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Fabrication of an Extra Sensitive Voltammetric Sensor Using Nanoparticles of Molecularly Imprinted Polymer for Determination of Ultra-Trace Promethazine in Plasma Sample

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In this work, the nanoparticles of promethazine-imprinted polymers (MIP) were synthesized by the ultrasonic assisted suspension polymerization in silicon oil. The MIP particles were then embedded in a carbon paste (CP) electrode in order to prepare the MIP (nano)-CP electrode. This electrode showed higher response to analyte, compared to the carbon paste electrode, modified with non-imprinted polymer (NIP (nano)-CP). Also, the sensitivity and selectivity of the MIP (nano)-CP were considerably better than those of the sensor prepared by the bulky MIP particles (MIP (micro)-CP). Various factors, known to affect the response behavior of selective electrode, were investigated and optimized. The sensor exhibited two distinct linear response ranges of 4×10^{-12}- 1×10^{-10} M and 1×10^{-9} -1×10^{-7} M with the sensitivities of 31.7 and 0.17 μA nM^{-1}, respectively. The lower detection limit of the sensor was calculated equal to 2.8×10^{-12} M (S/N). An interestingly low RSD equal to 0.81% was found for 5 separate determinations by the proposed sensor. The sensor was applied for promethazine (PMZ) determination in plasma samples without applying any sample pretreatment.

Keywords: Ultratrace, Molecularly imprinted polymer; Nanoparticles; Suspension Polymerization; Silicon oil; Promethazine

1. INTRODUCTION

Biological receptors including antibodies and enzymes have been widely used as the detection elements in a variety of chemical and biochemical sensors [1-3]. However, the use of artificial receptors in chemical/biological detections has become increasingly attractive, because the synthetic recognition systems such as molecularly imprinted polymers (MIPs) usually have lower costs, higher

physicochemical stability and better engineering possibility than the biological receptors [4-8]. Although, the MIPs possess molecular recognition ability similar to that of the biological receptors, traditional bulky MIP materials usually exhibit slow binding kinetics to the target species and suffer from the low rebinding capacity [9].

Employing of nanotechnology methods in the molecular imprinting strategy has attracted considerable research interest [10-14]. 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. Therefore, the forms of imprinted materials are expected to greatly improve the binding capacity and kinetics and site accessibility of imprinted materials. This is against the conventional bulky MIP particles which usually suffer from low rebinding kinetics [9].

Recently, we have published several voltammetric and potentiometric sensors for some compounds based on the MIP materials [15-20].

In the mentioned works, we used micro-sized MIP particles as the recognition elements of the sensors. It has been shown that a high sensitive, high selective, simple and cheap sensor could be prepared by the MIP technology. In order to improve further the analytically important characteristics of the MIP-based sensors, we aimed to apply nano-sized MIP particles for fabrication of the voltammetric sensor. Thus, in this work, the nanoparticles of molecularly imprinted polymer containing recognition sites of promethazine was prepared and then used for fabrication of a promethazine selective voltammetric sensor. It was found that the MIP size had explicit effect on the voltammetric sensor performance. The effect of different experimental parameters on the MIP (nano)-CP electrode, were investigated and optimized. The developed electrode was successfully applied for the analyte determination in serum sample without any time consuming pretreatment steps.

2. EXPERIMENTAL

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. AUTOLAB PGSTAT302 electrochemical analysis system and GPES 4.9 software package were used for the electrochemical impedance spectroscopy. Impedance measurements were performed at frequency range= mHz–1MHz, ΔEac= 50 mV and dc potential of 0.25 V (in the presence of redox couple of $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$). The measurements were performed in a three-electrode system: counter electrode (platinum), reference electrode (Ag/AgCl), and working electrode (investigated conventional electrodes). Vinyl benzene (VB), obtained from Sigma-Aldrich (Munich, Germany), was distillation under reduced pressure. Divinyl benzene (DVB) and ethylene glycol dimethacrylate (EGDMA), obtained from Fluka (Buchs, Switzerland), were also distilled under reduced pressure and stored at 4°C, until use. Promethazine, chlorpromazine, 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 the bulky PMZ imprinted polymer

The bulk polymerization method was used for the preparation of the micro-sized MIP particles. For the MIP preparation, 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, 20 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°C to start the polymerization process. After 12 h, the obtained polymer materials were ground and sieved. PMZ and unpolymerized monomers were removed by soxhlet extraction with 100 ml of methanol/acetic acid (80:20, v/v) by refluxing for 12 h. Then, the particles were suspended in acetone and allowed to settle for 4 h. The participated particles were discarded and those not precipitated were collected by centrifugation. The resulting MIP particles were dried under vacuum at 60 °C and were used in the following experiments. Non-imprinted polymer (NIP) particles were prepared analogously without the addition of PMZ during polymer material preparation. Scanning electron microscopy image of the prepared bulky MIP is shown in Fig. 1 (up).

2.3. Preparation of the nanosized-PMZ imprinted polymers

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 65 °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 washed with MeOH. Finally, the particles were dried in vacuum at 50°C overnight. The non-imprinted polymer nanoparticles were prepared and treated in the same manner in the absence of PMZ. The nano-MIP, prepared by this method was regarded as nano-MIP (2). Fig. 1 (down) illustrates the scanning electron microscopy image of the prepared MIP nanoparticles.

