Voltammetric Determination of Labetalol in Pharmaceuticals and Spiked Human Urine

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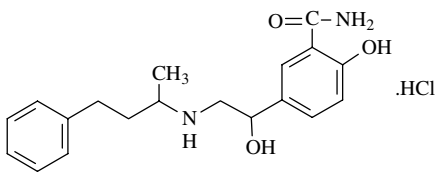
The electrochemical oxidation of labetalol was investigated by cyclic, linear sweep and differential pulse voltammetry at carbon paste electrode in Britton—Robinson buffers over the pH range 2.0—10.0. For analytical purposes, a well-defined adsorption-controlled anodic peak was obtained in Britton—Robinson buffer at pH 2.0. By anodic adsorptive linear sweep and differential pulse voltammetry, linear calibration plots were obtained in the ranges of 2.5×10^{-6}—1.0×10^{-5} mol dm^{-3} and 2.5×10^{-8}—1.0×10^{-5} mol dm^{-3} for both techniques, respectively. Detection limits were found 1.0×10^{-6} mol dm^{-3} for LSV and 1.0×10^{-8} mol dm^{-3} for DPV. Based on this study, two simple, rapid, selective, and sensitive voltammetric methods were developed for the determination of labetalol in tablet dosage form. The preconcentration/medium exchange/voltammetry approach was applied for the drug determination in spiked human urine.

Labetalol hydrochloride, 2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]benzamide hydrochloride, is an adrenergic β-receptor blocking agent used in the treatment of hypertension, which exhibits both α- and β-adrenoceptor blocking activity [1, 2], and because of its use as doping agent in sports, this drug has been added to the list of forbidden substances issued by the International Olympic Committee. Therefore, the development of an analytical method sensitive and selective enough for determining labetalol in both pharmaceutical and biological samples is of great importance. Several analytical methods have been developed to determine the concentrations of labetalol in biological fluids and pharmaceutical preparations based on spectrophotometry [3—8], spectrofluorometry [8, 9], potentiometry [10], thin-layer chromatography (TLC) [11, 12], high-performance liquid chromatography (HPLC) with UV [13] and electrochemical detection (ED) [14—16], liquid chromatography (LC) with mass spectrometric (MS) detection [17, 18], gas chromatography (GC) [19], micellar liquid chromatography [20], capillary electrophoresis [21, 22], and capillary liquid chromatography [23]. Although these methods have been successfully employed, they require long and tedious steps for the sample pretreatment.

Electroanalytical techniques have been used for the determination of a wide range of pharmaceuticals with the advantages that there is, in most instances, no need for derivatization, and that these methods are less sensitive to matrix effects than other analytical techniques [24].



The present work is concerned with a study of the voltammetric behaviour of labetalol at carbon paste electrode. Labetalol could be adsorbed on the carbon paste electrode and this phenomenon was put to analytical advantage in the design of an adsorptive anodic stripping voltammetric methods for the determination of labetalol in pharmaceutical and spiked human urine samples.

EXPERIMENTAL

Labetalol hydrochloride and its pharmaceutical dosage form were kindly provided by Glaxo Smith Kline. All the chemicals used were of reagent grade quality (Merck or Sigma) and were employed without further purification. Labetalol stock solution (1.0×10^{-3} mol dm^{-3}) was prepared daily by direct disso-

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lution in water. The working solutions under voltammetric investigations were prepared by dilution of the stock solution with selected supporting electrolyte. Britton—Robinson buffers of pH 2.0—10.0 were used as the supporting electrolytes.

The voltammetric measurements were carried out using a computer-driven AEW2 Analytical Electrochemical Workstation with ECprog3 Electrochemistry software (Sycopel, England) in combination with C-2 stand with a three-electrode configuration: a carbon paste (BAS Model MF-2010, 3 mm diameter) working electrode, an Ag/AgCl/3 M-KCl (BAS Model MF-2063) reference electrode, and a platinum wire (BAS Model MW-1032) counter electrode. Microcal Origin (v.5.10) software was used for the transformation of the initial signal. A CG 808 (Schott Geräte, Germany) digital pH-meter with glass combination electrode served to carry out the pH measurements.

Voltammetric analyses were carried out in 5.0 cm^3 of BR buffer. The accumulation potential (usually open circuit condition) was applied for a selected time while the solution was stirred at 2000 min^{-1}. The stirrer was then stopped, and after 5 s rest period, a linear sweep or a differential-pulse voltammogram was recorded between +0.3 V and +1.2 V. After background voltammograms had been recorded, aliquots of the drug standard were introduced and the adsorptive stripping cycle was repeated using a new electrode surface. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All data were obtained at ambient temperature.

