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Anodic adsorptive stripping voltammetry of the antihypertensive drug candesartan cilexetil at a glassy carbon electrode

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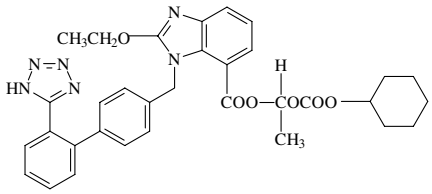
The electrochemical behaviour of candesartan cilexetil (CND) was investigated in an acetonitrile : supporting electrolyte mixture (30% acetonitrile) in the pH range 1.5–11.00 by cyclic, linear sweep, differential pulse (DPV), adsorptive stripping differential pulse (AdSDPV), square wave (SWV) and adsorptive stripping square wave (AdSSWV) voltammetric techniques. CND exhibited one wave and one peak to the anodic direction. The oxidation process was found to be irreversible and adsorption controlled. To obtain good sensitivity, the instrumental and accumulation variables were studied using DPV and SWV techniques. Two linear calibration plots were obtained for both techniques. The detection limits were 9.15×10^{-7} M and 7.94×10^{-6} M for AdSDPV and AdSSWV, respectively. The method was validated and successfully applied for the analysis of CND tablets.

1. Introduction

Candesartan cilexetil (CND) is a new angiotensin II receptor blocker with a high affinity for the AT_1 subtype angiotensin II receptor antogonist.

There have been relatively few methods for the analysis of CND alone in pharmaceutical dosage forms or in bulk form or biological media including HPLC (Stenhoff et al. 1999; Gonzalez et al. 2000, 2002), micellar electrokinetic chromatography (Hillaert et al. 2003), capillary zone electrophoresis (Hillaert and van den Bossche 2002) and spectrofluorimetry (Cagigal et al. 2001). No literature data was found on the electrochemical behaviour of CND in general or its voltammetric determination in particular.



In this study the oxidation process of CND on the glassy carbon disc electrode is described and analytical procedures for its determination are presented.

2. Investigations, results and discussion

In order to understand the electrochemical process occuring on the glassy carbon electrode cyclic and linear sweep voltammetry were carried out. For the quantitative determination of CND from tablets AdSDPV and AdSSWV techniques were realized.

CND was oxidized on glassy carbon electrode between pH 1.5 and 11.0, producing one well-defined peak and one or two waves (Fig. 1) depending on pH. At a concentration of 1×10^{-4} M CND the voltammograms were recorded at different scan rates ranging from 5 to 250 mVs^{-1}. The linear dependence of ip versus ν (scan rate) shows the adsorption controlled process according to eq. (1).

$$ip\ (\mu A) = 0.034\ \nu\ (mVs^{-1}) + 1.331 \qquad (1)$$
$$r = 0.991\,; \qquad n = 7$$

A linear dependence of the peak current vs the square root of the pre-concentration time was observed (r: 0.987). This behaviour is also confirmed for mass transport controlled by adsorption.

A 64 mV positive shift in the peak potential confirmed the irreversibility of the oxidation process. The tafel plots (log i vs E) was obtained with a scan rate of 5 mVs^{-1} begining from a steady-state potential in phosphate buffer at pH 5.03. The αn value of the anodic reaction from the slope of the linear part of the tafel plot was found to be 0.146. The exchange current density (i_o) is the forward and reverse electrode reaction rate at the equilibrium potential. This value was obtained as 2.46×10^{-9} A/cm^2 for this system. These values together with the absence of cathodic waves in cyclic voltammetry (Fig. 1) indicated the irreversibility of the oxidation reaction.

