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UV-Spectrophotometry and square wave voltammetry at nafion-modified carbon-paste electrode for the determination of doxazosin in urine and formulations

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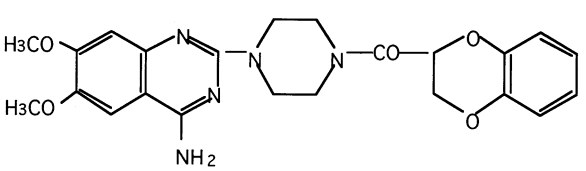
Abstract

By using several electrochemical techniques, the study of electroanalytical behaviour of antihipertensive Doxazosin at Nafion modified carbon paste electrode (NMCPE) has been carried out. The voltammetric peak is very pH dependent, reaching the maximum i_p at pH 6.8 (E_p −0.17 V), the reduction process being quasi-reversible and fundamentally controlled by adsorption. A method based on the control of adsorptive preconcentration of the Doxazosin on the NMCPE, before its voltammetric determination, is proposed. The detection limit reached using square wave voltammetry (SWV) as redissolution technique was 2.33×10^{-11} M and the variation coefficient at 2×10^{-9} M level was 3.54%. A spectrophotometric study of Doxazosin has also been made and two waves at 244 and 329 nm (pH 1.7), were obtained. The wave at 329 nm changes its height and position with the pH, allowing the pK'_a determination (6.94 ± 0.21) using different methods. The obtained detection limit was 0.5×10^{-6} M, and the variation coefficient at 1.5×10^{-5} M level was 0.99%. The UV spectrophotometric method is sufficiently accurate and precise to be applied in the Carduran tablets assay, while the voltammetric method (AdS-SWV) can in addition be used to determine the drug at trace level in human urine samples with good recoveries.

Keywords: Nafion carbon paste electrodes; Doxazosin; Electroanalytical behaviour; UV-spectrophotometry

1. Introduction



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Doxazosin is a drug used for the treatment of hypertension allowing once daily administration because of its long elimination half life. It is a selective α-adrenergic receptor antagonist used either alone or in combination with diuretics or β-adrenergic-receptor-antagonist [1].

Doxazosin is a quinazoline derivative and presents similar clinical effects to Prazosin [2] but its slow onset of hypotensive activity minimizes the first dose hypotensive effect seen with Prazosin [3]. In order to prevent adverse effects because of its big pharmacological activity, efficient screening procedures and methods for its quantitative determination at very low concentrations in biological samples are necessary. For this purpose, sensitive analytical methods such as liquid chromatography with fluorescent detection have been mainly selected [4–7] with detection limits in the range 0.5 to 1 ng ml^{-1}.

Electroanalytical techniques such as modern voltammetric ones are particularly suitable for the quantification of organic compounds with electroactive groups present in its molecule [8,9], using different types of electrodes which can be modified in order to improve its sensitivity and selectivity. The carbon paste electrode (CPE) is one of the most easily modifiable [10–12].

In previous papers, the reduction of Doxazosin molecule has been studied by us at the hanging mercury dropping electrode [13], and at CPE [14], the reduction peaks appearing at -1.3 V (pH 5.8) and at 0 V (pH 6.6), respectively. The detection limits reached [13,14] were 50 times smaller than the best value reported by the bibliography [7] using liquid chromatographic techniques.

Nafion polymers are a family of perfluorosulfonate cation-exchange resins, which are highly stable in aqueous solution [15]. These polymers are formed by hydrophilic ionic clusters with negative sulfonic groups, their counter ions, water molecules and significant amounts of polymer material; and this contributes to the selectivity for cations [16,17].

Nafion has almost ideal properties to be a chemical modifier: its chemical and electrochemical inertness, its insolubility in water and its hydrophilicity. Moreover this specific type of electrode has a rapid and reproducible preparation.

Nafion has been often used in the modification of CPE [18,19], glassy carbon electrodes [20], carbon fiber microelectrodes [21] and mercury film electrodes [22–24]. A very thin film of Nafion is ample to offer minimal obstruction to the diffusion of the analyte to the electrode, while preventing at the same time adsorption/desorption processes of organic species in biological fluids [25].