Procedure for Tablets Assay

Ten tablets of Trandate^{TM}, with declared amount of 100 mg of labetalol per tablet, were weighed and then crushed into a fine powder in a mortar. A suitable amount of this powder was accurately weighed, dissolved in deionized water and sonicated for 10 min. Aliquots of the clear supernatant liquor were added to the Britton—Robinson buffer of pH 2.0 in order to prepare sample solutions within the calibration range. The voltammetric procedure was continued as described above. The nominal content of the tablet was calculated using the corresponding regression equations of previously plotted calibration plots.

Procedure for Urine Assay

For the determination of labetalol in spiked human urine samples, the preconcentration/medium exchange/voltammetry scheme was adopted. A 1.0 cm^3 urine sample, spiked with increasing concentration of labetalol (1.0—50.0 $\mu g\ cm^{-3}$) was mixed with 9.0 cm^3 of Britton—Robinson buffer of pH 2.0. The mixture was transferred into the voltammetric cell. The solution was stirred at 2000 min^{-1}, and the carbon paste electrode was immersed in the solution for 5 min at open circuit condition (preconcentration step). The electrode was then washed with water and placed in the measurement cell containing 5.0 cm^3 of Britton—Robinson buffer of pH 2.0 and the differential pulse voltammogram was recorded following the optimized conditions. The electrode was renewed by series of cyclic anodic scans in a blank electrolyte solution. The electrode was then ready for use in a next measurement cycle. The peak current (μA) against the concentration of the drug in urine ($\mu g\ cm^{-3}$) was adjusted to a linear regression to derive a calibration curve. Quantification was achieved by referring to the calibration curve or to the regression equation.

RESULTS AND DISCUSSION

The oxidation of labetalol at carbon paste electrode was studied in aqueous medium in the pH range 2.0—10.0 using Britton—Robinson buffers as supporting electrolytes by means of cyclic voltammetry. Labetalol gave one anodic peak at pH $\leq$ 5.0. Above pH 5.0 this oxidation peak was split and ill-defined two waves were observed. The cyclic voltammogram for the oxidation of 5.0×10^{-5} mol dm^{-3} labetalol in Britton—Robinson buffer (pH 2.0) at carbon paste electrode at scan rate of 100 mV s^{-1} is shown in Fig. 1. In the forward scan, a single anodic peak is observed with no cathodic peak in the reverse sweep, which indicates that the labetalol oxidation is irreversible. The effects of the potential scan rate between 10 mV s^{-1} and 300 mV s^{-1} on the peak potential and peak current of labetalol were evaluated. The peak potential moves to more positive potentials with increasing the scan rate, which confirms the irreversibility of the process. The linear increase in the oxidation peak current with the scan rate showed that the electrode reaction is predominantly an adsorption-controlled process. A plot of $\log(i_p/\mu A)$ *vs.* $\log(\nu/(\text{mV s}^{-1}))$ gave a straight

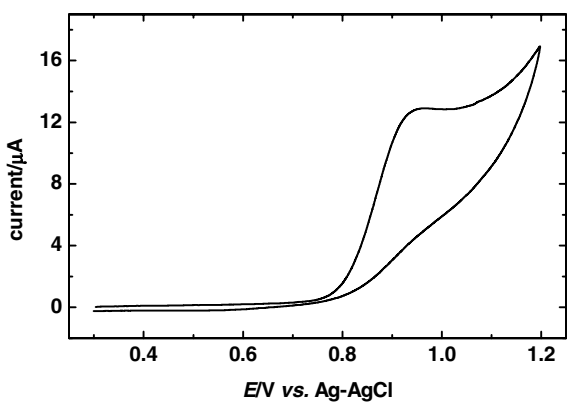

Fig. 1. Cyclic voltammograms for 5.0×10^{-5} mol dm^{-3} labetalol in Britton—Robinson buffer of pH 2.0 at carbon paste electrode, scan rate = 100 mV s^{-1}.

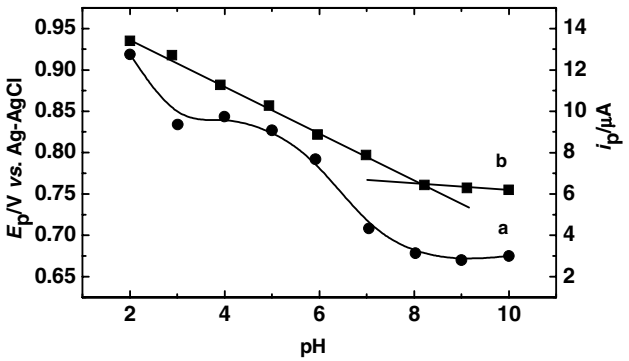

Fig. 2. Effect of pH on *a*) peak current and *b*) peak potential for 5.0×10^{-5} mol dm^{-3} labetalol solutions in Britton—Robinson buffer found out by means of linear sweep voltammetry at a carbon paste electrode, scan rate = 100 mV s^{-1}.

