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A novel high selective and sensitive metronidazole voltammetric sensor based on a molecularly imprinted polymer-carbon paste electrode

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ABSTRACT

The design and construction of a highly selective voltammetric sensor for metronidazole by using a molecularly imprinted polymer (MIP) as recognition element were introduced. A metronidazole selective MIP and a nonimprinted polymer (NIP) were synthesized and then incorporated in the carbon paste electrodes (CPEs). The sensor was applied for metronidazole determination using cathodic stripping voltammetric method. The MIP–CP electrode showed very high recognition ability in comparison to NIP-CPE. Some parameters affecting the sensor response were optimized and then the calibration curve was plotted. Two dynamic linear ranges of 5.64×10^{-5} to 2.63×10^{-3} mg L^{-1} and 2.63×10^{-3} to 7.69×10^{-2} mg L^{-1} were obtained. The detection limit of the sensor was calculated as 3.59×10^{-5} mg L^{-1}. This sensor was used successfully for metronidazole determination in biological fluids.

1. Introduction

Metronidazole is a nitroimidazole derivative and has been widely used for the treatment of protozoal diseases including trichomoniasis and giardiasis [1]. This drug is effective against trichomonas, Vincent's organisms, and anaerobic bacteria. Veterinarians also use metronidazole to treat bacterial infections as well as giardia in dogs and cats [2]. Metronidazole [3] contains a nitro group which is the electrochemically active reducible center. In the absence of oxygen the reduction process for nitroimidazoles is similar to that for nitrobenzene [4].

Several methods have been reported for determination of metronidazole which include potentiometric [5,6], polarographic [7,8], gas chromatography [9,10], supercritical fluid chromatography [11], thin layer chromatography (TLC) [12], high-performance liquid chromatography (HPLC) [13–16], voltammetric [17], derivative spectrophotometry [18–20], luminescence [21], and spectrophotometry [22–24]. However, these methods have some drawbacks such as time consuming, narrow range of determination, requirement of heating or extraction, the use of nonaqueous systems, stability of the colored product formed, etc. [25]. Hence, it is of primary importance to develop an alternative method for metronidazole determination with a high degree of selectivity and sensitivity.

Chemically modified carbon paste electrodes (CMCPE) have been continued to be of a major concern during the past decade. Furthermore a relatively large number of electrochemical research has been devoted to the development and applications of different types of CMCPE [26–31]. Modification of carbon paste electrodes with suitable materials facilitates the electrochemical reactions of the redox compounds to proceed without hindrance [32,33]. This phenomenon generally results increasing in selectivity and sensitivity of the determinations [34–36].

Molecularly imprinted polymers (MIPs) are extensively cross-linked polymers containing specific recognition sites with a predetermined selectivity for analytes. The procedure for synthesizing an MIP is based on the polymerization of a functional monomer and a cross-linking agent in the presence of a template. When the imprinted molecule is removed, the imprinted polymer with a high affinity for the template molecule is obtained. This affinity is due to the shape and the arrangement of the functional of the monomer units [37]. The MIPs are used as antibody like materials for high selectivity and sensitivity, chemical inertness, long-term stability, availability in large quantities, and insolubility in water and most organic solvents [38,39]. MIPs are promising materials continually being used in sensor fields such as recognition elements or modifying agents (instead of other commonly used modifiers). The application of MIPs in electrochemistry is rather recent and was directed to combine their intrinsic properties to selected electrochemical reactions, in order to improve the response of the electrode [40,41].

This study was led to the development of a new MIP modified electrode for the determination of metronidazole with improved qualities such as: simplicity of electrode preparation, a wider linear range, lower detection limit (DL), higher selectivity and more stability of the used modifier. The procedure was based on the reduction

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of metronidazole after its selective extraction in the carbon paste electrode. In general the measurement with MIP modified sensors were carried out via a three-step determination including analyte extraction in the electrode, electrode washing and finally electrochemical measurement. This procedure was time consuming. In order to reduce the analysis time the stripping voltammetric (SV) method was used. In this method the electrode washing step was omitted and two other steps (analyte extraction and electrochemicale measurements) took place in one step. The literature survey indicates that there is not any report on the use of stripping voltammetric method for measurement of analyte when MIP modified electrode was used. The developed sensor has been successfully applied for the determination of metronidazole in serum samples.

2. Experimental

2.1. Instruments and reagents

Electrochemical data were obtained with a three-electrode system using a Metrohm potentioastat/galvanostat model PGSTAT302. The differently prepared MIP or NIP involved carbon paste sensors was used as a working electrode. The surface of working electrode was 7.0 mm^2. A platinum wire and an Ag/AgCl electrode (saturated KCl) were used as the counter and reference electrodes respectively. Metrohm pH-meter (model 691) was also applied for pH measurements. The chromatography was carried out using the KNAUER instrument equipped with a power supplier, an autosampler and a UV sepectrophotometric detector. The HPLC was controlled by an EZ-Chrome Elite software. The separations were performed on a Eurospher 100-5C18 column (250 mm × 4.0 mm i.d.).