Some authors [26] incorporate the Nafion into the carbon paste during the mixing of the graphite and Nujol. Others applied the Nafion polymer layer on top of the surface of the CPE [19].

One important reason for the widespread application of Nafion modified electrodes in electroanalytical chemistry is their ability to preconcentrate positively charged molecules which increase the sensitivity of the method [16,17,27–29].

The accumulation mechanism of Nafion can be explained through an electrostatic interaction due to the hydrophilic negatively charged sulfonate groups in the polymer structure, whereas its ionic selectivity for hydrophobic organic cations is achieved through hydrophobic interactions with the hydrophobic fluorocarbons of the film [30].

Another method widely used for the determination of organic substances is UV spectroscopy [31–34], which allows the rapid and precise determination of substances [35–37]. Although the sensitivity of the spectroscopy is minor than that for voltammetric techniques, it is very useful to determine drugs in pharmaceutical formulations [38–42].

The aim of present work is to develop methods to detect Doxazosin in urine and formulations. A UV- spectrophotometric method could be applicable to the analysis of pharmaceutical formulations which contain Doxazosin, and a sensitive SW-voltammetric one could also be used to determine Doxazosin in urine, based on adsorption properties of Doxazosin at the NMCPE.

For this purpose, the redox behaviour of Doxazosin was studied with NMCPE by means of several voltammetric techniques. All factors that may influence both the preconcentration step and the voltammetric redissolution were studied to find out the most sensitive instrumental conditions.

2. Experimental

2.1. Reagents

The aqueous Doxazosin stock solutions (M.W. 547.58) were prepared by dissolving the pure product (Pfizer) 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-1,4-benzodioxin-2-yl) carbonyl]-piperazine monomethane sulfonate in de-ionized water. A Britton–Robinson buffer solution was used as supporting electrolyte (0.04 M in acetic, phosphoric and boric acids). The supporting electrolyte: $NaClO_4$ was added to Britton–Robinson to obtain a concentration of 0.01 M in the cell. The pH was adjusted with 2 M NaOH solution.

Graphite of ultra F purity (Ultracarbon, Bay City, MI) with a grain size < 33 mm, and Nujol (Aldrich, Milwaukee, WI) ($d = 0.838$ g cm^{-3}) as an aglutinant were used. A solution of Nafion in aliphatic alcohols (5% v/v) was provided by Aldrich. Dilutions of the Nafion stock solution were prepared using methanol.

LC-18 solid-phase extraction cartridges (1 ml) (Supelco, Bellefonte, PA) were used. All the other solutions were prepared from analytical-grade chemicals with de-ionized water with Milli-Q system (Millipore, Bedford, MA).

2.2. Instrumentation

An Autolab pStat 10 voltammetric analyzer (Eco Chemie, Utrech, Netherlands) coupled with a 663 VA stand Metrohm (Herisau, Switzerland) was used to carry out the voltammetric and adsorptive stripping experiments. An EXP8551 (Pentium 120) personal computer and the electrochemical software package GPES 4.4 (Eco Chemie) were also used. An Ag/AgCl (saturated KCl) reference electrode, a platinum counter electrode and a 'home made' Nafion modified carbon paste electrode (NMCPE) as working electrode were used. The pH was adjusted by means of PHM 92 laboratory pHmeter Radiometer. Spectral and absorbance measurements were made with a double beam Uvicon 992 spectrophotometer (Kontron Instruments S.p.A., Milan, Italy) with 1 cm matched quartz cells were used to do the spectrophotometric study over the spectral range 200– 900 nm at a scanning rate of 200 nm min^{-1}, against a corresponding blank.

2.3. Modification of the working electrode

The end of a polyethylene supporting tube (4.6 mm i.d.) was filled with the best quality carbon paste, prepared by mixing 1.0 g of graphite powder and 0.4 cm^3 of Nujol. To obtain a flat and shiny appearance surface, the end of the tube was rubbed gently and smoothed on a paper.