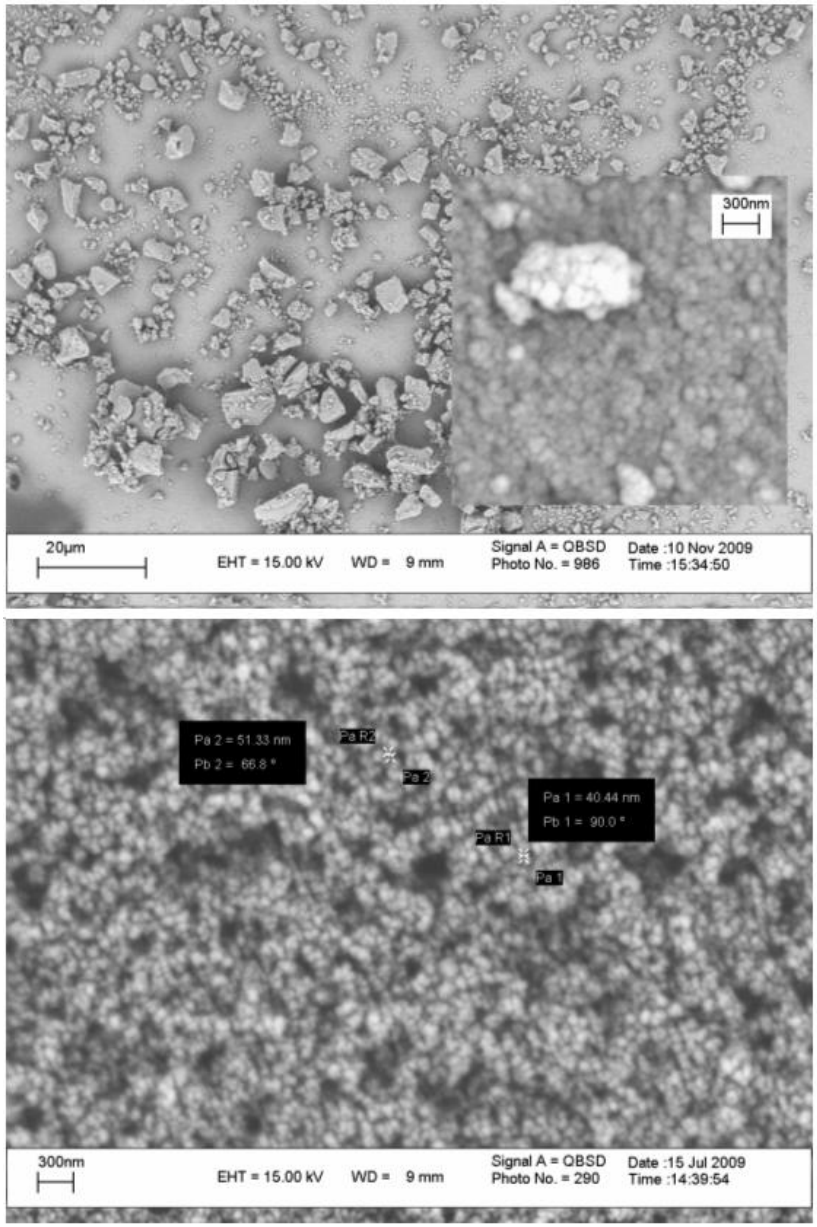

Figure 1. Scanning electron microscopy images of the prepared bulky MIP (up) and MIP nanoparticles (down)

2.4. Preparation of the sensors

The important part of any electrochemical sensor is selective sensing element [21-24]. For the construction of the sensor (MIP(nano/micro)-CP or NIP(nano/micro)-CP), 0.05 g of graphite was homogenized in a mortar with 0.01 g of promethazine-MIP or NIP as a sensing element for 10 min. Subsequently, n-eicosane (0.03 g) was melted in a dish in a water bath, heated at 45-50 °C. 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 °C. After cooling at room temperature, the excess of solidified material

was removed by the aid of a paper sheet. 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.

2.5. Electrochemical measurements

The electrochemical measurement was carried out according to the following sequentially procedure:

Extraction step: the prepared electrode was inserted into the analyte solutions containing phosphate buffer (pH=7). In the extraction period, all solutions were stirred at a fixed stirring rate for a determined time.

Washing step: the electrode was removed from the first solution and then inserted into the washing solution, composed of water/acetonitrile (98:5), remaining in this solution for 10 s.

Analyzing step: the electrode was placed in an electrochemical cell containing 10 ml of phosphate buffer (pH=7) and then the square wave voltammetry (SWV) technique was applied for determination of PMZ. The potential range of 0.2-1.2 V, having amplitude of 50 mV and frequency of 50 Hz, was used for SWV experiment.

3. RESULTS AND DISCUSSION

3.1. Comparison of different MIP and NIP based electrodes

Fig. 2 (I) and (II) show the voltammetric responses of the MIP (micro)-CP and MIP (nano)-CP electrodes to promethazine in two different concentrations of 1×10^{-7} and 3×10^{-11} M. NIP (micro)-CP, NIP (nano)-CP and CP were also tested in term of their sensitivities for promethazine. As can be seen, the MIP nanoparticles based sensor shows better response characteristics, compared to the sensor prepared by the micro-sized MIP particles. In every case, the response of the MIP based electrode is obviously higher than that of the relevant NIP based electrode, indicating the presence and proper performance of the selective recognition sites of the MIP. It is clear that the signal of MIP(nano)-CP electrode is about 2 times as much as that of NIP(nano)-CP electrode. Whereas, the response of MIP(micro)-CP is about 1.5 times as much as that of NIP(micro)-CP electrode. This is a good clue for the higher affinity of the recognition sites of the MIP nanoparticles, compared to those of the bulky MIP. When we used the CP electrode for promethazine determination an obvious signal was appeared. However, the CP electrode response is lower than both of the MIP and NIP based electrodes. In Fig. 2(II) the responses of the MIP and NIP based electrodes for ultra-trace PMZ are represented. As can be seen, no response is observed for the MIP(micro)-CP and NIP(micro)-CP electrodes whereas, an explicit voltammetric signal is produced by the MIP(nano)-CP. At this circumstance, the signal difference between the MIP (nano)-CP and NIP(nano)-CP electrodes is also enhanced. The high affinity of the recognition sites of MIP nanoparticles to the target molecules enables the MIP(nano)-CP electrode to be sensitive for PMZ even at ultra-trace concentrations.

