ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
24 (2001) 413–419

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Stripping voltammetric determination of indapamide in serum at castor oil-based carbon paste electrodes

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Received 21 February 2000; received in revised form 30 June 2000; accepted 25 July 2000

Abstract

The diuretic drug indapamide has been characterized voltammetrically at carbon paste electrodes by means of cyclic and differential pulse voltammetry. An adsorptive stripping method at carbon paste electrode modified with castor oil for trace determination of indapamide was described. A study of the variation of the peak current with solution variables such as pH, ionic strength, concentration of indapamide, possible interference, and instrumental variables such as scan rate, pulse amplitude, preconcentration time, accumulation potential, paste composition has resulted in the optimization of the oxidation signal for analytical purposes. By anodic stripping differential pulse voltammetry, the calibration plot was linear in the range 5×10^{-8}–1×10^{-7} M with a detection limit of 5×10^{-9} M at carbon paste electrode modified with castor oil in pH 4.0. The preconcentration medium-exchange approach was utilized for selective determination of indapamide in spiked serum. A detection limit of 15 ng ml^{-1} was obtained for dilute serum sample after 3 min accumulation and medium-exchange procedure.

Keywords: Indapamide; Carbon paste electrodes; Cyclic voltammetry; Differential pulse voltammetry; Serum analysis

1. Introduction

The most sensitive electrochemical procedures for the determination of trace concentrations of various pharmaceutical compounds have conventionally employed a two-step approach consisting of an initial preconcentration step during which the analyte is allowed to accumulate at the electrode surface under carefully controlled conditions and a subsequent measurement in which the accumulated analyte is then stripped-off and determined by a voltammetric method. This preconcentration/measurement sequence forms the basis of all of the so-called stripping techniques, which permits determination of electroactive compounds at very low concentration [1]. Carbon paste electrodes CPEs are considered to be one of the most useful basis for the selective accumulation of lipophilic organic analyte at electrode surface prior to voltammetric measurement [2]. It has been speculated that the extent of lipophilic preconcentration may be influenced by the nature of the pasting liquid or the paste additives [3–10]. Nujol, as a paste liquid in CPEs provides an excellent baseline and low residual current but it is poor solvent for many classes of organic com-

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PII: S0731-7085(00)00457-X

pounds. Kauffmann and co-workers have used lipid-modified carbon paste electrodes to determine a range of pharmaceuticals, including adriamycin, epirubicin and promethazine [3], antitumor drugs celiptium [4] and marcellomycin [5]. The lipid layer extracts the hydrophobic organic compounds from the contacting solution, while hindering the transport of hydrophilic molecules. The increased sensitivity associated with the preconcentration step is therefore accompanied by high degree of selectivity. Similarly, Lipid coated electrodes have been used to preconcentrate and quantify tricyclic antidepressants desimpramine, imipramine and trimipramine [6,7]. Carbon paste electrodes modified with fatty acids has been used for the sensitive and selective determinations of several cephalosporin antibiotics [8], folic acid [9] and the anti-inflammatory drugs piroxicam and tenoxicam [10]. Recently we use the castor oil as a new pasting liquid in carbon paste electrode for the sensitive and selective determination of the anti-inflammatory drug indomethacin [11]; based on the fact that indomethacin is readily dissolved in castor oil. Apparently, castor-oil as a binder can enhance the accumulation of several lipophilic compounds. Castor oil is a triglyceride of fatty acids (ricinoleic, oleic, linoleic, stearic and palmitic acids) and each acid can serve as a lipophilic modifier with high extractive capacity with a certain measure of selectivity towards a specific lipophilic compound. A complementary work appears to be necessary in order to evaluate how this new castor electrode fit into the scene of electroanalysis of lipophilic organic analytes.

Indapamide {4-chloro-*N*-(2-methyl-1-indolinyl)-3-sulfamolybenzamide} is a mild diuretic and antihypertensive agent The presence in the indapamide molecule of both a lipid-soluble methylindoline moiety [12] and oxidizable amide group make indapamide an interesting candidate for an anodic extractive stripping voltammetric method for analysis at castor oil modified carbon paste electrodes. The work presented here shows that the castor oil has a high affinity towards the lipophilic indapamide molecules but effectively rejected the anionic species (ascorbic or uric acid). This property has been exploited for developing a new method for determining indapamide at trace level in serum samples.

