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Original scientific paper

Screen printed carbon electrode modified with magnetic core shell manganese ferrite nanoparticles for electrochemical detection of amlodipine

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Abstract: In the present work, a rapid and sensitive detection of the amlodipine (AML) based on the modified screen printed carbon electrode (SPCE) is developed. At first, SPCE was modified with magnetic core shell manganese ferrite nanoparticles (MCSNP). Cyclic voltammetry (CV) was used to study the electrochemical behaviour of AML and its determination was conducted by applying differential pulse voltammetry (DPV). The results were showed that, the modified SPCE in comparison with unmodified SPCE exhibited the enhanced electrocatalytic activity toward the oxidation of AML. A single irreversible oxidation peak was observed at a potentials of 600 and 725 mV (on the scale of Ag/AgCl electrode) on the modified SPCE and unmodified SPCE, respectively. Under the optimized conditions, the anodic peak current of AML recorded by DPV varies linearly with AML concentration in the range 0.5–400 μM with a detection limit of 0.1 μM. The modified SPCE was used for the quantitative analysis of AML in AML tablet and urine samples and the results indicated the feasibility of the developed method for AML analysis in routine detection.

Keywords: amlodipine determination; magnetic core shell nanoparticles; screen-printed carbon electrode.

INTRODUCTION

The increased blood pressure is the leading risk factor for premature death, stroke and heart disease worldwide. In the year 2000, the world was estimated to have close to 1 billion people with hypertension and predicted an increase to 1.56 billion by 2025.[1]

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Amlodipine (3-ethyl-5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, AML) is a third generation dihydropyridine calcium channel blockers which is used alone or in combination with other medications for treating high blood pressure, certain types of vasospastic angina, hypertension, cardiac arrhythmias and coronary heart failure.[2] It inhibits the transportation of the calcium ions into the vascular smooth muscle and the cardiac muscle to protect the target organs. Moreover, it provides the significant neuroprotective effects under the ischemic damage in comparison with the first and second generation of calcium channel blockers.[3]

Owing to the therapeutic importance of AML, the development of the analytical procedures for AML determination with the high sensitivity and selectivity is not only of the pharmaceutical significance for point-of-care detection, but it is also important for industrial purposes.[4] For this purpose, the various methods including spectrophotometry,[5,6] high performance liquid chromatography (HPLC),[7–9] high performance thin layer chromatography (HPTLC),[10] gas chromatography (GC),[11,12] capillary electrophoresis (CE)[13,14] and enzyme-linked immune-sorbent assay[15] have been reported for AML determination in pharmaceutical formulations and biological fluids. Although these methods, sensitive for the determination of AML, with the exception of spectrophotometry, require the expensive instruments, laborious sample pre-treatment, highly skilled technicians, long analysis time and generate large amount of wastes, which make them unsuitable in quality control laboratories.

Recently, the voltammetric methods have been found as a highly-sensitive, convenient and effective tool for the analysis of important biomolecules including drugs in pharmaceutical formulations and human body fluids owing to their simplicity, low cost and relatively short analysis time as compared to the other routine analytical techniques.[16] Hence, the voltammetric determination of amlodipine on electrodes such as carbon paste[17] and glassy carbon electrode (GCE)[18–20] has also been reported.

The development of the screen-printed electrodes (SPEs) has become a major revolution in the construction of electrochemical sensors/biosensors.[21] The SPEs have been designed especially for the miniaturization of the electrochemical analytical systems.[22] SPEs are highly-versatile, easy to use, cost-effective analytical tools, also suitable to miniaturization.[23] Furthermore, a SPE avoids the cleaning process, unlike conventional electrodes such as a GCE.[24]

Nowadays, it continues to be of interest in the developments of the new materials capable to change the electrode surface with better analytical properties, including graphene, nanoparticles, and carbon nanotubes.[25–28] The nanomaterials, because of their unique properties, have been extensively developed. Nanoparticles can act as the conduction centres facilitating the transfer of electrons and provide the great catalytic surface areas.[29–33]

Among them, the nanosized metal particle modified electrodes have emerged as a promising alternative for the electroanalysis of organic and inorganic compounds.[34–40] The metal nanoparticles have some distinct advantages such as higher mass transport, lower influence of the solution resistance, low detection limit, and better signal-to-noise ratio over the conventional macroelectrodes.[41,42]

In the present work, we synthesized the magnetic core-shell manganese ferrite nanoparticles (MCSNP)[43] and the screen printed carbon electrodes were modified with MCSNP (MCSNP/SPCE). To the best of our knowledge, no study has been reported so far on the determination of amlodipine using MCSNP/ /SPCE.

