Anal. Bioanal. Electrochem., Vol. 12, No. 7, 2020, 1014-1024

Analytical & Bioanalytical Electrochemistry

Full Paper

In Situ Voltammetric Determination of Promethazine on Carbon Paste Electrode Modified with Nano-sized Molecularly Imprinted Polymer

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Received: 12 May 2020 / Received in revised form: 26 June 2020 / Accepted: 29 June 2020 / Published online: 31 July 2020

Abstract- A precise and simple in-situ voltammetric measurement of promethazine, based on the nano sized molecularly imprinted polymer (nano-MIP) was introduced. The nano-MIP was synthesized utilizing vinyl benzene and Divinylbenzene as the functional monomer and cross-linker respectively, and via the micro-emulsion polymerization method in silicon oil. The MIP particles were then embedded in a carbon paste electrode (CPE) in order to prepare the MIP-CP electrode. This electrode showed higher response to analyte, compared to the both bare CPE and modified with non-imprinted polymer. Also, the selectivity of the MIP-CPE was investigated using some of the cross reactants and the sensor was clearly selective towards the PMZ. Various factors, known to affect the response behavior of the sensor, were investigated and optimized. This sensor exhibited two distinct linear response ranges of 4×10^{-9}-4×10^{-7} M and $4\times10^{-}$ -7×10^{-6} M in optimum analysis conditions. Limit of detection was calculated equal to 1.4×10^{-9} M (S/N). An interestingly low RSD equal to 1.2% was found for 4 separate determinations by the proposed sensor. The sensor was applied for PMZ in-situ determination in plasma samples without applying any sample pretreatment.

Keywords- Imprinted Polymer nanoparticle; Promethazine; In-situ determination; Voltammetry; Carbon paste electrode; Micro-emulsion polymerization

1. INTRODUCTION

Molecular imprinting technique generates the recognition cavities with the steric and chemical information of template molecules in a crosslinked polymeric network. However,

the successfulness of molecular imprinting is extremely dependent on the bond nature of template-monomer complex, the form of imprinted materials, and the solidity of polymeric matrix. Although numerous imprinted materials were synthesized by various strategies, the imprinted materials ideally appropriate for the molecular recognition elements have yet to be explored, because of their small binding capacity and slow binding kinetics [1-3]. The imprinted nanomaterials with well-defined structures can practically been mounted onto the surface of devices in a required form for many applications in nanosensors and molecular detection. Primitive bulky MIPs need a grinding process to obtain small particles for many applications which leads to destruction of selective cavities in some cases, moreover the final particles are not small enough for lots of implementations. Recently, much attention has been paid on the preparation of MIP nanospheres because of the high yields and fairly simply control over size and distribution [4-6]. With the smaller sizes and better-defined morphologies, the MIP nanospheres achieved higher affinity and selectivity, and better site accessibility [7-12]. The molecular imprinting nanotechnology is expected to greatly enhance the molecular affinity of the MIP materials. Nanostructured, imprinted materials have a small dimension with extremely high surface-to-volume ratio, so that most of the imprinted sites are situated at the surface or in the proximity of surface.

Promethazine hydrochloride which belongs to the phenothiazine group, is a pharmaceutical compound widely used for its antihistaminic, sedative, antipsychotic, analgesic and anticholinergic properties. Electrochemical methods such as differential pulse polarography [13] voltammetry [14,15], potentiometry [16,17], and amperometry [18], have been widely applied for the determination of promethazine-HCl because of their simple, cost effective, and relatively short analysis times procedure.

MIP-based sensors are fabricated by assembling MIP materials onto the surface of transducer, and thus the analyte binding is transformed into a measurable signal. The electrochemical sensors are most commonly fabricated by installing MIP nanomaterials, as recognition elements, onto the surface of electrode. The changes of current and peak voltage at cyclic voltammetry upon the analyte binding can sensitively respond to the concentration and kind of analytes, respectively, because of the oxidation or reduction of analytes at the MIP-modified electrode. The design and development of portable field devices such as sensors rather than laboratory-based instruments in monitoring drugs at trace levels is prime challenge to analytical chemists. In view of the lack of selectivity of the conventional chemical sensors, molecularly imprinted polymers (MIP) recognition element based sensors are gaining wide acceptance now-a-days in the scientific community [19,20].

