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Fabrication of a Selective and Sensitive Electrochemical Sensor Modified with Magnetic Molecularly Imprinted Polymer for Amoxicillin

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In this work, a modified electrochemical sensor for the determination of amoxicillin (AMX) is introduced. A magnetic molecularly imprinted polymer (MMIP) was suspended in AMX solution and then collected on the surface of a magnetic carbon paste electrode (CPE) via a permanent magnet, situated within the carbon paste electrode, and then the voltammetry signals were recorded. It was confirmed that MMIP/CPE shows greater recognition ability than the magnetic non-molecularly imprinted (MNIP)/CPE. The influence of the operational parameters including pH, MMIP amount, extraction time and accumulation time was elucidated. The performance of the fabricated sensor was evaluated and the results indicated that the sensor has a high sensitivity in AMX detection, with a linear range from 0.0010-0.11 μ M and a limit of detection of 0.26 nM. The MMIP/CPE is simple to fabricate and easy to use and was successfully applied to the determination of AMX in pharmaceutical samples with recoveries between 98.8 and 103.2%, without the need of a sample pre-treatment steps.

Keywords: Modified electrodes, Molecularly imprinted polymers, Electrochemical sensors, Magnetic nanoparticles, Amoxicillin

INTRODUCTION

AMX is a beta-lactam antibiotic. It is bactericidal and is used in susceptible infections including actinomycosis, anthrax, biliary-tract infections, bronchitis, endocarditis, gastroenteritis, gonorrhoea, Lyme disease, mouth infections, otitis media, pneumonia, spleen disorders, typhoid and paratyphoid fever and urinary tract infections [1]. Because of its broad spectrum bactericidal activity, safety and efficacy, it was found widespread applicability in medicines in the form of capsules, tablets, injections, and powder for oral suspension [2] and treatment of humans and animals [3]. At present, some analytical methods have been used to determine AMX, such as spectroscopy [4], chromatography [5], surface plasmon resonance [6] and electrochemical method [7-9].

Because of the limitations of these methods, there is a need to develop a simple, sensitive, inexpensive, and rapid analytical method for the detection of AMX. Electrochemical methods have been found as a highly-sensitive, convenient and effective tool for the analysis of important biomolecules including active ingredients in pharmaceutical formulations and human body fluids [10,11]. Several strategies have been used to enhance sensitivity and selectivity of an electrochemical sensor. Molecularly imprinted polymers (MIPs) allow the construction of selective recognition sites in synthetic polymers which serve as synthetic mimics of antibody-antigen interactions. This technique, which allows high affinity and selectivity toward particular target compounds, has aroused great interest in recent years. A traditional electrochemical MIP sensor, which uses electro-polymerization modified molecularly imprinted film, is not suitable for mass detection and the process of electrode

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regeneration is difficult. For this reason, the MIP technique has been used together with magnetic nanoparticles to develop magnetic MIP or MMIP sensors. Magnetic nanoparticles can be easily isolated from samples using an external magnetic field without centrifugation. MMIPs have been successfully used as sorbents to recognize and separate the template. Additionally, MMIPs allow a controllable rebinding process and permit easy and quick magnetic separation. Fe₃O₄ nanoparticle-based MMIPs, due to their high performance in function-specific biological applications, smooth surfaces, narrow size distributions, large surface areas, high magnetic saturation to provide a maximum signal, and good dispersion in liquid media, have gained wide attraction. Fe₃O₄ particles are encapsulated inside MIPs; the resulting polymer material will be easily collected and isolated by an external magnetic field without additional centrifugation or filtration. Before MIP synthesis around the magnetic nanoparticles, magnetite is usually functionalized (surface-modified) with oleic acid or ethylene glycol, although other less common reagents have also been proposed for magnetite surface modification [12-15]. Therefore, the use of MMIPs allows electrochemical detection while eliminates the problem of regeneration difficulty occurring with traditional electrode systems.