EXPERIMENTAL

Apparatus and chemicals

The electrochemical measurements were performed by the Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System software. The screen printed carbon electrodes were purchased from Italsens Co. A Metrohm 710 pH meter was used for pH measurements. The scanning electron microscopy (Cam Scan MV2300) was used to explore the surface morphology of the MCSNP.

Amlodipine and all the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0–9.0. The magnetic core-shell manganese ferrite nanoparticles were synthesized in our laboratory as reported previously.[43]

Preparation of the electrode

The bare screen-printed electrode (0.0314 cm^2) was coated with MCSNP as follows. A suspension of MCSNP in 1 mL deionized water was prepared by dispersing 1 mg MCSNP with ultrasonication for 1 h, and a 2 μL aliquot of the MCSNP/H_2O suspension was casted on the carbon working electrodes, waiting until the solvent was evaporated at room temperature.

Preparation of real samples

Ten tablets of AML (labeled 10.0 mg per each tablet) were completely ground and homogenized, 400 mg of this powder was accurately weighed and dissolved with the ultrasonication in 20 mL of deionized water. Finally the mixture was filtered and the clear filtrate was transferred into a 100 mL volumetric flask and diluted to the mark using 0.1 M PBS with pH 7.0. Finally, a suitable volume of the resultant solution was transferred to the electrochemical cell and the resulting solution was used for the analysis of AML. The sample was spiked with the different amounts of AML and the contents were analyzed using the standard addition method in order to minimize any matrix effect. The amount of AML in the tablets can be detected by extrapolating the plot of signal (current) *vs*. concentration.

The urine samples of healthy volunteers were stored in a refrigerator immediately after the collection. Ten mL of the sample was centrifuged for 10 min at 3000 rpm. The supernatant was filtered out using a 0.45 μm filter. Then it was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine sample was spiked with the different amounts of AML. The AML contents were analyzed by the proposed method using the standard addition method in order to minimize any matrix effect.

RESULTS AND DISCUSSION

Characterization of MCSNP

The scanning electron microscope (SEM) is an important tool to characterize the surface morphology and is useful to determine the particle shape and size distribution of the nanoparticles. The SEM of MCSNP is shown in Fig. 1 and showed that MCSNP has relatively homogenous particles (≥100 nm).

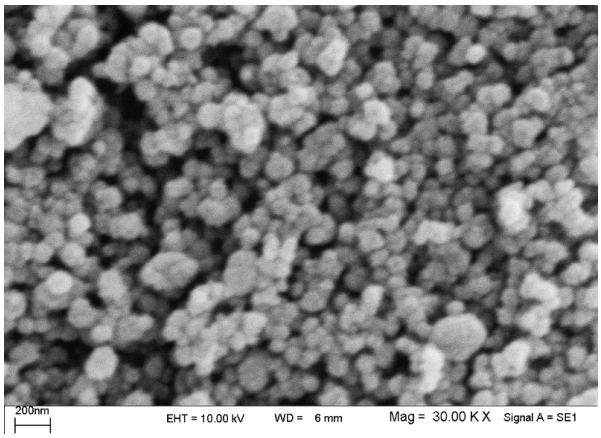

Fig. 1. SEM image of MCSNP.

The electrochemical behaviour of amlodipine at the surface of MCSNP/SPCE

The electrochemical behaviour of AML is dependent on the pH value of the aqueous solution. Therefore, the pH optimization of the solution seems to be necessary in order to obtain the best results for electro-oxidation of AML. Thus the electrochemical behaviours of AML were studied in 0.1 M PBS in different pH values (2.0–9.0) at the surface of MCSNP/SPCE by using CV. It was found that the electro-oxidation of AML at the surface of MCSNP/SPCE was more favoured under the neutral conditions than in acidic or basic medium. Thus, the pH 7.0 was chosen as the optimum pH for electro-oxidation of AML at the surface of MCSNP/SPCE.

Fig. 2 depicts the CV responses for the electro-oxidation of 400.0 μM AML at an unmodified SPCE (curve a) and MCSNP/SPCE (curve b). The peak potential due to the oxidation of AML occurs at 600 mV, which is about 125 mV more negative than that of the unmodified SPCE.