In this work, a precise and selective in-situ voltammetric measurement of promethazine, based on the nano sized molecularly imprinted polymer was introduced. To the best of our knowledge, MIP based voltammetric sensor has not been developed for promethazine in situ determination as yet. We aimed to apply nano-sized MIP particles in order to improve the

analytical characteristics of the sensor. In our previous report [15] we have shown that the washing step after extraction, can play an important role in sensor selectivity. In this work, it was interestingly found that when we are moving downward in MIP size from micro to nano scales, the high surface-to-volume ratio, as well as surface situated imprinted sites, powerfully reduce the washing step effect so that taking advantage of simplicity and speed of the in-situ method is preferred. The optimized sensor was successfully applied for the PMZ determination in serum samples without any time consuming pretreatment steps.

2. EXPERIMENTAL SECTION

2.1. Instruments and reagents

Electrochemical data was obtained with a three-electrode system using a potentiostat/galvanostat model PGSTAT302, Metrohm. The MIP/NIP based electrodes were used as the working electrodes. A platinum wire and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. Vinyl benzene (VB), obtained from Sigma-Aldrich (Munich, Germany), was distillation under reduced pressure. Divinyl benzene (DVB) obtained from Fluka (Buchs, Switzerland), was also distilled under reduced pressure and stored at 4°C, until use. Promethazine, chlorpromazine, clozapine, n-eicosane and 2,2′-azobisisobutyronitrile (AIBN) were supplied by Sigma-Aldrich (Munich, Germany) and used as received. Graphite powder was purchased from Fluka (Buchs, Switzerland). Other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Preparation of micro and nanosized MIP

The bulk polymerization method was used for the preparation of the micro-sized MIP particles. The complete method of preparation is mentioned in our previous report [15]. To remind briefly, 1 mmol of PMZ and 4 mmol of the functional monomers (VB) were poured in 50 ml of round bottom flask, containing 10 ml chloroform. Then, the mixture was left in contact for 5 min for prearrangement. Subsequently, 24 mmol of cross-linker (DVB) and 0.1 g of initiator AIBN were added. The mixture was purged with N_2 for 10 min and the flask was sealed under this atmosphere. It was then placed in a water bath, maintained at 60 ºC to start the polymerization process. After 12 h, the obtained polymer materials were ground and sieved. Scanning electron microscopy image of the prepared bulky MIP is shown in Fig. 1(a).

In order to prepare the MIP nanoparticles by suspension polymerization in silicon oil, 0.5 mmol of PMZ, 2 mmol of VB, 10 mmol of DVB and 0.05 g of AIBN were dissolved in 5 mL of acetonitrile. Then, 80 mL of silicon oil was purged with a stream of nitrogen gas for 15 min. The pre-polymerization mixture was added to the silicon oil and then dispersed at 800 rpm for 5 min. Next, the mixture was further mixed by ultrasonic waves. Then, the resulting mixture was put inside a water bath for 12 h, fixed at 60 °C, in order to start the

polymerization. The synthesized particles were filtered and washed several times with petroleum ether and toluene. To extract PMZ and the remained monomers from the polymer networks, the particles were subsequently washed with soxhlet extraction using ethanol solvent for 48 h. Fig. 1(b) illustrates the scanning electron microscopy image of the prepared MIP nanoparticles.

