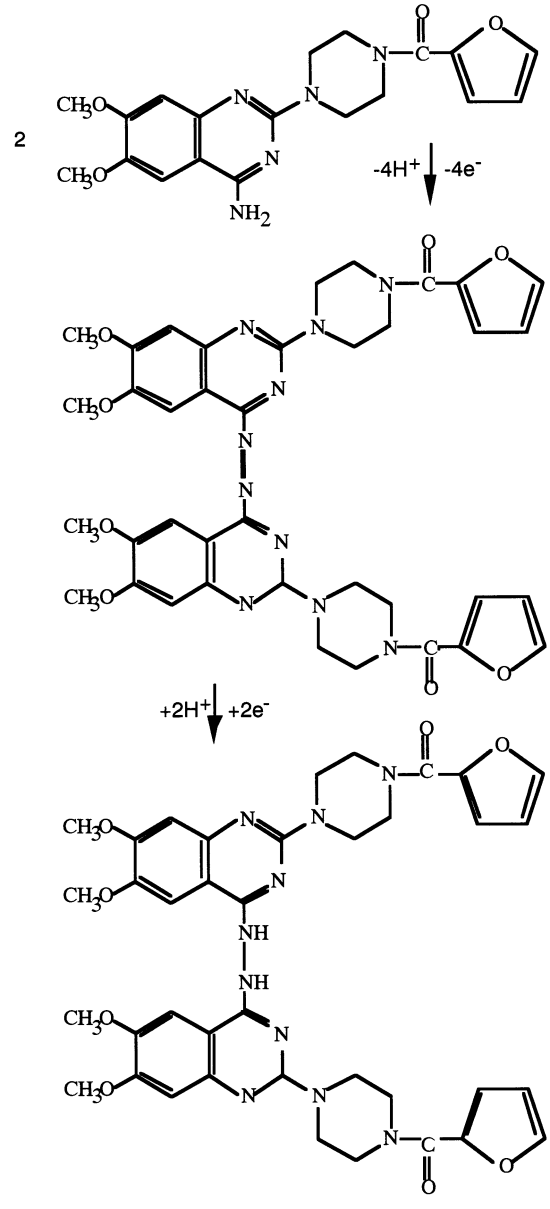
two lines: 6.92 ± 0.63 (95% confidence limit, $t = 2.45$).

Marquard non-linear least square method [48] was used to determine the charge transfer coefficient value α, supposing that the process was quasi-reversible and two electrons were exchanged. In Fig. 3, it can be observed that the simulated DP-voltammogram (B), obtained by using fit and simulation programs of GPES 4.4 software, was perfectly adapted to the experimental one (A) ($X^2 = 2.8 \times 10^{-2}$), and the obtained α value was 0.58.

In the two pH ranges (Fig. 2), the potential shifted to more negative values, and in the second one the slope was smaller than in the first one, indicating that a smaller number of protons were consumed in this region. The number of protons involved in the reduction process was 2, in the pH range between 1 and 7, being calculated through the slope of the plot E_p vs pH (59 m $H^+/n\alpha$).

3.1.3. Electrode process

Taking into account the results discussed above and that the amine function is the most easily oxidizable group present in the Prazosin molecule, the following interpretation might be proposed for the electrode process. In the preconcentration step, the adsorbed species are the oxidised form of Prazosin, which habitually gives dimer compounds, bonding the radicals formed through the oxidation of the amine group [49]. During the redissolution step, since the process is quasi-reversible, the reduction of the oxidised Prazosin form adsorbed might take place through the reduction of the –N=N– double bond [50]. According to this, the total process can be represented by the following equations:



