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Electrochemistry of moexipril: experimental and computational approach and voltammetric determination

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The electrochemistry of moexipril (MOE) was studied by electrochemical methods with theoretical calculations performed at B3LYP/6-31 + G (d)//AM1. Cyclic voltammetric studies were carried out based on a reversible and adsorption-controlled reduction peak at -1.35 V on a hanging mercury drop electrode (HMDE). Concurrently irreversible diffusion-controlled oxidation peak at 1.15 V on glassy carbon electrode (GCE) was also employed. Potential values are according to Ag/AgCl, (3.0 M KCl) and measurements were performed in Britton-Robinson buffer of pH 5.5. Tentative electrode mechanisms were proposed according to experimental results and *ab-initio* calculations. Square-wave adsorptive stripping voltammetric methods have been developed and validated for quantification of MOE in pharmaceutical preparations. Linear working range was established as 0.03–1.35 μM for HMDE and 0.2–20.0 μM for GCE. Limit of quantification (LOQ) was calculated to be 0.032 and 0.47 μM for HMDE and GCE, respectively. Methods were successfully applied to assay the drug in tablets by calibration and standard addition methods with good recoveries between 97.1 % and 106.2 % having relative standard deviation less than 10 %.

1. Introduction

Moexipril (MOE), chemically known as (3*S*)-2-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-6, 7-dimethoxy-1, 2, 3, 4-tetrahydroisoquinoline-3-carboxylic acid (Fig. 1), inhibits angiotensin-converting enzyme (ACE) activity, as an orally active non-sulfhydryl ACE inhibitor. Moexipril is commonly used alone or with thiazide diuretics in treatment of hypertension (Brogden and Wiseman 1998).

There are a number of analytical methods proposed for the determination of MOE. These include: RP-HPLC (Bhaskara and Lakshmana 2012), densitometry and HPLC (Elzbieta et al. 2010), HPLC-ES-MS (Kalasz et al. 2007), derivative spectrophotometric and HPLC (Beata et al. 2012; Erturk et al. 2003), GC-MS ionized by negative charge ion (Hammes et al. 1995), chemometric approach (Dinc et al. 2012), electrometric method (Elqudaby et al. 2013), MOE selective membrane electrode (Belal et al. 2009) and fluorescence (El-Hay et al. 2012).

Since MOE has electroactive groups in its chemical structure, investigation of its electrochemical characteristics may be of great importance. Electrochemical studies are generally useful for investigating many physical-chemical and redox properties of active species. These studies make it possible to evaluate the redox properties, characteristics of plausible mechanism pathways and influences of adsorption-diffusion to redox mechanism. These parameters are important for distribution, metabolism, and pharmacological, toxicological and pharmacokinetic properties of drugs. *Ab-initio* calculations were also found to be useful as a value added tool to enlighten possible oxidation-reduction mechanisms (Tasdemir et al. 2012; Pamuk et al. 2013; Zorluoglu et al. 2013). Voltammetric stripping techniques are generally used for electrochemical determination to lower the detection and determination limits. According to best of our literature knowledge, there is only one study about the electrochemistry of MOE (Attia 2010). The present study was designed to investigate the detailed electrochemical behavior of MOE on glassy carbon electrode (GCE) and hanging mercury drop electrode (HMDE). Tentative reaction mechanisms were also proposed. Computational studies were performed to support the proposed electrode mechanisms. In addition, it was also aimed to develop rapid, simple and novel voltammetric methods for direct determination of MOE in pharmaceutical dosage forms.

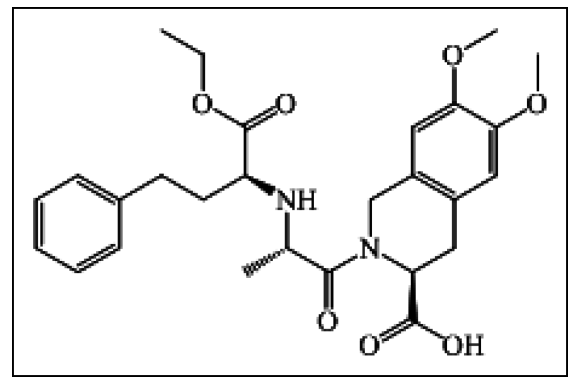


Fig. 1: Chemical structure of MOE.