Methacrylic acid (MAA) obtained from Sigma–Aldrich (Munich, Germany) was purified by passing it through a short column of neutral alumina followed by distillation under reduced pressure. Ethylene glycol dimethacrylate (EDMA) obtained from Fluka (Buchs, Switzerland) was distilled under reduced pressure in the presence of a hydroquinone inhibitor and stored at 4 °C. 2,2-Azobisisobutyronitrile (AIBN) was supplied by Sigma–Aldrich (Munich, Germany) and used as received. Graphite powder was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade and purchased from Merck (Darmstadt,Germany). Metronidazole, tinidazole, and furazolidone were purchased from Sigma Aldrich (Munich, Germany). Standard solution of metronidazole (0.01 mol L^{-1}) was prepared by dissolving 0.0171 g of metronidazole in 10 ml distilled water.

2.2. Preparation of the molecularly imprinted polymer (MIP)

The procedure adopted for preparation of the metronidazole imprinted polymer was based on that conventional bulk polymerization according to previous study [42]. In order to synthesis the molecularly imprinted polymer, 0.8 mmol of template molecule and 3.9 mmol of methacrylic acid and 8.5 ml of dry DMF were placed in a glass tube. Then the mixture was left for 10 min. Subsequently, EDMA (19.5 mmol) and AIBN (0.3 mmol) were added. The tube was sealed and the mixture was purged with nitrogen for 10 min. Polymerization took place in a water bath at 60 °C for 24 h. After polymerization, the glass tube was broken and the polymer was mechanically ground in a mortar. A steel sieve was employed to select particles with sizes <200 μm. After that the template was removed by soxhlet extraction with mixture of methanol and acetic acid (9:1) for 30 h. The complete removal of template from the polymer was traced by the differential pulse voltammetric method. The procedure to prepare the nonimprinted polymer (NIP) was the same as MIP procedure, however no template was presented in the polymerization media.

2.3. Preparation of the sensors

The bare carbon paste electrode was prepared by thoroughly mixing analytical grade graphite and n-eicosane, in a 65:35 (w/w%) ratio. The metronidazole modified CPE was prepared by mixing different percentages of graphite powder, n-eicosane, and MIP (or NIP). This mixture was mixed in a mortar for at least 10 min to become homogeneous. The paste was packed into an end of a Teflon holder in which electrical contact was made with a copper rod that runs through the center of the electrode body. The electrode surface was polished using a butter paper to produce reproducible working surface. Electrochemical behavior of metronidazole at these different electrodes was investigated using cyclic voltammetric technique. Best results were obtained at 66.7:20.0:13.3 (w/w%) ratio of graphite powder, n-eicosane, and MIP (or NIP). This optimized electrode composition was then used for the voltammetric determination of metronidazole.

2.4. General analytical procedure

A 10 ml volume of the (B.R) buffer solution with pH = 7 was introduced into electrochemical cell and deaerated with pure nitrogen for 10 min. An accumulation potential of 0.0 V was applied to the electrode for 40 s while the solution was stirred at 400 rpm. Then the stirring was stopped and after 10 s rest period differential pulse voltammogram in the range of 0.0 to −1.2 V were recorded and was used for background correction. Then, an appropriate volume of sample solution was added to the voltammetric cell. Afterward the solution was purged for 3 min and its voltammogram was recorded after 40 s accumulation at 0.0 V.

2.5. The measurement of metronidazole in biological fluids

Serum samples obtained from healthy volunteers were collected and stored frozen. An aliquot serum sample was fortified with metronidazole to achieve final concentration of 1.71×10^{-2} mg L^{-1} and treated with 0.2 ml methanol as serum protein precipitating agent. Then the volume was completed to 1.0 ml with the same serum sample. After vortexing for 30 s, the precipitated protein was separated by centrifugation for 3 min at 14,000 rpm. The clear supernatant layer was filtrated through a 0.45-μm milli-pore filter to produce a protein-free human serum. The urine samples used for measurements were centrifuged and diluted 20 times with water without any further pretreatment. An aliquot urine sample was fortified with metronidazole to achieve final concentration of 1.71×10^{-2} mg L^{-1}. Metronidazole content of both real samples were determined using voltammetric (proposed sensor) and HPLC. Calibration curve that was obtained by plotting the analytical signal (peak current or area under chromatogram) vs. concentration of standard solutions of metronidazole was used for determination of metronidazole for both methods.

In HPLC system the mobile phase consisted of acetonitrile–0.01 M phosphate solution (pH 4.7, 15:85, v/v) was degassed and filtered through 0.45 μm filter (Millipore, Saint-Quentin, Y velines, France). The flow rate was 1 ml min^{-1}. The detection wavelength was 318 nm. The injection volume was 20 μL and the run time was 10 min.

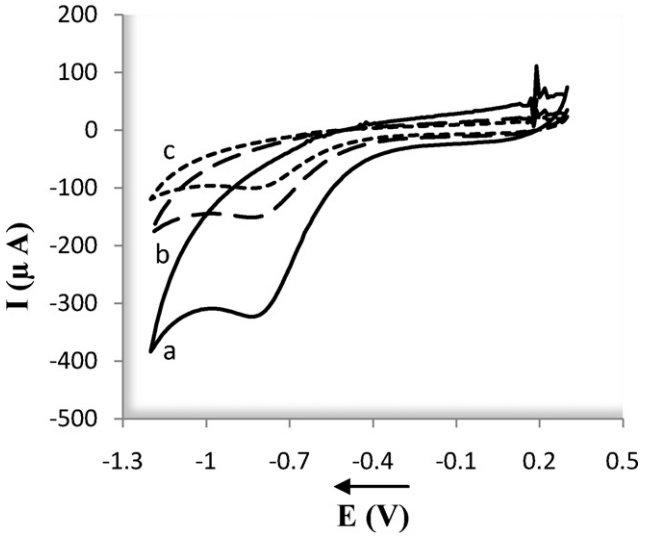

Fig. 1. Cyclic voltammograms of 8.6×10^{-1} mg L^{-1} of metronidazole at (a) MIP (b) NIP and (c) CP. Measurement conditions: pH = 7, scan rate = 60 mV s^{-1}.