The Nafion modified electrode (NMCPE) was prepared by pipetting 0.01 cm^3 of 0.05% Nafion solution on the surface of the previously prepared CPE. The resulting film was dried under a domestic hair-dryer. The electrical contact was assured by inserting a copper wire into the NMCPE and connecting it with a voltammetric analyzer through a banana plug.

Prior to each measurement a cathodic activation of the NMCPE was carried out, performing a sweep potential in Britton–Robinson buffer (pH 6.8) between 1.2 and -1.2 V, with the optimum operational parameters of the voltammetric technique used. Doxazosin is strongly adsorbed on the NMCPE, therefore the electrode surface must be cleaned by successive anodic scans in NaOH medium (0.1 mol l^{-1}), between -1.2 and 1.2 V, or preparing a new one.

2.4. Voltammetric procedure

For voltammetry measurements, 25 ml of the supporting electrolyte solution and the appropriate amounts of Doxazosin solution were added to the cell, and degassed with oxygen-free nitrogen for 10 min., maintaining the NMCPE out of the solution. Then, the electrode was introduced into the solution, and a preconcentration step was carried out at 0.5 V for 90 s with a stirring speed of 3000 rpm. After an equilibration period of 5 s a cathodic potential sweep was carried out between -1.2 V and 1.2 V using SWV (Osteryoung's method) as redissolution technique, with its optimum operational parameters (Table

1). All measurements were carried out at room temperature.

2.5. Spectrophotometric procedure

Doxazosin was dissolved in Britton–Robinson at pH 1.7 to obtain concentrations between 1×10^{-7} M and 7×10^{-5} M, and this solution was directly added to the quartz cell to perform the measurements at 244 nm, against a blank prepared with Britton– Robinson buffer pH 1.7.

To determine the apparent pK'_a, the variation of the absorbance with the pH was studied at 317, 329 and 341 nm. The pH solution was changed each 0.5 U, and near the pK'_a zone it was varied each 0.25 U. The temperature was kept constant at 25°C and the used Doxazosin solutions were 5×10^{-5} M for all the pH range considered.

2.6. Doxazosin assay in formulations

Both voltammetric and spectrophotometric techniques were used to determine Doxazosin in formulations. Five Carduran tablets (Pfizer, amount declared of Doxazosin per tablet 2 mg) were triturated in an agate mortar, pounded and finally dissolved in de-ionized water for 30 min. The excipient was separated by filtration and the residue washed three times with water. The solution and washed water was transferred quantitatively into a calibrated flask and diluted to a final volume of 500 ml with water. An aliquot of this solution was added to 25 ml B–R buffer (pH 6.8) into the voltammetric cell and the voltammogram was recorded following the voltammetric procedure. The UV- spectrophotometric determination of Doxazosin was made by adding an aliquot of the above mentioned solution to B–R buffer at pH 1.7 up to a volume of 25 ml, and then a quartz cell was filled with this solution. For both assays, the standard additions method was used.

Table 1
Optimum operational parameters selected for the voltammetric determination of Doxazosin solutions on the NMCPE

Parameters	Variation interval	SWV
pH	1.5–10.6	6.8
Ionic strength (M of $NaClO_4$)	0–0.5	0.01
Step (mV)	1–10	10
Pulse amplitude (mV)	10–100	60
Frequency (Hz)	25–200	175
Accumulation potential (V)	0.1–0.8	0.5
Accumulation time (s)	0–180	90
Equilibrium time (ms)	0–35	5
Stirring speed (r.p.m.)	0–3000	3000

2.7. Doxazosin assay in urine

The Doxazosin determination in urine was carried out by means of AdS-SWV. To avoid interferences due to urine compounds, a solid phase extraction using LC-18 cartridges was necessary. The cartridges were conditioned by passing through them 2 ml of 0.5% acetic acid in water. Then 1 ml of spiked urine was added, and two sequential washings were carried out, the first one adding 0.4 ml of 40% methanol in water, and the second one with 0.4 ml of 20% acetone in water. Finally, the elution of Doxazosin was carried out with 2 ml of 75% methanol in water.