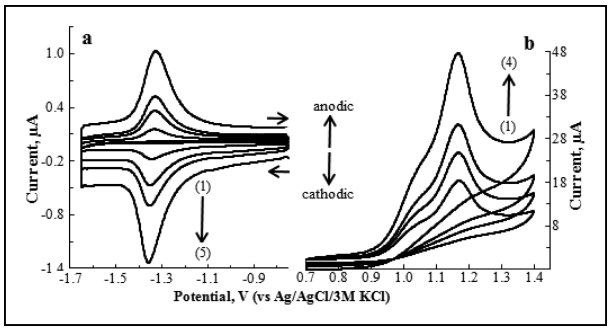


Fig. 2: CVs of MOE solutions in BR of pH 5.5, scan rate: 0.100 V/s on (a) HMDE (1) blank, (2) 0.10, (3) 0.35, (4) 0.65, (5) 1.00 mM (b) on GCE (1) 0.05, (2) 0.45, (3) 0.65, (4) 1.00 mM.

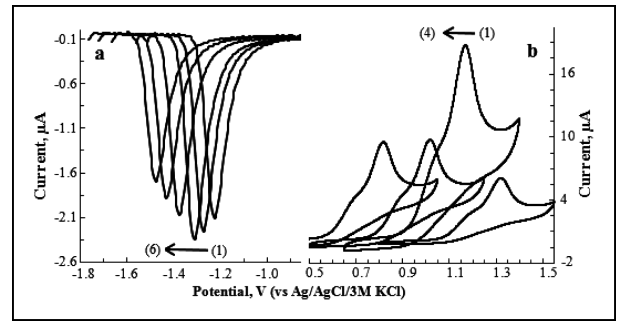


Fig. 3: (a) SWVs of 1.0 µM MOE solutions in BR of different pHs on HMDE pH values: (1) 3.5, (2) 4.5, (3) 5.5, (4) 6.5, (5) 7.5, (6) 8.5, scan increment is 3 mV and frequency is 25 Hz, (b) CVs of 50 µM MOE solutions in BR of different pHs on GCE pH values: (1) 3.5, (2) 5.5, (3) 8.5, (4) 10.0. scan rate 0.1 V/s.

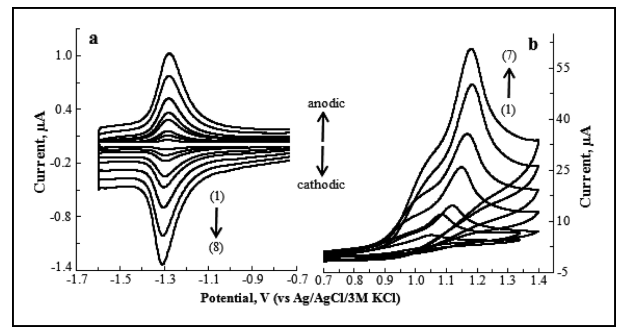


Fig. 4: Influences of scan rate on peak current and peak potential of MOE (a) on HMDE, scan rate values: (1) 0.050, (2) 0.090, (3) 0.175, (4) 0.300, (5) 0.425, (6) 0.500, (7) 0.750, (8) 1.00 V/s (b) on GCE scan rate values: (1) 0.02, (2) 0.05, (3) 0.10, (4) 0.25, (5) 0.50, (6) 0.75, (7) 1.00 V/s both in BR of pH 5.5.

2. Investigations and results

Electrochemistry of MOE and its diffusion - adsorption properties were studied on HMDE and GCE using cyclic voltammetry (CV), differential pulse voltammetry (DPV), square-wave voltammetry (SWV) and constant potential bulk electrolysis (BE).

2.1. Electrochemistry on GCE and HMDE

In CV studies, single well-defined reduction peak at -1.35 V on HMDE on negative-going potential scan and oxidation peak again at −1.35 V on reverse scan (Fig. 2a) was observed in Britton-Robinson buffer (BR) of pH 5.5. These peaks were not observed when only supporting electrolyte was scanned. These studies revealed a correlation between peak current and MOE concentration and current of reduction-oxidation couple increases linearly with concentration. As seen in Fig. 2a, oxidation and reduction peaks were symmetric in terms of their potentials, currents and areas. Having both reduction and oxidation peaks, nearly the same current and same area, mechanism seems to be reversible according to Brett and Brett (1996); Wang (2000), and Bond (2002). Symmetry of peak potential and peak current may be an indication of adsorption according to literature quoted by Brett and Brett (1996); Wang (2000), and Bond (2002). On the other hand, for GCE, a well-defined oxidation peak with linearly increasing current with increasing concentration of MOE at about 1.15 V was observed with pre-peak or shoulder at 1.05 V (Fig. 2b). No reduction response on reverse scan was observed in BR of pH 5.5 indicating an irreversible nature for oxidation on GCE. Pre-peak or shoulder may suggest the adsorption effect on mechanism.