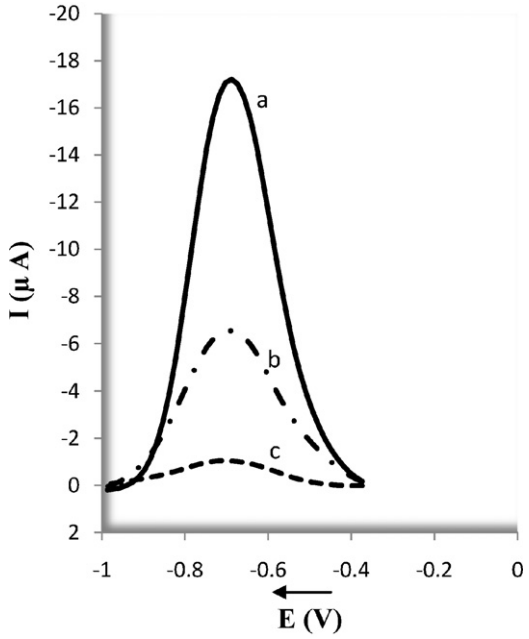

Fig. 2. DP voltammograms of 1.37×10^{-2} mg L^{-1} of metronidazole at the surface of (a) MIP, (b) NIP and (c) CP electrode. Measurement conditions: pH = 7, E_{ac} = 0.0 V, t_{ac} = 40 s.

3. Results and discussion

3.1. Electrochemical behavior of metronidazole

Preliminary cyclic voltammetric experiments were carried out to study the metronidazole voltammetric behavior at the CP, MIP–CP and NIP–CP electrodes (Fig. 1). The reduction system was characterized by a cathodic peak at the negative-moving step as well as by the absence of any anodic peak on the reverse scan to indicate that the reduction was irreversible. The reduction peak was attributed to the four-electron reduction of nitro group to the corresponding hydroxylamine according to the currently accepted mechanism for the electroreduction of aromatic and heteroaromatic nitro compounds [4,43]. This reduction peak was used for metronidazole monitoring.

In order to achieve a high sensitive sensor the selection of a proper electrochemical technique is of great importance. Therefore, differential pulse stripping voltammetry (DPSV) as a sensitive method was selected and used for further investigation. Thus the DP stripping voltammograms of 1.37×10^{-2} mg L^{-1} metronidazole at the surface of MIP–CP, NIP–CP and at bare CP electrodes were recorded. The results are presented in Fig. 2. As it is seen under the identical conditions metronidazole yields a very well defined reduction peak (at −0.68 V) at MIP–CP electrode in comparison to those obtained with other electrodes. Furthermore, no shift in the reduction peak potential of metronidazole was observed when three different electrodes were used. The reduction peak current was used for monitoring the drug by proposed modified electrode.

3.2. MIP–CP electrode selectivity

Molecular imprinted polymer sorbent was synthesized using metronidazole as a model template and MAA as a complementary functional monomer using noncovalant method according to previous report [42]. The constructed MIP particles via noncovalent approach usually contain selective sites with various affinities for template. Some of these sites are cavities with the sizes matchable with template molecule. These template recognition sites constructed with regular and perfect shape in the polymerization period and for that reason they have more affinity for metronidazole. Metronidazole molecules presented in such cavities are tightly absorbed by the MIP.

Molecularly imprinting polymer can act more selective than other imprinting materials as sol–gel. Besides, the proposed method for selective metronidazole sensor preparation is very simple and inexpensive. In order to test the selectivity of designed MIP–CP sensor, modified (MIP–CP) and unmodified carbon paste (CP) electrodes were prepared and inserted into the individual solutions of metronidazole and some metronidazole similar compounds such as tinidazole and furazolidone. Their results are compared in Fig. 3. It follows that all represented compounds contain nitrophenyl group similar to metronidazole which was responsible for electroactivity of these compounds. Fig. 3A shows the recorded CS voltammetric responses of the CP electrode inserted in the solutions of metronidazole and other tested compounds. As can be seen all of them have electrochemical signal in the metronidazole reduction potential range. It is also clear that voltammetric related current magnitude of tested compounds is as high as that of metronidazole. This indicates that these compounds can easily interfere in the metronidazole determination. When the carbon paste electrode was modified with synthesized MIP and carried out the previously explained CSV determination the results (Fig. 3B) showed that the presence of MIP in the carbon paste electrode makes the electrode very selective for metronidazole whereas the responses of all other tested compounds except metronidazole reach to very small while the concentration of metronidazole is 5 times lower than other tested compounds. These results approved the powerful ability of MIP–CP electrode for metronidazole determination purpose.