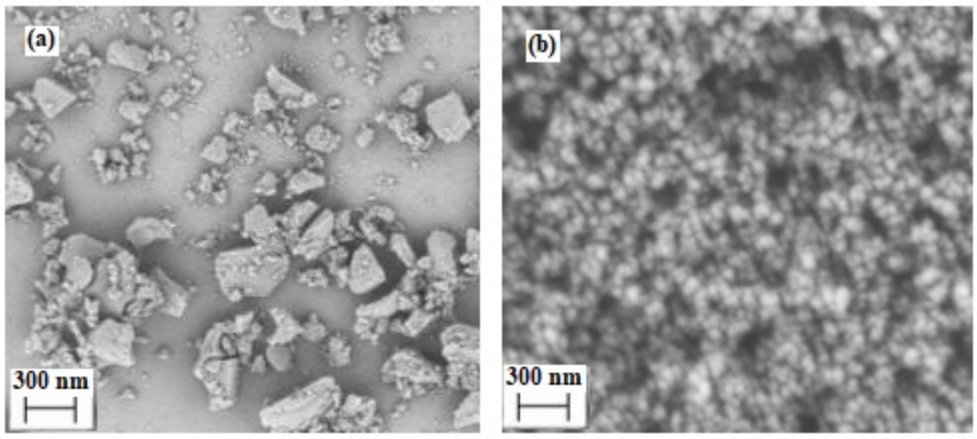

Fig. 1. Scanning electron microscopy images of the prepared micro-MIP (a) and nano-MIP (b)

2.3. Preparation of sensor

Sensor preparation was carried out as following:

In the first step, 0.058g of graphite was homogenized in a mortar with 0.012g of powdered promethazine MIP or NIP for 10 min. Subsequently, n-eicosane, 0.03g was melted in a dish in a water bath heated at 45–50 ºC. The graphite/MIP blend was then added to the melted n-eicosane and mixed with a stainless steel spatula. The final paste was used to fill a hole (2.00 mm in diameter, 3 mm in depth) at the end of an electrode body, previously heated at 45 ºC. The excess of solidified material was removed by the aid of a paper sheet after cooling at room temperature. The electrode can be reused after each experiment by moving the electrode surface on a paper in order to rub out a thin layer of the electrode surface.

2.4. Electrochemical measurements

The electrochemical measurement was carried out simply in one analyzing step without any separate extraction step. For this aim, three electrodes (including work, counter and reference electrodes) were placed simultaneously in an electrochemical cell containing 10 ml of phosphate buffer (0.3 M, pH=7) with different concentrations of PMZ and the square wave voltammetry (SWV) technique was applied after 800 sec. The potential range of 0.2-1 V, amplitude of 50 mV and frequency of 200 Hz, was used for SWV experiments.

3. RESULTS AND DISCUSSION

3.1. Comparison of CP, micro-MIP-CP & nano-MIP-CP electrodes

Fig. 2 demonstrates the cyclic voltammetry signals for bare CPE and MIP-modified electrodes with different size of polymer. As mentioned earlier, these experiments were performed in-situ, without using the washing step after extraction and also, this data is collected immediately after the electrodes placement in solution. It can be seen that the MIP nanoparticles based sensor shows significantly higher response, compared to the sensor prepared by the micro-sized MIP particles, indicating the accessibility and proper performance of the selective recognition sites of the nano-MIP. The notable active surface parallel to high affinity of the recognition sites of MIP nanoparticles to the target molecules, enables the nano-MIP-CP electrode to be sensitive for PMZ about three times as much as the micro-MIP-CPE.

The interesting point was the ability of nano-MIP-CP electrode to show its superiority over CPE even in a few seconds. While in the electrode containing micro-sized MIP, the insulating effect of polymer particles prevailed; to the extent that it even shows a decrease compared to bare CPE. This observation, encouraged us to utilize this in-situ method without any separate extraction and washing steps for developing the easy and fast PMZ determination using nano-sized MIP.

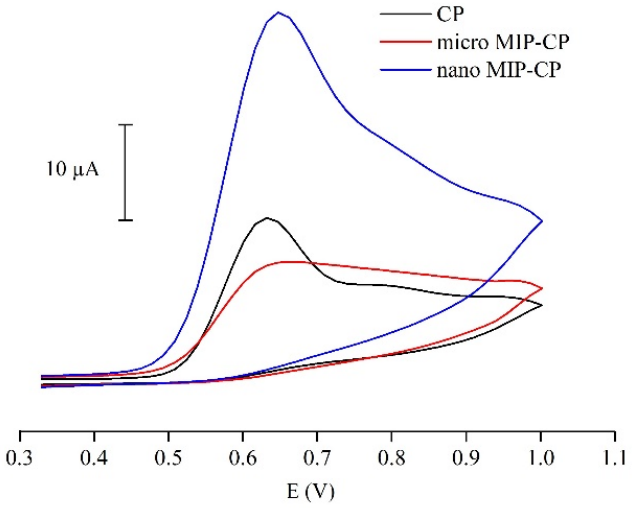

Fig. 2. Cyclic voltammetry responses of CPE, micro-MIP-CPE and nano-MIP-CPE at 0.1 M phosphate buffer pH=7, PMZ 10^{-4} M, scan rate = 0.1 V/sec

