ELECTROCHEMICAL BEHAVIOR OF MOCLOBEMIDE AT MERCURY AND GLASSY CARBON ELECTRODES AND VOLTAMMETRIC METHODS FOR ITS DETERMINATION

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Electrochemical oxidation and reduction properties of Moclobemide (MCB) were investigated at glassy carbon electrode (GCE) and hanging mercury drop electrode (HMDE). Diffusion-adsorption behavior and some extra electrochemical parameters such as diffusion coefficient, number of transferred electrons and proton participated to its electrode mechanisms on both electrode and surface coverage coefficient were calculated from the results of cyclic voltammetry and square-wave voltammetry. Reversible catalytic hydrogen wave mechanism was proposed at HMDE and single two-electron/two-proton irreversible oxidation mechanism controlled by adsorption with some diffusion contribution at GCE was proposed. Experimental parameters were optimized to develop new, accurate, rapid, selective and simple voltammetric methods for direct determination of MCB in pharmaceutical dosage forms and spiked human serum samples without time-consuming steps prior to drug assay. In these methods, the lowest limit of detection (LLOD) was found to be 0.0235 μM. Methods were successfully applied to determine the MCB content of commercial pharmaceutical preparations and spiked human urine. The methods were found to be highly accurate and precise.

Keywords: Cyclic voltammetry; Electrochemistry; Electron transfer; Stripping voltammetry; Moclobemide.

Moclobemide (MCB) chemically known as [*p*-chloro-*N*-(2-morpholinoethyl)-benzamide] shown in Fig. 1 is a new type of reversible and selective inhibitor of the enzyme monoamine oxidase subtype A (MAO-A). MCB is widely used and prescribed for the treatment of depression as a first benzamide antidepressant. Its inhibition of MAO leads to increased concentrations of the

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central monoamines, particularly noradrenalin and serotonin, and this effect may explain the mechanism of its antidepressant activity[1,2].

Few analytical methods have been described for determination of MCB in pharmaceutical samples or biological fluids. Described methods for MCB assay are based on the use of high-performance liquid chromatographic techniques with ultraviolet (UV)[3–8] and mass spectrometric (MS)[9,10] detection after solid-phase extraction process for human plasma samples. Spectrophotometric method[11] and Moclobemide-selective membrane electrode[12] have also been published for the assay of MCB in pharmecutical samples. Parameters of these methods are summarized in Table I. All chromatographic methods for the quantitation of MCB require tedious and time consuming pretreatment such as solid-phase extraction and require highly sophisticated instrumentation. Spectrophotometric methods are not suitable for determination of drug molecules in biological samples. To our best knowledge up to present time there is no electrochemical study dealing with electrochemical properties of MCB and voltammetric method for the assay of MCB in pharmaceutical formulation and biological samples.

Voltammetric techniques are used for the quantitative determination of a variety of organic and inorganic substances including drug active ingredients and excipients in pharmaceutical dosage forms and their possible metabolites in biological fluids. These techniques are also used to clarify the redox processes realized in various working medium.

Generally, voltammetric methods are sensitive, rapid, and economic when compared with chromatographic methods. It is also possible to determine various electro active species from mixtures by using voltammetric methods if their voltammetric signals are separated enough. Additionally, stripping techniques extends the use of voltammetric methods ensuring lower detection limits. In literature, there are many applications of voltammetric stripping methods to determine environmentally and biologically important substances[13–21].

The present study was designed to investigate the electrochemical reduction behavior of MCB at hanging mercury drop electrode (HMDE) and electrochemical oxidation behavior of MCB at glassy carbon electrode (GCE).

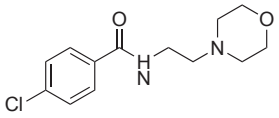


FIG. 1
Chemical structure of MCB

TABLE I
Literature summary for MCB

Method	Medium (column/mobile phase)	Linear range	LOD, LOQ	Application[a]	Ref.
HPLC-UV	Supelcosil LC-18 (120 mm × 4.6 mm, 5 mm), column 3% ammonium ion in water/ethanol and acetonitrile	45.45–500 ng/µl (169.1–1860.53 µM)	LOD: 10.37 ng/µl (38.59 µM)	Auromid tablet Serum	3
HPLC-UV	LiChrospher 60 Rp-select B column (250 mm × 4 mm, 5 µm), 35% of a mixture of acetonitrile/methanol (92:8 v/v) and 65% of 0.25 M sodium acetate buffer, pH 4.5	2.5–1000 ng/ml (0.01–3.7 µM)	LOQ: 2.5 ng/ml (0.01 µM)	Plasma	4
HPLC-UV	Nucleosil 100 C18 analytical column (125 mm × 4 mm, 5 µm), acetonitrile/water (25:75 v/v) adjusted to pH 2.7 with 85% ortho-phosphoric acid	20–2500 ng/ml (0.074–9.30 µM)	LOQ: 10 ng/ml (0.037 µM)	Aurorix tablet Plasma	5
HPLC-UV	Waters XTerra RP18 column (150 mm × 4.6 mm, 5 µm), 10 mM KH_2PO_4, 1% triethylamine (pH 3.9, _____/acetonitrile (83:17 v/v)	0.02–5.00 mg/l (0.074–18.60 µM)	LOD: 0.005 mg/l (0.019 µM)	Auromid tablet Serum	6
HPLC-UV-VIS	ODS column (250 mm × 4.6 mm, 5 µm)	15.6–2000 ng/ml (0.056–7.44 µM)	LOQ: 15.6 ng/ml (0.058 µM)	Aurorix tablet Plasma	7
HPLC-UV	5 mM NaH_2PO_4/acetonitrile/triethylamine (1000:350:10, v/v/v) Nova-pak C8 column and UV detection at 230 nm, acetonitrile/methanol/0.067 M phosphate buffer (pH 2.65)/0.4% triethyalmine	50–2000 ng/ml (0.19–7.44 µM)	LOQ: 10 ng/ml (0.037 µM)	Aurorix tablet Plasma	8
HPLC-MS	C18 column (50 mm × 4.6 mm), methanol gradient 5 mM acetate buffer (pH 3.9)	100–10000 ng/ml (0.37–37.21 µM)	LOQ: 5.7 ng/ml (0.021 µM)	Serum	9
HPLC-MS	Nova-Pak phenyl column (Waters, 150 mm × 32 mm, 4 µm), 0.2 M ammonium formate buffer (pH 3.57) and acetonitrile	0.01–5.2 µg/ml (0.037–19.35 µM)	LOQ: 0.01 µg/ml (0.037 µM)	Plasma	10
Spectro-photometric	Lewis base: MCB, Lewis acid: chloranilic acid (526 nm)	4–36 mg/100 ml (148.8–1339.6 µM)	–	Aurorix tablet	11
Moclobemide-Selective Electrode	Moclobemide-dipicrylamine ion pair liquid membrane electrode in nitrobenzene	1.00–1000 µM	LOD: 0.30 µM	Tablets	12