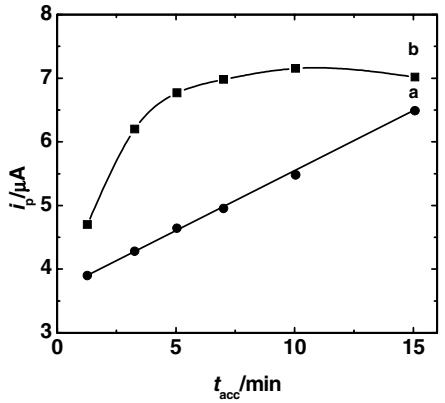

Fig. 3. Effect of the accumulation time on the peak current for *a*) 1.0×10^{-5} mol dm^{-3} and *b*) 5.0×10^{-6} mol dm^{-3} labetalol solutions in Britton—Robinson buffer of pH 2.0. Other conditions as in Fig. 2.

line with a slope of 1.11 (correlation coefficient 0.996). Slopes of 0.5 and 1.0 are expected for ideal reactions of solution and surface species, respectively [25].

The pH increase generated a decrease in peak current (i_p), which reaches its maximum value at pH 2.0 (Fig. 2, curve *a*), selected as optimum value to carry out quantitative determination of labetalol. The anodic peak potential is shifted to less positive values by increasing the pH with slope of 28 mV/pH-unit up to pH 8.0, then it remains practically pH-independent (Fig. 2, curve *b*). The intersection observed in the plot may be attributed to the acid-base constant of labetalol [26]. The possible sites of oxidation at labetalol molecule include the amide moiety, the phenolic group, and/or the secondary aliphatic amine group. Comparative study on salicylamide which is structurally related to labetalol was realized by cyclic voltammetry at the carbon paste electrode, as a function of pH, in order to identify the oxidation mechanism of labetalol. Taking into account that the cyclic voltammograms of salicylamide closely matched those of labetalol, we assumed that the oxidation process may be occurring on the amide and phenolic groups of salicylamide moiety of the molecule.

The dependence of the peak current, developed in Britton—Robinson buffer of pH 2.0, on accumulation time was investigated for two concentration levels: *a*) 5.0×10^{-6} mol dm^{-3} and *b*) 1.0×10^{-5} mol dm^{-3} labetalol, by means of linear sweep voltammetry. As shown in Fig. 3, the plot of i_p *vs.* t_{acc} for a 5.0×10^{-6} mol dm^{-3} solution was linear over the entire range of accumulation time tested (slope = 0.185 μA min^{-1}; correlation coefficient $r = 0.998$). For a 1.0×10^{-5} mol dm^{-3} solution, a full surface coverage is established after accumulation time $t_{acc} = 5$ min. Thus, the accumulation time of choice will be dictated by the sensitivity needed.

The effect of the accumulation potential on peak intensity was also evaluated for 5.0×10^{-6} mol dm^{-3} labetalol solution following 5 min accumulation time over the range 0.0 to +0.7 V and at open circuit potential. The peak stripping current is independent of accumulation potential, thus the adsorption stage was carried out at open circuit potential.

The quantitative evaluation is based on the dependence of the peak current on labetalol concentration. Under chosen conditions, the peak currents increased linearly with increasing amounts of labetalol by linear sweep and differential pulse voltammetry. The characteristics of the calibration plots are listed in Table 1. The detection (LOD) and determination limits (LOQ) of the procedures are also shown in Table 1, being estimated as: LOD = $3S_{y/x}/b$ and LOQ = $10S_{y/x}/b$ [27], respectively, where $S_{y/x}$ is the standard deviation of y-residuals and b is the slope of the calibration plot.

The repeatabilities of peak potential and peak current were tested by repeating four experiments on 5.0×10^{-6} mol dm^{-3} labetalol for both methods. The relative standard deviations were calculated to be 0.29 % and 0.18 % for peak potential and 0.95 % and 0.35 % for peak current using LSV and DPV techniques, respectively. The reproducibilities of peak potential and peak current were also tested by repeating four experiments on four different days with 5.0×10^{-6} mol dm^{-3} labetalol for both methods. The relative standard deviations were calculated to be 0.47 % and 0.26 % for peak potential and 1.34 % and 1.41 % for peak current using LSV and DPV, respectively.

