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Mesalazine Modified Carbon Paste Electrode for Voltammetric Determination of Amlodipine

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Abstract

In this study, Mesalazine modified carbon paste electrode (MESA-CPE) was developed and utilized to investigate the electrochemical behavior and determination of amlodipine (AML). The electroanalytical responses were evaluated by cyclic voltammetry, chronoamperometry and differential pulse voltammetry. The MESA/CPE showed good electrocatalytic activity with respect to the electrooxidation of AML with an over potential of 200 mV lower than that of the bare CPE. The sensor showed two linear dynamic ranges from 1.0 to 20.0 µM and 20.0 to 100.0 µM with a detection limit of 0.4 µM (S/N = 5). The MESA-CPE was applied for the determination of AML in some pharmaceutical formulations.

Keywords: Amlodipine, Mesalazine, Carbon paste electrode, Voltammetry, Electrocatalytic activity.

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Introduction

High blood pressure is the main cause of stroke and heart disease worldwide. Therefore, most people with high blood pressure need to be treated with antihypertensive [1]. AML, (IUPAC name: 3-ethyl 5-methyl 2- [(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro pyridine-3, 5-dicarboxylate) is used to lower their blood pressure. AML can be used alone or in combination with other heart medications [2, 3]. It blocks calcium from entering certain tissues and is the most commonly used drug for hypertensive patients in the world and directly protects neurons under ischemic damage [4]. The likelihood of some adverse effects such as peripheral edema, fatigue, palpitations, dyspepsia, and nausea is increased after drug ingestion [5]. Therefore, strict quality control of pharmaceuticals containing amlodipine is necessary, which requires the development of simple, rapid, and reliable analytical methods for the detection and quantification of this drug in both pharmaceuticals and biological samples.

Various analytical methods have been applied for the determination of AML in biological samples and pharmaceutical formulations like ultra violet spectroscopy [6], high-performance chromatography [7,8], a kinetic spectrophotometric method [9], high-performance thin layer chromatography [10, 11] and capillary electrophoresis [12]. The electrochemical methods have advantages over other methods because they are rapid, have short analysis time, and are low cost and easy to operate.

The research literature shows that the number of analytical methods developed using electrochemical techniques is widely increasing. In particular, developments in electrochemical sensors and nanotechnology show that these techniques enhance the selectivity and sensitivity of measurements due to the fast electron transfer, electrocatalytic character, and large working surface area. The different electrochemical sensors were reported for the determination of AML in tablets and biological samples such as CuO-NiO/ionic liquid modified CPE [13], nano-perovskite/glycine/carbon composite modified CPE [14], boron-doped diamond [15], Fe_3O_4@SiO_2/MWCNT nanocomposite modified CPE [16], Ag-$Ce_2(WO_4)_3$@CNF nanocomposite [17], $NiMoO_4$/chitosan electrode [18], multi-walled carbon nanotubes paste electrode in the presence of cationic surfactant cetyltrimethylammonium bromide (CTAB)[19], grass-like Pt-doped $NiCo_2O_4$ modified electrode [20] and gold nanorods, graphene oxide, functionalized carbon nanotubes nanocomposite [21]. Despite the advantages of the nanomaterials, the challenge in application of nanomaterials is to control not only the particle sizes but also the particle morphologies and shapes.

Mesalazine (MESA) (2-hydroxy-5-aminobenzoic) is an anti-inflammatory drug applied to treat inflammatory bowel diseases. MESA is very slightly soluble in water. The electrochemical behavior of MESA indicates that there is an oxidation peak around at 0.2 V [22]. In this work, MESA was utilized to modify the CPE (MESA-CPME) for the determination of AML using differential pulse voltammetry (DPV) in the pharmaceutical formulations. The proposed MESA-CPME has advantages such as simplicity, availability, wide linear dynamic range, high sensitivity, low cost, and low detection limit in determination of AML.