[a] All applications needed solid-phase extraction prior to HPLC analysis.

Proposing a tentative reaction mechanism was also aimed. Regarding these investigations, it is also aimed to develop rapid, simple and new validated methods for direct determination of MCB in pharmaceutical dosage form and human plasma. For reduction peak at HMDE square-wave voltammetry (SWV), differential pulse voltammetry (DPV), square-wave cathodic adsorptive stripping voltammetry (SWCAdSV), and differential pulse cathodic adsorptive stripping voltammetry (DPCAdSV) were used and for oxidation peak at GCE, square-wave voltammetry (SWV) was applied. Time consuming and expensive extraction procedures are not needed in sample preparation step. Thus, total time required for the analysis of MCB by these new methods is shorter than that of the previously published methods.

EXPERIMENTAL

Apparatus

All voltammetric measurements at HMDE were carried out using a CH-instrument electrochemical analyzer (CHI 760). The three electrode cell system incorporating the hanging mercury drop electrode (HMDE BAS CGME 1108) as a working electrode, platinum wire as an auxiliary electrode (BAS MW-1034) and an Ag|AgCl in 3.0 M KCl solution as a reference electrode (MF-2052 RE-5B) were used in all experiments. Voltammetric measurements at GCE were performed using a BAS 100W (Bio Analytical System, USA) electrochemical analyzer. The three electrode system contained a glassy carbon working electrode (BAS; MF 2012), with a platinum wire counter electrode and Ag|AgCl (3 M KCl) reference electrode (BAS-MF-2052 RE-5B) was used. Before each experiment GCE was polished manually with slurries prepared from 0.01 μm aluminum oxide on a smooth polishing pad (BAS velvet polishing pat), then rinsed with double-distilled water thoroughly.

All pH measurements were made with Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600; produced by Thermo Fisher Scientific) which had been calibrated with pH 4.13 and 8.20 stock standard buffer solutions before measurements. Double-distilled deionized water was supplied from Human Power I+, Ultra Pure Water System (Produced by ELGA as PURELAB Option-S). All measurements were performed at room temperature.

Reagents and Solutions

MCB was purchased from Sigma & Aldrich and Aurorix® tablets (150 mg MCB per tablet) as its pharmaceutical dosage form was purchased from local market in Ankara. All chemicals used were reagent grade.

Stock solutions of MCB (1×10^{-3} M) were prepared in methanol and kept in the dark in a refrigerator. MCB working solutions under voltammetric investigations were prepared by sufficient dilution of stock solution with selected supporting electrolyte and used within 24 h to avoid decomposition. Three different supporting electrolytes, namely phosphate buffer (0.2 M; pH 3.00–7.99), acetate buffer (0.2 M; pH 3.50–5.50), and Britton–Robinson buffer (0.04 M; pH 2.00–12.00) were prepared in double-distilled deionized water.

Procedure

For voltammetric measurements, known volume of MCB solution was pipetted into 10.0 ml selected supporting electrolyte. Voltammetric measurements were realized after purified nitrogen was passed through the cell for 5 min to remove dissolved oxygen. For adsorptive stripping measurements, a selected accumulation potential was then applied to the electrode for a selected accumulation time period, while the solution was stirred at 400 rpm. At the end of the accumulation time, the stirrer was stopped and a 5 s rest period was allowed for the solution to become quiescent. The voltammograms were then recorded by scanning the potential towards the positive direction at GCE for oxidation studies and negative direction at HMDE for reduction studies versus Ag|AgCl (3 M KCl) reference electrode by applying different waveforms and peak current was measured automatically by software.

Preparation of Aurorix® Tablets

Aurorix® tablets were used as pharmaceutical dosage form which contains 150 mg MCB per tablet. Ten Aurorix® tablets were accurately weighed and crushed to a homogeneous fine powder in a mortar and mixed. Approximate weight of one tablet was calculated. A powder sample, equivalent to one tablet was weighed and transferred into the calibrated flask containing about 100 ml of methanol and content of flask was sonicated for 10 min. After standing at room temperature, volume of the flask was completed to 250.0 ml with methanol. Then, to prepare final concentration required sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte for voltammetric studies. Quantitations in all proposed methods were performed by means of the calibration curve method from the related calibration equations.

Preparation of Spiked Human Serum

Drug-free human serum samples obtained from healthy volunteers were stored frozen until assay. After gentle thawing, 2.0 ml of an aliquot volume of serum sample was spiked with MCB in BR buffer to maintain 1×10^{-4} M concentration of MCB in serum and dissolved in acetonitrile to precipitate serum proteins. Then the mixture was vortexed for 25 s and then centrifuged for 10 min at 5000 *g* in order to eliminate serum protein residues and 2.0 ml from supernatant was taken and added into selected supporting electrolytes to attain the total volume of 10.0 ml. Sufficient volume (20, 35, 50, 75, 100, 125, 350, 500, 750, 850, 1000 µl) from this solution was taken and added to voltammetric cell contains 10.0 ml of selected supporting electrolyte. Quantitations were performed by means of the calibration curve method from the related calibration equations.