3. Results and discussion

3.1. Voltammetric study

3.1.1. Cyclic voltammetry

The cyclic voltammetric behaviour of Doxazosin on NMCPE (Fig. 1) was studied in Britton–Robinson buffer (pH 6.8) using a scan rate of 50 mV s^{-1} in the potential range from 1 to -0.5 V, after a preconcentration step of 90 s at 0.5 V and a stirring speed of 3000 rpm. In our work conditions, the first cathodic branch showed a wave at -0.17 V, due to the reduction of oxidized form of Doxazosin. To obtain this cathodic peak it was necessary to begin the scan at high anodic potentials (1.0 V) which improves after a deposition step at anodic potentials (0.5 V) before the scan. In the reverse scan, an oxidation peak appeared at -0.06 V as a consequence of the oxidation of the cathodic one. This process was not completely reversible because the difference between their potentials was $\Delta E = 0.116$ V, higher than the ex-

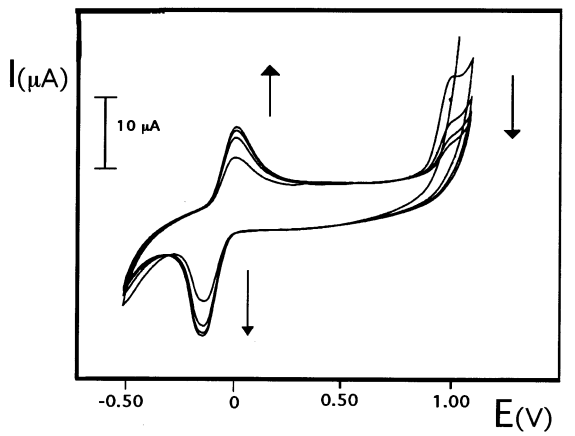

Fig. 1. Successive cathodic–anodic cyclic voltammograms of a 4×10^{-6} M Doxazosin solution in B–R buffer (pH 6.8), after a deposition step (0.5 V, 90 s and 3000 rpm) at a scan rate of 50 mV s^{-1}, using a NMCPE.

pected value of 0.059/n for a reversible process, and the relation between their intensities was $i_{pc}/i_{pa} = 2.85$, so the process is quasi-reversible. The most anodic peak (0.87 V) is formed as a consequence of the irreversible oxidation of the piperazine moiety present in the Doxazosin molecule, with a chemical follow-up reaction occurring [43], giving rise to a reaction product which causes the quasi-reversible couple. This is in accordance with the redox mechanism postulated by Kauffmann et al. [44] for the oxidation of piperazine ring present in the Trazodone molecule, in an ECE process.

As it can be observed in Fig. 1, the peak current of quasi-reversible couple increases as the number of scans increase, while the wave at more anodic potentials decreases, which confirms that the quasi-reversible electrochemical behaviour is related to Doxazosin oxidation and arises from the product of the followed-up reaction.The variation of $\log ip$ versus $\log v$, in the scan range 50–1000 mV s^{-1}, presented a slope of 0.89, close to 1, which was the expected value for a process controlled by adsorption. The equation obtained was:

$$\log I\ (\mu A) = -2.40 + 0.89 \log v\ (\text{mV s}^{-1})$$

$(r = 0.9983)$.

However, when the study of the variation of the $\log ip$ versus $\log v$ was made at bare CPE [14] it was found that, for slow rates, between 25 and 100 mV s^{-1}, the process had an important diffusive component because the slope was 0.34 close to the theoretical value of 0.5 for a diffusive process. For higher rates, between 200 and 800 mV s^{-1}, the adsorptive component was more important than the diffusive one obtaining a slope of 0.81, close to 1, this value being the theoretical one for an adsorptive process. So it could be concluded that the use of Nafion to modify the electrode increased the adsorptive component of the process in all scan rate range.

The effect of temperature on peak current was studied for 4×10^{-8} M Doxazosin solutions using DPV, and random values were obtained for the temperature coefficients calculated according to Meites [46]: $-1.63\%\ °C^{-1}$ (16–30°C), $4.57\%\ °C^{-1}$ (30–46°C) and $3.36\%\ °C^{-1}$ (46–58°C), which indicated the presence of an adsorptive component in the process.