Also, the MCSNP/SPCE shows much higher anodic peak current for the oxidation of AML compared to unmodified SPCE, indicating that the modification of SPCE with MCSNP has significantly improved the performance of the electrode toward the AML oxidation.

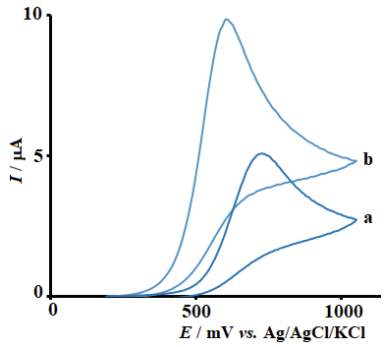

Fig. 2. Voltammograms of: a) unmodified SPCE and b) MCSNP/SPCE in the presence of 400.0 µM AML at pH 7 (at 50 mV s^{-1}).

Effect of scan rate

The effect of the potential scan rates on the oxidation current of AML (Fig. 3) have been studied by linear sweep voltammetry (LSV). The results showed that the increase in the potential scan rate induced the raise of the peak current. In addition, the oxidation processes are diffusion controlled as it is deduced from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$).

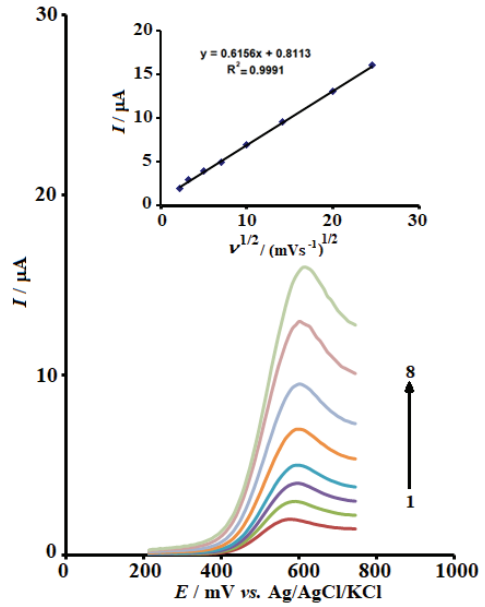

Fig. 3. LSVs of MCSNP/SPCE in 0.1 M PBS (pH 7.0) containing 200.0 µM AML at various scan rates; numbers 1–8 correspond to 5, 10, 25, 50, 100, 200, 400 and 600 mV s^{-1}, respectively. Inset: variation of anodic peak current *vs*. square root of scan rate.

The Tafel plot was drawn from the data of the rising part of the current voltage curve recorded at a scan rate of 5 mV s^{-1} for AML (Fig. 4). This part of the voltammogram, known as the Tafel region, is affected by the electron transfer

kinetics between substrate (AML) and MCSNP/SPCE. The Tafel slope of 0.1073 V was obtained, which agrees well with the involvement of one electron in the rate determining step of the electrode process[44] assuming the charge transfer coefficient $\alpha = 0.45$ for amlodipine.

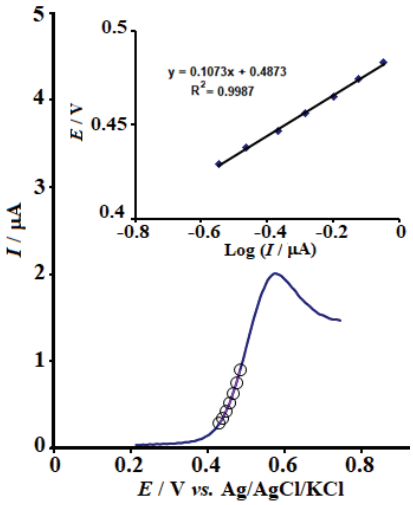

Fig. 4. LSV of MCSNP/SPCE (at 5 mV s^{-1}) in 0.1 M PBS (pH 7.0) containing 200.0 μM AML. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.

Chronoamperometric measurements

The chronoamperometric measurement of AML at MCSNP/SPCE was carried out by setting the working electrode potential at 0.8 V vs. Ag/AgCl/KCl (3.0 M) for the various concentrations of amlodipine (Fig. 5) and in PBS (pH 7.0). For the electroactive compounds (in this case AML) with a diffusion coefficient of D, the current observed for the electrochemical reaction, at the mass transport limited condition, is described by the Cottrell equation:[44]

$$I = nFAD^{1/2} c_b \pi^{-1/2} t^{-1/2}$$

where D and c_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of AML (Fig. 4a). The slopes of the resulting straight lines were then plotted *vs.* AML (Fig. 5b) concentrations. From the resulting slope and Cottrell equation, the mean value of the D was found to be 4.5×10^{-6} cm^2 s^{-1} for AML.