MMIP-based electrochemical sensors are fast and inexpensive, yet have high sensitivity and selectivity, and we reasoned that these sensors could be designed to allow detection of AMX [16,17]. In this study, MIPs were prepared on a magnetite surface, and AMX was linked to the cavities using binding sites of MIPs. The magnetic MIPs were then separated from the solution and deposited onto a magnetic CPE surface, and the bound AMX was measured. It was found that the MMIP-sensor has a high sensitivity and excellent specificity toward AMX and a good reproducibility, stability, and recovery. This study provides a method for construction of an electrochemical sensor and also introduces this method as an ideal approach for detection of AMX in various real samples.

EXPERIMENTAL

Apparatus and Chemicals

Fourier transform infrared (FT-IR) spectra were recorded in the range of 400-4000 cm⁻¹ on a

PerkinElmer, spectrum 100, FT-IR spectrometer. KBr pellets were used to prepare the samples for FT-IR measurements. Morphologies of the prepared composites were studied by a scanning electron microscope (SEM-EDX, Philips, XL30). X-ray powder diffraction (XRD, 38066 Riva, d/G. Via M. Misone, 11/D (TN) Italy) was employed to analyze the chemical components of the composites. Electrochemical experiments were carried out at room temperature using a Behpajoh potentiostat/galvanostat system (model BHP-2065). The electrochemical cell was assembled with a conventional three-electrode system: an Ag/AgCl as a reference electrode (Azar electrode) and a platinum disk as an auxiliary electrode. Different working electrodes including CPE and modified CPEs were used. The pH measurements were carried out using a Metrohm pH meter (model 713) with a combined pH glass electrode.

All chemicals and reagents used in this work were of analytical grade and used as received without further purification. AMX was prepared from Sigma-Aldrich. Deionized distilled water (DDW) was used to prepare all the solutions. The commercial pharmaceuticals available from a local pharmacy were subjected to the analysis. Britton-Robinson (B-R) universal buffer (0.04 M boric acid, 0.04 M acetic acid and 0.04 M phosphoric acid) was prepared in DDW and used as the supporting electrolyte.

Preparation of Fe₃O₄ Magnetite and MMIPs

The Fe₃O₄ magnetite was synthesized by the coprecipitation method: 0.01 mol FeCl₂·4H₂O and 0.02 mol FeCl₃·6H₂O were dissolved in 100 ml of water in a three-necked reactor (250 ml). The mixture was stirred equably and purged with nitrogen gas. When the temperature increased to 80 °C, 40 ml sodium hydroxide solution (2.0 M) was added to it. This reaction lasted for 1 h and remained at 80 °C. When the temperature dropped to the room temperature, the magnetic precipitates obtained were isolated from the solution by an external magnetic field and washed with deionized water several times until it was neutral.

The MMIP was synthesized as follows: the AMX (1.0 mmol) dissolved in 10 ml water:ethanol (9:1, v/v) and 8.0

mmol methacrylic acid were stirred for 30 min for the preparation of the pre-assembly solution. The Fe_3O_4 magnetite (1.0 g) was mixed with 1.0 ml oleic acid and stirred for 10 min. Then, 20 mmol ethylene glycol dimethacrylate (EGDMA) and the pre-assembly solution were added to the mixture of Fe_3O_4 and oleic acid. This mixture was subjected to ultrasound for 30 min for the preparation of the pre-polymerization solution. After that, the polyvinylpyrrolidone (PVP) (0.4 g) was dissolved in 100 ml ethanol in a three-necked round-bottomed flask. The mixture was stirred at 300 rpm and purged with N_2 gas while the temperature increased to 60 °C. The pre-polymerization solution was added into the three-necked flask, and then 0.1 g azobisisobutyronitrile (AIBN) was also added to it. The reaction was allowed to proceed at 60 °C for 24 h. After the polymerization, the polymers were separated by the external magnetic and washed with methanol:acetic acid (8:2, v/v) for several times, then washed with methanol for several times. Then, the polymers were washed with water three times again and dried at 60 °C. The MNIP was prepared and processed similarly as above, except that the template molecule AMX was not added [18].