3.1.2. Influence of pH on the reduction peak

DPV was used to study the effect of pH on peak current and peak potential for the reduction of 4×10^{-6} M Doxazosin solutions (Fig. 2).

The peak current reaches its maximum value at pH 6.8, which was selected as optimum value to carry out its quantitative determination (Fig. 2A). The peak potential shifted to more positive values up to pH 6.9 with a slope of 3.0 mV pH^{-1}; if the

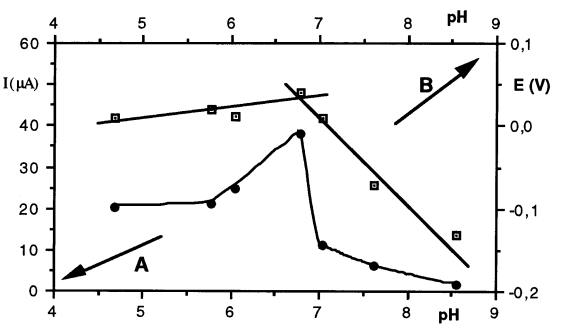

Fig. 2. Variation of peak current (*A*) and peak potential (*B*) with pH for 4×10^{-6} M Doxazosin solutions by means of SWV on the NMCPE. For operating conditions, see voltammetric parameters in Table 1.

pH was increased the potential shifted to more negative values with a higher slope: 96.8 mV pH^{-1} indicating that the number of protons consumed in the second range was higher than in the first one (Fig. 2B). The two equations obtained were:

$(4.5 < pH < 6.8)$

$$Ep(V) = -5.44 \times 10^{-2} + 1.30 \times 10^{-2}\ pH$$

$(r = 0.8598)$

$(6.8 < pH < 8.5)$

$$Ep(V) = 0.68 - 9.68 \times 10^{-2}\ pH$$

$(r = 0.9810)$

The polarographic pK'_a of Doxazosin obtained by the intersection of the two lines was 6.76 ± 0.92 (95% confidence limit, $t = 2.57$); this is in accordance with the obtained value ($pK'_a = 6.89 \pm 0.57$), using DPV on the bare CPE [14].

By using fit and simulation programs of software package GPES 4.4 the α value could be calculated [45]. Marquard non linear least square method was used to determine the α value supposing that the process was quasi-reversible and two electrons were exchanged. The obtained simulation curve was perfectly adapted to the experimental one ($X^2 = 5.23 \times 10^{-3}$), and the obtained α value was 0.52. Taking into account that the slope of the plot Ep versus pH was 59 $mH^+/n\alpha$, the number of protons involved in the redox process was calculated. In the 4.5 to 6.8 pH range the number of protons was 0, while for $pH > 6.8$ two protons were exchanged in the reduction process.

3.1.3. Adsorption process

The selection of suitable conditions for the deposition of Doxazosin onto the NMCPE surface for a 4×10^{-8} M solution was carried out by means of SWV, with its optimum operational parameters (Table 1), which have been selected previously. On the other hand, the effect of increasing the concentration of Nafion on the MCPE surface was also studied in the range from 0.01 to 2% (Nujol:methanol); and a maximum value for peak current was reached, when

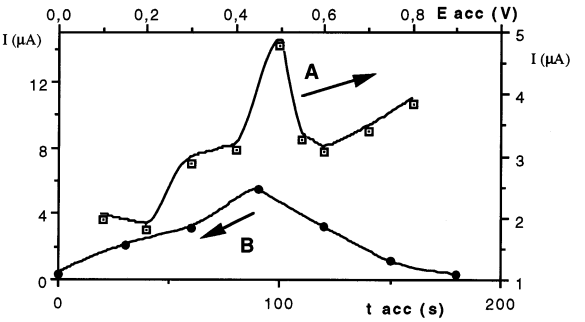

Fig. 3. Influence of accumulation potential (A) and accumulation time (B) on peak current for 4×10^{-8} M Doxazosin solutions at pH 6.8 by means of SWV on the NMCPE. For operating conditions, see voltammetric parameters in Table 1.