2.1.1. Effect of pH on electrochemical behavior

In detailed electrochemical investigation effects of pH on peak potential and peak current were initially studied using SWV on HMDE only for reduction side and using CV on GCE between pH 3.5 and 10.0. As seen from Fig. 3a, reduction potential on HMDE shifted to more negative potentials with increasing pH in given range. Changing the peak potential with pH is the evidence of proton(s) in reduction mechanism. When peak potential (Ep) was taken into account as a function of pH, linear equation given in Eq. (1) was established:

$$\text{Ep, V} = -(1.04 \pm 0.11) - (0.05 \pm 0.008)\text{pH}; \quad R^2 = 0.9961 \tag{1}$$

According to slope value of Ep - pH graph, reduction process is Nernstian and identical number of protons and electrons participated in the mechanism according to Tasdemir et al. (2012); Pamuk et al. (2013), and Zorluoglu et al. (2013). Shifting of peak potential to more negative (more cathodic) potentials with increasing pH may suggest protonation before electron transfer. Similar studies were carried out on GCE and shifting of oxidation peak potential to less positive potential with increasing pH between 3.5 and 10.0 was observed (Fig. 3b). In this case peak potential found to be changed by pH as follows:

$$\text{Ep, V} = (1.56 \pm 0.13) - (0.07 \pm 0.009)\text{pH}; \quad R^2 = 0.9854 \tag{2}$$

According to Eq. (2), an identical number of protons and electrons is present in the oxidation of MOE on GCE. Having less positive oxidation potentials with increasing pH values may be concluded as deprotonation process before electron transfer. After these investigations, peak current, peak shape and symmetry were taken into account and optimum pH was selected as 5.5 for both HMDE and GCE.

2.1.2. Effect of potential scan rate on electrochemical behavior

Afterwards, effect of potential scan rate on peak potential was investigated while MOE concentration was held constant as 0.1 mM for HMDE and 0.05 mM for GCE. It is clear from Fig. 4a that the potential of the reduction-oxidation peak couple is nearly independent from scan rate. Having reduction and oxidation peak nearly with the same current, same peak area and having scan-rate-independent potential is the evidence for a reversible mechanism on HMDE. On the other hand as seen from Fig. 4b, oxidation peak potential shifts to more anodic values with increasing scan rate, this could be evidence of irreversible or quasi-reversible mechanism. Since there is no reduction peak on reverse scan, mechanism is irreversible. Dependency of