Experimental

Materials

All chemicals used such as graphite powder, mineral oil, hydrochloric acid, methanol, sodium hydroxide were of analytical grade (Merck and Sigma). Amlodipine (>98.7 %) was supplied from ARYA pharmaceutical Co., Iran. The commercial pharmaceutical tablets containing AML labeled 5.0 mg and combined dosages of AML:VAL labeled 5:80 mg were obtain from local drugstore.

Apparatus

An Autolab potentiostat/galvanostat type 302 N (Metrohm, Switzerland) carried out all the voltammetry experiments. The CPE and MESA-CPE as working electrode, a platinum wire as auxiliary electrode and Ag/AgCl/KCl 3.0 M as reference electrode were used. The pH measurements were performed using a pH meter (model481, Metrohm, Switzerland).

Preparation of working electrode

The MESA-CPME was prepared by mixing 0.37 g of graphite powder, 0.03 g of mesalazine and 0.1 g of mineral oil in a mortar with a metal spatula. The MESA paste was inserted inside a plastic cylindrical tube (inner diameter 2.5 mm) and a copper wire was used for the electrical contact. A weighing paper smoothed the MESA-CPME surface.

Electrochemical measurements

A stock solution (1.0 mM) of AML was prepared in methanol. The stock solution was diluted with the phosphate buffer (PB) before electrochemical measurements. The PB (0.1 M) was prepared by mixing $NaH_2PO_4.H_2O$ (0.2 M) and $Na_2HPO_4.2H_2O$ (0.2 M) NaOH (0.2 M) and

HCl (0.1 M) solutions were used to adjust the pH of PB. Cyclic voltammetry (CV) was used for studying electrochemical properties of the CPE and MESA-CPME in5.0 mM $[Fe(CN)_6]^{3-/4-}$solution containing 0.1 M KCl. The cyclic voltammograms were recorded ranging from–0.3 and 0.6 V at a scan rate range of 5-200 mV s^{-1}. The chronoamperometry (CA) and differential pulse voltammetry (DPV) were used for electrochemical investigation and determination of AML, respectively. DPV was performed at a potential range from 0.0 of 0.5 V and the chronoamperometric responses were recorded at a potential step of 0.4 V.

Determination of AML in formulation tablets

Two brands of tablets containing AML were purchased from the local drugstore. Ten tablets from each brand were weighed and carefully powdered with a mortar and pestle. Then, the appropriate amount of powder was dissolved in 5.0 ml methanol, filtered, and transferred in a 5.0 ml volumetric flask, and diluted with methanol. The required volume was diluted with PB (0.1 M) and transferred into an electrochemical cell. The standard addition method was used for the determination of AML in tablets.

Results and discussion

Electrochemical properties of modified electrode

At first, the cyclic voltammetry technique were utilized to study of the electrochemical behavior of the MESA-CPME and CPE in 5.0 mM $[Fe(CN)_6]^{3-/4-}$solution containing 0.1 M KCl (not shown). The results showed that redox peak currents related to $K_4[Fe(CN)_6]$ for MESA-CPME compare to bare CPE is increased. Also, peak separations (ΔEp) are found 320 and 260 mV for CPE and MESA-CPME, respectively. The results indicated that MESA-CPME can improved electron transfer in the redox process. The effective surface area (A) was evaluated by the Randles-Sevcik equation [23]:

$$I_{pa} = (2.69 \times 10^5)\, n^{2/3}\, C\, A\, D^{1/2}\, \upsilon^{1/2} \qquad (1)$$

where D is the diffusion coefficient, I_{pa} is the peak current, n is the electron number, C is the concentration of $K_4Fe(CN)_6$,A and D are the surface area of the electrode and diffusion coefficient, respectively. The effective surface area values were obtained 0.04 and 0.07$cm^2$for CPE and MESA-CPME, respectively.

The cyclic voltammograms of MESA-CPME at various scan rates (5 to 100 mV s^{-1}) in 0.1 M PB (pH 8) are shown in Figure 1. As can be seen, mesalazine showed an irreversible oxidation peak with a peak potential value at about 200 mV at scan rates. The anodic peak currents showed a linear relationship proportional to the scan rate (inset of Figure 1), demonstrating an adsorption-controlled process. Moreover, by increasing of scan rate, a single reduction peak was observed in the reverse scan at a much more negative potential (–50 mV) than would be expected for the reversible process. This behavior emphasizes the presence of a chemical reaction after an electrooxidation of MESA (EC mechanism) and a quasi-reversible electrode process at a high scan rate.