The peak potential of the anodic process moved to less positive potentials by raising the pH. The variation of peak intensity and peak potential was studied for 2×10^{-4} M CND by cyclic, linear sweep, DPV and SWV between pH 1.5 and 11.0. All obtained curves were similar to each other. For this reason, only the DPV graph is shown in Figs. 2a and 2b. The plot of the peak potential versus pH showed a straight line (Fig. 2a) between pH 2 and 6, which can be expressed by the following equations (Britton-Robinson buffer):

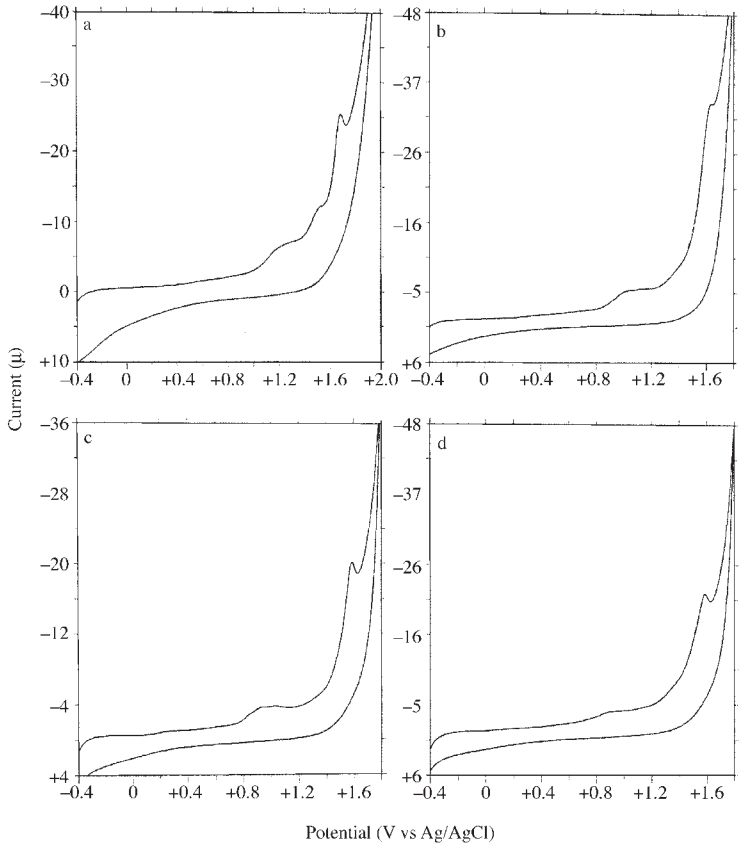


Fig. 1: Cyclic voltammograms of 2×10^{-4} M CND in Britton-Robinson buffer at pH 2.00 (a); Britton-Robinson buffer at pH 5.00 (b); phosphate buffer at pH 7.00 (c); phosphate buffer at pH 9.00 (d). Scan rate 100 mVs^{-1}

$$Ep\,(mV) = 1661.6 - 15.6\,pH\,; \qquad r: 0.979 \qquad (2)$$

The peak potential vs pH was nearly independent above pH 6 according to the following equation:

$$Ep\,(mV) = 1563.6 - 0.60\,pH\,; \qquad r: 0.297 \qquad (3)$$

Linearity was observed in the pH range between 2.0 and 6.0, giving a negative slope of 15.6 mV per pH unit. The intersection observed in the plot at pH 6.0 can be explained by changes in protonation of the acid-base function in the molecule. The intersection point of the curve is found to appropriate with pKa value of CND molecule which is pKa = 6.0 (Cagigal. et al. 2001).

The amin group of the benzimidazole moiety is one of the oxidizable groups in CND. The related step was obtained as the main oxidation peak at more positive potentials. The amin function of the tetrazole moiety is another oxidizable group in the CND molecule. The waves related to this group were seen before the main peak, which is obtained at less positive potentials than the main peak.

The anodic oxidative behaviour of CND is compared with those of benzoic acid, lansoprazol and omeprazol chemically related with the benzimidazole moiety. No oxidation wave or peak was obtained with benzoic acid. Also CND is compared with losartan and valsartan related to the amin function of the tetrazole moiety which is the other oxidizable group in the molecule. According to the results obtained, the main peak of CND was related to the amin group of the benzimidazole moiety.

Our results also revealed a good aggreement with the redox mechanism postulated for lansoprazol and omeprazol and suggested that the main oxidizable group of CND can be determined electrochemically by oxidation of the amin group of the benzimidazole moiety (Radi 2003).