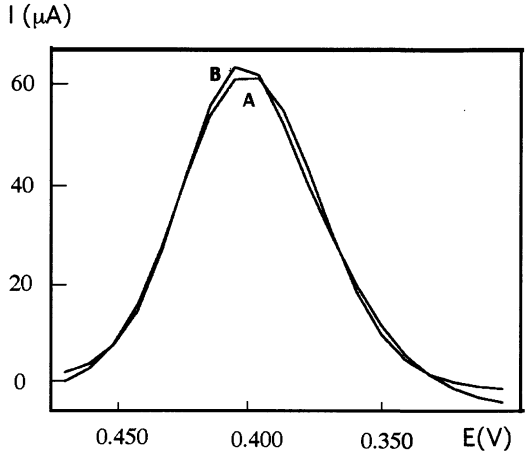


Fig. 3. Experimental DP-voltammogram (A) and simulated one (B) obtained for a quasi-reversible process, $n = 2e$, $\alpha = 0.58$ and ($X^2 = 2.8 \times 10^{-2}$). Other conditions in Table 1.

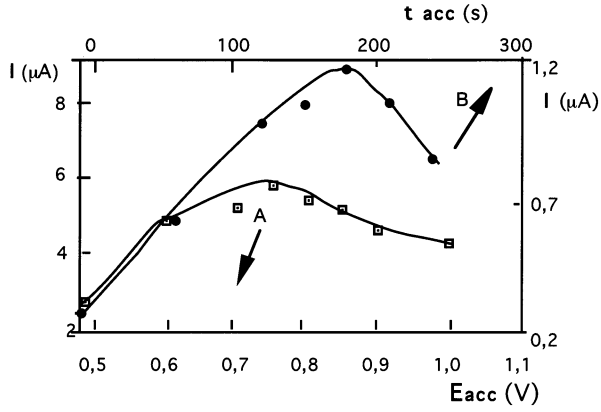


Fig. 4. Influence of accumulation potential (A) and accumulation time (B) on peak current for a 4.0×10^{-6} M Prazosin solution at pH 6.0 by means of AdS-DPV on the NMCPE. For operating conditions, see Table 1.

3.1.4. Adsorption process

The selection of suitable conditions for the deposition of Prazosin onto the NMCPE surface for a 8.0×10^{-8} M solution was carried out by means of DPV with its optimum operational parameters (Table 1).

To optimize the electrode composition, a bare carbon paste electrode was prepared, and then 0.01 ml of different proportions of a nafion/methanol solution (0.01–2%) were added to the electrode surface and dried, leading to the maximum peak intensity at 0.05%. For slower and higher values the peak intensity decreased slowly.

The variables that affected the deposition procedure were: initial sweep potential, deposition potential, stirring speed, accumulation time and equilibrium time.

The initial potential of cathodic sweep was a very important variable because its value significantly influenced the peak height when the voltammogram was begun at 1000 mV, so this value was selected.

Prazosin was deposited on the electrode at open circuit and at different potentials between 500 and 1000 mV. The maximum peak height was obtained for a deposition potential of 750 mV, diminishing progressively for higher or smaller deposition potentials (Fig. 4).

The deposition time was studied at two different Prazosin concentrations: 2.0×10^{-10} (Fig. 4) and 2.0×10^{-8} M. The optimum deposition times were 180 and 150 s, which indicated that an increase in Prazosin concentration diminished the time necessary to saturate the electrode surface as expected. If a bare CPE was used, the optimum deposition time for a 2.0×10^{-10} M Prazosin solution increased by two (360 s) and the peak current was three times smaller. The diminution of t_{acc} of Prazosin on the NMCPE may be explained because it possesses aromatic rings which have three consecutive carbon atoms without hydroxyl groups [39]. There were also hydrophobic interactions between the hydrophobic part of the Prazosin and the hydrophobic fluorocarbons of the nafion film. The union takes place through the SO_3H– group on the polymeric structure of the nafion film [51].

After the deposition stage was carried out, an equilibrium time was applied, and the highest peak was obtained for 20 s, although few changes in the peak height were observed for other deposition times.

The stirring speed was varied between 0 and 3000 rev./min giving the maximum peak height at 2000 rev./min, which was selected to do the measurements, although between 1500 and 3000 rev./min the peak height remained nearly constant.