RESULTS AND DISCUSSION

Electrochemical reduction behavior was studied at HMDE and oxidation behavior was characterized in detail at GCE. In these studies, electrochemical behavior, diffusion and adsorption properties of MCB were studied using cyclic voltammetry (CV) and square-wave voltammetry (SWV).

Electrochemical Behavior of MCB at HMDE and GCE

In CV studies, a single well-defined reduction peak at HMDE at about –1.45 V when pH is 10.0, and oxidation peak at GCE at about 0.9 V when pH is 6.0 was observed (Fig. 2). No peak was observed when only blank BR was scanned at the same conditions. Besides, anodic and cathodic peak intensities increase with increasing concentration of MCB (Fig. 2, insets). It may be concluded that the reduction peak at HMDE and the oxidation peak at GCE should be caused by MCB molecules. As could be seen from Fig. 2a, there is also an anodic peak at reverse scan at HMDE suggesting the reversible nature of electroreduction of MCB. On the other hand, there is no cathodic peak recorded at reverse scan at GCE as could be seen in Fig. 2b suggesting the irreversible nature of electrooxidation of MCB.

To investigate the electrochemical behavior of MCB, firstly influences of potential scan rate on peak current and peak potential were studied. The cathodic peak current ($i_{p,c}$) at HMDE and anodic peak current ($i_{p,a}$) at GCE were investigated for 0.7 mM MCB in the 0.005–1.0 V/s potential scan rate range. As could be seen from Fig. 3a and inset A of Fig. 3a, both anodic and cathodic peak current (in μA) at HMDE were linearly changed with changing scan rate (in V/s). Cathodic peak current at HMDE obeys the relation of $i_{p,c} = 1.1\ v - 0.001$ with $R^2 = 0.9997$ and anodic peak current obtained at reverse scan of cathodic peak obeys the relation of peak $i_{p,a} = 0.9\ v + 0.01$ with $R^2 = 0.9950$. On the other hand, current of anodic oxidation at GCE is

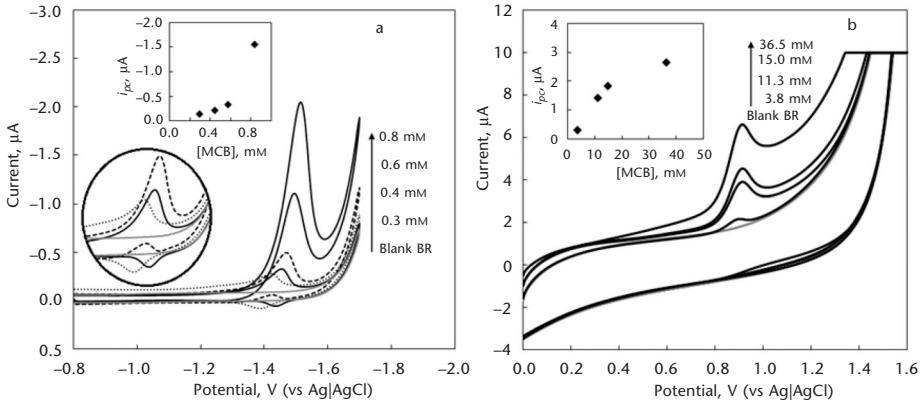


FIG. 2
Cyclic voltammograms of MCB solutions with different concentrations at HMDE in BR of pH 10.0, scan rate 100 mV/s (a) and at GCE in BR of pH 6.0, scan rate 100 mV/s (b). Insets: dependences of peak current on MCB concentration

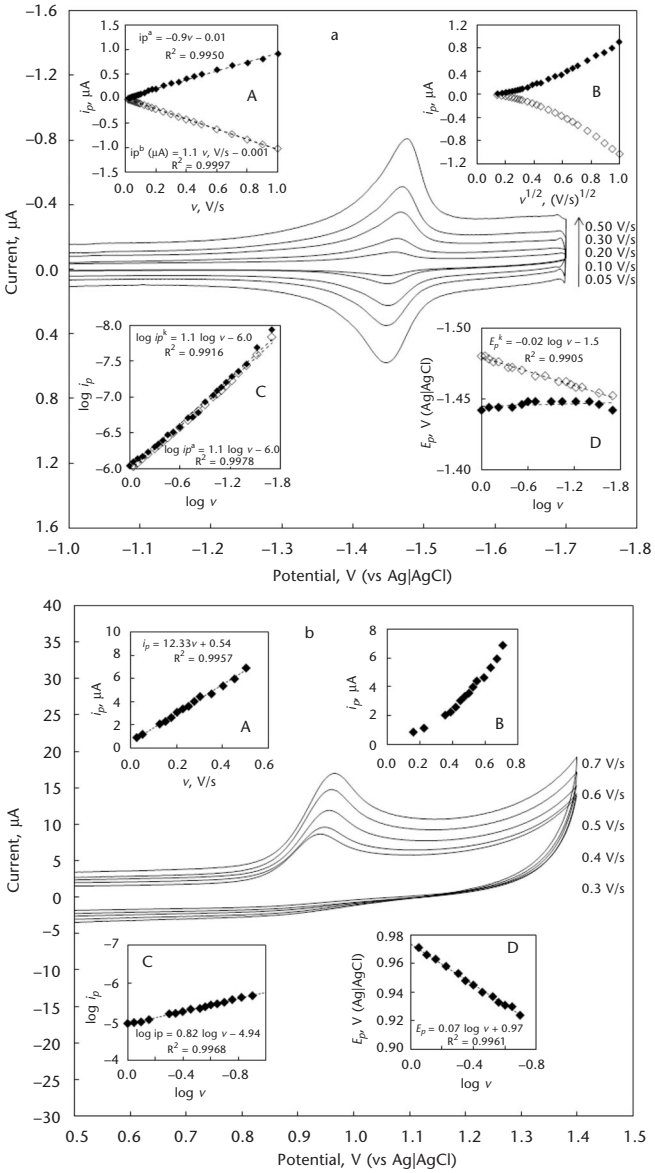


FIG. 3

Influences of scan rate on peak current and peak potential at HMDE in BR of pH 100 for [MCB] = 0.3 mM (a) and at GCE in BR of pH 6.0 for [MCB] = 0.7 mM (b). Insets for both electrodes: peak current vs scan rate (A), peak current vs square root of scan rate (B), logarithm of peak current vs logarithm of scan rate (C), peak potential vs logarithm of scan rate (D)

also found to change linearly with scan rate by obeying the equation $i_{p,a}$ = 12.33 v + 0.54 with R^2 = 0.9957 (Fig. 3b, inset A). Linear dependence of the cathodic peak current and the anodic peak currents at HMDE and the anodic peak current at GCE upon the scan rate confirmed an adsorption behavior at both electrodes.