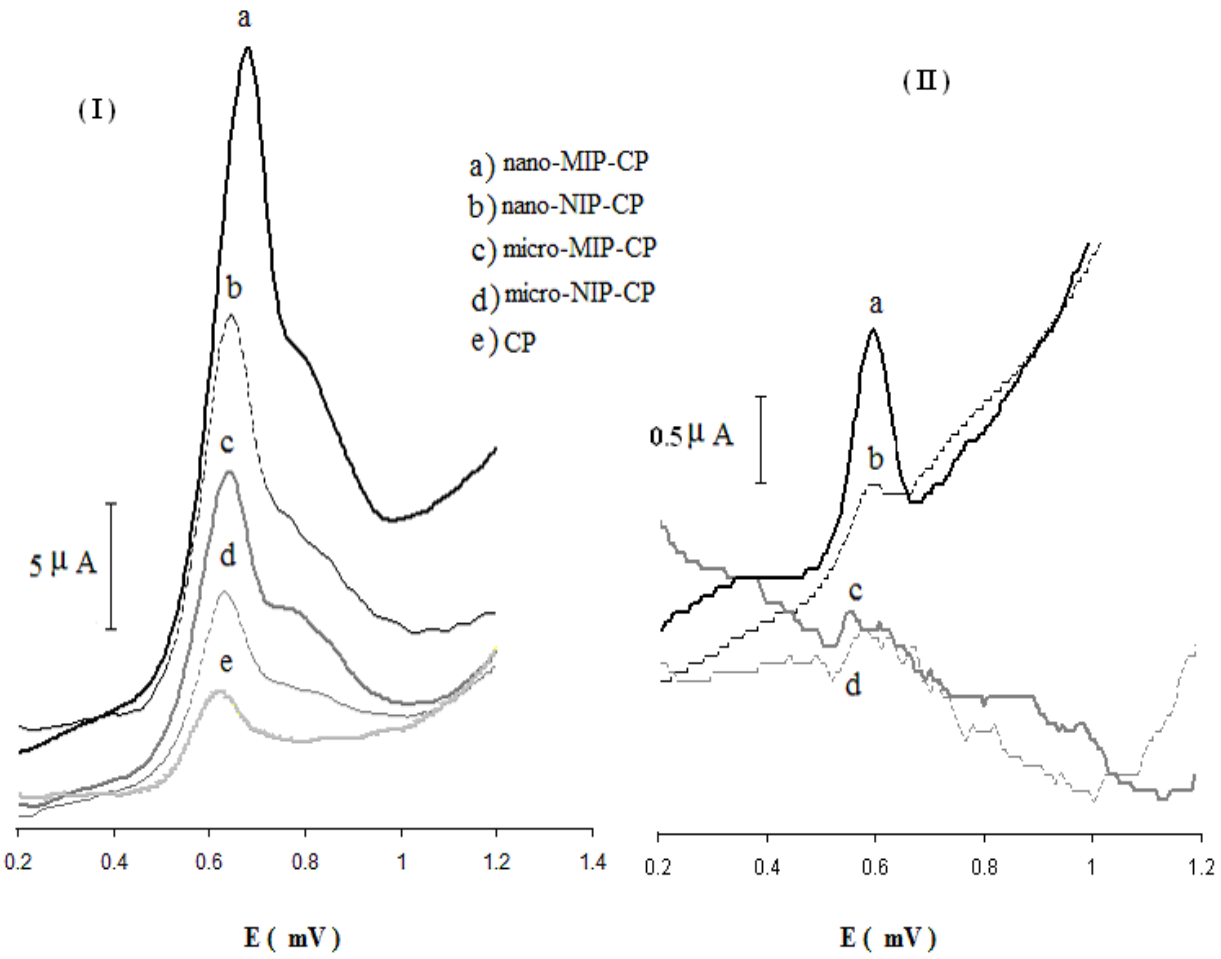

Figure 2. Square wave voltammetry signals obtained from the electrodes of (a) MIP(nano)-CP, (b) NIP(nano)-CP, (c) MIP(micro)-CP, (d) NIP(micro)-CP, (e) CP in different PMZ concentrations of (I) 1×10^{-7} M and (II) 3×10^{-11} M, extraction time= 7 min, stirring rate=400 rpm

3.2. Selectivity of the MIP(nano)-CP electrode

In order to test the selectivity of the sensor, the response of the MIP(nano)-CP electrode for promethazine and chlorpromethazine was checked. The obtained results (as the cyclic voltammetry signals) are illustrated in Fig. 3. Although, chemical structure of promethazine and chlorpromethazine are highly alike; the response of the electrode for promethazine is noticeably higher than that for the analogue compound. On the other hand, some previous studies [25] have shown that the response of a glassy carbon electrode for chlorpromethazine is noticeably higher than that of promethazine. Moreover, we also found that the response of a carbon paste electrode to chlorpromethazine is higher than that to promethazine at the same conditions. This indicates that the MIP-CP electrode affinity for prometazine (compared to chlorpromethazie) is higher than that is illustrated in the related voltammograms in Fig. 3.