At present, only few studies dealing with diuretics electrochemical behavior have been reported. Voltammetric analyses at a carbon paste electrode have been described for bumetanide [13], xipamide [14] and indapamide [15]. A differential pulse adsorptive stripping voltammetry of diuretic torsamide at hanging mercury drop electrode have been reported [16].

2. Experimental

2.1. Apparatus

Cyclic voltammetric experiments were performed with an Oxford portable potentiostat in conjunction with Philips PM-8043 X-Y recorder. Differential pulse voltammograms were recorded on a Sargent-Welch (model 4001) voltammetric analyzer. A three-electrode cell was employed incorporating a Nujol-based CPE (Nj-CPE) or a castor oil-based CPE (Ct-CPE), an Ag/AgCl/(3 M KCl) reference electrode and a glassy carbon counter electrode.

Mass transport was achieved with a Sargent-Welch magnetic stirrer and 1 cm long stirring bar. A stirring rate of 400 rev min^{-1} was used in all studies.

All pH measurements were made with a SCHOTT GerÄte digital pH-meter with a glass combination electrode.

The paste was prepared by thoroughly hand mixing 1.2 g of graphite powder (Aldrich 1–2 μm) with 0.8 g of the Nujol (Sigma) or castor oil (ADWIC, Egypt) in an agate mortar with pestle. The paste was packed into the well of a Teflon sleeve of homemade electrode (3.5 mm, i.d. 2 mm deep). A platinum wire embedded in the paste provided electrical connection. The paste was smoothed on a clean paper.

2.2. Reagents

A stock solution of 1×10^{-3} M indapamide was prepared fresh daily in pure methanol. Britton-Robinson buffers (acetic, phosphoric and

boric acids, all at 0.04 M; pH adjusted with 0.2 M NaOH) were used as supporting electrolytes. All solutions were prepared from AnalaR-grade reagents in doubly distilled water.

2.3. Procedure

The preconcentration step was performed by immersing the carbon paste electrode in a stirred 20 ml sample solution for a given period of time, either at open circuit potential or at a potential range from -0.3 to $+0.3$ V. The stirring was then stopped and after a delay period of 15 s to settle the solution and decrease the background current, cyclic or differential pulse voltammogram was recorded in the anodic direction. In experiments involving medium-exchange, the electrode was removed from the sample (after accumulation), rinsed slightly with water, and re-immersed in a second cell containing an electrolytic blank solution, while the voltammetric scan was initiated. The remaining accumulated species was removed from the surface by holding the electrode at $+1.0$ V for 2 min. A renewed carbon paste surface was used for each measurement.

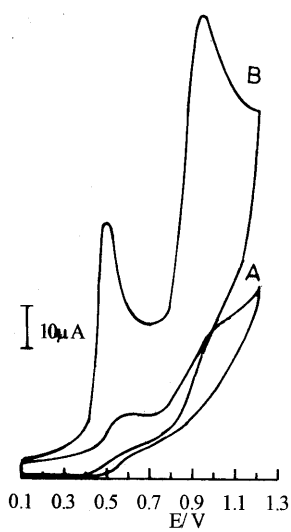

Fig. 1. Cyclic voltammograms for 2.5×10^{-5} Mindapamide in Britton-Robinson buffer pH 4.0; at Nj-CPE (A); and Ct-CPE (B); scan rate: 50 mV s^{-1}

2.3.1. Determination in serum

For determination of indapamide in serum, the preconcentration /medium exchange/voltammetry scheme was adopted. For calibration graph, serum (500 μl) samples containing the drug in a range of 25–300 ng ml^{-1} were mixed thoroughly with 9.5 ml of 0.2 M perchloric acid by sonication. The solution was stirred at 400 rpm at open circuit conditions, and the modified electrode was immersed for 3 min (preconcentration step). The electrode was then washed with water, dried, and placed in the measurement cell containing Britton-Robinson buffer, pH 4.0 (20 ml) and the differential pulse voltammogram was recorded with pulse amplitude of 25 mV and sweep rate of 10 mV s^{-1} between 0.0 and 1.2 V.