The developed LSV and DPV techniques for labetalol were applied to Trandate^{TM} tablet. The labetalol content of commercially available tablets, prepared as described in Experimental, was determined directly using the LSV and DPV techniques. There is no need for any extraction procedure before voltammetric analysis. The amount of labetalol in tablets was calculated by reference to the calibration plot. The results obtained (Table 2) were statistically comparable with those given using the official method [28]. Trandate^{TM} tablets also contain the inactive ingredients: corn starch, FD&C Yellow No. 6, hydroxypropyl-

Table 1. Characteristics of Labetalol Calibration Plots in Britton—Robinson Buffer of pH 2.0

	LSV	DPV
Linearity range/(mol dm^{-3})	2.5×10^{-6}—1.0×10^{-5}	2.5×10^{-8}—1.0×10^{-5}
Slope/(μA/(mol dm^{-3}))	0.238	3.645
Intercept/μA	4.272	0.093
Correlation coefficient	0.999	0.999
RSD of slope	0.005	0.078
RSD of intercept	0.027	0.348
LOD/(mol dm^{-3})	1.0×10^{-6}	1.0×10^{-8}
LOQ/(mol dm^{-3})	3.3×10^{-6}	3.3×10^{-8}

Table 2. Application of the Proposed Voltammetric Method to the Determination of Labetalol in Trandate^{TM} Tablets (100 mg/tablet)

	Proposed method		Reference method [27]
	LSV	DPV	
Labeled amount/mg	100	100	100
n	5	5	5
$\overline{x}$/%	99.72	99.80	99.61
s/%	0.13	0.15	0.18
CL/%	±0.16	±0.19	0.12
t-test of significance	1.11	1.91	$\{t(P = 0.05)\} = 2.78$
F-test of significance	1.92	1.44	$\{F(P = 0.05)\} = 6.39$

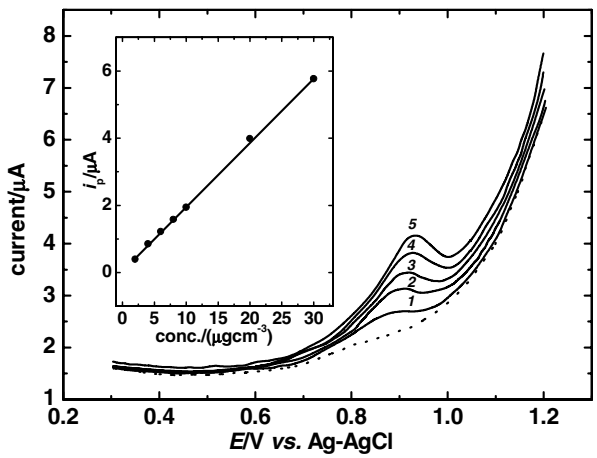

Fig. 4. Differential pulse voltammograms obtained after medium exchange for the determination of labetalol in spiked human urine samples: Dotted line represents the blank; (*1*—*5*) urine spiked with increasing concentration of labetalol: *1.* 2.0 μg cm^{-3}, *2.* 4.0 μg cm^{-3}, *3.* 6.0 μg cm^{-3}, *4.* 8.0 μg cm^{-3}, and *5.* 10.0 μg cm^{-3}. Pulse amplitude 50 mV, pulse width 50 ms, and scan rate 10 mV s^{-1}. Inset is the calibration plot.

Table 3. Application of the Proposed DPV to the Determination of Labetalol in Spiked Human Urine Samples

Added conc.	Found conc.	Recovery
μg cm^{-3}	μg cm^{-3}	%
2.0	2.13	106.50
4.0	4.05	101.25
6.0	5.96	99.33
8.0	8.12	101.50
10.0	10.13	101.30
$\overline{x}$		101.97
S.D.		2.68

methylcellulose, lactose, magnesium stearate, methylparaben, pregelatinized corn starch, propylparaben, talc, and titanium dioxide. These excipients did not interfere with the assay.

Fig. 4 illustrates the preconcentration/medium exchange/differential pulse voltammetric response to human urine samples (1.0 cm^3 each) spiked with increasing concentration of labetalol and mixed with 9.0 cm^3 of Britton—Robinson buffer of pH 2.0 after 5 min accumulation at open circuit condition. The peak current was linearly related to the labetalol concentration within the range 1.0—50.0 μg of labetalol per cm^3 of urine (1.0×10^{-6}—5.0×10^{-5} mol dm^{-3}) according to the regression equation: $i_p/\mu A = 0.052 + 0.191\ C/(\mu g\ cm^{-3})$, $r = 0.998$; standard deviations for slope and intercept of the calibration curve were 0.0370 and 0.002, respectively. The detection limit was 0.7 μg cm^{-3}. The proposed method was then applied to spiked human urine samples. The results listed in Table 3 are satisfactorily accurate and precise.

CONCLUSION

Application of linear sweep and differential pulse voltammetric methods using a carbon paste electrode to pharmaceutical dosage form of labetalol is possible after a simple dilution step. The analyses were performed without any interferences from the excipients in tablets. Developed procedure has also been used for urine samples, with good recoveries obtained at the levels tested. The developed methods are simple, fast, and less-cost tool for labetalol analysis.

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