Fabrication of Magnetic CPE

Magnetic CPE was prepared by thoroughly hand mixing of graphite powder with an appropriate amount of paraffin oil in a mortar using a pestle (75:25, w/w%). A portion of the composite mixture was packed firmly into a piston-driven carbon paste electrode holder in which a cylindrical magnet was placed on the back of paste. The paste was carefully packed into the syringe tip to avoid possible air gaps, which often enhance the electrode resistance. The external surface of the carbon paste was smoothed with a soft paper. A new surface was produced by scraping out the old surface and replacing the new carbon paste. MMIP/CPE and MNIP/CPE were obtained by accumulation of MMIP and MNIP on the surface of magnetic carbon paste electrode.

Determination of AMX

For AMX determination, 15 mg of MMIP was dispersed in 50 ml of AMX solution, adjusted to pH = 7.0 and were shaken continuously using a vortex mixer. This stage lasted

for 5 min and led to the extraction of AMX in the MMIP. Afterwards, the magnetic CPE was inserted into the solution, leading to accumulation of MMIP on the electrode surface. This stage was completed within 120 s and then the differential pulse and cyclic voltammetry signals were recorded in the potential range of 0.4-1.2 V [19].

Samples Preparation

The commercial pharmaceutical capsule of AMX was bought from local pharmacies. Each capsule contained a dose of 500 mg AMX as a substantial and electrochemically active component of the tablet (without any other known additive drugs), as the manufacturer guarantees in drug information leaflet. Six capsules were completely grinded and homogenized, and 30 mg of the powdered tablet was dissolved in DDW with intensive stirring of a magnetic stirrer for 20 min to ensure that tablets were dissolved completely. The mixture was filtered through a filter paper to obtain a clear filtrate and then quantitatively transferred into 100 ml volumetric flask. To obtain final concentrations in the range of calibration curve, the sample solutions were suitably diluted with supporting electrolyte.

RESULTS AND DISCUSSION

Characterization

Figure 1A shows the SEM of Fe_3O_4 nanoparticles and MMIP. The average diameter of Fe_3O_4 nanoparticles is 20-30 nm with a spherical shape, the aggregation of the nanoparticles can be discerned clearly. In Fig. 1A, the MMIP structure can be observed. The dispersity of MMIP is also improved, and the average size is increased. It can be seen that the surface of MMIP exhibits a porous and rough structure. Also, its SEM image demonstrated the small cavities of template molecules. Created cavities in the leached polymer can be related to the removal of AMX from polymer particles after the leaching process.

FTIR spectra of Fe_3O_4 and MMIP are shown in Fig. 1B. For Fe_3O_4 , the presence of Fe-O bond vibration at about 570 cm^{-1} is obviously observed. This pattern corresponds to the Fe-O bonds, which is reported to belong to bulk magnetite. At the spectrum of MMIP, we could see the peaks of the C-O group at about 1200 cm^{-1} , the C=O group at about 1730 cm^{-1} , and the C-H group of $-\text{CH}_2-$ and $-\text{CH}_3$ at about 2990