Different amounts of $NaClO_4$ were added to the solution, in order to study the ionic strength influence on the peak height, and the maximum peak was obtained for a 0.01 M solution of $NaClO_4$. When the $NaClO_4$ concentration was increased, the peak diminished progressively with the $NaClO_4$ concentration.

3.2. Spectrophotometric study

The influence of pH was studied between 1.8 and 12.6, and two absorption bands appeared in the pH range. The band at 246 nm was independent of the pH, but the second one underwent a batochromic shift moving between 329 nm (pH 1.8) and 343 nm (pH 12.6), and an isosbestic point appeared close to 347 nm (Fig. 5).

For the second band, the influence of pH on the absorbance at three wavelengths (341, 329 and

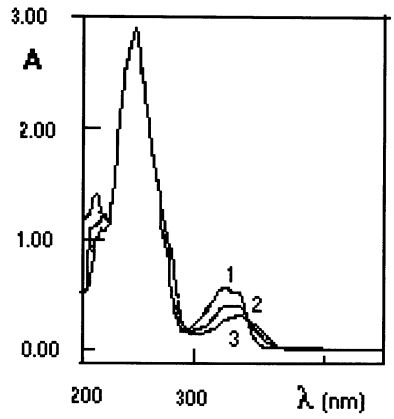


Fig. 5. Absorption UV-spectra of a 5.0×10^{-5} M Prazosin solution at different pH values: (1) 1.8, (2) 7.1, and (3) 11.6.

317 nm) was investigated. Two ranges with a pH-independent behaviour were found, the first between pH 1.8 and 5.5, and the second between pH 9.5 and 12.6. For intermediate pH values (5.5–9.5) the absorbance changed with pH, and this allowed us to determine a spectrophotometric apparent constant pK'_a using different graphic methods: Strentöm–Goldsmith [52] and Sommer [53], and the numerical one, Letagrop Spefo [54].

In Strentöm–Goldsmith's method, $pH = pK_a + \log [(A - A_1)/(A_0 - A)]$, and this pH was represented vs $\log [(A - A_1)/(A_0 - A)]$, A_0 being the absorbance in the basic plateau, A_1 the absorbance in the acidic one and A the absorbance measured. This method was applied at the three wavelengths studied: 341, 329 and 317 nm, and three straight lines were obtained with slopes close to 1. The intercept of these lines with pH axis indicated the pK'_a value of Prazosin. The pK'_a values were: 7.14 (341 nm), 7.09 (329 nm) and 7.12 (317 nm). The mean value was 7.12 ± 0.13 (95% confidence limit, $t = 4.30$).

Table 2
pK'_a values obtained using different methods in UV spectrophotometry for a 2.5×10^{-5} M Prazosin solution at different wavelengths

Method	341 nm	329 nm	317 nm
Strentöm–Goldsmith	7.14	7.08	7.12
Sommer acidic	7.06	7.02	6.99
Sommer basic	7.30	7.22	7.26
Letagrop Spefo	7.10	7.06	7.07

Sommer's method was applied to determine pK'_a knowing the absorbance (A_1) of the acidic plateau ($C_T/A = \varepsilon_0^{-1} + \varepsilon_0^{-1}K_a^{-1}(A - A_1)\ [H^+]/A$). In this case C_T/A vs $(A - A_1)\ [H^+]/A$ was a straight line whose slope was $\varepsilon_0^{-1}K_a^{-1}$ and the intercept was ε_0^{-1}. The mean value obtained was 7.09 ± 0.53 (95% confidence limit, $t = 4.30$). This method allowed the determination of pK'_a too ($C_T/A = \varepsilon_1^{-1} + \varepsilon_1^{-1}K_a^{-1}\ (A - A_0)/[H^+]A$), knowing the absorbance of the basic plateau (A_0) through the slope ($\varepsilon_1^{-1}K_a^{-1}$) of the representation C_T/A vs $(A - A_0)/[H^+]A$. The terms C_T, ε_0 and ε_1 are the total concentration and the molar absorption coefficients of non-protonated and protonated species, respectively. The mean value was 7.26 ± 0.16 (95% confidence limit, $t = 4.30$).