Different supporting electrolytes, such as sulphuric acid, Britton-Robinson and phosphate buffer were examined. The peak current-potential curve is the most useful analytical signal for both techniques in phosphate buffer at pH 5.03 (30% acetonitrile). The effect of pH on both peak potential and peak current of the main peak of CND in the range of pH 1.5–11.0 was examined in the different supporting electrolytes. Therefore, phosphate buffer at pH 5.03 was selected as supporting electrolyte for the analytical studies. The effect of accumulation time on the peak current of 6×10^{-5} M CND concentration in phosphate buffer at pH 5.03 was studied and the results are shown in Fig. 3. The peak current increased with the accumulation time increasing until 90 s. For both techniques, a similar ip-time curves were observed. On this basis, 90 s deposition times were used for AdSDPV and AdSSWV techniques. The maximum peak current values were obtained with an accumulation potential of + 1.45 V. The interfacial accumulation of CND on glassy carbon electrode is indicated from the DPV of 6×10^{-5} M CND in phosphate buffer at pH 5.03 recorded before and after 90 s accumulation at + 1.45 V (Fig. 4). After a short pre-concentration time, a sharp anodic peak appeared at about +1.60 V. As can be seen in Figs. 4a

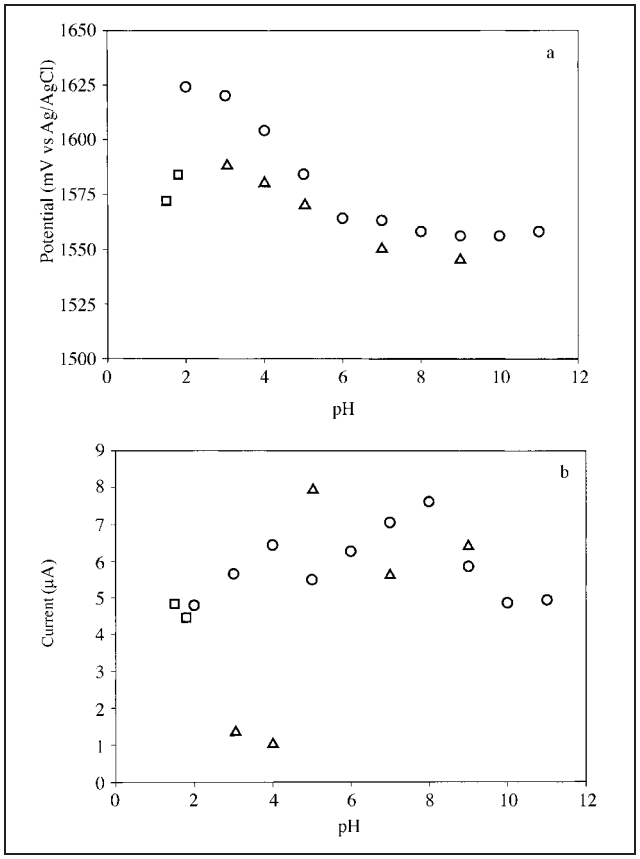


Fig. 2: Effects of pH on CND peak potential (a) and peak current (b); CND concentration 2×10^{-4} M. H_2SO_4 (□); Britton-Robinson (○); and phosphate (△) buffers

and b only an ill defined anodic wave was obtained without applying a pre-concentration step. Under defined experimental conditions the linear dependence of ip versus CND concentration was achieved from 1×10^{-5} to 6×10^{-5} M and 6×10^{-5} to 1×10^{-4} M for AdSDPV and $1 \times 10^{-5} - 4 \times 10^{-5}$ M and $4 \times 10^{-5} - 1 \times 10^{-4}$ M for AdSSWV with two linear segments at pH 5.03 phosphate buffer. Table 1 summarizes the characteristics of the calibration equations established for both techniques. The validation parameters, LOD (limit of detection) and LOQ (limit of quantification) are shown in Table 2, which were calculated on the peak current using the following equations:

$$\mathrm{LOD} = 3s/m \quad (4)$$

$$\mathrm{LOQ} = 10s/m \quad (5)$$

Where s, the noise estimate, is the standard deviation of the peak currents (three runs) of the sample, m is the slope of the related calibration graphs (Swartz and Krull 1997; Riley and Rosanske 1996).