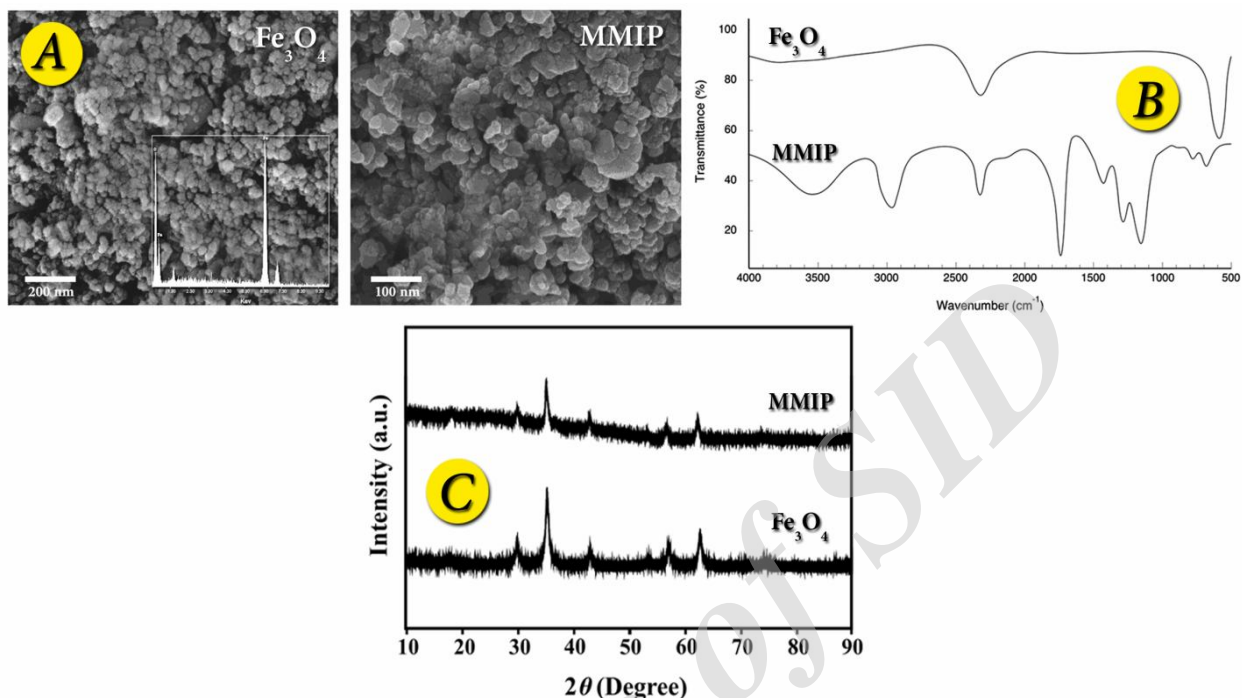


Fig. 1. Comparison of (A) SEM images, (B) FTIR spectra and (C) XRD patterns for Fe₃O₄ and MMIP.

cm⁻¹. All these evidences indicated that the methacrylic acid layer has been successfully formed on the surface of Fe₃O₄ [20].

The XRD patterns of Fe₃O₄ and MMIP were investigated, and the results are shown in Fig. 1C. In the 2θ range of 10-90°, six characteristic peaks for magnetite (at 2θ about 30, 35, 43, 53, 57 and 63) were observed for the two samples, which matched well with the database of magnetite in the Joint Committee on Powder Diffraction Standards (JCPDS) International Center for Diffraction Data (JCPDS Card 19-629). Moreover, the peak positions were unchanged upon coating the polymer layers and polymerization, indicating that the crystalline structure of the magnetite was essentially maintained [21].

Electrochemical Behavior

To compare the performance of the prepared modified electrodes, the electrochemical response of AMX was first studied on the surface of various electrodes using cyclic voltammetry. Figure 2 illustrates the cyclic voltammetric responses of 0.1 μM AMX in the B-R buffer (pH = 7.0)

with the scan rate of 100 mV s⁻¹ on the surface of the CPE, MNIP/CPE and MMIP/CPE respectively. Cyclic voltammograms at the CPE did not exhibit any voltammetric peak. However, as shown in Fig. 2, at MNIP/CPE, an anodic peak appears. Therefore, it has been proved that the use of the Fe₃O₄, obviously improves the sensitivity of AMX detection. A further enhancement of peak current response was observed for the MMIP/CPE. This significant difference between MMIP and MNIP electrodes proves the ability of synthesized MIP to selective recognition of AMX. This difference in the peak currents might result from the imprinted cavities in the MIP's and the functional groups on the cavities produced by the template molecules.