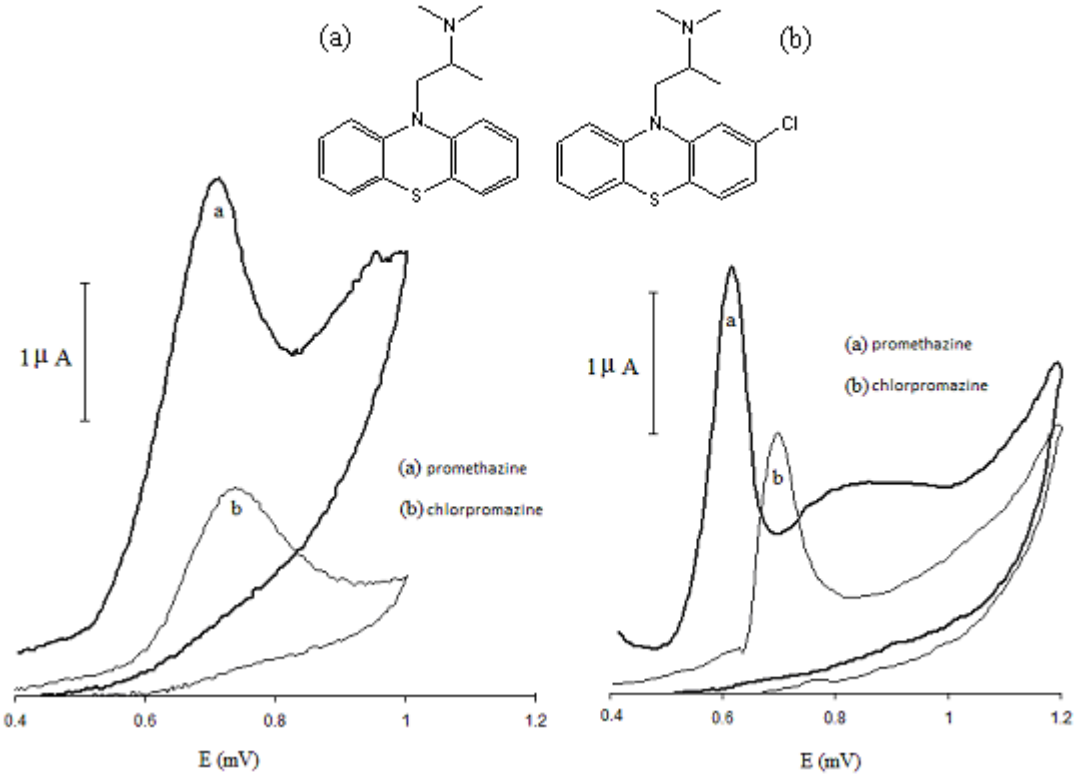

Figure 3. Cyclic voltammetry responses of the MIP (micro)-CP (left side) and MIP(nano)-CP (right side) to promethazine (a) and chlorpromethazine(b); scan rate= 0.05V.s^{-1}MIP (micro)-CP: [promethazine]= 1×10^{-6}M, [chlorpromethazine] = 1×10^{-6}M; MIP (nano)-CP: [promethazine]= 1×10^{-7}M, [chlorpromethazine] = 1×10^{-7}M

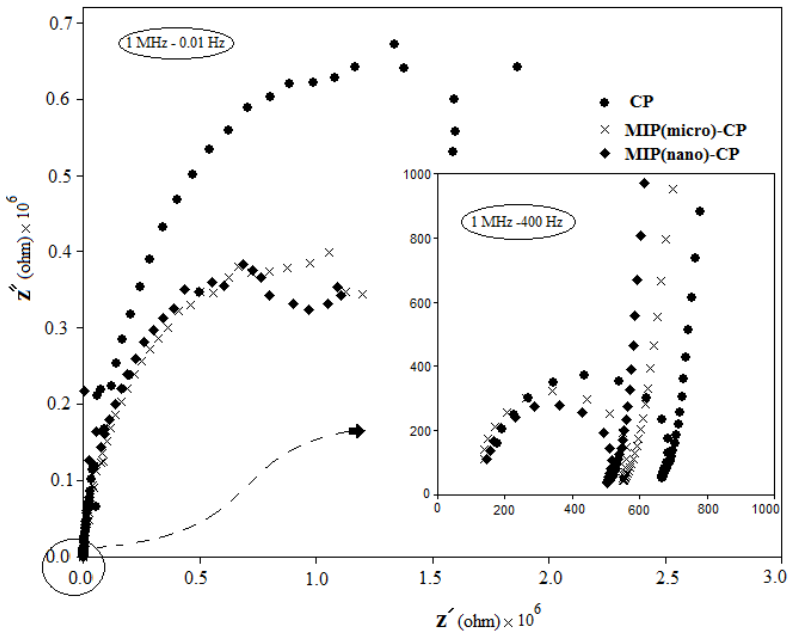

Figure 4. Electrochemical impedance spectroscopy diagram of the CP, MIP(micro)-CP and MIP(nano)-CP electrodes in the PMZ solution (1×10^{-5} M) and phosphate buffer; applied potential = 0.75 V, potential amplitude = 0.05 V, frequency range = 0.01Hz- 1MHz