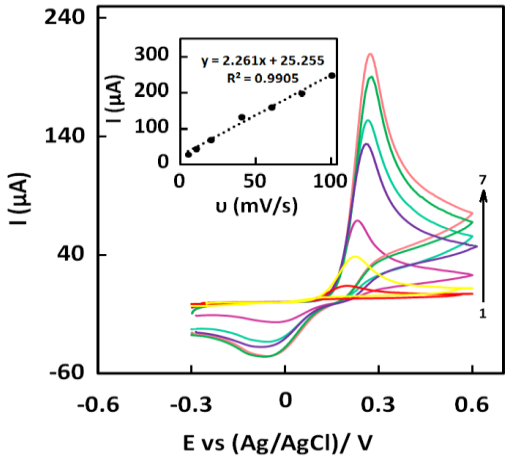

Figure 1. Cyclic voltammograms of MESA-CPME in PB (0.1 M, pH 8) on at different scan rates (υ): 1) 5.0, 2) 10.0, 3) 20.0, 4) 40.0, 5) 60.0, 6) 80.0 and 7) 100.0 mV/s.

Electrochemical behavior of AML

The electrochemical behavior of AML at the surface of CPE and MESA-CPME was evaluated using the voltammetric method. The CVs in the absence and presence of 100 µM AML in the 0.1 M PB at pH 8, at the surface of the CPE and MESA-CPME were shown in Figure 2. The CV responses indicated that electrooxidation peaks of AML at the surface of the CPE and MESA-CPME appear at about 0.52 and 0.3 V respectively. As seen, the oxidation peak potential of AML at MESA-CPME shifted by about 220mV toward the negative values compared with that at a bare CPE. In addition, a very large enhancement in the anodic current was observed at the surface of the MESA-CPME in the presence of AML. The results suggest that the electrocatalytic process occurs at MESA-CPME which can be applied to the determination of AML.

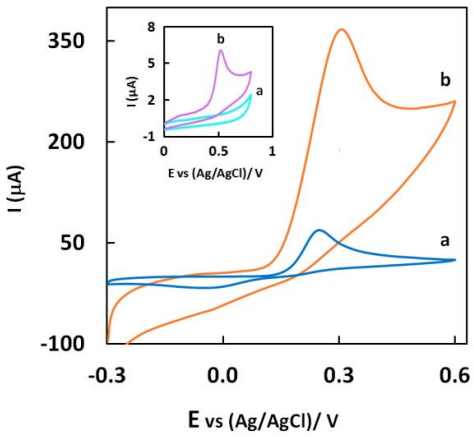

Figure 2. The CVs of MESA-CPME in the (a) absence and (b) presence of 100 µM AML. Inset: CVs of CPE in the (a) absence and (b) presence of 100 µM AML in PB (0.1 M, pH 8) at a scan rate of 40 mV/s.

Effect of pH

The influence of pH was tested using PB in the range of 4.0-9.0 by CV (Figure 3A).It can be seen that by an increase of pH value, the oxidation peak potential shifted to less positive values, as well as the oxidation peak current increased. From Figure 3B, the relationship between peak potential (Ep) and pH was obtained Ep (V) = 0.0521 pH + 0.7157 (R^2 =0.9898). The slope value (52.0 mV/pH) demonstrated equal numbers of electrons and protons involved in the electrochemical process. Moreover, according to Figure 3B, the higher oxidation peak current was found at pH 8. Therefore, PB (pH 8.0) was selected as the optimum pH for the determination of AML.