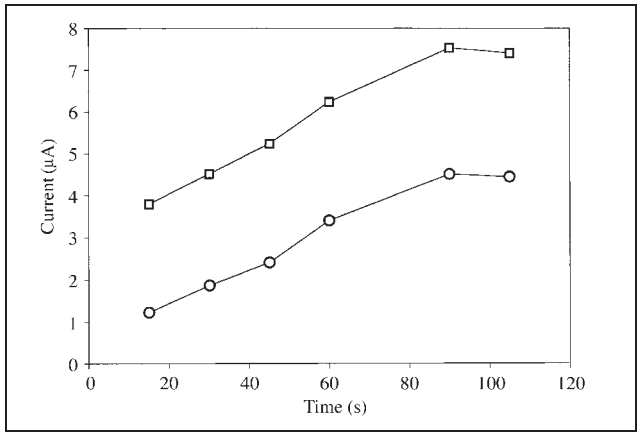


Fig. 3: The effect of preconcentration time on CND stripping current

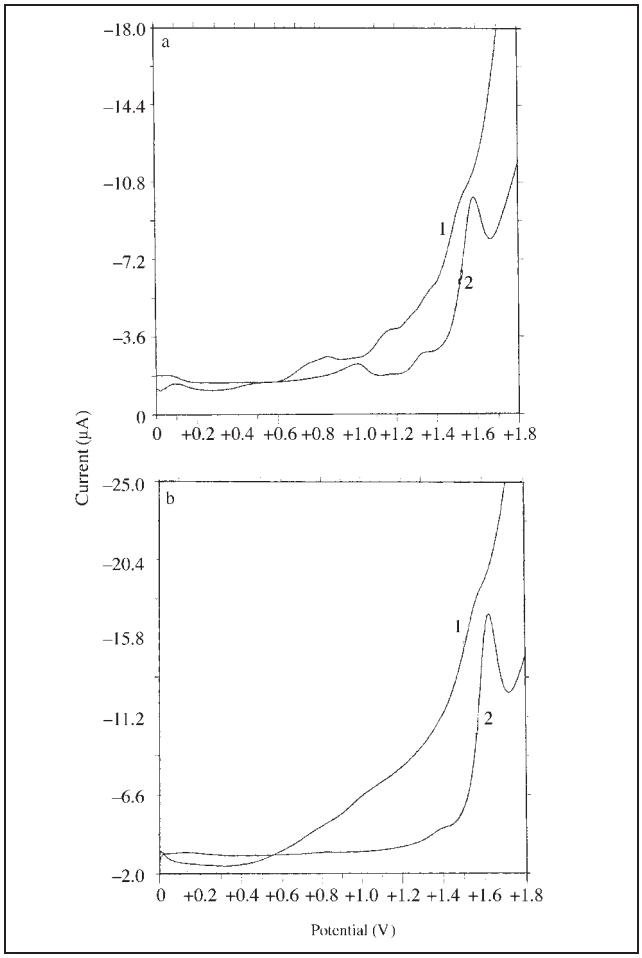


Fig. 4: Differential pulse and AdSDP (a) and square wave and AdSSW (b) voltammograms obtained for the determination of 6×10^{-5} M CND in phosphate buffer at pH 5.03 without preconcentration time (1) and after 90 s preconcentration times (2)

Table 1: Statistical and analytical parameters of the proposed methods in phosphate buffer at pH 5.03

	AdSDPV		AdSSWV	
	First equation	Second equation	First equation	Second equation
Linearity range (M)	$1 \times 10^{-5} - 6 \times 10^{-5}$	$6 \times 10^{-5} - 1 \times 10^{-4}$	$1 \times 10^{-5} - 4 \times 10^{-5}$	$4 \times 10^{-5} - 1 \times 10^{-4}$
Slope (μA M^{-1})	2.50×10^4	9.84×10^4	2.29×10^4	1.34×10^4
Intercept (μA)	0.103	−4.20	0.065	−4.17
Correlation coefficient	0.999	0.997	0.999	0.998
SE of slope	7.44×10^2	8.28×10^3	1.11×10^2	5.73×10^3
SE of intercept	2.701×10^{-2}	0.676	3.04×10^{-3}	0.421