Comparison of responses of the MIP(micro)-CP and MIP(nano)-CP electrodes is particularly interesting. As can be seen, in the case of the MIP(nano)-CP, the promethazine oxidation current peak is separated obviously from that of chlorpromazine. Whereas, in the case of the MIP(micro)-CP the voltamograms of both compounds are broaden, thus overlapping the related signals. This observation is due to the homogeneity of the recognition sites of the MIP nanoparticles, compared to the micro-sized MIP particles. The voltamogram of the MIP(micro)-CP is broaden because of different activation energies, consumed for the oxidation of the PMZ molecule present in the sites with different energies. On the other hand, the majority of the PMZ molecules are adsorbed on the selective sites of the MIP nanoparticles with approximately the same adsorption energies. Thus, narrower energy range is required for oxidation of PMZ, existing in these selective cavities. This can result in the narrower voltamograms for both compounds in the MIP(nano)-CP electrode.

Fig. 4 shows the impedance spectra (Nyquist plots) of the CP, MIP (micro)-CP and MIP (nano)-CP electrodes in the presence of phosphate buffer and PMZ solution. It seems that the charge transfer resistance of PMZ on the MIP (nano)-CP electrode is slightly lower than that of the MIP (micro)-CP. The inset of Fig. 4 represents the Nyquist plots of the mentioned electrodes at high frequency region. This section in the plots, describe the bulk resistance and geometrical capacitance, created by the graphite particles/n-eicosane composite. In general, a bulk resistance in parallel with a geometrical capacitance accounts for the conductive pathways across the binder/graphite or graphite/MIP/binder composite. It can be seen that the bulk resistance of the examined electrodes obeys the order of CP> MIP (micro)-CP > MIP (nano)-CP. This indicates that the surface of the MIP(nano)-CP is more conductive than that of the MIP(micro)-CP electrode. This can be adopted as another reason for the narrower oxidation peaks of the tested compounds on the MIP(nano)-CP electrode.

3.3. The role of washing step on the electrode performance

It is well known that both mechanisms of surface adsorption and recognition via the imprinted cavities are important for up-taking of target molecules by the MIP materials. The first mechanism has nonselective nature, while the later way is responsible for the MIP selectivity to the target molecules. Since the surface adsorption phenomenon eliminates the selectivity of the MIP, it is necessary to distinguish the selective recognition from the nonselective surface adsorption. For this aim, after extraction of analyte in the electrode, it was washed with the aqueous solution containing a low percent of ethanol. The effect of the washing on the SWV signal of the MIP(nano)-CP, NIP(nano)-CP and CP electrodes is shown in Fig. 5. As it is clear, the electrodes washing does not noticeably influence the PMZ signal in the MIP(nano)-CP. However, the response of the NIP(nano)-CP and CP electrodes is considerably decreased by the washing. These results indicate that the MIP(nano)-CP electrode has more affinity to PMZ, compared to the NIP(nano)-CP and CP electrodes.

The washing process can remove the weakly and nonspecifically absorbed PMZ molecules from the electrode surface. This occurrence can be clearly seen in the case of both NIP-CP and CP.

However, the PMZ molecules incorporated in the selective sites of the MIP are not removed easily by the washing process.

It was also found that the neutral water containing a little amount of an organic solvent decreased the NIP(nano)-CP signal, whereas the MIP(nano)-CP signal was influenced considerably by the mentioned mixture. Different organic solvent such as ethanol, methanol, acetone and acetonitrile were added to water (5% in water) and tested as a washing solution. The response difference between the MIP(nano)-CP and NIP(nano)-CP in the case of ethanol was the highest among all the tested solvents. Thus, ethanol was selected as an organic solvent to mix with water.

The time of the electrode incubation in the washing solution was also investigated. The response difference between the MIP(nano)-CP and NIP(nano)-CP was maximum when the washing was performed for 7 s. Thus the time of 7 s was chosen as optimal washing time.

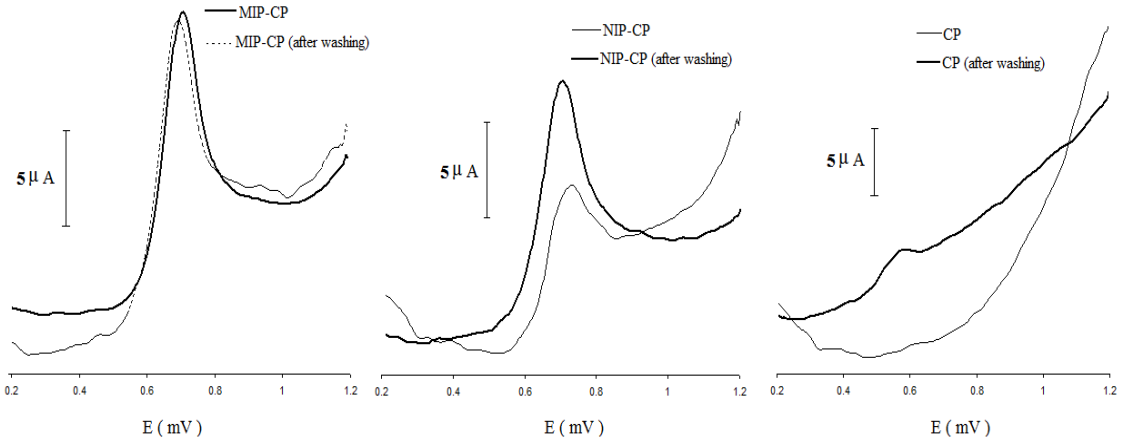

Figure 5. The effect of the electrode washing on the SWV responses of different electrodes; incubation time =10 min, [PMZ] = 5×10^{-8} M, extraction volume =30 mL