3.2. Technique selection

In order to achieve the highest voltammetry response, two more sensitive techniques of Differential Pulse Voltammetry (DPV) and Square Wave Voltammetry (SWV) was considered. The result is shown in Fig. 3. (left). As it is clear, SWV method can provide us

the considerably higher signal compare to its competitive method. Therefore the SWV technique was selected for further investigation.

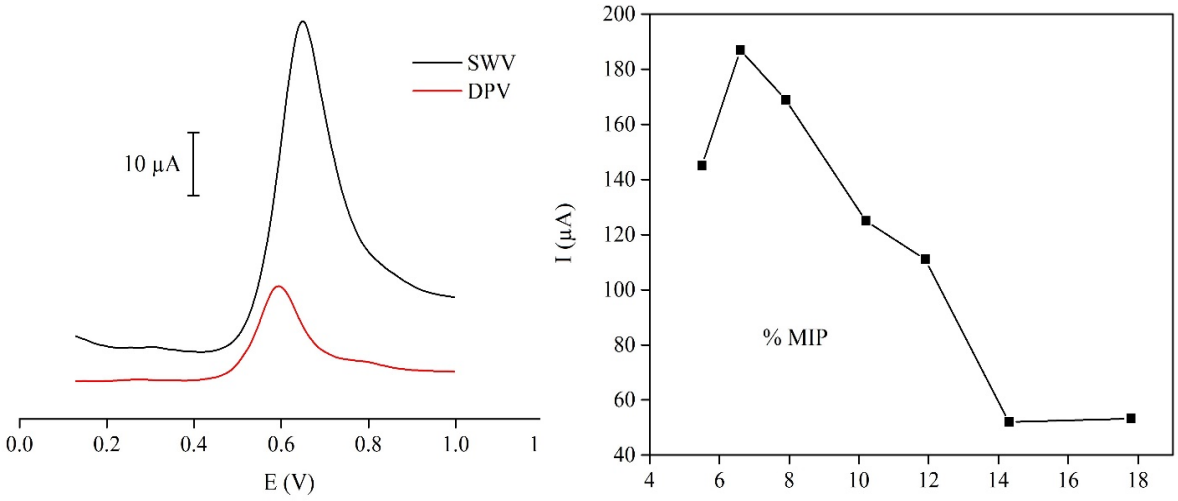

Fig. 3. Comparison of SWV and DPV current signals for PMZ assay using nano-MIP-CPE (left) in phosphate buffer (0.3 M, pH=7), [PMZ] = 5×10^{-6} M, freq. = 200 Hz, time = 550 sec; The effect of MIP content on sensor response (right); in phosphate buffer (0.3 M, pH=7), [PMZ] = 5×10^{-5} M, freq. = 200 Hz, time = 650 sec

3.3. MIP-CP composition optimization

The role of the MIP content in the developed electrode is very important, since it provide recognition sites for the target molecule. The amount of the MIP, present in the electrode composition, influences the performance of the sensor. An enhancement in the MIP content of the electrode can cause the increase in electrode signal because of increasing the number of selective sites at the electrode surface. However, further increase in the MIP content may lead to a destructive effect on the sensor response due to the insulating effect of the MIP particles. Fig. 3 (right) shows the effect of the MIP content of the electrode on its signal. According to this figure, the current signal of the nano-MIP-CP electrode is maximized at MIP content of 6.6% and at higher MIP content, the signal was declined sharply.

3.4. PMZ analysis condition optimization

The effects of different analysis conditions (such as equilibrium time, sample pH, buffer concentration and frequency) on the nano-MIP-CPE performance were investigated and shown in Fig. 4. The first parameter was incubation time that is the time, in which the electrode was remained in analyte solution before recording the SWV signal. Fig. 4(a) shows that the current signal was increased gradually up to 650 sec with the notable jump in 800

sec, while it remained nearly constant after that. Thus the incubation time of 800 sec was selected as optimum value.