Effect of pH on the Electrochemical Oxidation of AMX

The effect of pH on the response of AMX was evaluated using differential pulse voltammetry employing MMIP/CPE. The effect of pH on the peak potential and peak current of AMX was investigated over the pH range of

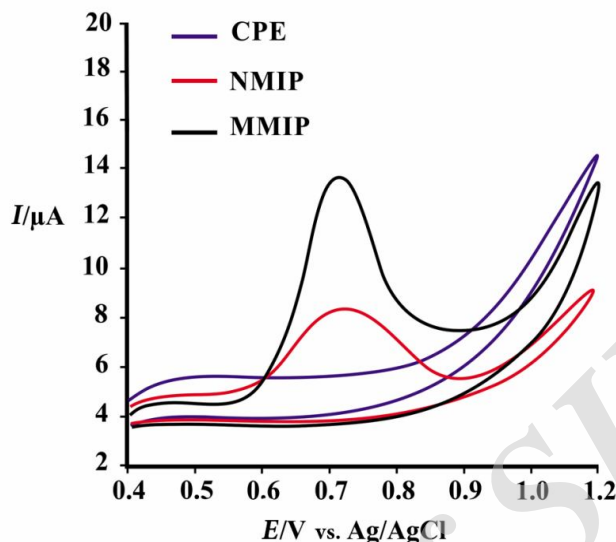
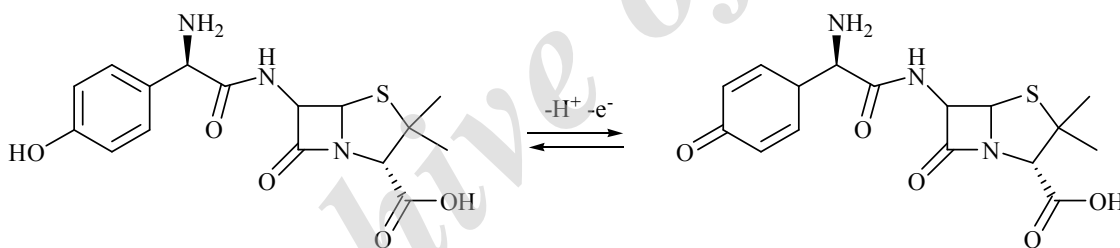


Fig. 2. CVs for 0.1 μM AMX in B-R buffer solution (pH = 7) on the surface of various electrodes. Scan rate 100 mV.



Scheme 1. Suggested oxidation mechanism for AMX

2.0-8.0 using B-R buffer. As shown in Fig. 3B, with increasing pH, the current increases and highest peak currents are obtained at pH 7.0 and then decreases with increasing pH. Figure 3C shows that the peak potential of AMX is also pH dependent and the potentials have shifted negatively when the pH of the solutions increases due to the participation of protons in the electrode reaction. This dependence is linear over the pH range of 3.0-7.0. The linear regression equation is as below:

$$E = -0.064 \text{ pH} + 1.17 \quad (R^2 = 0.997) \quad (1)$$

The slope of 0.064 obtained on MMIP/CPE is almost similar to that shown in previous electrochemical

investigations [22], indicating/suggesting that the number of electrons and protons are equal in the oxidation of AMX. The reaction mechanism for the oxidation of AMX is as given in Scheme 1 [8].

Effect of Scan Rate

Investigating the effect of the scan rate on the oxidation peak current and peak potential can evaluate the kinetics of the electrode reaction. CV experiments were carried out at different scan rates to investigate the electron-transfer process of the selected molecules on the MMIP/CPE. All the CV experiments for AMX were conducted in B-R buffer at pH = 7.0. The CVs were recorded from 10 -175 mV s⁻¹ and the results are shown in Fig. 4. The evaluation of the