Simultaneously the response of the proposed electrode for determination of 1.71×10^{-2} mg L^{-1} of metronidazole was compared when a lengthy three-step procedure along with fast cathodic stripping voltammetric procedure were used. As it is obvious from Fig. 4, CSV method affords not only higher sensitivity but also lower analyzing time. Thus stripping method was recommended for further uses.

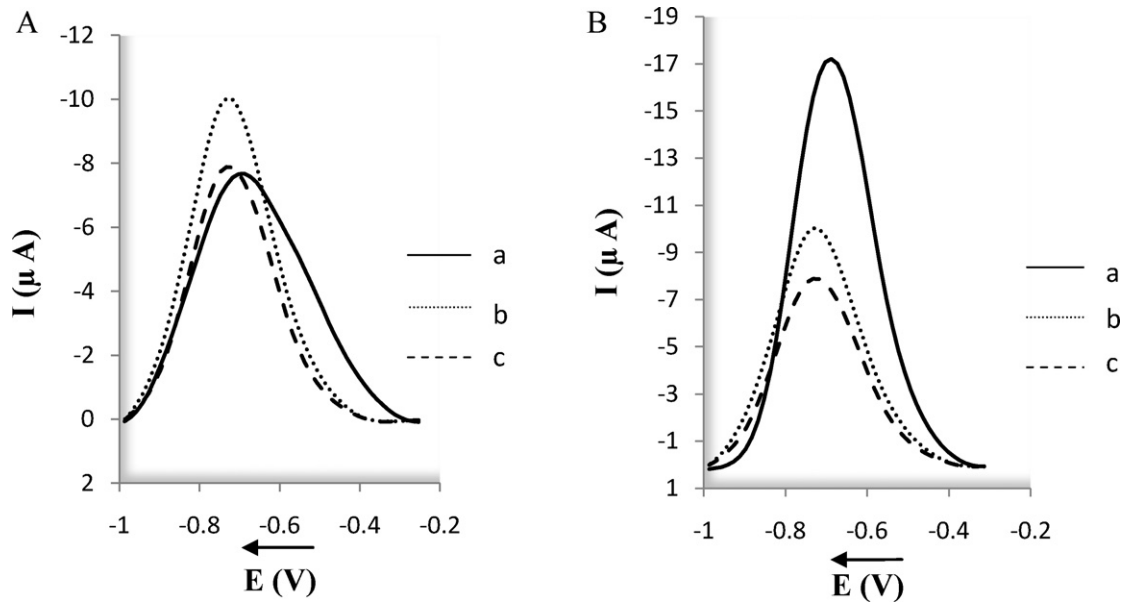

Fig. 3. (A) The response of CP electrode immersed in to the solutions containing 5.13×10^{-2} mg L^{-1} of each (a) metronidazole (b) furazolidone and (c) tinidazole. (B) The responses of MIP–CP electrode for (a) mtronidazole (1.0×10^{-2} mg L^{-1}), (b) furazolidone (5.13×10^{-2} mg L^{-1}) and (c) tinidazole (5.13×10^{-2} mg L^{-1}) after extraction of the mentioned compounds in the MIP–CP electrode. Measurement conditions: stirring rate = 400 rpm, pH = 7, t_{ac} = 40 s, E_{ac} = 0.0 V, scan rate = 60 mV s^{-1}, puls amplitude = 50 mv, puls width = 40 ms.

3.3. Optimization of parameters for metronidazole detection

3.3.1. MIP–CP composition optimization

MIP–CP composition optimization was carried out with one variable at a time in the fixed conditions of extraction and stripping voltammetric determination. The obtained responses were used for conclusion. For initial optimization purposes the MIP–CP electrodes were prepared with fixed amounts of carbon and n-eicosane and different amounts of MIP. The resulted electrodes at each case were used for metronidazole extraction and determination. The obtained results show that the maximum response for the prepared sensor appeared in the MIP amount of 0.01 g. Increasing the amount of MIP in the MIP–CP electrode can increase the sensor response due to providing more recognition sites on the electrode surface. However, enhancement the MIP amount more than a threshold level (0.01 g) lead to a decrease in the prepared sensor response probably because of decreasing the electrode surface conductivity. Similar experiments were also carried out in order to investigate the effect of carbon and n-eicosane amounts on the electrode response. From the results, the optimum amount of carbon and n-eicosane were defined as 0.05 and 0.015 g, respectively. At first, increasing the carbon content of MIP–CP electrode leads to an increase in the electrode response because of electron transferring capability enhancement of the electrode. However, after a certain point, further increase in carbon content reduces the signal that may be due to decrease of the MIP content on the electrode surface. The optimum amount of n-eicosane is also necessary for the MIP–CP electrode preparation. Presence of higher amounts of binder (n-eicosane more than 0.015 g) in the MIP–CP electrode leads to a