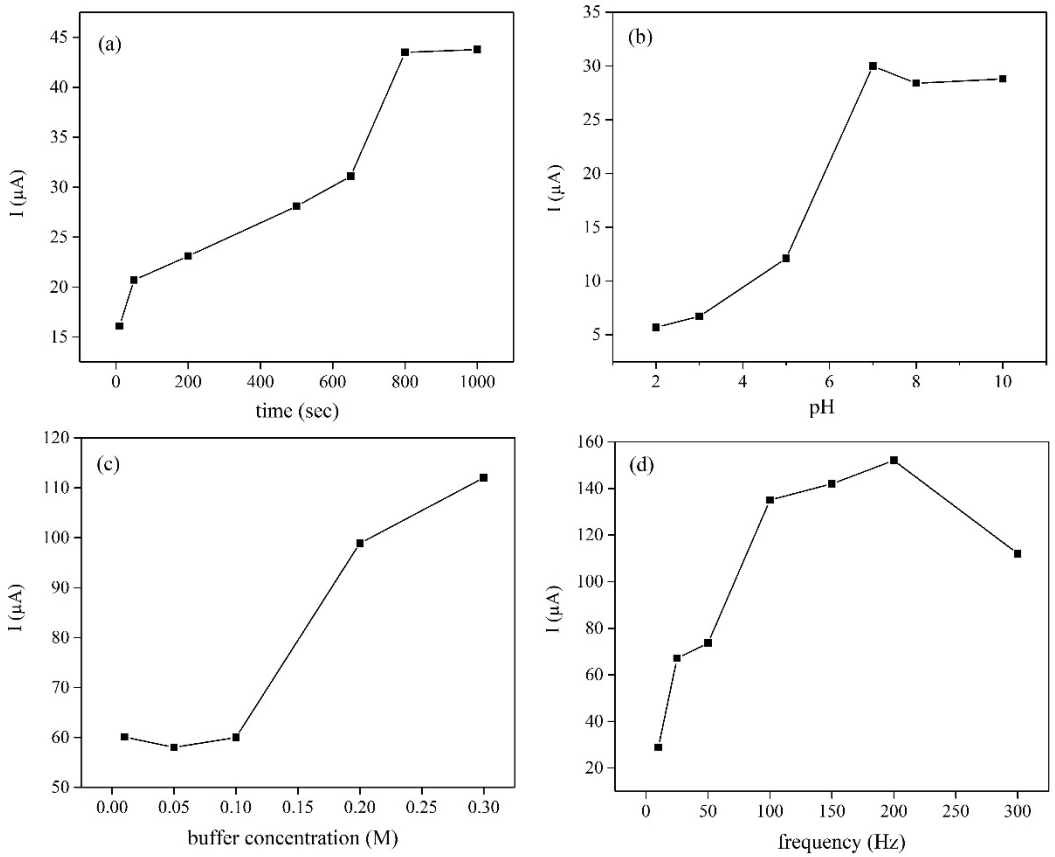

Fig. 4. The effects of different parameters of incubation time (a), analysis pH (b), buffer concentration (c) and SWV frequency (d) on the nano-MIP-CP electrode

According to Fig. 4(b), the optimum solution pH for in-situ determination of PMZ was reached at pH = 7, and nearly stabilized in higher pH values. Buffer concentration is one of the other important parameters which affects the sensor response. As can be seen in Fig. 4(c), the electrode signal reaches to the maximum amount at the highest concentration of the buffer (0.3 M). As it is shown in Fig. 4(d), the frequency of the SWV influences the final signal of the electrode. The frequency of 200 Hz, leads to a maximum in nano-MIP-CPE current signal.

3.5. Analytical characterization

The striking feature of MIP based techniques is their high selectivity. This selectivity is especially interesting when the target molecule response is compared with that of analogue compounds, having very similar chemical structure to the target molecule. Fig. 5 illustrates the sensor response to the PMZ and its two other cross-reactants with the same concentrations. Although, chemical structure of promethazine and chlorpromazine are

notably similar; the response of the electrode for promethazine is still higher than that for the analogue compound.