3.4. The effect of the MIP content on the MIP (nano)-CP electrode performance

The role of the MIP 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 increase in the MIP content of the electrode can lead to an enhancement in the electrode signal because of increasing the number of selective sites in 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. This has been established in our previous studies [7-10]. Fig. 6 shows the effect of the MIP content of the electrode on its signal. According to this figure, thesignal of the MIP (nano)-CP electrode is maximized at MIP content of 12%. In the previous work [19] we found the same optimum point for the MIP-(micro)-CP electrode. However, compared to that electrode, the MIP(nano)-CP is active in wider range of the MIP content of the electrode. For example, even in the MIP content of 4% a considerably high response can be seen for the MIP(nano)-CP electrode whereas, in the MIP content of 8 % the signal completely disappears for the MIP(micro)-CP [19]. This is due to the fact that MIP nanoparticles provide more recognition sites

in the electrode surface, compared to the bulky MIP particles. On the other hand, at higher MIP contents (more than 12%) the observed signal for the MIP nanoparticle based electrode is considerably more than that of the MIP(micro)-CP [19], indicating lower unwanted insulating effect of the MIP nanoparticles. This can be attributed to the uniformly distribution and dispersion of the fine MIP nanoparticles in the electrode composition due to the very small sizes of them. The result represented in the inset of Fig. 4 can be used as a witness for this statement.

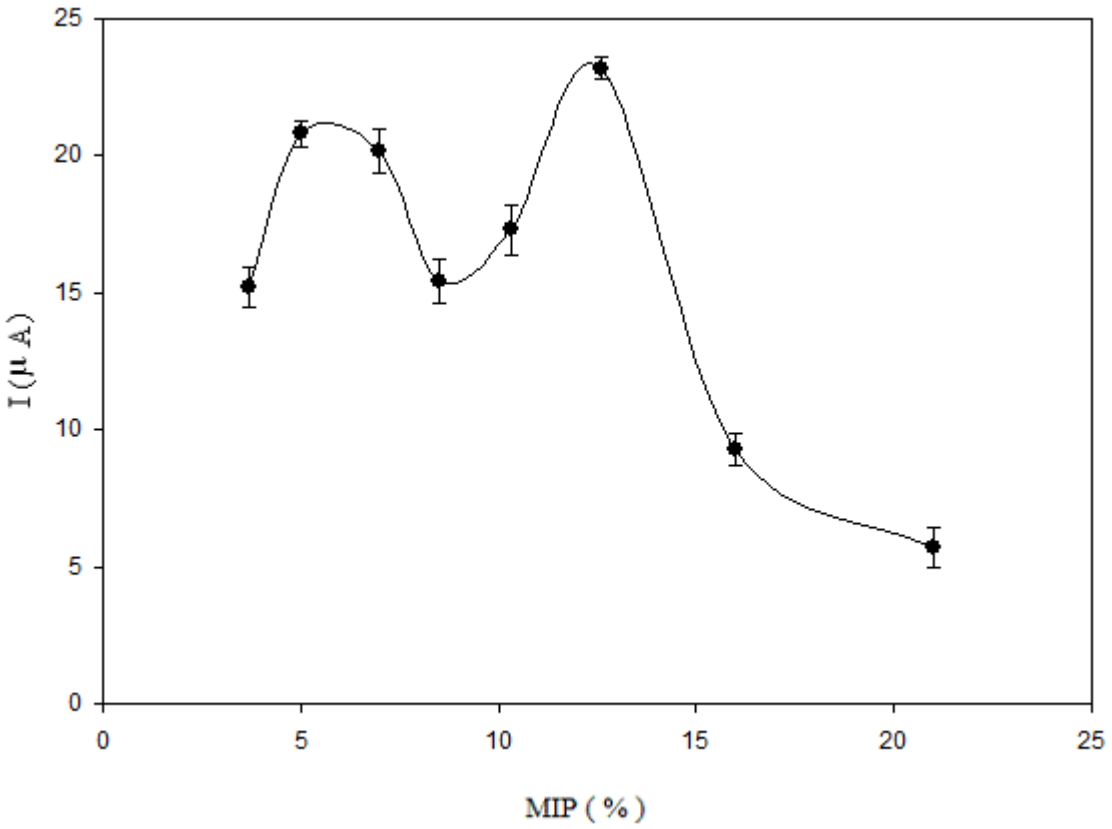

Figure 6. The effect of MIP content on the SWV response of the MIP (nano)-CP electrode; [PMZ] = 1×10^{-7} M, extraction time=pH, extraction pH= 7

3.5. The effect of different parameters on the sensor performances

The effect of different parameters such as pH of promethazine solution, stirring speed, time of extraction and promethazine solution volume on the MIP(nano)-CP were studied. The obtained results are illustrated in Fig. 7. As it is clear, the MIP(nano)-CP signal remains constant and maximum in the pH range of 7-12 (Fig. 7(I)). Compared to the MIP(micro)-CP [19] that has shown steady state extraction condition in the pH range of 9-12, this is a wider range. Fig. 7(II) shows the effect of extraction time on the MIP(nano)-CP. As can be seen, after 2 min, the adsorption of PMZ in the MIP(nano)-CP reaches to a constant amount. This is considerably lower than 10 min extraction time, required for the MIP(micro)-CP electrode [19]. This is a clear witness for the higher rebinding kinetic

of target molecules in the selective sites of the MIP nanoparticles compared to that of the micro-sized MIP.