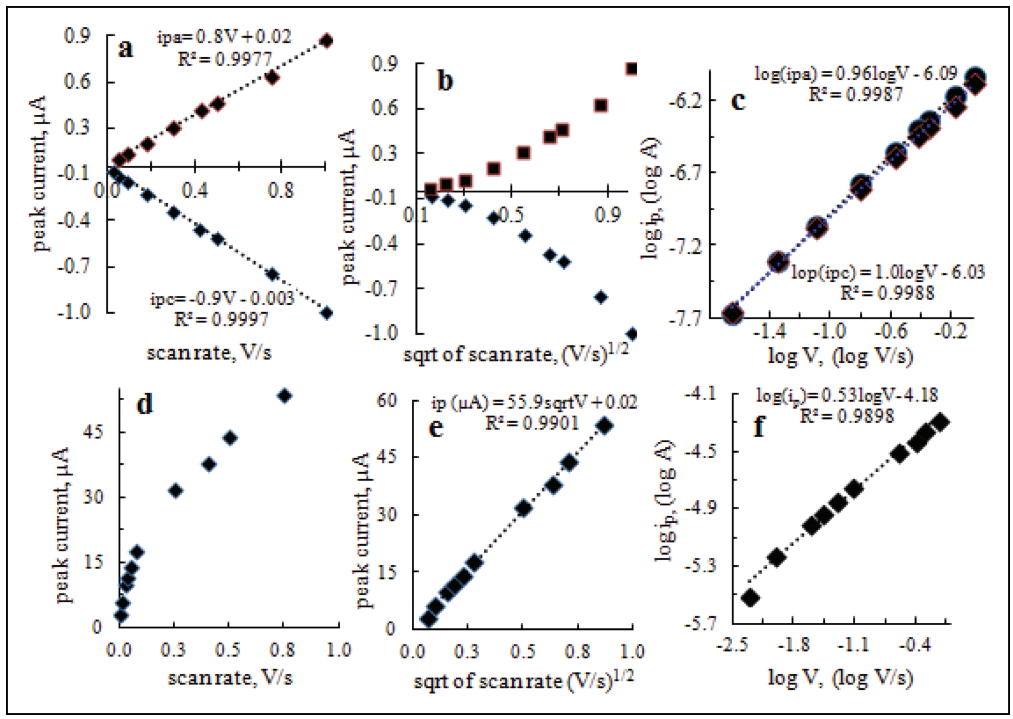


Fig. 5: Effect of potential scan rate on peak current (a) peak currents vs. scan rate (b) peak current vs. square-root of scan rate (c) logarithm of peak current vs. logarithm of scan rate for HMDE and (d) peak currents vs. scan rate (e) peak current vs. square-root of scan rate (f) of peak current vs. logarithm of scan rate for GCE, all conditions are the same with that of Fig. 4.

oxi dation peak potential of MOE to logarithm of scan rate was found to be expressed as:

$$Ep,V = (0.78 + 0.05) + (0.039 + 0.002)\log v; \quad R^2 = 0.9812 \qquad (3)$$

The equation given in the literature (Brett and Brett 1996; Bond 2002) was used and value for αn, (n is number of electrons) was calculated to be 1.22 for GCE.

Effect of scan rate on peak current was also studied. Peak current of reduction-oxidation couple on HMDE changes linearly with scan rate by nearly have the same dependencies (Fig. 5a) whereas current of this couple has no linear dependency to square-root of scan rate (Fig. 5b). More importantly, plot of logarithm of peak current (A) *versus* logarithm of scan rate (V/s) has a slope value of 1.0 for reduction and 0.96 for oxidation on HMDE (Fig. 5c). Studies on GCE showed that oxidation peak current is not changed linearly with increasing scan rate (Fig. 5d), but it changes linearly with the square-root of scan rate (Fig. 5e) and lastly, slope of the plot of logarithm of peak current versus logarithm of scan rate was found to be 0.53 (Fig. 5f). As a results of all these investigations reversible reduction-oxidation processes on HMDE should have surface-confined and adsorption-controlled mechanism, whereas oxidation on GCE should take please on electrode-solution interface and controlled by diffusion (Tas demir et al. 2012; Pamuk et al. 2013; Zorluoglu et al. 2013).

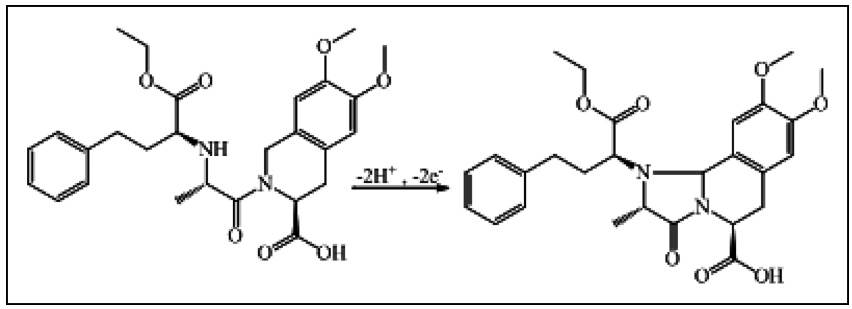
2.1.3. Bulk electrolysis (BE) and number of electrons

BE studies at 1.5 V was carried out to find the number of electrons in the oxidation mechanisms on GCE, by following the Faraday's equations (Brett and Brett 1996; Wang 2000; Bond 2002) number of electrons was found to be two for oxidation on GCE. Similar studies on HMDE were performed at -1.5 V. Although flowing of remarkable current was observed by applying constant potential for several hours on HMDE, the peak potential and peak current were not affected by BE. Thus, the number of electrons involved in the mechanism was not calculated by Faraday's law. Instead, it was calculated by following the approach and equation given in the literature for adsorbed species (Brett and Brett 1996; Wang 2000; Bard and Faulkner 2001; Bond 2002) and it was found to be two for each MOE molecule.