A plot of logarithm of cathodic peak current (in A) versus logarithm of scan rate (in V/s) at HMDE gave a straight line with a slope of 1.1 (R^2 = 0.9916) (Fig. 3a, inset C) and for anodic oxidation at GCE this slope was found to be 0.8 (R^2 = 0.9968) (Fig. 3b, inset C). Slope value for reduction at HMDE is found to be very close to the theoretical value of 1.0 for adsorbed species, and reduction peak at HMDE shifted to more cathodic potential with increasing concentration which confirmed that reduction process at HMDE is controlled mainly by adsorption.

Also the plot of peak current versus square root of scan rate was constructed and this graph is not linear even if scan rate is extremely low or extremely high (Fig. 3a, inset B). These results show that electrode reaction is controlled by adsorption. The slope value for GCE is less than the theoretical value for adsorption but higher than that for diffusion process which may be attributed to partial involvement of the diffusion and adsorption control for electrode process.

In electrochemical studies carried out at HMDE there is an oxidation peak at reverse scan and this peak is very symmetric with reduction peak and indicates the reversible nature of electrode reaction. For an ideal reversible electrochemical mechanism, it is expected that the ratio of anodic peak current to cathodic peak current should be unity and peak potential is not affected by scan rate[12]. For studies performed on HMDE, the peak potential was not affected by potential scan rate (Fig. 3a) and the ratio ($i_{p,a}/i_{p,c}$) was calculated as 1.0 for 0.005 to 1.0 V/s potential scan rates. A value of this ratio is concentration dependent and takes smaller value than 1.0 when the concentration of MBC is lower than 0.5 mM. Thus, it may be concluded that MCB initiates a reversible reduction and oxidation couple on HMDE and this electrochemical reaction is controlled mainly by adsorption. There is no reduction peak observed at reverse scan on GCE and on the other hand, peak potential was linearly shifted to more positive values with increasing scan rate (Fig. 3b, inset D) confirming the irreversible nature of the oxidation process of MCB on GCE. For totally irreversible oxidation process on the base of linear relationship between the peak potential ($E_{p,a}$) and logarithm of scan rate the slope of the straight line is equal to $RT/n\alpha F$, where α is charge transfer coefficient, n is the number of electron in rate determining step and other terms are commonly known constants. For oxida-

tion studies on GCE, the plot of $E_{p,a}$ versus log v was found to be linear and it was expressed as E_p (V) = 0.07 log v + 0.97 (R^2 = 0.9961) and $n\alpha$ calculated as 0.4. Moreover, for irreversible electrochemical reaction, the half peak width ($W_{1/2}$) is equal to 62.4/$n\alpha$, where α is the charge transfer coefficient and n is the number of transferred electrons. For MCB, half peak width in cyclic voltammogram was estimated to be around 133 mV, according to above equation, $n\alpha$ value was calculated as 0.47.

Additional studies were also performed to prove the adsorption behavior of MCB at HMDE. Accordingly, the value of the ratio of cathodic peak current to concentration ($i_{p,c}/C$) decreases with increasing concentration, value of the ratio of cathodic peak current to multiplication of concentration and scan rate ($i_{p,c}/Cv$) is nearly constant with increasing scan rate, and value of the ratio of cathodic peak current to multiplication of concentration and square root of scan rate ($i_{p,c}/Cv^{1/2}$) increases with increasing scan rate for reduction and oxidation processes.

In electrochemical studies, pH is one of the variables that commonly and strongly influence the electrochemical behaviors of molecules. Effects of pH on peak potential and peak current were studied using CV and SWV techniques between pH 2.0 and 11.0. As could be seen from Fig. 4a, potential of reduction-oxidation couple at HMDE is not affected by pH. But at pH values lower than 8.0, reduction peak could not be investigated. This behavior may be explained by two possibilities: (i) at lower pH values (in acidic solutions), MCB is not electroactive molecule at HMDE, (ii) MCB is electroactive molecule in both acidic and basic solutions but in acidic region peak poten-

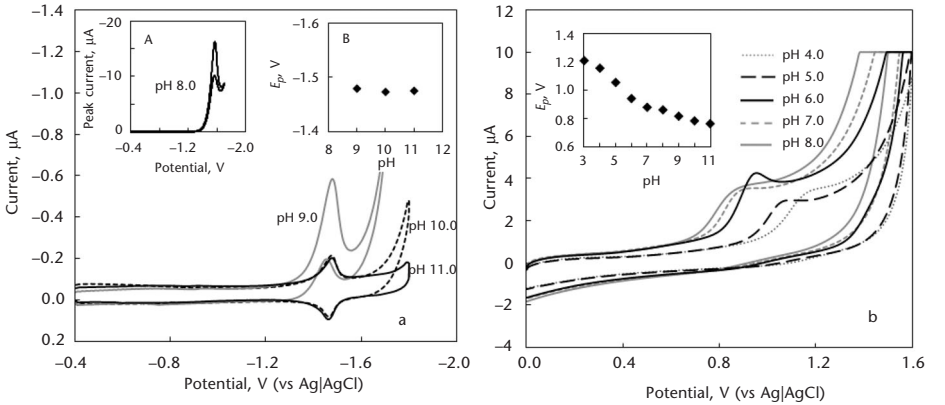


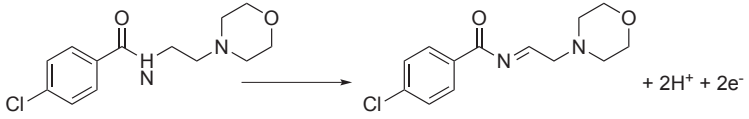
FIG. 4
Effect of pH on peak current and peak potential at HMDE (a) and at GCE (b). Insets: voltammograms of MCB at pH 8 (A in a), peak potential vs pH (B in a, inset in b)

tial is more cathodic than hydrogen evaluation potential on HMDE. Inset graphs of Fig. 4a support the second possibility. As could be seen, in peak current at pH 8.0 is much greater than those in higher pH values, which may be caused by hydrogen evaluation.