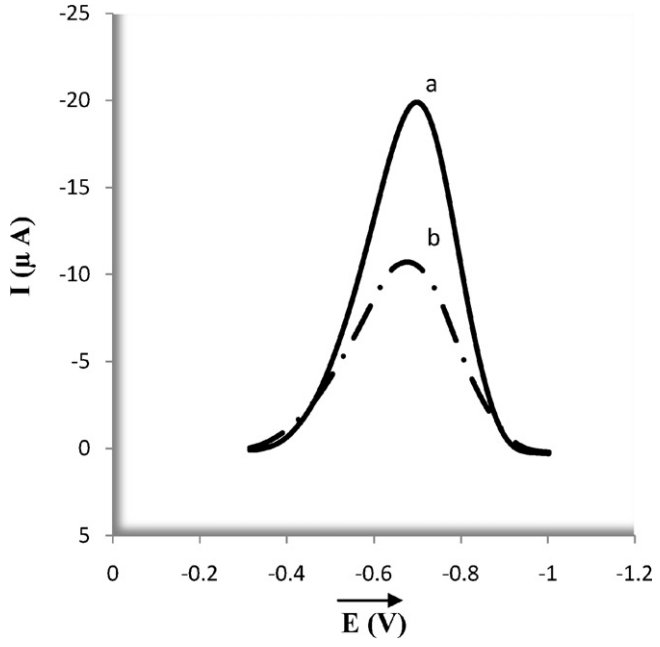

Fig. 4. Comparison of the response of MIP–CP electrode in 1.71×10^{-2} mg L^{-1} metronidazole solution (a) CSV procedure (pH = 7, t_{ac} = 40 s, E_{ac} = 0.0 V, stirring rate = 400 rpm, scan rate = 60 mV s^{-1}) and (b) a three steps procedure (extraction conditions: pH = 7, extraction time = 7 min, stirring rate = 400 rpm. Electrochemical measurement conditions: pH = 7, scan rate = 60 mV s^{-1}).

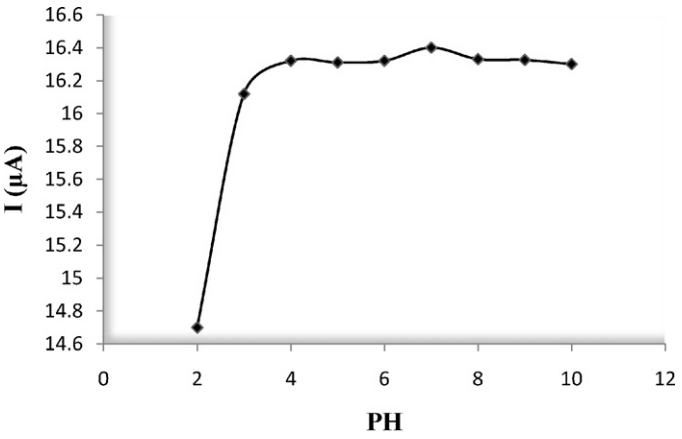

Fig. 5. Optimization of pH for metronidazole extraction in the MIP–CP electrode (Extraction conditions: metronidazole (1.37×10^{-2} mg L^{-1}), extraction time = 7 min, stirring rate = 200 rpm. Electrochemical measurement conditions: pH = 2.5).

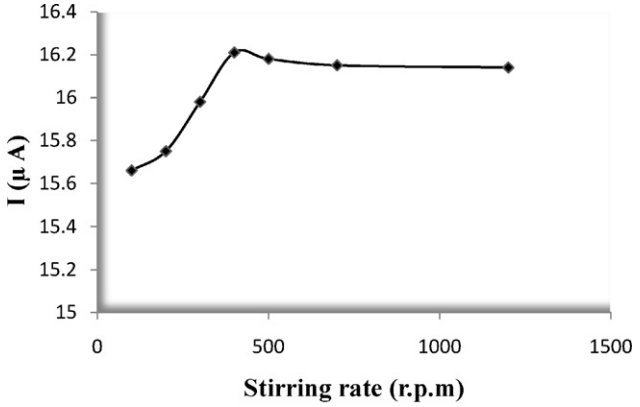

Fig. 6. Optimization of stirring rate for metronidazole extraction in the MIP–CP electrode (measurement conditions: metronidazole (1.37×10^{-2} mg L^{-1}), pH = 7, $E_{ac} = -0.2$ V, $t_{ac} = 30$ s).

decrease in electrode response. This is because the electrode surface conductivity is decreasing, due to the insulating effect of the binder.

3.3.2. Effect of pH

The effects of the pH of test solution on the performance of MIP have been reported previously [42]. According to this report the adsorption of the analyte on the MIP sorbent is pH independent at pHs higher than 3. Farias et al. [44] have reported that the metronidazole molecule was protonated at pHs below the metronidazole's pK_a value (2.5), whereas at higher pH values it would be neutral. Therefore, the interaction between the molecule and the binding sites of the MIP would be favored at pH greater than the pK_a value. Thus, the effect of pH on the adsorption of the analyte on the MIP–CP electrode was studied by varying the pH in the range of 2–10. After 7 min the electrodes were removed from the metronidazole solution and DP voltammograms were recorded. The results are shown in Fig. 5. Accordingly the adsorption of the analyte on the MIP–CP was pH independent at pHs higher than 3 and confirmed the previous report [42]. Similarlly the effect of pH on the stripping response of 1.37×10^{-2} mg L^{-1} metronidazole adsorbed at the surface of MIP–CP electrode was investigated. The obtained results showed that the best reduction peak with high peak current was obtained at pH around 7. Thus, pH = 7 was selected as the optimum pH for extraction and electrochemical measurement.