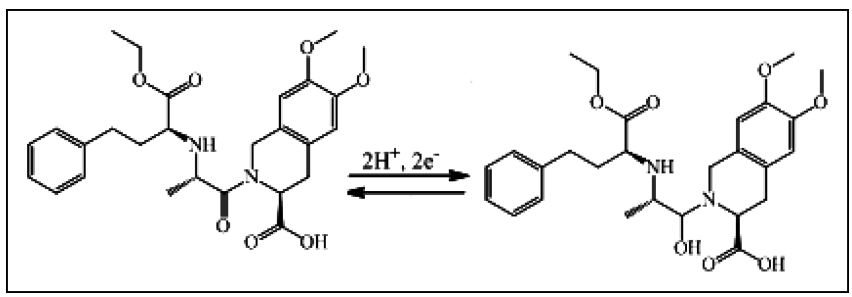
2.1.4. Theoretical investigation and tentative electrode mechanisms

An electron flows from the electrode into the lowest unoccupied molecular orbital (LUMO) of the molecule as reduction of molecule occurs. When the oxidation takes place, electron from the highest occupied molecular orbital (HOMO) of molecule is transferred to electrode. Consequently, the arrangement of HOMO and LUMO is important to determine the most relevant part/atoms of the molecule for electrode mechanism to be proposed more accurate way. For this reason, in order to predict HOMO and LUMO, geometry of MOE was optimized first, using semi empirical methods (AM1). These methods are fast but often fail to predict accurate energy values of compounds. Hence a more accurate basis set was used to obtain energy values that match experimental accuracy. Accordingly, single point energy calculation processes were performed at B3LYP/6-31 + G (d) and HOMO and LUMO of MOE together with their corresponding energies are depicted in Fig. 6.

According to Fig. 6, less tightly held electrons in the molecule (HOMO) lie mainly on the methyl moieties bound to etheric oxygens and on the amino residue with six-membered-ring contains N atom. Amino residues are known to be oxidized. The proposed mechanism is irreversible which initiated by removal of a proton from NH group and involves a total of $2e^-/2H^+$ and formation of five-membered ring. Another alternative to its oxidation is the formation of six-membered-rind by removing one electron and one proton from the terminal methoxy groups but this kind of oxidation is highly improbable. The most probable mechanism was shown in Scheme 1.



Scheme 1: Possible oxidation mechanisms of MOE on GCE.



Scheme 2: Possible reduction mechanisms for MOE on HMDE.

LUMO, which will be the easiest route to the addition of electrons to the molecule is located around carbonyl oxygen adjacent to nitrogen atom on six-membered-ring and ester oxygen. Most negatively charged parts of the molecule are again the same centers. First possible reduction mechanism is the reduction of carbonyl group to corresponding alcohol (Scheme 2) initiated by protonation of most negatively charged part of molecule indicating that this is a classical acid catalyzed reaction. The reduction of carbonyl group will be more favorable at low pHs. Similarly, protonation step will be more difficult in higher pHs and higher potential will be needed as investigated in pH studies. The second possible reduction is the protonation of the ester oxygen followed by electron transfer and finally fragments into the corresponding alcohol and aldehyde. But this obviously would be an irreversible reaction which will not meet with our experimental findings.

2.2. *Voltammetric determination of MOE*

In an effort to develop a voltammetric method for MOE determination, quantitation of peak current resulting from the reduction on HMDE and oxidation on GCE were examined. Square-wave (SWV) and differential pulse (DPV) techniques were applied first without using stripping mode. In such studies, SWV method was found to be more suitable and reproducible than DPV on both electrodes. Then, to get more sensitive methods, square-wave anodic adsorptive stripping voltammetry (SWAAdSV) on GCE and square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) on HMDE were applied.

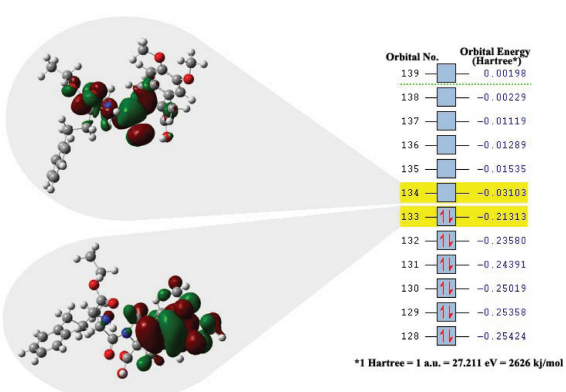


Fig. 6: Frontier molecular orbitals mapped on optimized molecular structure of MOE and their corresponding energies calculated at B3LYP/6-31 + G (d)//AM1.