On the other hand, potential of oxidation peak at GCE was affected by pH (Fig. 4b); potential versus pH graph is linear at two regions. In acidic solutions, peak potential changes linearly with pH by obeying the correlation of: E_p (V) = –0.1 pH + 1.6 (R^2 = 0.9988) and in alkaline solutions, relation between peak potential and pH could be expressed with following equation: E_p (V) = –0.03 pH + 1.1 (R^2 = 0.9811). These linear lines intercepted at pH of 7.1 (Fig. 4b, inset) and this pH value could be related to pK_a value of MCB. Slope of these equations should be equal to $2.303RT\partial/nF$, where ∂ is the number of protons involved in electrode reaction, n is the number of electrons transferred in electrode reaction and other terms are commonly known constants[20]. From this relation, ∂/n value could be calculated as 0.5 for basic solutions and as 1.7 for acidic solutions. Since all further studies at GCE were carried out at pH 6.0, the effect of pH on peak potential in the pH range from 5.5 to 7.5 was used and the ratio of protons to electrons participated in the mechanism would be found as 1.0. Using these findings, oxidation mechanism at GCE may be proposed as in Scheme 1.

In mechanistic studies at HMDE, to find out the numbers of electrons transferred in electrode reaction (n), equations given in literature[20,21] and references cited therein, were used in CV results and number of electrons in electrochemical step was found as 1.0 ± 0.1.

The surface coverage of adsorbed substance (Γ) was calculated from the slope of curve of peak current (in A) versus scan rate (0.025–1000 V/s) according to equation given in the same references and it was found as 6.0×10^{-11} mol/cm^2 for reduction process at HMDE and 6.8×10^{-11} mol/cm^2 for oxidation process at GCE. From these values, it is easy to say that each MCB molecule occupies an area of 2.8 nm^2 at HMDE surface and 2.4 nm^2 at GCE surface. Using these findings for HMDE, reduction mechanism may be proposed as: (i) MCB molecules are adsorbed to electrode and this adsorption

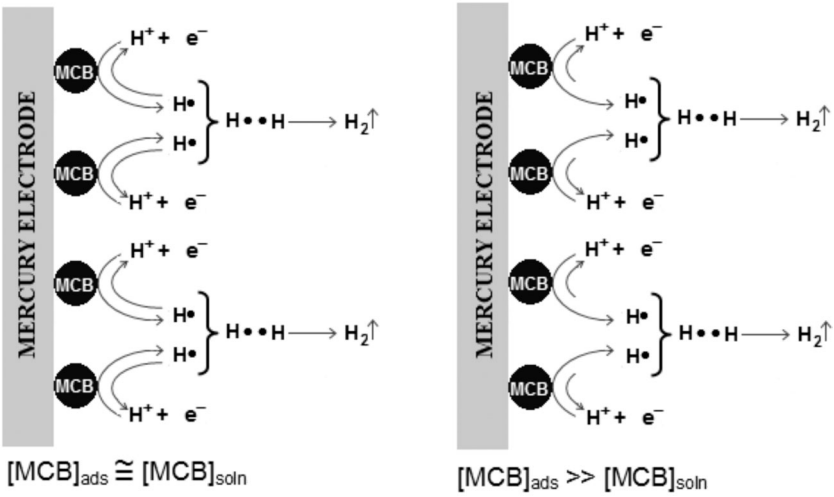


SCHEME 1
Proposed oxidation mechanism of MCB at GCE

modifies the mercury surface. MCB-modified surface of mercury behaves as a catalyst for reduction of protons. When the concentration gradient between the surface and bulk solution is high enough (i.e. $[MCB]_{ads} >> [MCB]_{soln}$, as shown in the right-hand side of Scheme 2), reduced ions are rapidly diffused to solution from the surface and the ratio of anodic peak current to cathodic peak current decreases and deviates from unity, but when the concentration gradient between the surface and bulk solution is not high enough to force the diffusion of reduced ions into the bulk solution (i.e. $[MCB]_{ads} \cong [MCB]_{soln}$, as shown in the left-hand side of Scheme 2), reduced ions would not diffuse to the solution rapidly from the surface and the ratio of anodic peak current to cathodic peak current would not deviate unity, as expected for reversible mechanism. Existence of a lone pair of electrons on the structure of MCB may support this mechanism, because to bind the proton from solution to surface there should be a lone pair of electrons located on surface.

Voltammetric Determination of MCB

In order to develop voltammetric methods, quantitation of peak current resulting from the electroreduction of MCB at HMDE and electrooxidation of MCB at GCE were examined using SWV and DPV techniques. Due to the



SCHEME 2
Proposed catalytic hydrogen reduction mechanism at MCB-adsorbed HMDE

adsorptive behavior of MCB, more sensitive cathodic and anodic adsorptive stripping techniques were also applied. Favorable results can be acquired for SWV, DPV, SWCAdSV, and DPCAdSV techniques for studies carried out at HMDE and SWV for those at GCE. For all techniques, variation of voltammetric peak current of MCB and its shape with instrumental conditions such as frequency (f), scan increment (ΔE_i), pulse amplitude (ΔE_a), accumulation time (t_{acc}), and accumulation potential (E_{acc}) were investigated. The optimization studies were carried out for 0.5 μM MCB in a BR buffer of pH 9.0 at HMDE and in a BR buffer of pH 6.0 at GCE. As a result, optimum parameters for cathodic peak in SWV were found as follows: f = 25 Hz, ΔE_a = 25 mV and ΔE_i = 4 mV, and for DPV ΔE_a = 50 mV, pulse with 0.06 s and ΔE_i = 5 mV with and without accumulation mode. Besides, the optimized parameters for anodic peak at GCE in SWV were found as follows: f = 15 Hz,