3. Results and discussion

The anodic cyclic voltammogram for the oxidation of indapamide in Britton-Robinson buffer (pH 4.0) at Nj-CPE and Ct-CPE are shown in Fig. 1. In the forward scan, two anodic peaks owing to the oxidation of amide group is observed. In the reverse sweep no cathodic peak is observed which indicates that the indapamide oxidation is irreversible. It is clear that the characteristic of the voltammetric response depends on the composition of the electrode. At Nj-CPE the oxidation peaks observed were broad in nature with peak potentials ≈ 50 mV more positive in comparison to Ct-CPE. The Ct-CPE gives almost a 4-fold increase in peak current compared to Nj-CPE. This may be connected to the facile oxidation of the accumulated drug. Differences between carbon pastes in the oxidation of organic compounds have been reported by Kauffmann et al. [5].

As shown in Fig. 2 differential pulse voltammogram of indapamide exhibits two distinguishable anodic peaks at pH 4.0 corresponding to the cyclic voltammetric peaks. Differential pulse voltammograms of the modified electrode recorded at different pulse amplitude (10–75 mV) and potential scan rate (2–20 mV s^{-1}) shows that the two peaks increase, broaden and shift to lower potential values on increasing the amplitude or

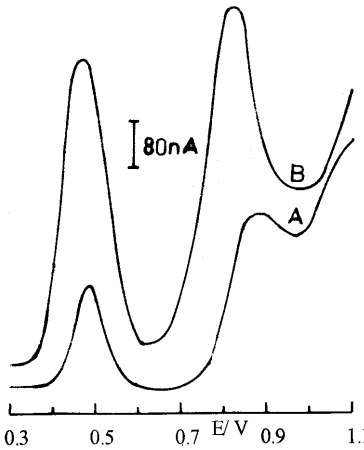

Fig. 2. Differential pulse voltammograms of 1×10^{-6} Mindapamide in Britton-Robinson pH 4.0 at Nj-CPE (A); and Ct-CPE (B); scan rate: 10 mV s^{-1}; pulse amplitude 25 mV.

the potential scan rate. The waveform employing amplitude of 25 and 10 mV s^{-1} was used in all subsequent work as a compromise between sensitivity, speed and resolution.

The effects of the potential scan rate ν on the peak current i_{p1} and peak potential E_{p1} was studied after 3 min preconcentration time. Linear sweep voltammetry at different scan rates, ν (5–200 mV s^{-1}), showed that the peak current i_{p1} was proportional to square root of scan rate $\nu^{1/2}$ at Ct-CPE, as predicted for a diffusion-controlled regime. This may be the diffusion of dissolved molecules from the oil layer to the graphite particle surface. Moreover, E_p shifted to more positive potentials when the scan rate increased which confirm the irreversibility of the oxidative process. The peak current i_{p1} at Ct-CPE was found to gradually decrease in the subsequent scans indicating that the extractive accumulation and the redox reaction are followed by slow diffusion of the reaction product from the electrode interior back to the interface [17].

The effect of pH on the oxidation of indapamide at Ct-CPE and Nj-CPE was studied over the pH range 2.0–12.0 at the same concentration (1×10^{-6} M) by means of linear sweep voltammetry. At both electrode indapamide yields two main irreversible oxidation processes, which shifts towards less positive potentials as the pH increased. The E_{p1} versus pH plot exhibits two linear intervals with break approximately at pH 7.0. The value of $\Delta E_p/\Delta$pH over the pH range 2.0–7.0 was close to -60 mV. At pH > 7.0 the process is nearly independent of pH. The peak current is also dependent on the pH, implying the involvement of protons in the current-limiting electrode process. The peak current (i_{p1}) has its maximum value at pH 4.0, which was used in the subsequent examination of other dependencies.

The pH of the solution has also a marked effect on the preconcentration step, as shown by the stripping current peaks recorded in the blank electrolyte (pH 4.0) after 3 min accumulation from Britton-Robinson buffers over pH range 2.0–12.0. The best accumulation is attained at pH 4.0, which was selected for accumulation step. This pH value was also recommended for the measurement step.

The influence of ionic strength on the efficiency of the accumulation of 1×10^{-6} M indapamide solution was studied at Ct-CPE. The ionic strength was varied by changing the NaCl from 0.001 to 0.05 M in Britton-Robinson buffer of pH 4.0. The results showed that increasing ionic strengths were found to be of a less significance on the degree of accumulation. This indicates that the process responsible for accumulation of the drug at the electrode surface is not electrostatic in nature. The predominance of hydrophobic/hydrophilic interactions between the electrode surface and the drug may, therefore, be considered. The stripping peak remained almost constant; for this reason the ionic strength recommended for analytical purposes is given by the Britton-Robinson buffer (0.04 M).