3.3.3. Effect of stirring rate

Since the electrode contacting area with the metronidazole containing solution was partially small, the analyte extraction into the electrode must be accompanied with stirring the solution. In order to optimize the stirring rate in the extraction period, metronidazole was extracted in the prepared MIP–CP electrodes at the assorted stirring rates whereas the other extraction parameters such as time, pH and concentration remained constant. Fig. 6 shows the drug signal variation against the stirring rates. The higher the stirring rate, the greater the electrode response was for metronidazole. This indicated the high effect of stirring on the metronidazole extraction in the case of the MIP–CP electrode. The growth in metronidazole voltammetric response with an increase in stirring rate continued noticeably till 400 rpm but after that it appeared that the metronidazole extraction enhancement was not so much and the variation of extraction with stirring rate changing was small. Thus the value of 400 rpm as optimum for this optimization purpose was selected.

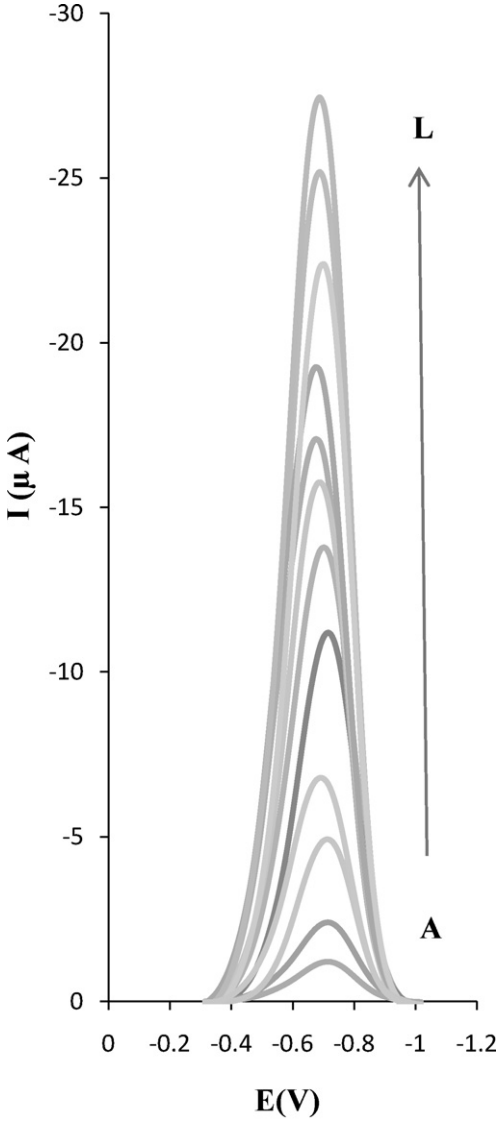

Fig. 7. DPSV signal variation with changing the metronidazole concentration onto MIP–CP electrode. (A) 5.64×10^{-5} mg L^{-1}, (B) 2.56×10^{-4} mg L^{-1}, (C) 6.49×10^{-4} mg L^{-1}, (D) 1.026×10^{-3} mg L^{-1}, (E) 2.052×10^{-3} mg L^{-1}, (F) 2.63×10^{-3} mg L^{-1}, (G) 9.40×10^{-3} mg L^{-1}, (H) 1.45×10^{-2} mg L^{-1}, (I) 2.96×10^{-2} mg L^{-1}, (J) 4.41×10^{-2} mg L^{-1}, (K) 5.78×10^{-2} mg L^{-1}, and (L) 7.69×10^{-2} mg L^{-1}). Measurement conditions: stirring rate 400 rpm, pH = 7, $t_{ac} = 40$ s, $E_{ac} = 0.0$ V, scan rate = 60 mV s^{-1}, pulse amplitude = 50 mV, pulse width = 40 ms.

3.3.4. Effect of accumulation potential, accumulation time and scan rate

Accumulation potential is an important parameter for stripping techniques and has non-negligible influence on the sensitivity of determination. The effect of accumulation potential on the stripping peak current of metronidazole was examined over the potential range of 0.2 to −0.35 V. The plot of stripping peak current as a function of preconcentration potential indicated that the maximum peak current was occurred at 0.0 V. Thus, accumulation potential of 0.0 V was chosen for subsequent uses.

At the same time the influence of accumulation time on the stripping peak current of 1.37×10^{-2} mg L^{-1} metronidazole was also investigated. Variation of the accumulation time showed that the peak current of metronidazole increased with increasing the accumulation time, gradually leveling off at period longer than 40 s, presumably due to saturation of the electrode surface. Thus deposition time of 40 s was used throughout, because