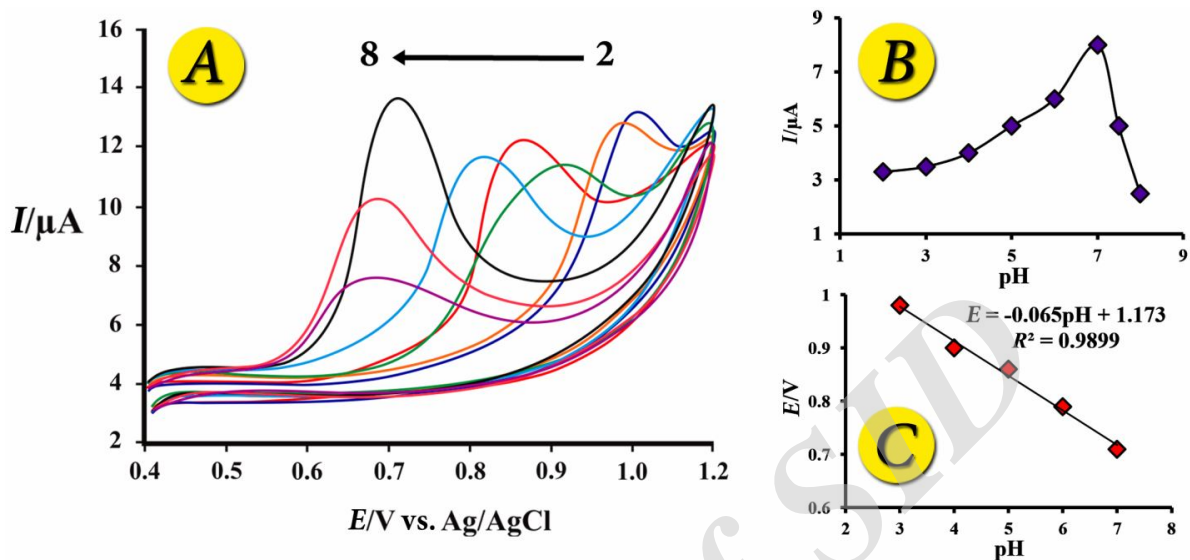


Fig. 3. (A) CVs for MMIP/CPE in 0.1 μM AMX at various pH values (pH 2, 3, 4, 5, 6, 7, 7.5 and 8). (B) Effect of pH on the peak current of AMX (C) Effect of pH on the peak potentials of AMX.

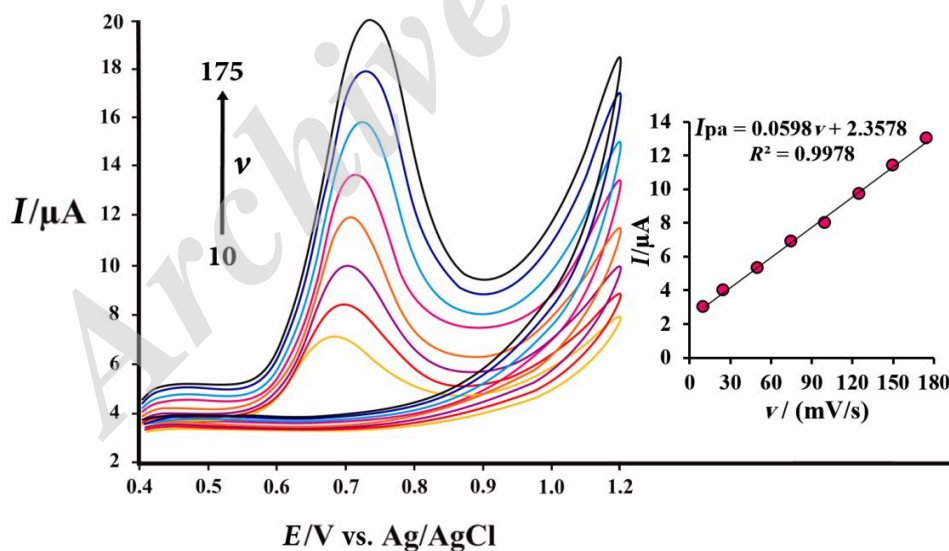


Fig. 4. CVs for MMIP/CPE in B-R buffer of pH 7 containing 0.1 μM of AMX with scan rates of 10, 25, 50, 75, 100, 125, 150 and 175 mV s^{-1} . Insets show the linear relationship of the anodic peak current versus scan rate.