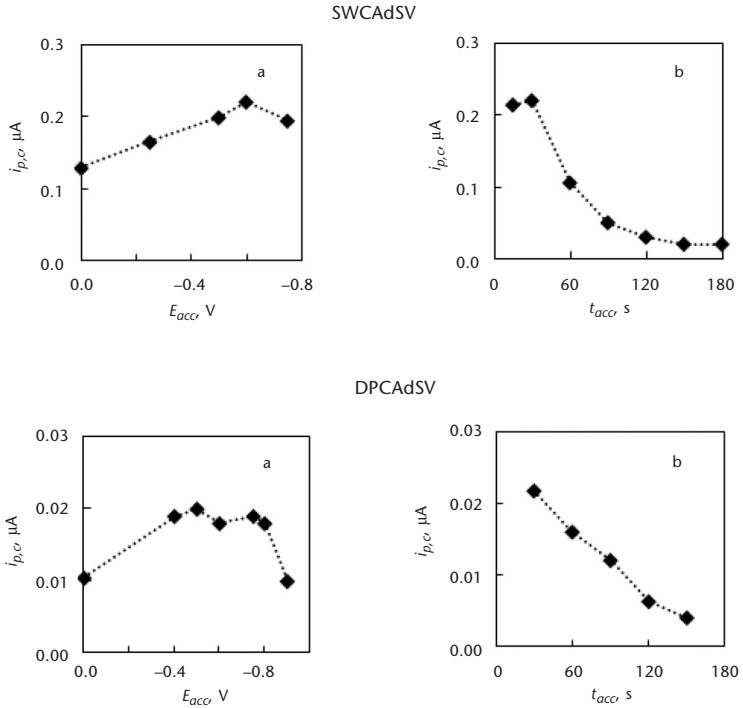


FIG. 5
Optimization of stripping parameters of MCB at HMDE for SWCAdSV and DPCAdSV. For both methods: accumulation potential (a), accumulation time (b)

ΔE_a = 25 mV and ΔE_i = 4 mV with and without accumulation mode. Meaningful results could not be obtained with DPV technique at GCE.

Type of supporting electrolyte also affects the peak response of the MCB. Various electrolytes such as BR, phosphate and acetate buffer solutions were examined to find the best conditions for quantification of MCB. BR gave the highest peak current and better peak shape than other mentioned buffers. Therefore, BR was selected for further works. The effect of pH on peak current and peak potential was given in early stage of manuscript.

Adsorptive stripping techniques were examined at both HMDE and GCE and the effects of accumulation time (t_{acc}) and accumulation potential (E_{acc}) on the cathodic and anodic peak currents for 0.5 μM MCB were investigated. However, after optimization of stripping variables for GCE, the lower limit of the dynamic linear range for anodic adsorptive stripping techniques was found to be very close to the value obtained without stripping method especially with SWV technique. Therefore, no further studies were performed with anodic adsorptive stripping techniques on GCE. Thereby, voltammetric stripping methods were carried out on HMDE.

As shown in Fig. 5a, for SWCAdSV studies at HMDE, peak current increases as the accumulation potential increased from 0.0 V to –0.6 V. The maximum peak current was achieved at the accumulation times of 30 s. Therefore, optimum accumulation potential and accumulation time for SWCAdSV studies were chosen as –0.6 V and 30 s, respectively.

For DPCAdSV studies, cathodic peak current increases linearly with increasing accumulation potential from 0.0 V to –0.4 V, then it was nearly held constant until –0.8 V and after reaching –0.8 V, cathodic peak current decreased gradually (Fig. 5b). The peak current decreases continuously with increasing accumulation time longer than 30 s. Therefore, the optimum accumulation potential and accumulation time for DPCAdSV studies were chosen as –0.5 V and 30 s, respectively.

Using these optimized conditions, the applicability of the proposed voltammetric procedures for determination of MCB was examined. Peak currents were measured as function of MCB concentrations at least five times under the optimized operational parameters. Average of five serial measurements for each MCB concentration was used as a peak current. Calibration graphs for MCB were obtained to estimate the analytical characteristics of methods and calibration graphs for each method were constructed (Figs 6 and 7).

Moreover, linearity was checked by preparing standard solutions at more than 10 different concentrations for each proposed method. For all voltammetric methods, it was found that when the MCB concentration was

higher, peak current deviated from linearity. This result was interpreted as due to the strong absorption behavior of MCB, and saturation of electrode surfaces with drug molecules. The characteristics of the calibration plots are summarized in Table II.

Validation of the proposed methods for the quantitative assay of MCB was done by evaluation of linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability (within-day), reproducibility (between-day), specifity, recovery, precision, and accuracy.

The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident from the values of the correlation coefficients and standard deviations (Table II) for all proposed methods.