To calculate numerically the pK'_a value, the Letagrop Spefo program was used. The pK'_a values found were: 7.30 (341 nm), 7.06 (329 nm) and 7.07 (317 nm), so the mean value was 7.14 ± 0.58 (95% confidence limit, $t = 4.30$).

Table 2 shows all the medium values found, with the different techniques used. Considering all the obtained values, the mean total value was 7.12 ± 0.21 (95% confidence limit, $t = 3.18$). This value was in accordance with the polarographically one obtained by us: $pK'_a = 6.92 \pm 0.48$. The results achieved by both techniques have been compared through the Student's t-test ($P = 0.05$, $n = 3$), in order to see if they are significantly different; and a value of $t_{calculated} = 1.31$ has been obtained, being smaller than the $t_{theoretical}$ value 2.78; therefore, both methods are statistically the same.

The hydrolysis of the drug was studied in acidic (pH 1.8), neutral (pH 7.5) and basic media (pH 12). In acidic and neutral media, the band position and absorbance did not change with time. In basic media, however, a small variation of the absorbance was observed with time, and this allowed us the determination of the hydrolysis constants. Their values for the direct and inverse hydrolysis process at pH 12.8 were: $K_{h_1} = 1.23 \times 10^{-1}$ and $K_{h_{-1}} = 2.13 \times 10^{-1}$ at 344 nm, and $K_{h_1} = 1.80 \times 10^{-11}$ and $K_{h_{-1}} = 3.76 \times 10^{-1}$ at 250 nm.

Table 3
Regression data of calibration lines for the quantitative determination of Prazosin by AdS-DPV and AdS-SWV with a NMCPE[a]

Parameter	AdS-DPV		UV-Spectrophotometry	
	First range	Second range	329 nm	246 nm
LR (M)	0.04–2 nM	2–40 nM	1–50 μM	0.1–15 μM
b (A l mol^{-1})	226.22	144.47	8.92×10^{3}	5.23×10^{4}
a (A)	9.07×10^{-8}	1.73×10^{-7}	8.80×10^{-3}	1.23×10^{-2}
Sb (A l mol^{-1})	1.14	2.36	43.59	105.73
Sa (A)	1.13×10^{-9}	4.60×10^{-8}	8.98×10^{-4}	6.73×10^{-4}
Syx (A)	2.34×10^{-9}	8.43×10^{-8}	2.00×10^{-3}	1.65×10^{-3}
r	0.9999	0.9991	0.9999	0.9999
n	9	9	10	11
LOD (M)	3.1×10^{-11}	–	6.7×10^{-7}	0.9×10^{-7}

[a] LR, linear range; b, slope; a, intercept; Sb, standard deviation of the slope; Sa, standard deviation of the intercept; Syx, error standard deviation; r, correlation coefficient; n, data number; LOD, detection limit.

3.3. Calibration graphs and statistical parameters

3.3.1. Voltammetric study

After optimization of the variable values for the AdS-DPV, the variation of peak current with concentration was studied.

Two linear ranges were obtained, the first between 4.0×10^{-11} and 2.0×10^{-9} M, and the second between 2.0×10^{-9} and 4.0×10^{-8} M (Table 3), with a detection limit of 3.0×10^{-11} M, according to the expression $3Syx/b$ [55]. The reproducibility of the method was studied for 8.0×10^{-10} and 8.0×10^{-9} M Prazosin solutions with variation coefficients of 4.08 and 3.78% ($n=10$) respectively.