Table 2: Necessary validation parameters of CND by proposed methods

	AdSDPV	AdSSWV
LOD (M)	9.15×10^{-7}	7.94×10^{-6}
LOQ (M)	3.05×10^{-6}	2.38×10^{-6}
Repeatability of peak current* (RSD%)	1.097	1.86
Repeatability of peak potential* (RSD%)	0.57	0.12
Reproducibility of peak current** (RSD%)	1.89	1.92
Reproducibility of peak potential** (RSD%)	0.605	0.616

* Mean values represent four different CND standards
** Between-day reproducibility was determined from four different runs over a one-week period

Table 3: Assay results from CND tablets (Atacand®) and mean recoveries in spiked tablets

Labelled claim (mg)	16.00	16.00
Amount found (mg)[a]	16.02	15.90
RSD %	0.43	1.05
Bias %	−0.125	0.625
t-test of significant	$t_{theoretical}$: 2.31	$t_{calculated}$: 0.171
F-test of significant	$F_{theoretical}$: 5.63	$F_{calculated}$: 0.110
Added (mg)	12.00	12.00
Found (mg)[a]	11.939	11.936
Recovery %	99.49	99.47
RSD % of recovery	1.01	1.08
Bias %	0.508	0.533

[a] Each value is the mean of five experiments.

Precision, accuracy, repetability and reproducibility of both methods were evaluated by within-day and between-day determinations of CND at 8×10^{-5} M (n = 4). The results are shown in Table 2. Sample solutions recorded after 4 days did not show any appreciable change in assay values.

On the basis of the results obtained, the proposed AdSDPV and AdSSWV methods were applied to the direct determination of CND in tablet dosage forms without any sample extraction, filtration or evaporation step and after an adequate dilutions. The proposed methods were successfuly applied for the assay of CND in tablet dosage forms (Table 3). The calculated values of t and F test were less than that of the theoretical t and F values showing that there are no significative differences between both of the proposed methods, according to a 95% of the confidence level (Swartz and Krull 1997; Riley and Rosanske 1996).

Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analysed formulations of CND. Excipients presented in tablet dosage forms did not interfere with the analysis (Table 3). Both the procedures represent a good alternative for quality control, because the preparation of the sample is easy and the excipients do not interfere with the determination and consequently, seperations, evaporation or extraction procedures are not needed.

3. Experimental

3.1. Equipment

Voltammetric measurements were carried out in a BAS® 100 W electrochemical analyser (Bioanalytical system, USA) with a suitable software programme of BAS for totaly automated control of the experiments and data acquisition. A 10 mL capacity BAS cell equipped with a three-electrode system was used. The working electrode was a glassy carbon disc electrode, BAS® model ($\phi = 3$ mm). An Ag/AgCl (KCl 3 M, BAS) reference electrode and platinum wire auxiliary electrode (BAS) was used. Before each measurement, the glassy carbon electrode was polished manually with alumina ($\phi = 0.01$ μm) in the presence of bi-distilled water on a damp smooth polishing cloth (BAS velvet polishing pad). All measurements were done at room temperature. The pH measurements were carried out with a pH meter Model 538 (WTW, Austria) using a combined electrode (glass electrode-reference electrode) with an accuracy of ± 0.05 pH.

For analytical applications, the following parameters were employed:
DPV: pulse amplitude, 50 mV; pulse width, 0.05 s; scan rate, 20 mVs^{-1}.
AdSDPV accumulation time, 90 s; accumulation potential, 1450 mV; 50 mV pulse amplitude; scan rate 20 mVs^{-1} and quiet time, 10 s.
SWV: pulse amplitude 25 mV; frequency, 15 Hz, potential step 4 mV.
AdSSWV: pulse amplitude 25 mV; frequency, 15 Hz, potential step 4 mV; accumulation time, 90 s, accumulation potential 1450 mV, quiet time, 10 s. Constant stirrer speeds, 600 rpm for AdSSWV and 1000 rpm for AdSDPV were used.

3.2. Reagents

Candesartan cilexetil and its pharmaceutical dosage form Atacand® tablets were kindly provided by AstraZeneca Pharm. Ind. (Istanbul, Turkey). Model compounds omeprazol, lansoprazol were kindly supplied from Eczacıbaşı Pharm. Ind.; losartan and valsartan were also kindly provided by Merck Sharp & Dohme and Novartis Pharm. Ind, respectively. All chemicals for preparation of buffers and supporting electrolytes were of analytical reagent grade (Merck or Sigma).