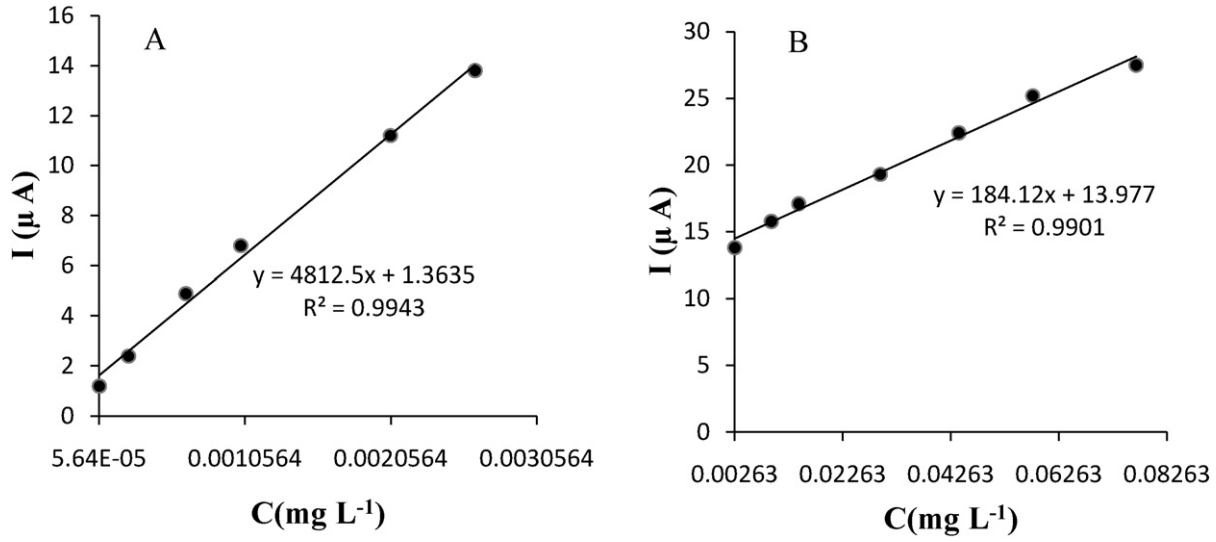

Fig. 8. The linear calibration curves of the dependence of the peak current from Fig. 7 on the metronidazole concentration for two concentration ranges: (A) 5.64×10^{-5} to 2.63×10^{-3} mg L^{-1} and (B) 2.63×10^{-3} to 7.69×10^{-2} mg L^{-1}.

as it combined good sensitivity and relatively short analysis time.

Afterward the effect of scan rate on the stripping peak current was also tested in the range 10–100 mV s^{-1}. The current was found constant at the scan rates higher than 60 mV s^{-1}. Therefore, a scan rate of 60 mV s^{-1} and accumulation time of 40 s were selected for further studies.

3.4. Analytical characterization

To evaluate the interference of different species in the determination of metronidazole, a systematic study was carried out with different interferents in deaerated medium to avoid interference from dissolved oxygen. For 1.71×10^{-2} mg L^{-1} of metronidazole, the results showed that over 100-fold excess concentration of glucose, sodium chloride, ascorbic acid, cyctine and alanine had no influence on the current response of metronidazole (signal change below 5%).

The obtained optimal conditions and parameters were used for the measurement of calibration curves. Differential pulse cathodic stripping voltammetry with an accumulation potential (E_{ac}) of 0.0 V and subsequent stripping step from 0.0 to −1.2 V were applied. The voltammetric responses of the MIP-electrode are shown in Fig. 7. After 40 s accumulation (t_{ac}) the proposed electrode exhibited two working concentration ranges of 5.64×10^{-5} to 2.63×10^{-3} mg L^{-1} and 2.63×10^{-3}–7.69×10^{-2} mg L^{-1} (Fig. 8). The linear regression equations are as follows:

$$I_p\,(\text{mg L}^{-1}) = 1.363 + 4812.0\,C_{\text{Metronidazole}} \quad (r^2 = 0.994) \qquad (1)$$

$$I_p\,(\text{mg L}^{-1}) = 13.97 + 184.1\,C_{\text{Metronidazole}} \quad (r^2 = 0.990) \qquad (2)$$

The limits of detection (LOD) and quantification (LOQ) were calculated using the relation ks/m [45], where $k = 3$ for LOD and 10 for LOQ, s represented the standard deviation of the peak currents of the blank ($n = 12$) and m represented the slope of the first calibration curve for metronidazole. Both LOD and LOQ values were found to be 3.59×10^{-5} and 1.17×10^{-4} mg L^{-1} respectively. These values indicated the sensitivity of the proposed method.

When the concentration of metronidazole was controlled at 1.37×10^{-2} mg L^{-1}, good repeatability was observed with relative standard deviation (R.S.D.) of 3.8% for seven parallel detections. This level of precision is suitable for the routine quality control analysis of the drug in pharmaceutical dosage form and biological fluids. The sensor retained a response of 97% of the initial current after 10 days stored in ethanol at room temperature. Consequently it showed no obvious decline after 20 times. Reproducibility of proposed electrode was investigated by using CSV. Six freshly packed electrodes were prepared on six consecutive days and the peak current values of a solution containing 1.37×10^{-2} mg L^{-1} of metronidazole was measured for each electrode. The obtained result showed a relative standard deviation R.S.D. of CS voltammogarm's currents for five replicate less than 4.5%.

To study the metronidazole recognition ability of MIP, the cathodic stripping voltammetric (CSV) response of MIP–CP and NIP–CP electrodes were tested at different metronidazole concentrations. After 40 s accumulation at potential 0.0 V differential pulse voltammograms were recorded. Based on Fig. 9, the DP signal of MIP–CP electrode (black color) was significantly increased with increasing the concentration of metronidazole. On the other hand in a solution containing different metronidazole concentrations, no significantly change was observed when NIP–CP electrode used (gray color). This indicates that the MIP in the carbon paste electrode can extract metronidazole intensively in comparison to NIP–CP.