3.3.2. Spectrophotometric study

The variation of the absorbance with concentration was studied (pH 1.8) for the two waves obtained (246 and 329 nm), and a linear regression was obtained for each band. The regression parameters for the two lines appear in Table 3. The detection limits reached were 0.9×10^{-7} M at 246 nm and 6.7×10^{-7} M at 329 nm. At 329 nm, the linear calibration range was wider (10–500×10^{-7} M) than that obtained at 246 nm (1–150×10^{-7} M), although the sensitivity was higher at 246 nm (slope ratio 5.08). The variation coefficients for a 1.5×10^{-5} M Prazosin solution were 0.97 and 1.14% ($n=10$) at 329 and 246 nm respectively. These results show that the measurements at 246 nm are more suitable than those 329 nm.

4. Determination of Prazosin in spiked urine and in Minipres tablets

4.1. Prazosin assay in Minipres tablets

Following the procedures described above, Prazosin was determined in Minipres tablets using UV-spectrophotometry and AdS-DPV (Fig. 6), without sample preparation and after an adequate dilution. For both techniques, five aliquots of the solution were analyzed by the standard additions method. The results obtained are listed in Table 4.

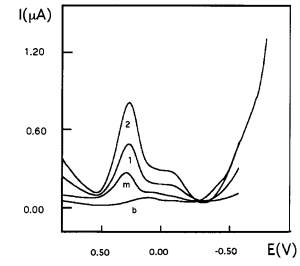


Fig. 6. Voltammetric curves (AdS-DPV) obtained for the determination of Prazosin in human urine samples: b, blank; m, urine spiked with Prazosin at 8.0×10^{-9} M level, 1 and 2: two standard additions of 250 μl of a 1.0×10^{-6} M Prazosin solution. For operating conditions see Table 1.

Table 4
Mean recoveries obtained for five determinations of Prazosin in Minipres tablets and spiked urine samples at two concentration levels, using AdS-SWV at the NMCPE and UV-spectrophotometry

Sample	Prazosin added	Prazosin found	Recovery (%)
Tablets (mg)			
AdS-DPV	2.00	1.97	98.50
UV-spectrophotometry	2.00	1.98	99.00
Urine (nM)			
AdS-DPV	0.80	0.795	99.38
AdS-DPV	8.00	7.910	98.88

The variation coefficients were 4.83% for AdS-SWV and 1.18% for UV-spectrophotometry. The determination of Prazosin in five Minipres tablets gave a mean value of 1.98 ± 0.25 mg (95% confidence limit, $t = 2.78$) for UV-spectrophotometry, and 1.97 ± 0.26 mg (95% confidence limit, $t = 2.78$) for AdS-SWV, both in good accordance with the declared value of 2 mg (Table 4).

4.2. Prazosin assay in urine

The determination of Prazosin in spiked urine samples was carried out at two different levels of concentration: 0.8×10^{-9} and 8.0×10^{-9} M, following the solid phase extraction and voltammetric procedures described above. The recoveries obtained were 99.38 and 98.88% with variation coefficients of 5.16 and 4.90% respectively ($n = 5$) (Table 4).

5. Conclusions

In this work, we have described the quantitative determination of Prazosin using AdS-DPV and UV-spectrophotometry in aqueous medium.

The UV-spectrophotometric method proposed provides an alternate procedure for the quality control of Prazosin-containing pharmaceutical preparations. The LOD reached (25.2 ng ml^{-1}) is three magnitude orders lower than that obtained (1.0 μg ml^{-1}) using UV-spectrophotometry in methanolic-acid medium [11,12] and visible-spectrophotometry [13].

From electroanalytical studies, it was concluded that Prazosin is irreversibly oxidized at high positive potentials (919 mV at pH 6.0), giving rise to the formation of a product which demonstrates an adsorptive and quasi-reversible electrochemical behaviour at less positive potentials.

The use of a nafion-MCPE resulted in a large increase in peak current and diminished the deposition time by a half compared to bare CPE, providing an efficient barrier to negatively charged interfering compounds which may be present in biological samples.