Stock solutions of CND (1×10^{-3} M) and all other stock solutions were prepared in acetonitrile and kept in the dark in a refrigerator.

The working solutions were prepared by serial dilution of the stock solution with selected supporting electrolytes ($1 \times 10^{-5} - 1 \times 10^{-4}$ M) and contained 30% acetonitrile. As supporting electrolytes sulphuric acid (0.1 M and 0.5 M), phosphate buffer (0.2 M, pH 3.05–9.00), acetate buffer (0.2 M, pH 3.5–5.7) and Britton-Robinson buffer (0.04 M, pH 2–11) were used.

The calibration curves for AdSDPV and AdSSWV were constructed by plotting the peak current against the sample concentration. The precision and accuracy of the techniques are found in a quantitative fashion by the use of Bias % (relative error).

The ruggedness and precision were determined within day (n = 4) and between days (n = 4). Relative standard deviations were calculated to check the ruggedness and precision of the method.

All solutions were protected from the sunlight and used within 24 h to avoid decomposition. However, voltammograms of the sample solutions recorded after 96 h after preparation did not show any appreciable change in assay values.

3.3. Tablet assay

Ten Atacand® tablets (each tablet contains 16 mg CND) were weighed and ground to a homogeneous fine powder in a mortar. A weighed portion of this powder equivalent to 10^{-3} M of CND was transferred to a 50 mL calibrated flask and completed to volume with acetonitrile. The contents of the flask were sonicated for 10 min, to ensure dissolution of the drug. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with acetonitrile: phosphate buffer solutions at pH 5.03 in order to obtain a final solution of 30 : 70 acetonitrile : phosphate buffer. These solutions transferred to a 10 mL voltammetric cell and both the AdSDPV and AdSSWV were recorded as in pure CND.

The amount of CND per tablet was calculated using the related linear regression equations obtained from the calibration curve of pure CND.

3.4. Recovery experiments from tablets

In order to see whether the tablet excipients show any interference with the analysis, known amounts of the pure compound were added to the tablet formulations of CND. The recovery experiments also showed to the accuracy of the proposed method. For this procedure, known amounts of pure compound were added to an earlier analyzed tablet formulation of CND. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of pure drug.

References

Cagigal E, Gonzalez L, Alonso RM, Jimenez RM (2001) pKa determination of angiotensin II receptor antagonists (ARA II) by spectrofluorimetry. J Pharm Biomed Anal 26: 477–486.

Gonzalez L, Alonso RM, Jimenez RM (2000) A high performance liquid chromatographic method for screening angitonsin II receptor antagonists in human urine. Chromatographia 52: 735–740.

Gonzalez L, Lopez JA, Alonso RM, Jimenez RM (2002) Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr A 949: 49–60.

Hillaert S, De Beer TRM, De Beer JO, Van den Bossche W (2003) Optimization and validation of a micellar electrokinetic chromatographic method for the analysis of several angiotensin-II-receptor antagonists. J Chromatogr A 984: 135–146.

Hillaert S, Van den Bossche W (2002) Optimization and validation of a capillary zone electrophoretic method for the analysis of several angiotensin-II-receptor antagonists. J Chromatogr A 979: 323–333.

Matsumori A (2003) Efficacy and safety of oral candesartan cilexetil in patients with congestive heart failure. Eur J Heart Failure 5: 669–677.

Radi A (2003) Anodic voltammetric assay of lansoprazole and omeprazole on a carbon paste electrode. J Pharm Biomed Anal 31: 1007–1012.

Riley CM, Roosanske TW (1996) Development and validation of analytical methods. Elsevier Science Ltd, New York.

Stenhoff H, Langerstrom PO, Andersen C (1999) Determination of candesartan cilexetil, candesartan and a metabolite in human plasma and urine by liquid chromatography and fluorimetric detection. J Chromatogr B 731: 411–417.

Swartz ME, Krull IS (1997) Analytical development and validation. Marcel Dekker, New York.