1×10^{-2} cm^3 of the 0.05% modifier was deposited onto the MCPE surface.

The effect of changing the ionic strength was studied by adding different amounts of $NaClO_4$ to the cell at pH 6.8. The concentration of $NaClO_4$ in the cell was varied between 0 and 0.5 M and the highest peak was obtained for 0.01 M, so this concentration was used to determine the rest of the variables.

The deposition of Doxazosin on the electrode surface was carried out at open circuit and at several potentials in the range between 0.1 and 0.8 V. When the peak potential increased to more positive values, the peak intensity increased up to 0.5 V, (Fig. 3A). For higher potential values the intensity decreased, so 0.5 V was selected as optimum deposition potential.

The stirring speed was varied between 0 and 3000 rpm and the peak intensity increased linearly following this equation:

$$I\ (\mu A) = 1.48 + 1.49 \times 10^{-3}\ v\ (rpm)$$

$(r = 0.9947)$.

The peak intensity reached the maximum value at a deposition time of 90 s. when this variable was changed in the range 0–180 s. (Fig. 3B). If a bare CPE was used, the optimum deposition time increased by four (360 s); so the use of Nafion diminished the deposition time.

Doxazosin is a good candidate to be preconcentrated onto the NMCPE surface, since it possesses aromatic rings which have three consecutive car-

bon atoms without hydroxyl groups [18]. The union takes place through the SO_3H-group on the polymeric structure of the Nafion film [30]. There were too hydrophobic interactions between the hydrophobic part of the Doxazosin and the hydrophobic fluorocarbons of the Nafion film. Both types of interactions favored the adsorption of the Doxazosin onto the electrode surface.

3.2. Spectrophotometric study

The study of the influence of pH was studied between 1.0 and 12.5 pH values, and in all range studied two waves appeared. The wave at 244 nm is independent of the pH, but the other wave undergoes a batochromic shift, moving between 329 nm (pH = 1.0) and 341 nm (pH = 12.5) (Fig. 4).

For this last wave, the influence of pH on the absorbance was studied at three wavelengths: 341, 329 and 317 nm, and it was observed that in the ranges between pH 1 and 4.5, and pH 8.5 and 11.2, the wave shows a pH-independent behaviour. In turn, there was a pH dependent zone between pH 4.5 and 8.5, that indicates the presence of a spectrophotometric apparent constant pK'_a. With these data the pK'_a of Doxazosin was calculated using several graphic methods: Strentöm–Goldsmith [47] and Sommer [48] and the numerical method Letagrop Spefo [49].

In the Strentöm–Goldsmith method, pH was represented versus $\log[(A-A_1)/(A_0-A)]$ where A_0 is the absorbance in the basic plateau and A_1 is the absorbance in the acidic one. For the three studied wavelengths: 341, 329 and 317 nm, straight lines were obtained with slopes close to 1, and the intercept of these lines with pH axis indicated the pK'_a value of Doxazosin. The different pK'_a found values were: 6.98 (341 nm), 6.87 (329 nm) and 6.80 (317 nm) (Table 2). The mean value was 6.88 ± 0.39.

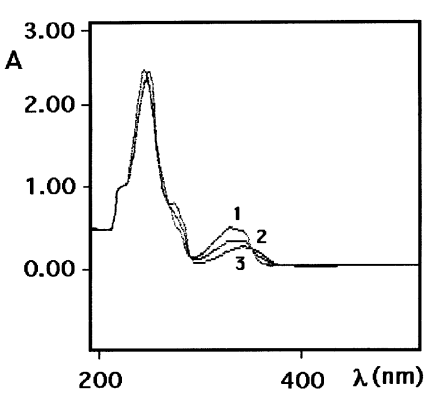

Fig. 4. Absorption UV-spectra of a 5×10^{-5} M Doxazosin solution at different pH values: (1) 1.7; (2) 7.0; and (3) 11.5.