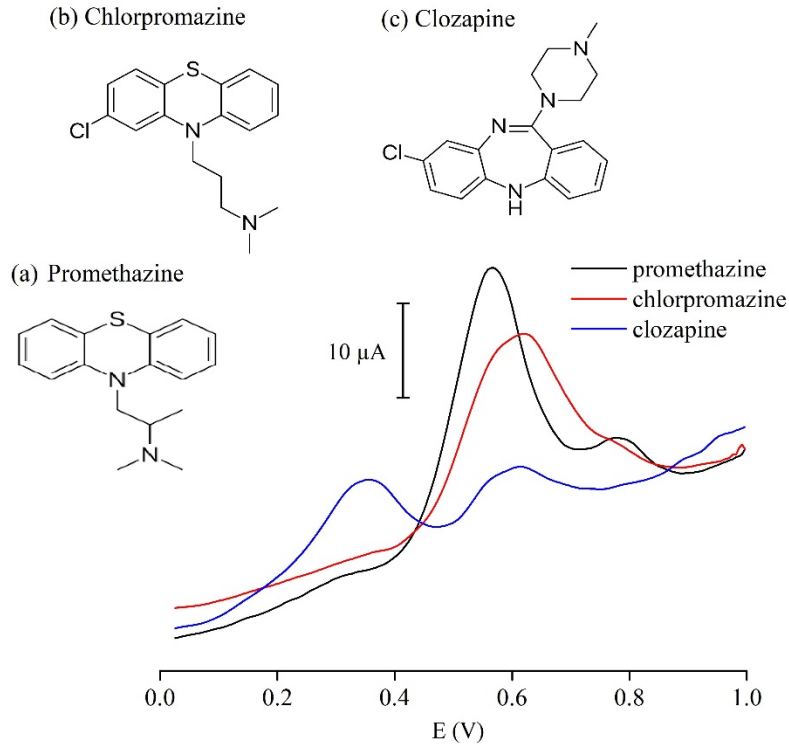

Fig. 5. Square wave voltammetry signals for the MIP-CPE to promethazine (a), chlorpromazine (b) and clozapine (c); [concentration] = 4×10^{-6} M, amplitude =50 mV and frequency =200 Hz.

Table 1. Interference levels of some species in PMZ assay by developed sensor

Species	**Interference level**
SO_4^{2-} , Cl^-	<120
K^+ , Na^+	<240
Fe^{2+} , Cu^{2+} , Zn^{2+}	<80
Phenol	100
Aniline	100
Ascorbic acid	150
Para-Nitrophenol	100

On the other hand, some previous studies [21] have shown that the response of a glassy carbon electrode for chlorpromazine is noticeably higher than that of promethazine. Moreover, we also found that the response of a bare CPE to chlorpromazine is higher than that to promethazine at the same conditions. These are the strong evidence that proves the

high affinity of nano-MIP-CPE to its target molecule (PMZ). Also, the sensor response to the clozapine as other cross-reactant is remarkably weaker compare to the PMZ response, which indicates that it does not have the ability to compete with target molecules to occupy the PMZ selective cavities at the electrode surface.

After the optimization and establishment of the determination method, the interference effect of various species was examined. The tolerance limit was defined as the maximum concentration of foreign species that caused a relative error of 5% in the analytical signal and the obtained results, showing a mole ratio of interfering agent per PMZ, are given in Table 1.

Fig. 6 is showing the Calibration curve plotted for promethazine determination with MIP-CP using SWV technique. The plot consists of two linear dynamic range of 4×10^{-9}-4×10^{-7} M and 4×10^{-7}-7×10^{-6} M in optimum analysis conditions.