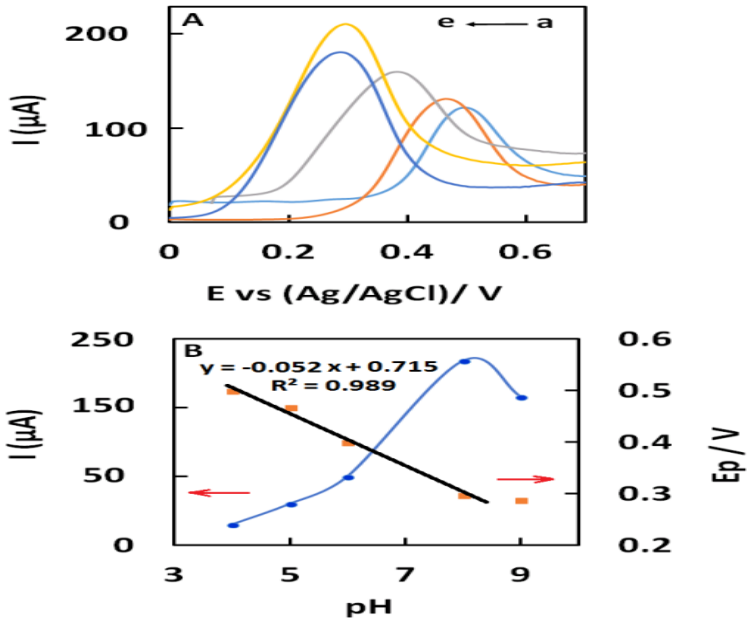

Figure 3. (**A**) DPVs of 100 µM AML on MESA-CPME at various pH values: a to e are 4, 5, 6, 8 and 9 respectively. (**B**) The relationships between the peak potential (E_p) and the oxidation peak current (I_{pa}) with pH.

Effect of scan rate

Figure 4A shows the cyclic voltammograms of the MESA-CPME at different scan rates in 0.1M PB(pH 8.0)containing 100 µM AML. As seen, in Figure 4B, a linear relationship between anodic peak currents versus the square root of the scan rates was observed, which indicates that the electrocatalytic process is controlled by AML diffusion to the electrode surface.

$$I_{pa}\ (\mu A) = 18.85\ \upsilon\ (mV\ s^{-1}) - 8.2056\ (R^2 = 0.993\ ) \quad (2)$$

The electrooxidation peak potentials shift slightly toward the positive direction by increasing of scan rate (Figure 4A). This behavior confirms the kinetic limitation of the electrochemical reaction. The number of electrons in the overall reaction can be obtained from the slope of the IP versus v1/2 plot (Figure 4B). According to the following equation for totally irreversible diffusion-controlled processes [23]:

$$I_P = 3.01\times10^5 n[(1-\alpha)n_\alpha]^{1/2} ACD^{1/2}v^{1/2} \quad (3)$$

and considering (1−α) nα= 0.39 (see below), D= 7.76×10−5 cm2 s−1 (obtained by chronoamperometry shown below), and A = 0.07 cm2, it is estimated that the total number of electrons involved in the anodic oxidation of AML is n = 1.6 (≈2). Figure 4C shows the Tafel plot drawn using the data derived from the rising part of the voltammogram at a scan rate of 5 mVs−1.The number of electrons involved in the rate-determining step, nα, can be estimated from the slope of the Tafel plot [23]. A Tafel slope of 0.150 V per decade was obtained, indicating that one electron process was involved in the rate-determining step, assuming a charge transfer coefficient of α = 0. 61. Based on the results, Figure 5 can be describing the mechanism of electrooxidation of AML.

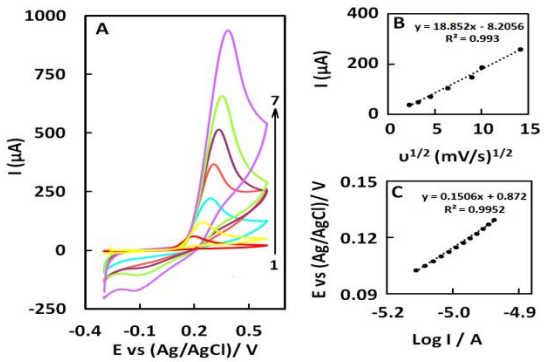

Figure 4(A) Cyclic voltammograms of AML (100 µM) on MESA-CPME in PB (0.1 M, pH 8) with different scan rates (*υ*): 1) 5.0, 2) 10.0, 3) 20.0, 4) 40.0, 5) 80.0, 6) 100.0 and 7) 200.0 mV/s. (B) linear dependence of I_p versus square root of the scan rate. (C) The tafel plot derived from the CV at scan rate of 5 mV/s.