The AdS-DPV at NMCPE method proposed is a rapid, accurate and very sensitive procedure for the determination of Prazosin at trace levels. The LOD reached 3.1×10^{-11} M (13.0 pg ml^{-1}) is only comparable to the value reached using the radioreceptor assay (21.0 pg ml^{-1}) [24] and it is clearly lower than the other reported methods. Besides, the voltammetric method is simpler, faster and requires less expensive equipment than the radioreceptor assay and LC-methods.

As the reduction peak appeared at low negative potentials, well separated from background interferences, it could be used for the quantitative determination of Prazosin by means of HPLC and FIA with electrochemical detection.

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References

[1] R.N. Brogden, R.C. Heel, T.M. Speight, G.S. Avery, Drugs 14 (1997) 163–197.

[2] N.A. Awan, M.K. Evenson, K.E. Needham, D.T. Mason, Am. Heart J. 102 (1981) 626–634.
[3] R.M. Graham, Am. J. Cardiol. 53 (1984) 16–20.
[4] T.B. Levine, Am. J. Cardiol. 55 (1985) 32–35.
[5] D. Cambridge, M. Davey, R. Massingham, Br. J. Clin. Pharmacol. 59 (1977) 514–515.
[6] R.M. Graham, H.P. Oates, L.M. Stoker, G.S. Stokes, J. Pharmacol. Exp. Ther. 201 (1977) 747–752.
[7] J.C. Doxey, C.F. Smith, J.M. Walker, J. Pharmacol. 60 (1977) 91–96.
[8] British Pharmacopoeia 1988, Vols. I and II, HMSO, London, 1988.
[9] United States Pharmacopoeia, XXII revision, US Pharmacopoeial Convention, Rockville, MD, 1990.
[10] K. Nikolic, K. Velasevic, Arch. Farm. 38 (1-2) (1988) 3–6.
[11] B. Panzova, M. Ilievska, G. Trendovsica, B. Bogdanov, Int. J. Pharm. 70 (1991) 187–190.
[12] M.E. Mohamed, H.Y. Aboul-Enein, Pharmazie 40 (1985) 358.
[13] K. Sreedhar, C.S.P. Sastry, M. Narayana Reddy, D.G. Sankar, Talanta 43 (1996) 1847–1855.
[14] T. Daldrup, F. Susanto, P. Michalke, Fresenius Z. Anal. Chem. 308 (1981) 413–427.
[15] P. Llisunde, P. Korte, J. Anal. Toxicol. 15 (1991) 71–78.
[16] H. Soini, M.L. Riekkola, M.V. Novatny, J. Chromatogr. 680 (2) (1994) 623–634.
[17] W.J. Batchman, J. Liq. Chromatogr. 9 (1986) 1033–1049.
[18] D.J. Gisch, B. Feibush, B.T. Hunter, T.L. Ascah, Biochromatography 4 (1989) 206, 210, 212–215.
[19] S.S. Zarapkar, S.J. Vaidya, V.R. Bhate, Indian Drugs 29 (6) (1992) 284–285.
[20] T.V. Saprygina, Y.R. Petrosyan, A.B. Kazaryan, V.G. Kukes, Khim-Farm. Zh. 23 (9) (1989) 1143–1146.
[21] E.M. Niazy, Y.M. El- Sayed, S.H. Khidr, J. Liq. Chromatogr. 18 (5) (1995) 977–987.
[22] A.J. Fletcher, R.S. Addison, R.H. Mortimer, G.R. Kannell, J. Liq. Chromatogr. 18 (14) (1995) 2911–2923.
[23] A. Rathinavelu, A. Malave, J. Chromatogr. Biomed. Appl. 670 (1) (1995) 177–182.
[24] S. Yamada, C. Tanaka, M. Suzuki, T. Ohkura, R. Kimura, K. Kawabe, J. Pharm. Biomed. Anal. 14 (3) (1996) 289–294.