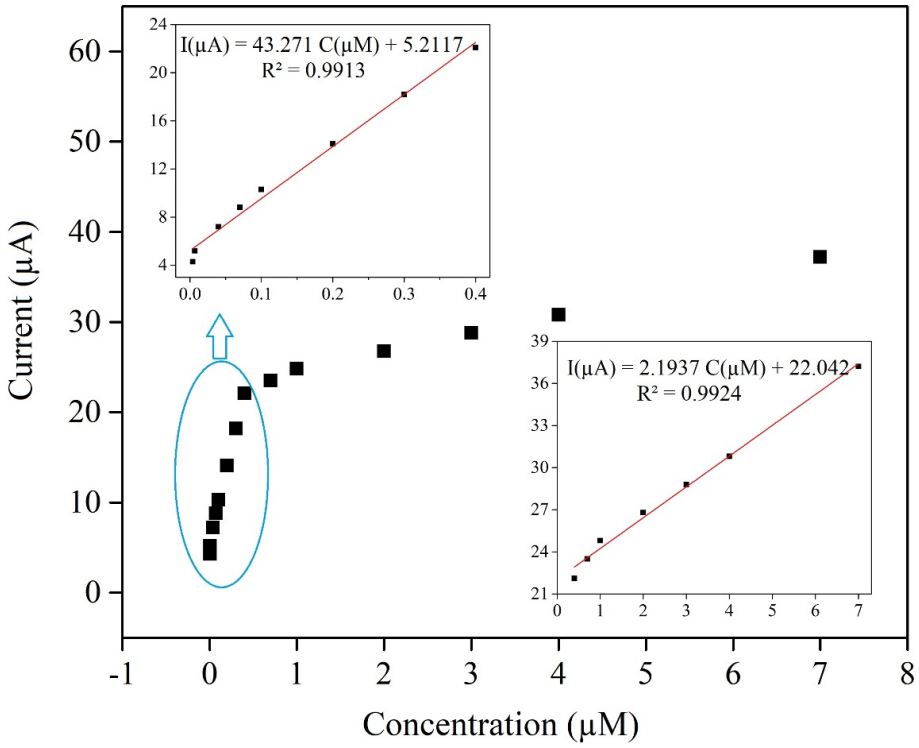

Fig. 6. Calibration curves obtained from different concentrations of PMZ using MIP-CPE in phosphate buffer (0.3 M, pH=7) at optimum conditions; Percentage of MIP 6.6% of total weight, amplitude 50 mV and frequency 200 Hz.

3.6. PMZ assay in human serum

The sensor was applied for PMZ measurement in spiked human serum samples. For this aim, different amounts of PMZ solution were injected into the serum samples, adjusting the PMZ content of the solutions equal to 10.0×10^{-7} and 0.1×10^{-6} M. The sensor was then applied for determination of the mentioned spiked solutions and results are represented in Table 2.

The acceptable percent of recoveries, certify the applicability of this electrode for real sample analysis contain these low amounts of PMZ.

Table 2. Promethazine measurement in serum samples

Sample [a]	Add (M)	Found (M)	Recovery (%)
1	10^{-6}	1.04×10^{-6}	104
2	10^{-7}	0.095×10^{-7}	95

[a] Number of each sample assayed = 4

4. CONCLUSION

Nanostructured, imprinted polymers have a small dimension with extremely high surface-to-volume ratio, so that most of template molecules are situated at the surface and in the proximity of materials surface. Therefore, the forms of imprinted materials are expected to greatly improve the binding capacity, kinetics and site accessibility of imprinted materials.
In this work a precise, rapid, simple and low-cost method for promethazine determination in plasma samples is introduced. For this aim, carbon paste electrode modified with nano-sized Molecularly Imprinted Polymer was applied as the working electrode using Square Wave Voltammetry (SWV) technique. MIP nanoparticles, containing PMZ selective sites, were synthesized by the method of ultrasonic assisted suspension polymerization in silicon oil. In our last work [6] it was proved that the selective election of the PMZ in a separate extraction step and the washing of electrode before electrochemical determination enhanced the selectivity of the sensor because of removing weakly adsorbed compounds. But this time, the benefit of in-situ high speed determination was preferred, and the %RSD=1.2 which was found for 4 separate determinations by this sensor, illustrated the excellent accuracy of the method. The effect of different factors on the sensor response was investigated and the proper conditions were selected. The optimized sensor exhibited two linear range of 4×10^{-9}-4×10^{-7} M and 4×10^{-7}-7×10^{-6} M with the slopes of 43.3 and 2.2 $\mu A.\mu M.l^{-1}$ respectively. The detection limit was calculated equal to 1.4×10^{-9} M. This sensor was applied successfully for promethazine determination in serum samples without applying any sample pretreatment.

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