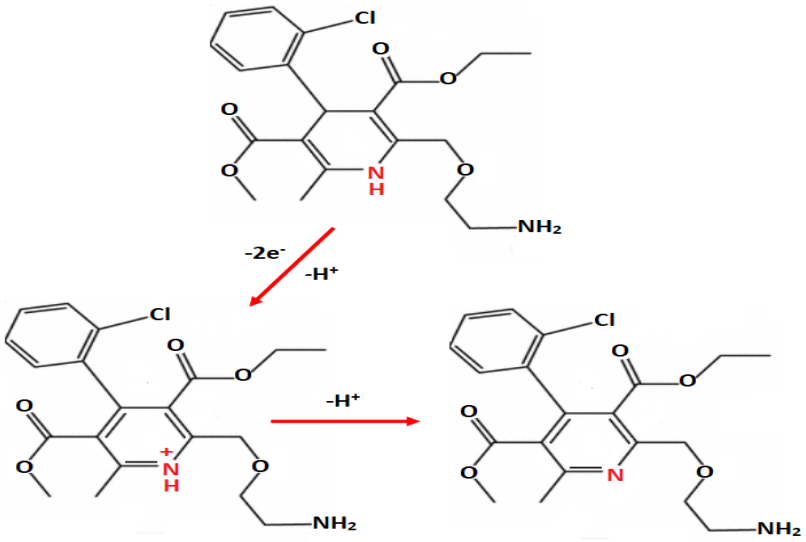

Figure 5. Electrooxidation mechanism of AML.

Chronoamperometry

The chronoamperometry was used to calculate diffusion coefficients of AML in PB (pH 8) at the potential step of 0.5 V (Figure6A). The various concentration of AML (50-250 µM) was used in this work. The Cottrell equation [23] was used to calculate diffusion coefficient of AML:

$$I_{pa} = n\,F\,A\,C\,D^{1/2}\pi^{-1/2}t^{-1/2} \qquad (4)$$

Where F is the Faraday constant, Ipa is the current, D is the diffusion coefficient, n is the number of electrons (n= 2 for AML electrooxidation), A is the effective surface area (0.07cm–2), t is the time and Cis the analyte concentration. Figure 6B, shows a linear relationship between Ipa vst–1/2. The plots for different of AML were indicated in Figure 6C, inset b. The diffusion coefficient of AML was obtained to be 7.76× 10–5 cm2/s.