Calibration plot and limit of detection

The electro-oxidation peak current of AML at the surface of the MCSNP/ /SPCE can be used for the determination of AML in solution. Since, DPV has the advantage of an increase in sensitivity and better characteristics for analytical applications, therefore, the DPV experiments were performed using MCSNP/SPCE

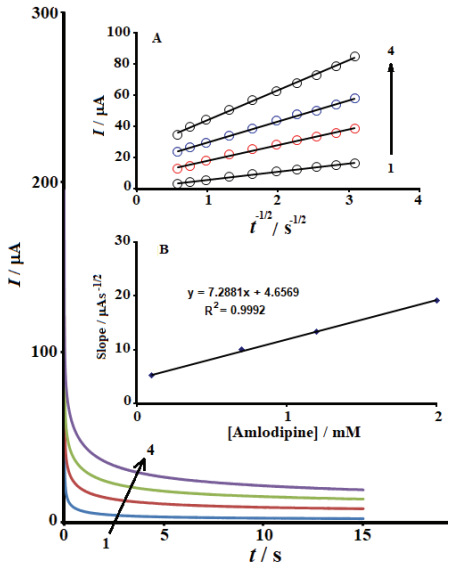

Fig. 5. Chronoamperograms obtained at surface of MCSNP/SPCE in 0.1 M PBS (pH 7.0) for different concentration of AML. The numbers 1–4 correspond to 0.1, 0.7, 1.2 and 2 mM of AML. Insets: Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–4 (A), and plot of the slope of the straight lines against AML concentration (B).

in 0.1 M PBS containing the various concentrations of AML (Fig. 6). The results show the electrocatalytic peak currents of AML oxidation at the surface of MCSNP/SPCE which were linearly dependent on the AML concentrations, over the range of 5.0×10^{-7}–4.0×10^{-4} M (with a correlation coefficient of 0.9991). The

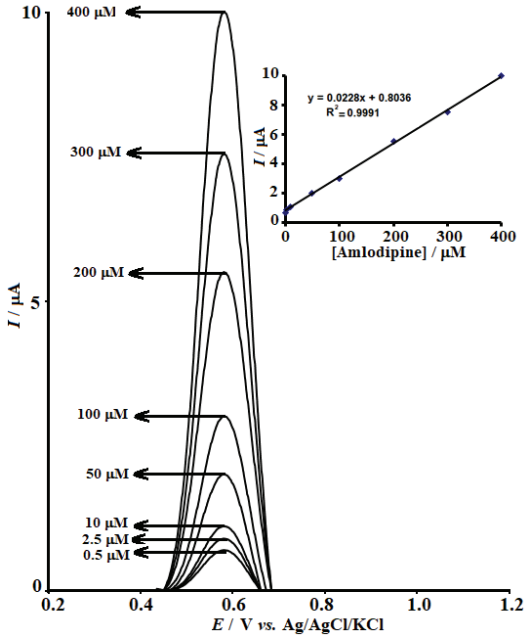

Fig. 6. DPVs of MCSNP/SPCE in 0.1 M PBS (pH 7.0) containing different concentrations of AML (0.5, 2.5, 10, 50, 100, 200, 300 and 400 μM). Inset: The plot of the peak current as a function of AML concentration in the range of 0.5––400 μM.

detected limit, based on the three times the standard deviation of the blank (3*s*) was 1.0×10^{-7} M. These values are comparable with the values reported by other research groups for electro-oxidation of AML at the surface of chemically modified electrodes (see Table I).

Table I. Comparison of the performances of methods used in detection of AML

Electrode	Method	Modifier	*LOD* / μM	*LDR* / μM	Ref.
SPCE	SWV	DNA	0.149	0.066–2.0	4
EPPGE[a]	SWV	MWCNT[b]	0.001	0.005–1.2	16
GCE	DPV	–	12	8.1–41	20
Diamond electrode	DPV	BDDE[c]	0.07	0.2–38.0	45
Carbon paste electrode	SWASV[d]	TiO_2 nanoparticles	0.003	0.01–1	46
GCE	DPV	–	0.8	4–100	47
Diamond electrode	SWV	BDDE	0.0764	0.497–28.6	48
Carbon paste electrode	SWV	MWCNT	0.049	0.58–5.9	49
SPCE	DPV	MCSNP	0.1	0.5–400	This work