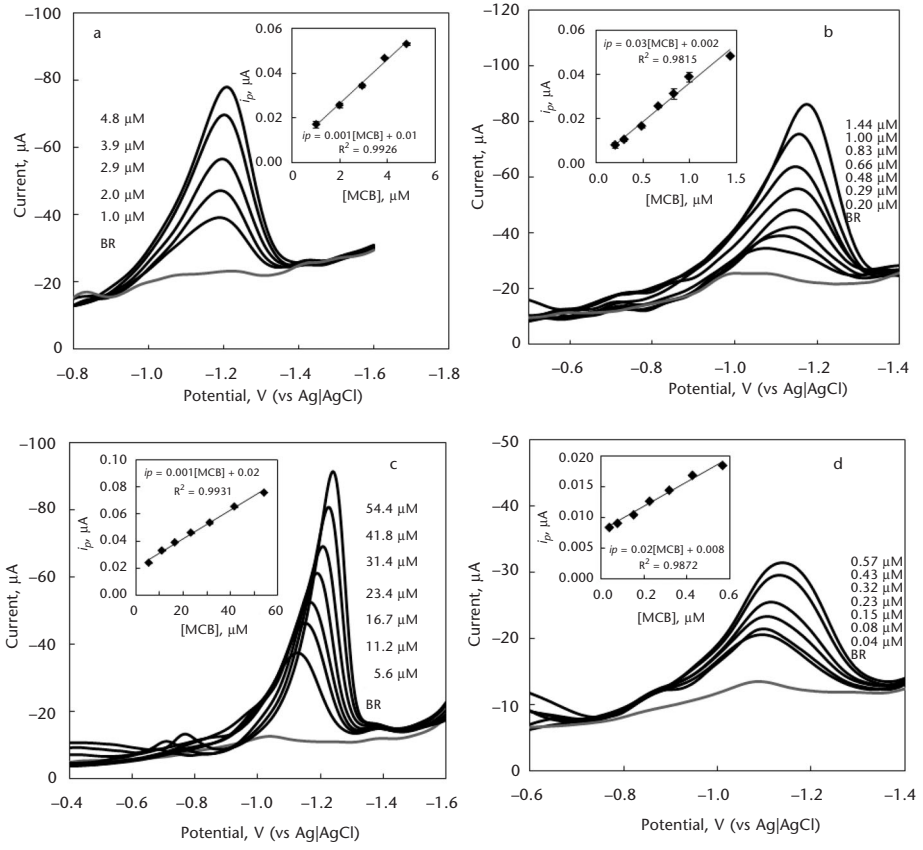


FIG. 6
Calibration dependences at HMDE for SWV (a), SWCAdSV (b), DPV (c), and DPCAdSV (d). Insets: calibration curves for related methods

TABLE II
Regression data of the calibration curve

Parameter	HMDE				GCE
	SWV	SWCAdSV	DPV	DPCAdSV	SWV
Dynamic linearity range, μM	1.0–4.8	0.2–1.4	5.6–54.4	0.04–0.6	3.8–30.4
Slope, μA/μM	0.001	0.030	0.001	0.020	0.065
Intercept, μA	0.010	0.002	0.020	0.008	0.161
Correlation coefficient, R^2	0.9926	0.9815	0.9931	0.9872	0.9985
Standard deviation (SD of slope	0.001	0.002	0.00004	0.001	0.0013
SD of intercept	0.0016	0.002	0.0011	0.0001	0.029
LOD, μM	0.5	0.2	3.7	0.02	1.5
LOQ, μM	1.6	0.5	11.1	0.07	4.5
Repeatability of peak current (RSD, %)[a]	0.88	1.33	0.93	1.46	3.3
Repeatability of peak potential (RSD, %)[a]	0.15	0.31	0.23	0.27	1.3
Reproducibility of peak current (RSD, %)[a]	1.13	1.57	1.04	1.52	3.8
Reproducibility of peak potential (RSD, %)[a]	0.76	0.48	0.42	0.84	1.6

[a] For five serial measurements.

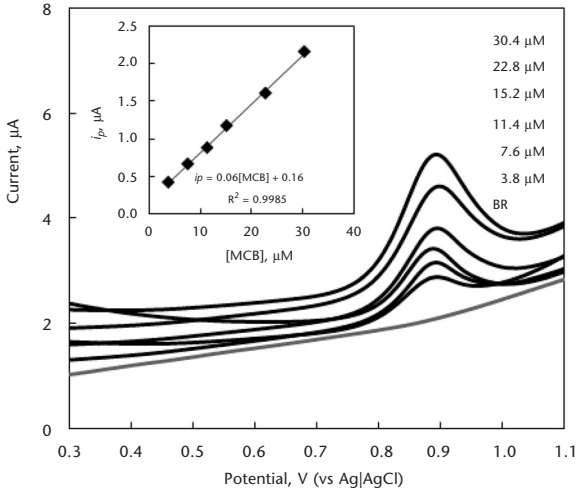


FIG. 7
Calibration dependences at GCE for SWV. Inset: calibration curve for related concentrations

Limit of detection and limit of quantitation values for MCB were calculated using the relations: LOD = $3s/m$ and LOQ = $10s/m$ [22], where s is the standard deviation of intercept of calibration curve and m is the slope of the related calibration curve. LOD and LOQ values for each proposed methods are given in Table II.

MCB Assay in Tablets and Human Serum

In order to evaluate the applicability of the proposed methods to pharmaceutical preparations and biological samples, MCB was determined in Aurorix® tablets and spiked human serum samples by using direct calibration method. When a portion of the tablet solution was added to BR at optimum pH value, a cathodic peak around –1.5 V and an anodic peak around