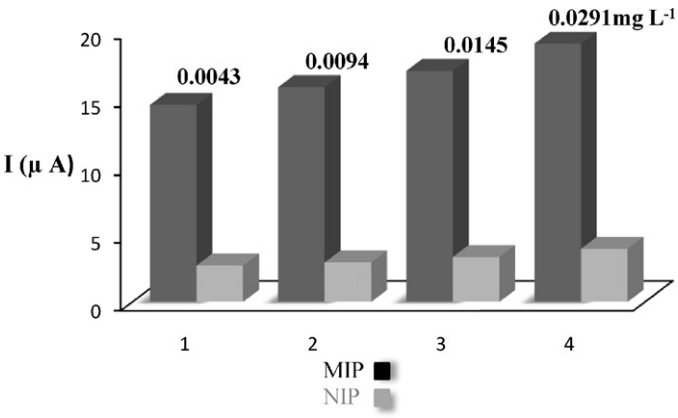

Fig. 9. The response of sensors based on MIP (black) and NIP (gray) for different metronidazole concentrations. Measurement conditions: 0.1 M BR buffer, pH = 7.0, t_{ac} = 40 s, E_{ac} = 0.0, stirring rate = 400 rpm, pulse amplitude = 50 mV, pulse width = 40 ms and scan rate 60 mV s^{-1}.

Table 1
Determination of metronidazole in real samples. ($n = 3$).

Sample	Voltametric method				HPLC method			
	Added ($mg\,L^{-1}$)	Found ($mg\,L^{-1}$)	Recovery (%)	R.S.D. (%)	Added ($mg\,L^{-1}$)	Found ($mg\,L^{-1}$)	Recovery (%)	R.S.D. (%)
Blood	0.0171	0.0164	96.0	3.1	0.0171	0.0173	101.0	1.8
serum	0.0256	0.0259	101.3	2.7	0.0256	0.0235	94.0	1.1
Urine	0.0171	0.016	94.0	2.9	0.0171	0.0162	94.6	2.1
	0.0256	0.0263	99.2	3.6	0.0256	0.0249	97.3	1.3

Table 2
Comparison of characteristic of proposed sensor with recent literature data on the determination of metronidazole obtained under different experimental conditions.

Method	Linear range ($mg\,L^{-1}$)	Limit of detection ($mg\,L^{-1}$)	R.S.D. (%)	pH	Reference
1. Voltammetry of metronidazole at Co/GC	6.84×10^{-2} to 17.1	3.42×10^{-2}	–	1.7	[46]
2. Reduction of metronidazole at GC	3.42×10^{-1} to 103	1.88×10^{-1}	1.7	10.0	[47]
3. Adsorptive stripping voltammetry of metronidazole	1.71×10^{-3} to 1.71×10^{-1}	4.28×10^{-4}	-	4.6	[48]
4. Voltammetry of metronidazole at a carbon fiber microdisk	1.71×10^{-1} to 3.76	8.55×10^{-2}	3.7	9.0	[49]
5. Eletrochemical reduction MTZB at CP modified electrode	1.71–171	7.52×10^{-1}	–	7.0	[50]
6. Electrocatalytic determination of metronidazole	8.55×10^{-2} to 1.71 and 3.42–137	2.57×10^{-2}	<5.0	2.0	[51]
7. Determination of metronidazole at GC electrode modified with single wall carbon	1.71×10^{-2} to 34.2	1.08×10^{-2}	6.0	1.0	[52]
8. Voltammetry of the interaction of metronidazole with DNA	8.55×10^{-3} to 10.3	3.42×10^{-3}	4.5	9.0	[3]
9. voltammetric determination of metronidazole at a nanomaterial thin film coated GC electrode	4.28×10^{-3} to 1.71	1.03×10^{-3}	4.8	9.0	[53]
This work	5.64×10^{-5} to 7.69×10^{-1}	3.59×10^{-5}	<4.0	7.0	

3.5. Real sample analysis

To demonstrate the applicability of the proposed method for the analysis of real sample, two biological fluids including human serum and urine were analyzed. The analyzed samples did not contain metronidazole, as a result they had to be spiked with the analyte at a certain concentration. Spiking of the samples was achieved first by injecting a metronidazole stock solution and then it was homogenized. Metronidazole content of both samples were determined using the modified MIP–CP electrode and HPLC technique. The obtained results were summarized in Table 1. It follows that the recovery of metronidazole was found to be between 94.0 and 101.0% using voltammetric methode which was comparable with those obtained with HPLC. Moreover the relative standard deviation of the proposed method was less than 3.6%, indicating the acceptable precision of the voltammetric determination of metronidazole using the modified MIP–CP electrode. For that reason the proposed procedure should be applicable to the analysis of real samples with different matrices. Thus the cathodic adsorptive stripping voltammetry offered a practical potential for trace determination of metronidazole with high selectivity, sensitivity, simplicity and speed which has not been presented together in the previously reported systems (Table 2).

4. Conclusions

The new electrode incorporating the MIP as a recognition element in carbon paste showed high selectivity and sensitivity towards metronidazole. The MIP functioned as both preconcentrator and high selective recognition element in the carbon paste structure. The proposed sensor was used successfully for metronidazole determination in the biological fluids. It can be concluded that using the cathodic stripping voltammetry with a short accumulation time instead of three steps procedure reduces the analysis time.

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