[25] G.C. Barker, A.W. Gardner, Z. Anal. Chem. 173 (1960) 79–88.
[26] P. Zuman, Organic Polarographic Analysis, Pergamon Press, Oxford, 1964, pp. 219–251.
[27] M.Ü. Özgür, S. Aycan, S. Islimyeli, Pharmazie 50 (6) (1995) 435–436.
[28] G. Altiokka, M. Tuncel, Pharmazie 52 (5) (1997) 401–402.
[29] E.Y. Zachowski, M. Wojciechoworski, J. Osteryoung, Anal. Chim. Acta 183 (1986) 47–56.
[30] M.R. Smyth, W. Franklin Smyth, Analyst 103 (1978) 1227–1234.
[31] W. Murray, A.G. Ewing, R.A. Durst, Anal. Chem. 59 (1987) 379A–390A.
[32] J. Wang, B.A. Freiha, Anal. Chem. 55 (1983) 1285–1294.
[33] L. Hernández, E. González, P. Hernández, Analyst 113 (1988) 1175–1183.
[34] M.N. Szentirmay, C.R. Martin, Anal. Chem. 56 (1984) 1898–1902.
[35] S. Moane, M.R. Smyth, M. O'Keeffe, Analyst 121 (1996) 779–784.
[36] W.H. Chan, H. Huang, Analyst 121 (1996) 1727–1730.
[37] S. Moane, J.R. Barreira Rodriguez, A.J. Miranda Ordieres, P. Tuñon Blanco, R.M. Smyth, J. Pharm. Biomed. Anal. 14 (1995) 57–63.
[38] D. Boyd, J.R. Barreira Rodriguez, P. Tuñón Blanco, M.R. Smyth, J. Pharm. Biomed. Anal. 12 (1994) 1069–1074.
[39] D. Boyd, J.R. Barreira Rodriguez, A.J. Miranda Ordieres, P. Tuñón Blanco, Analyst 119 (1994) 1979–1984.
[40] E.R.M. Kedor-Hackmann, E.A.S. Gianotto, M.I.R.M. Santoro, Anal. Lett. 30 (10) (1997) 1861–1871.
[41] D. Agbaba, S. Eric, K. Karljikovic-Rajic, S. Vladimirov, D. Zivanov-Stavic, Spectrosc. Lett. 30 (2) (1997) 309–319.
[42] U. Hachuta, J. Planar Chromatogr. Mod. TLC 10 (2) (1997) 131–132.
[43] T. Aman, M.S. Shaid, A. Rashid, Y. Khokhar, Anal. Lett. 30 (8) (1997) 1517–1529.
[44] A.A. Alwarthan, A.M. Al-Obaid, J. Pharm. Biomed. Anal. 15 (7) (1997) 911–916.
[45] E. Laviron, J. Electroanal. Chem. 112 (1980) 11–23.
[46] A.J. Bard, L.R. Faulkner, Electrochemical Methods. Fundamentals and Applications, Wiley, New York, 1980, pp. 528–529.
[47] L. Meites, Polarographic Techniques, 2nd edn, Wiley Interscience, New York, 1965, p. 140.
[48] C.M.A. Brett, A.M. Oliveira, Electrochemistry: Principles, Methods and Applications, Oxford University Press, Oxford, 1993, p. 412.
[49] E. Steckhan, in: H. Lund, M.M. Baizer (Eds.), Organic Electrochemistry, Part IV-15, 3rd edn, Marcel Dekker, New York, 1991, pp. 586–587.
[50] H. Lund, in: H. Lund, M.M. Baizer (Eds.), Organic Electrochemistry, Part III-9, 3rd edn, Marcel Dekker, New York, 1991, pp. 422–423.
[51] J. Zhou, E. Wang, Anal. Chim. Acta 249 (1991) 489–496.
[52] W. Strentöm, N. Goldsmith, J. Phys. Chem. 30 (1926) 1683–1690.
[53] L. Sommer, Folia Fac. Sci. Nat. Univ. Pukynianae Brno. 5 (11) (1964) 1–35.
[54] L.G. Sillén, B. Warnquist, Arkiv Kemi. 31 (1969) 377–390.
[55] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 2nd edn, Ellis Horwood, Chichester, UK, 1988, p. 103.