peak currents as a function of scan rate revealed a linear relationship (see insets in Fig. 4), indicating that the electrode reaction of AMX is an adsorption-controlled irreversible process. The regression equations are as

follows:

$$I(\mu\text{A}) = 0.059 v + 2.35 \quad (R^2 = 0.997) \quad (2)$$

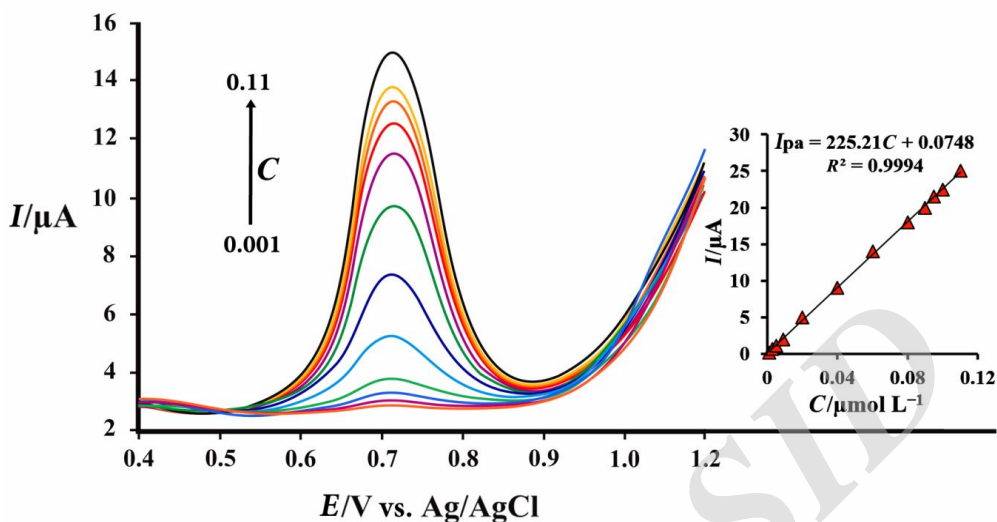


Fig. 5. DPVs of different concentrations of AMX in B-R (pH = 7) and under optimal conditions. Inset shows the plot of peak current as a function of AMX concentration (from a to l): 0.001, 0.003, 0.005, 0.009, 0.02, 0.04, 0.06, 0.08, 0.09, 0.095, 0.10 and 0.11 μM .

Influence of MMIP Amount, Extraction Time and Accumulation Time

The effect of MMIP dosage on the extraction of AMX was investigated by adding a known quantity of the MMIP, in the range of 1-20 mg of its powdered form, into the individual beakers containing 50 ml of 0.05 μM AMX solution. The resulting suspension was immediately stirred with a magnetic bar for 5 min. The peak current increased with increasing the MMIP amount up to 15 mg. This observation can be explained by the higher number of available MIP sites for AMX molecules at higher MMIP dosages. Further increase of the MMIP dosage did not affect the extraction of AMX. Hence, the optimum dosage of MMIP was found to be 15 mg.

The contact time is one of the prime factors influencing the target analytes extraction. The results showed that when MIPs were isolated immediately without a contact process into the samples, the analytes were hardly adsorbed. Stirring caused an increase in the adsorption rate. Stirring time for adsorption was optimized in order to minimize the time required for sample processing. The experimental results indicated that quantitative recovery of AMX in 50 ml sample solution is achieved when the stirring time is more than 5 min. In subsequent experiments, this time was used

for extraction.

The influence of accumulation time of MMIP on the CPE surface on the peak current of AMX was also investigated. Variation of the accumulation time showed that the peak current of AMX increased with increasing the accumulation time, gradually leveling off at a period longer than 120 s, presumably due to saturation of the electrode surface. Thus, deposition time of 120 s was used.

Analytical Performance

The MMIP/CPE was used for the assessment of the analytical utility of the proposed method as a means for the analysis of trace amounts of AMX. The DPV method using the suggested modified electrode was applied as a method featuring high sensitivity, very low detection limit and good selectivity in the presence of other compounds in B-R buffer with pH 7.0. Quantitative evaluation is based on the linear correlation between the peak current and concentration. Linear calibration curves were obtained for AMX in the range of 0.001-0.11 μM with a regression equation of $I_p (\mu\text{A}) = 225.21 C (\mu\text{M}) + 0.074$ and $R^2 = 0.999$ (Fig. 5). The detection limit (based on $3S_b/m$) of the procedure was found to be 0.26 nM.