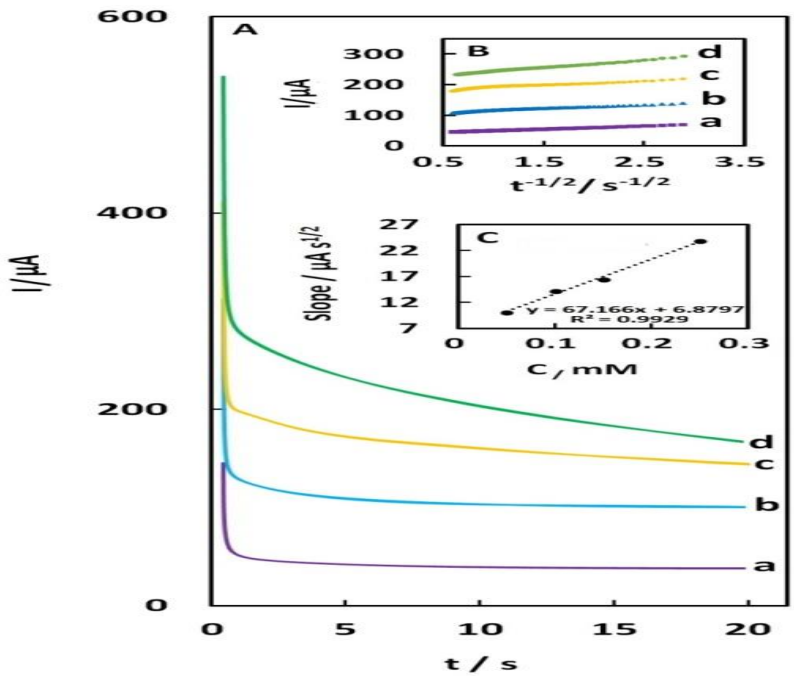

Figure 6. (**A**) Chronoamperometric response of the MESA-CPME in PB (0.1 M, pH 8) for different concentration of AML (0.05-0.25 mM) for a potential step of 0.5 V Ag/AgCl. The traces of a-d correspond to 0.05 to 0.25 mM of AML. **B)** Plots of I *vs* $t^{1/2}$ obtained from chronoamperograms a-d. **C)** Plot of the slope of the straight lines against the AML concentration a-d.

Determination of AML

DPV is better than that cyclic voltammetry in sensitivity and resolution. Therefore, this method was employed for determining AML on the MESA-CPME. Figure 7 shows the differential pulse voltammograms of AML at MESA-CPME in concentration range of 1.0 – 100.0 µM and the variation of oxidation peak currents versus AML concentration. As seen in Figure 7A, two linear segments from 1.0 to 20.0 µM and 20.0 to 100.0 µM were observed. The linear regression equations were calculated as Ip (µA) = 1.9014 C (µM) + 7.256 (R2=0.9902) and Ip (µA) = 0.4150 C (µM) + 35.8533 (R2= 0.9958) respectively. Moreover, the limits of detection (LOD) and quantitation (LOQ) were calculated using the relation ksb−1, where k = 3 for LOD and 10 for LOQ, b and s are the slope of the calibration curve and the standard deviation, respectively. The LOD and LOQ values for the determination of AML were obtained 0.4 and 2.1 µM respectively. The comparison of the analytical performance of MESA-CPME with other reports is presented in Table 1. As can be seen, the detection limit and linear calibration range for AML determination of this work are comparable and or better than those obtained by other reports.

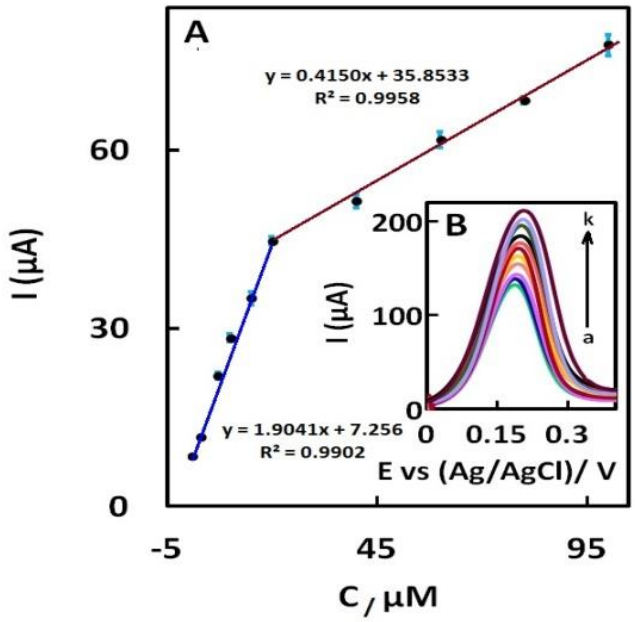

Figure 7. A) Plot of differential pulse voltammograms peak current versus AML concentration. B) Differential pulse voltammograms of MESA-CPME in the solution containing AML at different concentrations: a) 0, b) 1.0, c) 3.0, d) 7.0, e) 10.0, f) 15.0, g) 20.0, h) 40.0, i) 60.0, g) 80.0 and k) 100.0µM.

Table 1. The comparison of different methods for AML determination.