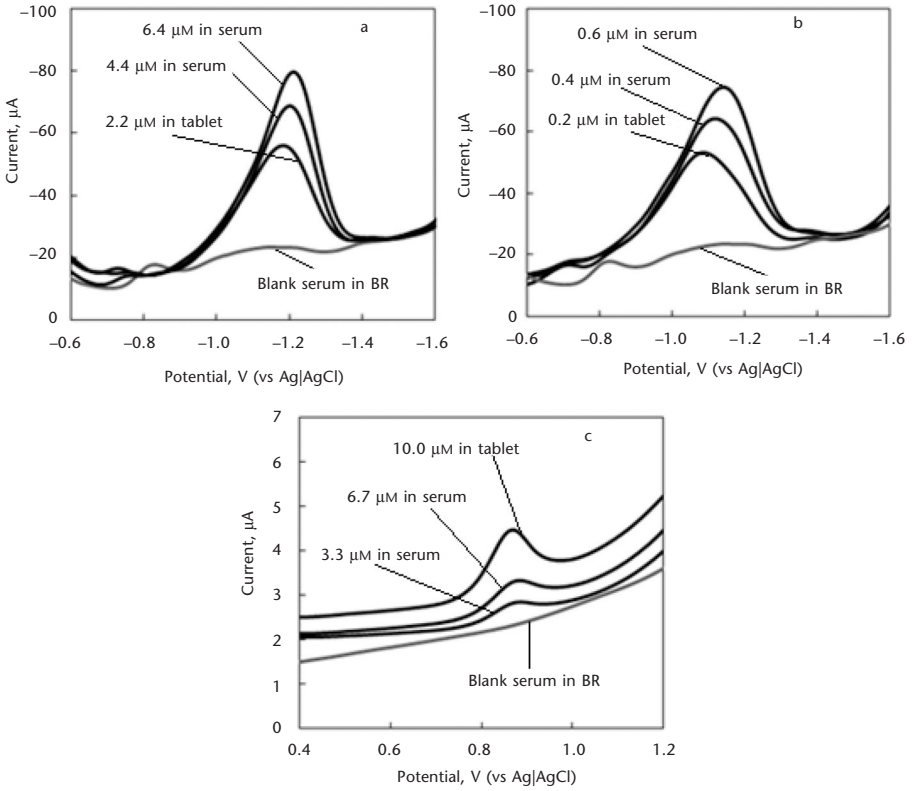


FIG. 8
Voltammograms for various concentration of MCB in tablet solutions and serum samples at HMDE for SWV (a), HMDE for SWCAdSV (b), and at GCE for SWV

0.9 V were recorded. The peak currents of these peaks increased with increasing concentration of tablet solution (Fig. 8). MCB amounts in tablet solutions were calculated using direct calibration method and results are presented in Table III.

As can be seen from Table III, the mean results of the applications with all techniques for both electrodes were found very close to the declared value of 150 mg MCB per tablet. In order to compare the precisions and evaluate the difference of proposed methods, tablet analysis results were examined using *F*- and student *t*-tests. According to *F*-test results the variances between methods were found to be insignificant at 95% confidence level indicating that no significant differences exist between the performances of the proposed methods regarding their precision. Besides, according to *t*-test results as mentioned in Table III, it could easily be conclude for all proposed methods that there is no significant difference between the found and labeled MCB amounts. These results indicate that the content of MCB in the pharmaceuticals can be safely determined using these methods without interference from other substances present in the tablet. The recovery studies of standard additions to commercial pharmaceuticals were carried out in order to provide further evidence of validity of the methods. The results related to these studies are presented in Table III. It can be seen from this table that the mean recoveries and RSD values for DPV, SWV, SWCAdSV, and DPCAdSV at HMDE, SWV at GCE are in the range of 98.2–101.1%, which is a good evidence of validity of methods.

TABLE III
Results of applications of proposed methods for Auroxis® tablets

Parameter	HMDE				GCE
	SWV	SWCAdSV	DPV	DPCAdSV	SWV
Labeled amount, mg	150	150	150	150	150
Found amount, mg[a]	150.7 ± 2.0	150.8 ± 2.7	150.0 ± 1.5	148.9 ± 1.2	149.7 ± 2.9
Standard error	0.8	1.1	0.6	0.5	1.1
Added, mg	18.3	2.5	40.9	1.4	27.8
Founded, mg[a]	18.3 ± 0.4	2.4 ± 0.1	41.4 ± 0.5	1.4 ± 0.02	27.5 ± 1.4
Recovery, mg[a]	99.8 ± 1.9	99.3 ± 2.3	101.1 ± 1.2	98.2 ± 1.7	99.1 ± 5.2
RSD of recovery, %	1.8	2.1	1.1	1.7	4.5
t-value[b]	0.94	0.73	0.03	–2.35	–0.27

[a] Value = average ± $ts/N^{1/2}$, (N = 5 at 95% confidence level); [b] t_{critic} = 2.78

Besides, recovery studies in spiked human serum samples were performed using direct calibration method. In these applications firstly voltammetric base lines for MCB-free serum samples in BR solutions were taken and it was found that there is no voltammetric signal in the potential range in which studies for MCB were carried out (Fig. 9). It was concluded from this investigation that there is no any interference effect of any possible species found in human serum. As could be seen from Table IV, recovery values in applications to spiked serum were found to be in the range between 99.9 and 102.6% and differences between spiked concentration and calculated concentration using related calibration equation are insignificant at 95% confidence level. Precision of measurements for serum samples are in a good agreement since RSD values are less than 7.0%.

These results showed that the proposed methods could be applied to MCB assay in tablet dosage form and human serum without any pretreatment.

TABLE IV
Results of applications of proposed methods to spiked human serum

Parameter	HMDE				GCE
	SWV	SWCAdSV	DPV	DPCAdSV	SWV
Concentration added, μM	2.2	0.5	11.0	0.4	15.0
Concentration found, μM[a]	2.3 ± 0.2	0.5 ± 0.02	11.3 ± 0.2	0.4 ± 0.02	15.1 ± 0.6
Recovery, %[a]	102.6 ± 7.7	100.8 ± 4.0	102.4 ± 2.2	99.9 ± 4.6	100.9 ± 3.8
RSD of recovery, %	6.7	3.5	1.9	4.0	3.3

[a] Value = average ± $ts/N^{1/2}$, (N = 5 at 95% confidence level).

CONCLUSION

Electrochemical characteristics of MCB at HMDE and GCE were studied for the first time. To understand the mechanism of action for drug molecules and target/related organs redox properties and electrochemical parameters could be meaningful. Determination of drug molecules themselves or any related species from serum and any other biological samples after various time of inhaling may also be important. Five voltammetric techniques have been developed for determination of MCB in tablet dosage forms and human serum. The proposed methods are sensitive, precise, accurate, and rapid enough to be used in routine analysis. In addition, no sophisticated

instrumentation like HPLC and prior tedious extraction process is required. Furthermore, percentage of recovery results indicates that the developed methods can be applied for quantitation of MCB without interference from other ingredients.

There is no significant difference between the detection limits of proposed methods and reported chromatographic and spectrophotometric methods (Table I). All developed methods can be applied to the detection of MCB in tablet dosage form. Besides, it can be stated that developed adsorptive stripping methods may be more suitable for the determination of MCB in biological medium where the detection of lower concentration is required with an insignificant matrix effect.

There is no official method present in any pharmacopoeias related to determination of MCB. Consequently, the proposed methods have the potential of a good analytical alternative for determining MCB in pharmaceutical formulations and human serum. Also, they can be adopted for pharmacokinetic studies as well as for quality control laboratory studies.

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