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

Method	341 nm	329 nm	317 nm
Strentöm–Goldsmith	6.98	6.87	6.80
Sommer acidic	6.88	6.90	6.90
Sommer basic	6.99	7.00	6.96
Letagrop Spefo	7.01	7.01	7.00

Sommer method was then applied to determine the pK'_a, knowing the absorbance (A_1) of the acidic plateau, taking into account that $\varepsilon_0^{-1} K_a^{-1}$ is the slope of the plot C_T/A versus $(A-A_1)\ H^+/A$; ε_0 being the molar extinction coefficient for the basic species. The mean obtained value was 6.89 ± 0.05. This method permitted too the determination of pK'_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 versus $(A-A_0)/H^+A$ (Table 2). The mean value being 6.98 ± 0.09.

The Letagrop Spefo program was also used to determine numerically the pK'_a value (Table 2). The different pK'_a found values were: 7.01 (341 nm), 7.01 (329 nm)and 7.00 (317 nm) (Table 2). In this case, the mean value was 7.01 ± 0.02. Considering all the obtained pK'_a values with the different methods, the mean total value was 6.94 ± 0.21. This value is very close to the polarographically calculated value (pK'_a 6.76 ± 0.92).

The presence of hydrolysis processes was studied in acid, neutral and basic medium, and it was observed that in acid (pH 1.7) and neutral mediums (pH 7.0) the peak position and absorbance did not change with the time. However, in basic media, the absorbance diminished lightly with the time. The rate constants for the direct and inverse hydrolysis process at pH 11.5 were:

$Kh_1 = 7.08 \times 10^{-4}$ and $Kh_{-1} = 2.21 \times 10^{-2}$ at (343 nm), and $Kh_1 = 2.37 \times 10^{-4}$ and $Kh_{-1} = 1.48 \times 10^{-2}$ at (248 nm).

3.3. Calibration graphs and statistical parameters

3.3.1. Voltammetric study

Under chosen experimental parameters (Table 1), the variation of peak current with the Doxazosin concentration was studied by means of AdS-SWV.

A linear relation between peak intensity and Doxazosin concentration was found in the range 4×10^{-11}–2.8×10^{-9} M (Table 3). The detection limit, defined as $a + 3\ Syx$ [50], was 2.33×10^{-11} M and a coefficient of variation 3.54% ($n = 10$) was found for 2×10^{-9} M Doxazosin solutions.

A second linear calibration range was obtained between 4×10^{-9} and 40×10^{-9} M (Table 3) with small sensitivity, the slope ratio being 1.69.

The obtained sensitivity and detection limits are quite similar to those found using mercury electrode [13], CPE and TMCPE [14], although the linear calibration range is wider and the accumulation time decreases for NMCPE.

3.3.2. Spectrophotometric study

The variation of absorbance with concentration was studied at pH 1.7 for the two peaks obtained (244 and 328 nm), and the corresponding linear regression equations appear exposed in Table 3.

The detection limits reached, according to $a + 3S_{yx}$ [50], were 0.5×10^{-6} M at 244 nm and 1.4×10^{-6} M at 328 nm.

The linear calibration range at 328 nm was wider ($2–50 \times 10^{-6}$ M) than the obtained at 244 nm ($1–15 \times 10^{-6}$ M); on the contrary, the sensitivity is bigger at 244 nm, the slope ratio being 5.87. The variation coefficients for a 1.5×10^{-5} M Doxazosin solution were 0.99 and 0.85 ($n = 10$) at 244 and 329 nm, respectively.

4. Doxazosin assay in real samples

Doxazosin was determined in 2 mg Carduran tablets and spiked urine at different concentration levels, and the procedures used have been described above.

4.1. Doxazosin assay in Carduran tablets

Without sample preparation but the adequate dilution of analite present in the solution of Carduran tablets, UV-spectrofotometry and AdS-SWV can be used for the determination of Doxazosin. Five aliquots of this solution were analyzed by the standard additions method. Re-

Table 3
Regression data of calibration lines for quantitative determination of Doxazosin AdS-SWV with a Nafion modified carbon paste electrode and by UV-spectrophotometry[a]