2.2.1. *Optimization of experimental variables*

The nature of supporting electrolyte affected the peak response of the MOE. Thus, various buffer as supporting electrolyte such as BR, phosphate and acetate were examined to find the better supporting electrolyte for quantification. BR gave the highest peak current and better peak shape than the other buffers. Therefore, BR was selected for further studies. The effect of pH was also investigated. Peak current, peak shape and peak symmetry were taken into account and then optimum pH was selected as 5.5 for both GCE and HMDE, as emphasized before.

For all techniques, variation of peak current and its shape with instrumental conditions such as frequency (f), scan increment (ΔE_i), pulse height (ΔE_a) were investigated using 1.0 μM MOE in a BR at optimum experimental conditions. As a result, optimum instrumental parameters were found as follows: $f = 25$ Hz, $\Delta E_i = 3$ mV, and $\Delta E_a = 45$ mV.

Accumulation potential and time for adsorptive stripping techniques were also optimized for both electrodes using 0.1 μM MOE. As a result, 0.5 V and 180 s for GCE and − 0.5 V and 210 s were found optimal for HMDE.

Applying these optimized conditions, the applicability of the proposed voltammetric procedures for the determination of MOE was investigated. Peak currents were measured as a function of MOE concentration in quintuplicate under the optimized operational parameters and average of these five serial measurements was used as a peak current. Calibration graphs for MOE were obtained to estimate the analytical characteristics of methods. Results are given in Figure 7a for HMDE and in 7b for GCE.

2.2.2. *Validation of proposed methods and determination of MOE in tablets*

The proposed voltammetric methods were validated investigating the following parameters: Linearity range, accuracy and

Table 1: Regression data of the calibration curve

Regression Parameter	GCE	HMDE
Linearity range, μM	0.2–20.0	0.03–1.35
Slope of calibration curve, ALmol^{-1}	0.017	0.62
Intercept, μA	0.02	0.12
Standard deviation (SD) of regression, μA	0.008	0.02
SD of slope, μALmol^{-1}	0.005	0.017
SD of intercept, μA	0.0008	0.002
Limit of detection (LOD), μM	0.14	0.009
Limit of quantification (LOQ) μM	0.47	0.032
Determination coefficient, R^2	0.9928	0.9932
Within-day repeatability of peak current[a], (% RSD)	8.97	4.86
Between-day repeatability of peak current[a], (% RSD)	12.3	8.32
Within-day repeatability of peak potential[a], (% RSD)	2.48	2.31
Between-day repeatability of peak potential[a], (% RSD)	4.76	3.45

[a] *Relative standard deviation for 5 serial measurements*

Table 2: Recovery of MOE from tablet samples

Electrode	Nominal value, mg	Values calculated, mg	Recovery[a], %	RSD[b], %
GCE	7.5	7.0; 7.1; 7.3; 7.4; 7.6	97.1 ± 3.9	3.3
HMDE	7.5	7.2; 7.35; 7.5; 7.5; 8.12	99.9 ± 6.6	5.3

[a] *value = average ± ts/√N (N = 5 and at 95 % confidence level)*
[b] *relative standard deviation for 5 serial measurements*

precision, sensitivity, limits of detection (LOD) and quantitation (LOQ), reproducibility and repeatability of current and potential according to literature (Süslü et al. 2010; Tasdemir et al. 2010). Linearity was checked by preparing standard solutions at twelve different concentration levels for each electrode. Five serial measurements were taken for each concentration and subsequent to evaluation of the required statistical test (Q-test), the average was used as a peak current of related concentration. The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident from the coefficient of determination (R^2) (Fig. 8a and 8b)

Slope value of corresponding calibration curve (di_p/dC) was evaluated as sensitivity of proposed methods and 0.62 ampere per each molar of MOE was established on HMDE whereas 0.017 ampere per each molar of MOE was found for GCE.

Calibration characteristics were interpreted in least-square approach and some validation parameters were calculated including LOD and LOQ values of proposed methods and results are given in Table 1.

In order to evaluate the applicability of the proposed methods to pharmaceutical preparations, MOE was determined in commercial tablets by calibration method. As shown in Table 2, mean results of each application for both electrodes lie between 97.1 % and 99.9 with RSD value less than 10.0 %. These results indicate the validity of proposed methods.

In order to test whether excipients have a significant effect on performance of the proposed methods and retest the accuracy and precision, standard addition method was used. In these applications a tablet solution was prepared with known concentration and five different volumes of standard MOE solutions were spiked to the tablet solution, voltammetric measurement was performed and recovery values were calculated by using peak current and calibration parameters As could be seen in Table 3 recovery values are between 103.1 % and 106.2 % with RSD values less than 10.0 %. The differences between spiked and calculated concentrations are insignificant at 95 % confidence level confirms the validity of proposed methods.