The effect of accumulation potential on the extraction efficiency was also investigated at a potential range from -0.3 to $+0.3$ V or at open circuit potential following 3 min preconcentration from 1×10^{-6} M indapamide solution at pH 4.0. The stripping peak currents at the Ct-CPE surface appear to be fairly independent of the accumulation potential. This may be due to the non-faradaic nature of the extraction process. Considering these data, open circuit condition was selected for further study.

Fig. 3 shows the dependence of the peak heights on accumulation time for 5×10^{-7} mol dm^{-3} indapamide in Britton-Robinson buffer of pH 4.0 for (A) Nj-CPE; and (B) Ct-CPE. For Ct-CPE, the peak current is proportional to accumulation time up 3 min. After this period, the curve bends over indicating saturation of the electrode surface. At Nj-CPE, there was no significant accumulation of indapamide at the electrode surface. The intersection of the graphs with the peak current axis may be attributed to the accumulation the indapamide molecules during the slow scan between the switching potentials. Such nonlinear time profile for Ct-CPE was expected for a preconcentration step of extractive nature [18].

In order for the extractive stripping voltammetry to posses significant analytical utility, it must exhibit linear concentration dependence, which is both well characterized and highly reproducible. The stripping differential-pulse peak current of indapamide (measured from the straight line connecting the side minima), following 3 min preconcentration at open circuit condition, increases linearly with concentration from 5×10^{-8} to 1×10^{-7} M with a slope of 833 nA μM^{-1}. At concentration $> 1 \times 10^{-7}$ M, a curvature of the calibration graph was observed. The curvature presumably indicates the saturation of the electrode surface. The detection limit (estimated as concentration corresponding to the signal to noise ratio of 3) was 5×10^{-9} M. The repeatability of the peak current at new surfaces as measured by the relative standard deviation (RSD) was 4.2% and at same electrode after consecutive accumulation and cleaning step, was 2.2% ($n = 5$).

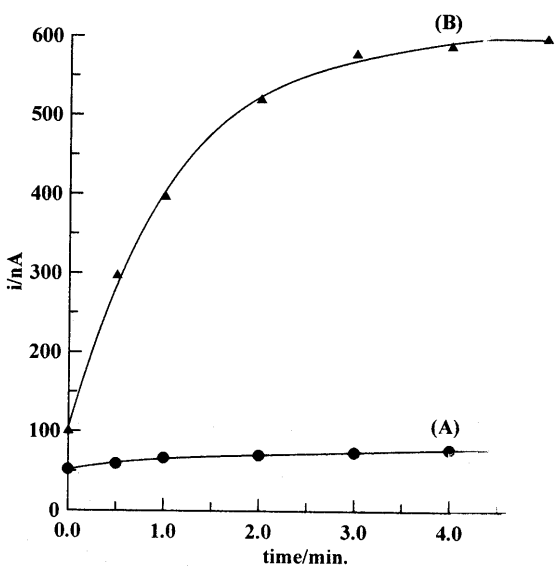

Fig. 3. Effect of accumulation time on peak current for 5×10^{-7} M indapamide at at Nj-CPE (A); and Ct-CPE (B); other conditions as in Fig. 2.

3.1. Analytical application

While the sensitivity enhancement associated with the interfacial accumulation is significant, the main advantage of the method is its inherent selectivity towards the surface-bound analyte. For this purpose the working electrode with the extracted drug was transferred from the complex sample to an electrolytic blank solution between the preconcentration and measurement steps. The selectivity improvement obtained by such medium-exchange procedure is illustrated in Fig. 4. For example, the voltammogram recorded in a solution containing 1×10^{-5} M ascorbic acid shows a large and defined anodic peak, due to the oxidation of ascorbic acid (Fig. 4A). No peak was recorded after medium-exchange experiment (Fig. 4B). The voltammogram recorded in a sample containing 1×10^{-5} M ascorbic acid and 5×10^{-7} M indapamide does not permit quantification of the latter due to the large overlapping ascorbic acid oxidation peak (Fig. 4C). Using medium-exchange procedure, in contrast, the ascorbic acid interference is eliminated, thus allowing convenient quantitation of indapamide (Fig. 4D). Similarly, the addition of uric acid (not shown) did not affect the determination of indapamide after medium-exchange.