[a]Edge plane pyrolytic graphite electrode; [b]multi-walled carbon nanotubes; [c]boron-doped diamond electrode; [d]square wave adsorptive stripping voltammetry

Interference studies

The influence of various substances as the compounds potentially interfering with the determination of amlodipine was studied under the optimum conditions. The potentially interfering substances were chosen from the group of substances commonly found with AML in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5 % in the determination of AML. According to the results, l-lysine, glucose, uric acid, l-asparagine, l-serine, l-threonine, l-proline, l-histidine, l-glycine, l-phenylalanine, lactose, saccarose, fructose, benzoic acid, methanol, ethanol, urea, caffeine, dopamine, epinephrine, norepinephrine, serotonin, ascorbic acid, Mg^{2+}, Al^{3+}, NH_4^+, F^-, SO_4^{2-} and S^{2-} did not show interference in the determination of AML (equal molar).

Real sample analysis

Finally, MCSNP/SPCE was applied for the measurement of AML in AML tablet, and urine samples. For this purpose, the measurement of AML in the real samples was carried out (Table II). Also, the recovery of AML from samples spiked with the known amounts of AML was assessed. The results showed that the added AML was quantitatively recovered from the real samples. These results demonstrate the applicability of the MCSNP/SPCE for the measurement of AML in the real samples. Also, the reproducibility of the method was demonstrated by the relative standard deviation (*RSD*).

The amount of AML in the tablets were found to be 10.02 mg/tablet. It was found that there was no significant difference between the results obtained by the proposed voltammetric method with the MCSNP/SPCE and the declared value

on the tablet label (10.00 mg/tablet). The *t*-test was applied to the result, showing that there was no significant difference at the 95 % confidence level.

TABLE II. The application of MCSNP/SPCE for determination of AML in real samples ($n = 5$)

Sample	Spiked, μM	Found, μM	Recovery, %	*RSD* / %
AML tablet	0.0	10.7	–	3.0
	20.0	31.0	101.0	2.5
	40.0	50.1	98.8	2.7
	60.0	72.3	102.3	3.2
Urine	0	Not detect	–	3.1
	20.0	20.7	103.5	2.4
	40.0	39.1	97.7	2.5
	60.0	61.4	102.3	2.5

CONCLUSIONS

In this work, the use of the magnetic core shell nanoparticles as a modifier of SPCEs, a novel sensor has been developed, that provides a sensitive method for the determination of AML. The proposed protocol demonstrated herein a novel, simple, portable, inexpensive and easy-to-use fabrication method for the measurement of AML concentration in tablets and urine samples with good analytical performance. Due to the unique properties of the magnetic core shell nanoparticles, the sensor exhibited the remarkable electrochemical activity toward the oxidation of AML. Under the optimized conditions, the differential pulse voltammetry exhibited the linear dynamic ranges from 0.5–400 μM with the detection limit of 0.1 μM.

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ИЗВОД

ШТАМПАНА ЕЛЕКТРОДА ОД УГЉЕНИЧНЕ ПАСТЕ МОДИФИКОВАНА МАГНЕТИЧНИМ НАНОЧЕСТИЦАМА МАНГАН-ФЕРИТА ТИПА ЈЕЗГРО–ОМОТАЧ ЗА ЕЛЕКТРОХЕМИЈСКУ ДЕТЕКЦИЈУ АМЛОДИПИНА

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У раду је приказан развој модификоване штампане електроде од угљеничне пасте за брзу и осетљиву детекцију амлодипина. Модификација је извршена магнетичним наночестицама манган-ферита типа језгро–омотач. Електрохемијско понашање амлодипина је испитивано цикличном волтаметријом и диференцијалном пулсном волтаметријом. Показано је да модификована електрода има већу активност за оксидацију амлодипина од немодификоване електроде. На волтамограму је запажен један иреверзибилни струјни пик оксидације амлодипина на потенцијалима 600 и 725 mV (на скали Ag/AgCl електроде) на модификованој и немодификованој електроди, редом. Под оптимизова-

ним условима струјни пик оксидације амлодипина регистрован диференцијалном пулсном волтаметријом зависи линеарно од концентрације у опсегу 0,5–400 μM уз границу детекције од 0,1 μM. Модификована електрода је коришћена за квантитативну анализу амлодипина у таблетама и узорцима урина. Добијени резултати су показали да се описана метода може користити за рутинску детекцију амлодипина.

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