Parameters of the proposed methods were statistically compared with those of one standard method given in the literature

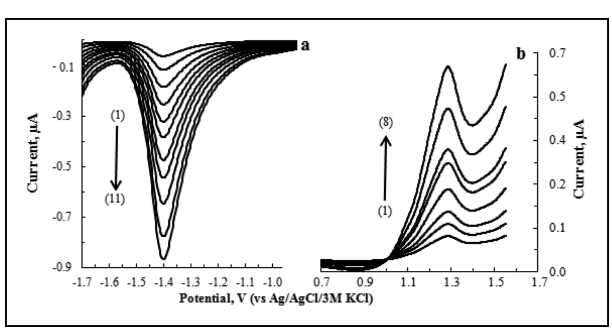


Fig. 7: Calibration dependencies of MOE when $f = 25$ Hz, $\Delta E_i = 3$ mV, and $\Delta E_a = 45$ mV on (a) HMDE concentrations: (1) 0.03, (2) 0.06, (3) 0.12, (4) 0.25, (5) 0.35, (6) 0.45, (7) 0.60, (8) 0.70, (9) 0.85, (10) 1.00, (11) 1.35 μM (b) GCE, concentrations: (1) 0.2, (2) 0.5, (3) 1.0, (4) 2.2, (5) 4.9, (6) 7.8, (7) 11.5, (8) 20.0 μM.

Table 3: Results of standard addition method

Electrode	A[a]	B[b]	C[c]	Recovery[d], %	RSD[e], %
		0.15	0.41		
		0.25	0.58		
HMDE	0.3	0.35	0.70	103.1 ± 8.5	6.6
		0.45	0.78		
		0.55	0.91		
		0.5	1.7		
		1.5	2.3		
GCE	1.0	2.5	3.7	106.2 ± 10.4	7.9
		3.5	4.9		
		4.5	6.1		

[a] *concentration of tablet solution prepared, μM*
[b] *concentration of standard MOE solution added, μM*
[c] *calculated concentration of proposed method, μM*
[d] *value = average ± ts/√N (N = 5 and at 95 % confidence level)*
[e] *relative standard deviation for 5 serial measurements*

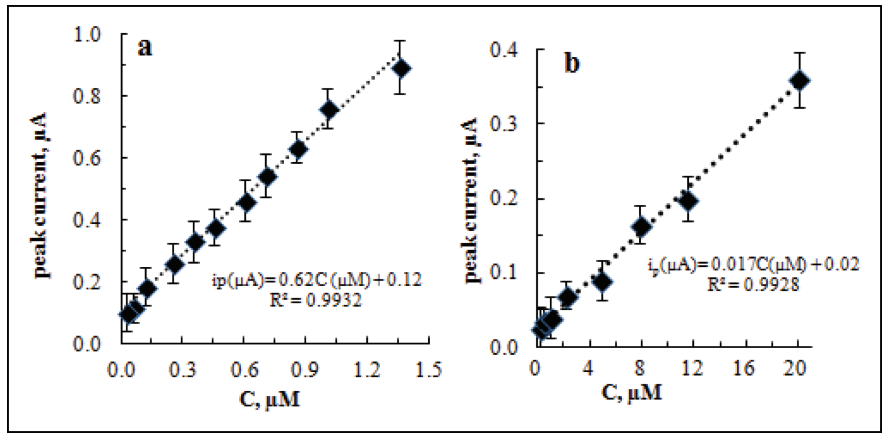


Fig. 8: Calibration line for (a) HMDE, and (b) GCE conditions are the same with Fig.7.

(Hammes et al. 1995) for 95% confidence level and results were given in Table 4. Experimental values of both Student-t and F values were found to be less than critical values for 95% confidence level meaning that differences between gas chromatographic method and proposed methods are insignificant by means of accuracy and precision.

2.3. Conclusion

Electrochemical characteristics of MOE with computational calculations support were studied for the first time on bare electrodes. Redox properties and electrochemical parameters of drug molecules may be of crucial importance in understanding the mechanism of action against their target/related organs. Determination of drug molecules in pharmaceuticals and biological samples are also of great importance. In this study, precise, accurate, rapid and sensitive voltammetric methods which require neither sophisticated instrumentation nor tedious extraction processes have been proposed. Consequently, proposed methods have the potential of a good analytical alternative for determination of MOE in various samples. Also, they can be adopted for pharmacokinetic studies as well as for quality control laboratory studies.