The repeatability and reproducibility of the MMIP/CPE

Table 1. Results for AMX Determination (μM) in Various real Samples Obtained by the Proposed Method Using DPV Method under the Optimal Condition

Samples	Added (nM)	Found (nM)	Recovery (%)
	0.00	34.00	-
Capsule	30.00	63.64	98.8
	50.00	85.61	103.2

Table 2. Comparison of some Characteristics of the Proposed Electrode with those Reported Previously

Electrode	Method	Linear range (μM)	Detection limit (μM)	Ref.
ZnO NRs/gold/glass electrode	CV	50-250	19	[23]
Pt-Pd bimetallic NPs-SWCNTs / GCE	DPV	0.001-1 and 1-6	0.00089	[9]
DMBQ/ZnO/CNTs/CPE	SWV	0.002-720 and 1.0-950	0.0008	[24]
Modified CPE	SWAdSV	0.5-250	0.00035	[7]
Ni/CR/CPE	Amperometry	8-100	5	[8]
PGA/GLU modified electrode	SWV	2.0-25.0	0.92	[25]
MWCNTs/GCE	Adsorptive Stripping cyclic voltammetry	0.6-8	0.2	[22]
MMIP/CPE	DPV	0.001-0.11	0.00026	This work

were carried out for $0.05 \mu\text{M}$ CF. The relative standard deviation (RSD) was 2.3% for five measurements with the same electrode and the RSD of 2.6% was obtained for six different modified electrodes with the same test solution. The stability of the electrode was also tested and the peak current only decreased less than 4% after the electrode was

stored at room temperature for 18 days.

Interference Study

In order to investigate the selectivity of the prepared electrode for the determination of AMX, several species were checked as potentially interfering materials in their

analysis. The potentially interfering substances were chosen from the group of substances commonly found with AMX in the pharmaceuticals and/or in the biological fluids. The tolerance limit was taken as the maximum concentration of foreign species that caused a relative error of approximately $\pm 5\%$ for the determination of $0.08 \mu\text{M}$ each of AMX plus the potential interfering substances at pH 7. The interference study was conducted by placing the modified carbon paste into a solution containing target analytes at optimum conditions. It was found that 700-fold of SO_4^{2-} , PO_4^{3-} , SO_4^{2-} , NO_3^- , Cl^- , Ca^{2+} , Mg^{2+} , Cu^{2+} , Cd^{2+} , K^+ , Na^+ have no influence on the signal of $0.08 \mu\text{M}$ of AMX. A 200-fold excess of tartaric acid, fructose, glucose, sucrose, valine, starch had no effect on the signals of AMX, and also ascorbic acid, uric acid, aspartic acid, dopamine showed no changes in the signals until an 80-fold excess was used. In addition, isoniazid, pyrazinamide, and rifampicin did not interfere until a 20-fold excess was achieved. These results suggested that the determination of AMX in pharmaceutical formulations at MMIP/CPE was not significantly affected by the most common interfering species.

Determination of AMX in Real Samples

The applicability of the prepared sensor was tested by determination of AMX in real samples. The results presented in Table 1 indicated that the modified electrode has good efficiency in the determination of AMX with satisfactory results. MMIP/CPE was further utilized to detect AMX content in commercial pharmaceutical samples. The recoveries were determined by spiking the samples with a certain amount of standard solutions of AMX, and the results were found to be 97.4-103.2%.

CONCLUSIONS

Herein, we have demonstrated the development of advanced MMIP-based sensors for electrochemical detection of AMX. MMIP can be assembled on the surface of CPE by a permanent magnet, leading to the formation of uniformly MIP film on the carbon paste electrode with high sensitivity. The developed method exhibited good analytical performance in terms of sensitivity, reproducibility, and recovery. The data obtained support the notion that the method, combining molecular imprinting and

electrochemistry, is a potential route for the creation of cost-effective, miniaturized sensors for the detection of species in pharmaceutical samples. The data presented in Table 2 compare the analytical characteristics of the proposed method for the determination of AMX with those reported previously. The proposed method can provide comparable linear range and lower detection limit with a simple electrode preparation procedure.

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