As it is shown in Fig. 7 (III), the stirring rate of PMZ solution influences the final signal of the electrode. The stirring rate of 200 rpm leads to a maximum in the extraction of PMZ in the MIP(nano)-CP electrode. According to Fig. 7 (IV), the optimum extraction volume for nano-MIP based electrode is 50 mL, whereas it was 40 mL for MIP(micro)-CP [19]. This means that the MIP(nano)-CP electrode can compete with higher aqueous solution for PMZ up-taking and this can increase further the enrichment factor of the electrode.

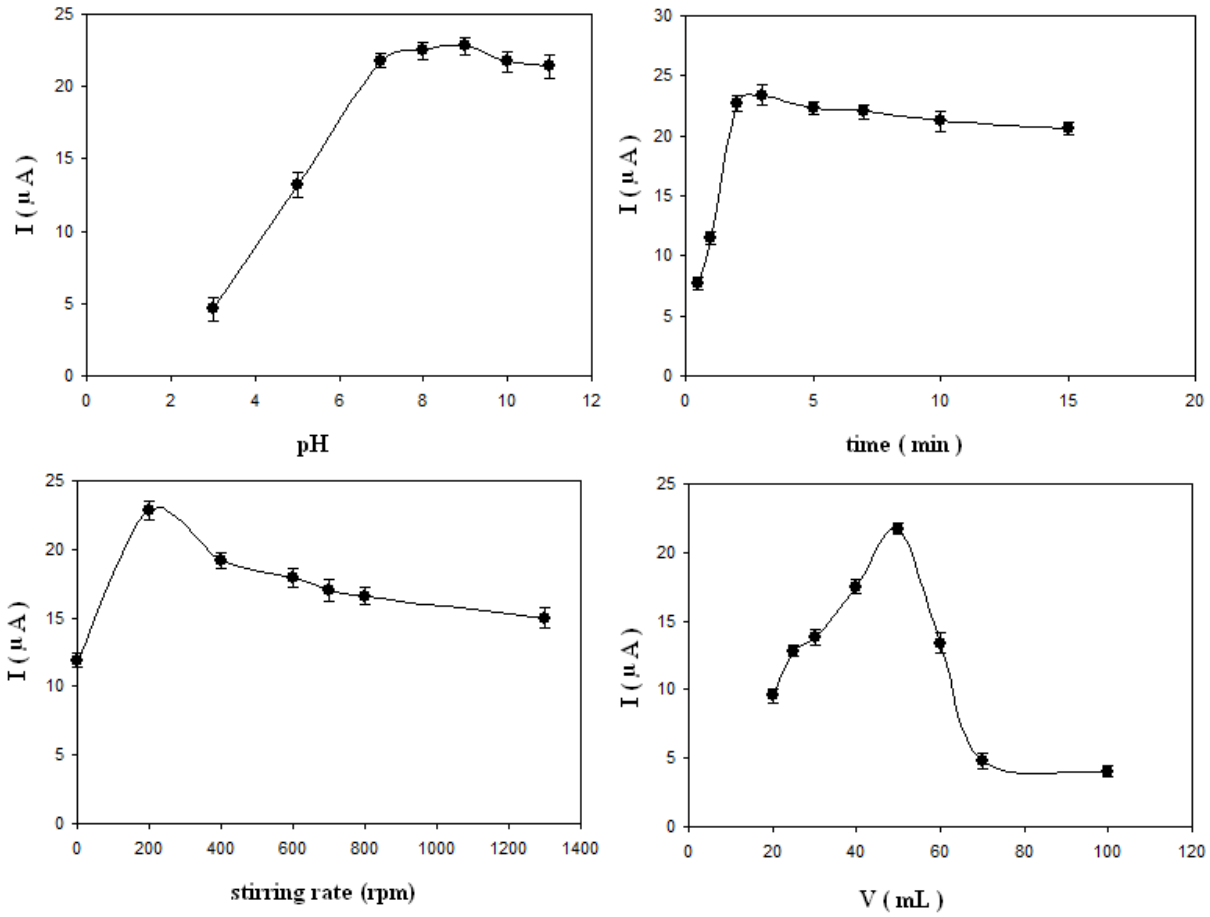

Figure 7. The effects of different parameters of (I) extraction pH, (II) extraction time, (III) stirring rate of extraction solution and (IV) extraction sample volume on the MIP(micro)-CP and MIP(nano)-CP electrodes

3.6. Analytical characterization

After establishment of the determination method, the interference effects of various species were examined. The tolerance limit was established as the maximum concentration of foreign species that caused a relative error of 5% in the analytical signal. The obtained results, showing a mole ratio of interfering agent per PMZ that produces 5% error in the PMZ determination, are given in table1.