3. Experimental

3.1. Apparatus

Voltammetric measurements on both electrodes were carried out using CHI760D (CHI instruments, USA) electrochemical work station. Three electrode system consisted of working electrodes (hanging mercury drop electrode (HMDE); BAS CGME 1108, 0.0145 cm^2 and glassy carbon electrode (GCE); BAS, MF 2012, 0.071 cm^2), reference electrode (Ag/AgCl; 3 M KCl; MF-2052, RE-5B) and a Pt auxiliary electrode (BAS MW-1034) were used. Prior to each experiment, GCE was polished manually with slurries prepared from 0.01 micron aluminum oxide on a smooth polishing pad (BAS velvet polishing pad), then rinsed with double-distilled water thoroughly.

pH Measurements were made with a Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600; Thermo Fisher Scientific). Double-distilled deionized water was supplied from Ultra-Pure Water System (ELGA as PURELAB Option-S). All measurements were performed at room temperature.

Table 4: Results of statistical comparisons of proposed methods with GC-MS (Hammes et al. 1995) for 95% confidence level

	GC-MS	GCE	HMDE
N	8	5	5
Average recovery, %	97.21	97.07	100.45
Standard deviation	1.55	2.67	3.22
	critical value		2.23
Student-t	for GC-MS/GCE		0.09
	for GC-MS/HMDE		1.61
	critical value		4.74
F	for GC-MS/GCE		2.93
	for GC-MS/HMDE		4.33

3.2. Reagents and solutions

MOE standard was achieved as its HCl salt from Adeka Drug Coll. All chemicals used were of analytical grade and used without further purification.

MOE stock solutions (5.0 mM) were prepared in absolute ethanol and kept in the dark and below 4 °C. Working MOE solutions were prepared by a sufficient dilution of stock solution with supporting electrolyte of optimum pH and used within the day to avoid decomposition. Phosphoric acid (Riedel-de-Haen, Honeywell Specialty Chemicals Seelze GmbH, Germany), boric acid (Riedel-de-Haen, Honeywell Specialty Chemicals Seelze GmbH, Germany) and acetic acid (Merck KGaA, Darmstadt, Germany) were used in the preparation of BR solution in which each component had an analytical concentration of 0.04 M. Double-distilled deionized water was used in preparations of all the solutions. All chemicals were used as received.

3.3. Procedure

For voltammetric measurements, a known volume of MOE solution was pipetted into 10.0 mL supporting electrolyte and after degassing with argon for 5 min. Voltammograms were recorded by scanning the potential towards the positive direction on GCE (oxidation studies) and negative direction on HMDE (reduction studies) versus reference electrode.

A three-electrode combination system for bulk electrolysis (BE) with mercury pool (55.4 cm^2) and glassy sieve as working electrode, coiled platinum wire as an auxiliary electrode (BAS MW-1033 (23 cm)) and Ag/AgCl reference electrode (BAS MF-2052 RE-5B in 3.0 M KCl) was used. In BE studies 15 mL of 1.0 mM solutions were used for both electrodes.

3.4. Preparation of tablet samples

Commercial tablets were also achieved from the same company and were used as pharmaceutical dosage form that contains 7.5 mg MOE per tablet. Ten tablets were accurately weighed and crushed into a homogeneous fine powder in a mortar and mixed. Average weight of one tablet was calculated. A powder sample, equivalent to one tablet was weighed and transferred into the calibrated flask containing *ca.* 100 mL of absolute ethanol then content was sonicated for 10 min. After standing at room temperature for *ca.* 30 min, flask volume was completed to 250.0 mL with double-distilled water. Preparation of final concentration was established as: Required sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the supporting electrolyte solution. Quantitations in all proposed methods were performed by means of the calibration curve method from the related calibration equations.

3.5. Computation

Theoretical calculations were performed to support the proposed mechanism for electrode processes. All calculations were performed with the Gaussian 09 suite of programs (Frisch et al. 2009). The geometry of MOE was fully optimized at AM1 level. Frequency calculations were computed at the same

level to verify that the optimized geometry is a real minimum on the potential energy surface without any imaginary frequency. Single point energy calculation was done using AM1-optimized geometry at DFT/B3LYP level of theory, with the popular polarized basis set, 6-31 + G (d) which adds d functions on heavy atoms.

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