Electrode	Technique	Linear range (µM)	LOD (µM)	Ref.
CuO-NiO/IL/CPE	DPV	0.1-100	0.06	[13]
Boron-doped diamond electrode	DPV	6-38	0.07	[15]
Fe_3O_4@SiO_2/MWCNT/ CPE	SWV	0.25-500	0.15	[16]
MWCNT paste electrode/ CTAB	SWV	0.58–5.9	0.049	[19]
MWCNT/gold electrode	SWASV[a]	24-34	4.2	[24]
$NiMoO_4$/CHIT/GCE	DPV	0.1-374.5	0.012	[25]
GCE	DPV	4-100	0.8	[26]
GCE	DPV	1-35	0.31	[27]
ZrO_2/GCE	DPV	10-200	2	[28]
Poly (Gly)[b]/GCE	DPAdSV[c]	0.5-25	0.08	[29]
Pt-NiO/MWCNTs/GCE	DPV	1-250	0.092	[30]
MWCNT/CuNPs-CPE	AdSWV[d]	0.02.0 - 6.3	0.0005	[31]
MESA/CPE	DPV	1.0 - 20.0 20.0 100.0	0.4	This work

Analytical features

The intra-day and inter-day precisions were investigated for determining AML (100 µM) in terms of relative standard deviation (RSD) for five measurements at MESA-CPME. The RSD values of AML were about 98.2 % and 100.2 % for intra-day and inter-day, respectively. The long-term stability of the MESA-CPME was obtained about two mounts for determination of AML. The reproducibility of the modified electrode was examined by three different MESA-CPMEs. The reproducibility presented a satisfactory RSD value of 4.9 %.

Real sample analysis

To examine the practical ability of MESA-CPME, recovery determination of AML in Tablet is performed. Recovery results can also be evaluated as a measure of selectivity. For this reason, in order to know whether the excipients or ingredients (such as gelatin, colloidal silicone dioxide, cellulose, lactose, starch, magnesium stearate, sodium lauryl sulfate, titanium dioxide) in the pharmaceutical formulations showed any interference with the analysis, a recovery test was done by the standard addition method. The recovery results are shown in Table 2. The average recovery result was 99.3 %. These data showed that there was no interaction of excipients in the analysis of AML in pharmaceutical formulations by the proposed method and it can be concluded that the proposed electrochemical sensor could be utilized for the reliable determination of AML.

Table 2. Results for the determination of AML.

Sample	Standard added (µM)	Total concentration (µM)	Found (µM)	Recovery (%)	RSD (%)	Bias (%)
AML5 mg (Amlodipine ARYA 5)	0	100.0	99.3	99.3	0.3	0.7
	5	105.0	99.0	98.2	0.4	1.8
	10	110.0	98.9	99.7	0.4	0.3
AML:VAL 5/80 mg (Valzomix Abidi)	0	100.0	98.8	100.2	0.9	0.2
	5	105.0	99.6	99.6	0.6	0.4
	10	110.0	98	99.0	0.3	1.0

Conclusions

MESA is used for the electrooxidation of AML in PB of pH=8. The modified electrode decreases anodic over potential for oxidation of AML with respect to bare electrode and

increases the anodic peak current in CV and DPV. The modified electrode by MESA show low detection limit, good sensitivity, reproducibility and long-term stability in determination of AML. Some kinetic parameters such as diffusion coefficient and electron transfer coefficient of AML were obtained using electrochemical approaches.

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List of abbreviations

Adsorptive square wave voltammetry (ASWV)

Amlodipine (AML)

Carbon paste electrode (CPE)

Cetyltrimethylammonium bromide (CTAB)

Chronoamperometry (CA)

Chitosan (CHIT)

Cyclic voltammetry (CV)

Differential pulse adsorptive stripping voltammetric (DPASV)

Differential pulse voltammetry (DPV)

Glassy carbon electrode (GCE)

Glycine (Gly)

Ionic liquid (IL)

Limits of detection (LOD)

Limits of quantitation (LOQ)

Mesalazine (MESA)

Mesalazine modified carbon paste electrode (MESA/CPE)

Multiwall carbon nanotubes (MWCNT)

Phosphate buffer (PB)

Square-wave anodic stripping voltammetry (SWASV)

Valsartan (VAL)