Fig. 5 illustrates the preconcentration/medium exchange/differential pulse voltammetric response to different concentrations of indapamide in serum samples mixed with 9.5 ml perchloric acid (0.2 M), after 3 min accumulation at open circuit condition. The electrode response was linearly related to the indapamide concentration within the range 50–250 ng ml^{-1} according to the regression equation: $\{i_{p1}$ (nA) = 0.7146 C (ng

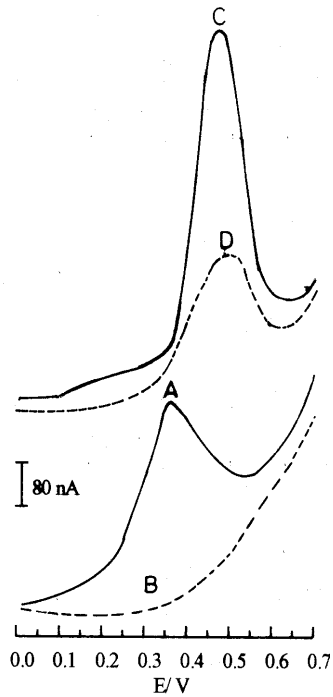

Fig. 4. Linear sweep voltammograms in Britton-Robinson buffer pH 4.0, after 3-min preconcentration at open circuit condition for 1×10^{-5} M ascorbic acid (A and B); 1×10^{-5} M ascorbic acid $+ 5 \times 10^{-7}$ M indapamide (C and D). A and C, direct measurement and B and D, medium exchange.

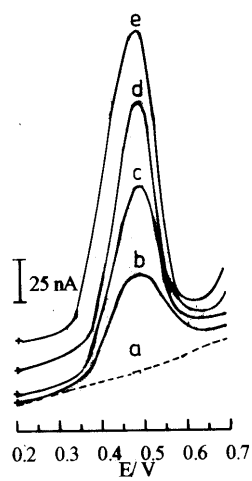

Fig. 5. Adsorptive differential pulse voltammograms obtained after medium exchange for the determination of indapamide in serum samples: (a) blank; (b, c, d and e) serum spiked with increasing concentration of indapamide; (b) 50 ng; (c) 100 ng; (d) 150 ng; and (e) 200 ng per one ml of serum.

$ml^{-1}) + 25.045$, $r = 0.996\}$; standard deviations for slope and intercept of the calibration curve were 0.0396 and 0.0243, respectively. The detection limit was 15 ng ml^{-1}. Extending the accumulation time can lower the detection limit. The detection limit is low enough to reach the level expected in plasma after therapeutic dose [19]. The repeatability of total analytical process was determined from multiple measurement at each of the serum samples ($n = 5$). An average deviation of 3.0% was obtained.

The selectivity of the proposed method was tested in the presence of other sulphonamidic diuretics. The oxidation peak of indapamide proceeded at a potential of 0.50 V at pH 4.0 which is well distinguished from those of xipamide ($E_p =$ 1.15 V), furosemide (1.08 V), bumetanide (0.90 V), clopamide (0.95 V) and toresamide (1.10 V). Therefore the presence of these diuretics did not affect the analysis of indapamide.

In addition, it was found possible to determine indapamide in the presence of their principal metabolites, glucuronide and sulfate conjugates of indapamide. The hydroxylation of indoline ring with conjugation may be the major route of metabolism [19]. The hydroxylation and conjugation render the indoline ring moiety hydrophilic [20]. The castor oil has a high affinity towards the lipophilic indapamide molecules but effectively rejected the hydrophilic metabolic species. Indapamide can therefore be determined with selectivity when it is present in a mixture with their metabolic analogues.

In conclusion, the present work demonstrates that highly sensitive electrochemical measurement of the drug indapamide is feasible utilizing its extraction on to castor oil-based carbon paste electrode. Short preconcentration period permit convenient measurement of nanomolar concentrations. The medium-exchange protocol (involving transfer of the electrode to blank solution between the accumulation and measurement steps) further enhance the selectivity by precluding the contribution from non-accumulated species with similar redox potential in biological fluids with no sample pre-treatment.

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