Parameters	AdS-SW		UV-Spectrophotometry	
			328 nm	244 nm
LR	(0.04–2.8) nM	(4–40) nM	(2–50) μM	(1–15) μM
b	174.97	102.99	8.89×10^{3}	5.22×10^{4}
a	1.38×10^{-7}	3.22×10^{-7}	1.00×10^{-2}	1.00×10^{-2}
Sb	0.53	1.59	90.24	606.81
Sa	6.08×10^{-10}	3.27×10^{-8}	1.86×10^{-3}	3.87×10^{-3}
Syx	1.36×10^{-9}	4.77×10^{-8}	4.15×10^{-3}	9.45×10^{-3}
r	0.9999	0.9996	0.9996	0.9994
r^2	0.9998	0.9992	0.9992	0.9988
n	9	5	10	11
DL	23.3 pM	–	1.4 μM	0.5 μM

[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; DL, detection limit.

Table 4
Mean recoveries obtained for five determinations of Doxazosin in spiked urine samples and Carduran tablets, using AdS-SWV at the NMCPE and UV-spectrophotometry[a]

Sample	Doxazosin added[a]	Doxazosin found	Recovery (%)
Tablets (mg)			
AdS-SWV	2.00	1.97	98.50
UV-spectroph.	2.00	1.98	99.00
Urine, spiked (nM)	0.400	0.399	99.75
SPE	4.000	4.015	100.38

[a] The amount added was equal to the declared content of the pharmaceutical preparation.

sults obtained (Table 4) gave a mean value of 1.98 ± 0.01 mg (UV-spectroph. at 244 nm) and 1.97 ± 0.16 mg (AdS-SWV) which is in accordance with the declared amount (2 mg).

4.2. Doxazosin assay in urine

A solid-phase extraction with C-18 cartridges was necessary to determine Doxazosin in urine by means of AdS-SWV using the standard additions method and following the voltammetric procedure described above. Two different concentration levels 4×10^{-10} and 4×10^{-9} M Doxazosin in the cell were used. The mean recoveries, at this concentration levels, were 99.75 and 100.38% for 0.4 and 4 nM, respectively (Table 4 and Fig. 5).

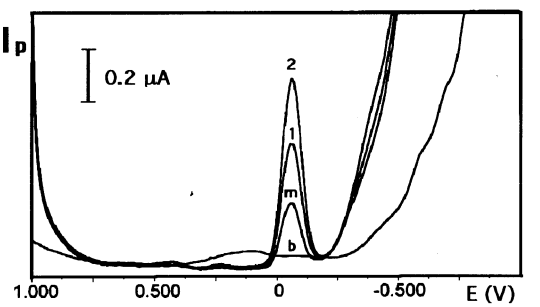

Fig. 5. Voltammetric curves (AdS- SWV) obtained for the determination of Doxazosin in human urine, b: blank, m: urine spiked with Doxazosin at 4×10^{-10} M, 1 and 2: two standard additions of 100 μl of 1×10^{-7} M Doxazosin solution (for conditions see Table 1).

5. Conclusions

The UV-spectrofotometric results obtained in this work let us propose an effective method to determine Doxazosin in Carduran tablets. This method is simple, precise and affordable; also it requires no complex pretreatment of the active principle to be determined.

Besides this work describes a study of the electroanalytical behaviour of Doxazosin at NMCPE. This study confirms the adsorptive and quasi-reversible reduction (pH 6.8) of the oxidized form of Doxazosin followed by a chemical reaction, which resulted in a ECE mechanism. The presence of Nafion in the composition of the MCPE resulted in a considerable diminution of accumulation time compared to CPE [14] and HMDE [13] results. Accumulation studies carried out at trace levels on the reduction peak showed an increase in linear range compared to those obtained by means of HMDE [13] and CPE [14]. The AdS-SWV at NMCPE method proposed is an accurate, sensitive and rapid procedure for the determination of Doxazosin in urine at trace levels, avoiding the use of toxic mercury electrode. The limit of detection reached is quite similar than to those obtained by means of HMDE [13] and TMCPE [14], and lower than those chromatographic proposed (0.5–1.0 ng l^{-1}) [4–7]. All the methods exposed in this paper make a viable alternative to existing analytical methods for routine analysis.

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