Table 1. Interference levels of the tested species in the determination of PMZ by the developed method

Species	Interference level
Co^{2+}, SO_4^{2-}, Cl^-	<400
K^+, Na^+	<400
Ca^{2+}	<200
Fe^{2+}, Zn^{2+}, Cu^{2++}, Ni^{2+}	170
Phenol	200
Aniline	200
para-nitrophenol	200
Benzoic acid	300
Nitrobenzene	200
Ascorbic acid	<250

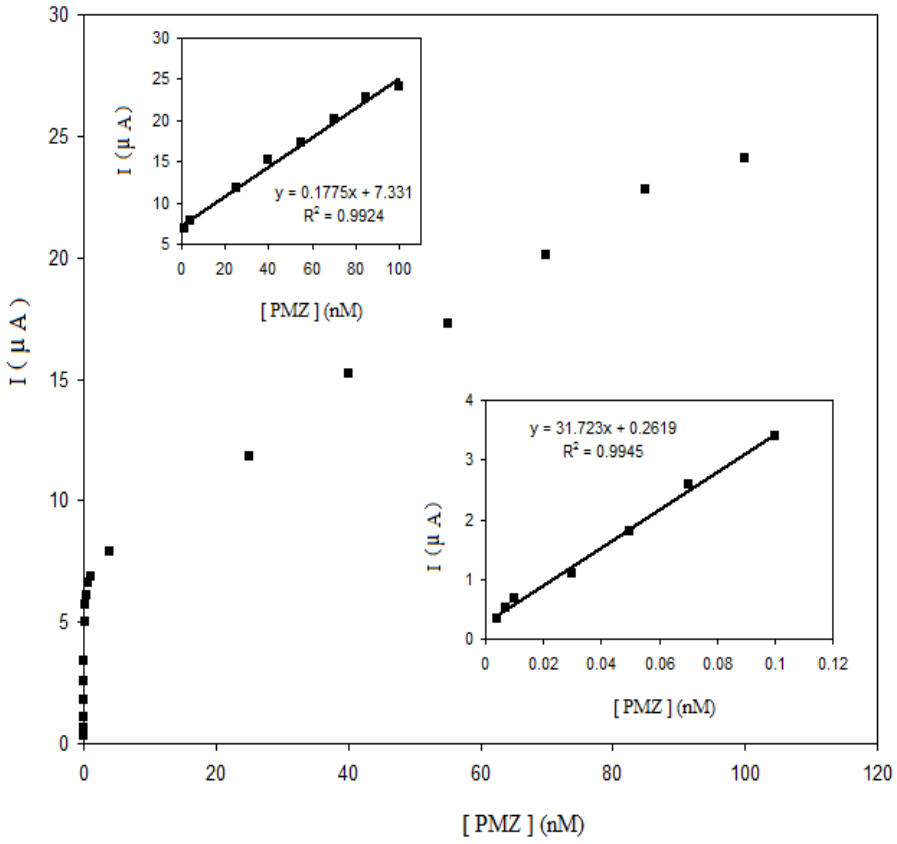

Figure 8. Calibration curve for the MIP(nano)-CP electrode at the optimized conditions; insets are two linear ranges of the calibration curve

According to these results, the electrode is highly resistant against the tested interfering species. The calibration graph of the MIP (nano)-CP sensor is represented in Fig. 8. As can be seen, this electrode exhibits two distinct linear response ranges of 4×10^{-12} -1×10^{-10} M and 1×10^{-9} -1×10^{-7} M with the sensitivities of 31.7 and 0.17 µA nM^{-1}, respectively. The lower detection limit of the method was

calculated equal to 2.8×10^{-12} M. Relative standard error percent of 5 separate determinations by the proposed sensor was found to be 0.81%.

Table 2. Comparison of analytical characteristics of the MIP(nano)-CP electrode with some other previously reported PMZ voltammetric sensors

Method	Analytical Characterizations				
	Linear range (M)	Slope (μAL/n M)	Detection limit (M)	RSD%	[Ref.]
MIP(nano)-CP	4.0×10^{-12}-1.0×10^{-10} 1.0×10^{-9}-1.0×10^{-7}	31.7 0.177	1.2×10^{-12}	0.81	This work
MIP(micro)-CP	4.0×10^{-7}-7.0×10^{-9}	0.04	3.2×10^{-9}	3.6	19
Pretreated Glassy Carbon Electrode modified with DNA	4.7×10^{-10}-9.3×10^{-9}	10.1	3.0×10^{-10}	-	26
Boron-Doped Diamond electrode using adsorptive square wave voltammetry	5.9×10^{-7} -4.7×10^{-6}	0.12	4.6×10^{-8}	3.87	27
vadsorption/extraction at a wax-impregnated graphite electrode	5.0×10^{-7}-1.0×10^{-4}	-	5.0×10^{-8}	-	28
Multi-walled carbon Nanotubes coated gold electrode	5.0×10^{-8}-1.0×10^{-5}	-	1.0×10^{-8}	1.53	29

A number of analytical characteristics of the MIP (nano)-CP and other electrochemical sensors, previously reported for the PMZ determination, are given in table 2. It can be seen that the MIP (nano)-CP sensor is better than MIP (micro)-CP electrode and other previously reported PMZ sensors, regarding the analytical characteristics. The detection limit of the MIP (nano)-CP is about 260 times lower than that of the MIP (micro)-CP. Also, the detection limit of the MIP (nano)-CP is 26 times lower than that of DNA based sensor (as the best PMZ sensor shown in the table).

Also, the sensitivity of the MIP (nano)-CP electrode is about 792.3 times higher than that of the MIP (micro)-CP. This electrode exhibits a sensitivity that is about three times higher than that of the sensor with the highest sensitivity (among those shown in the table 2). A considerably lower standard error of the MIP(nano)-CP electrode is particularly interesting, because the standard errors of determinations by a carbon paste based electrodes are usually high. This low standard deviation,

compared to that of the MIP (micro)-CP electrode, can be related to the smaller and more uniform size of the nano-MIP particles that leads to a more repeatable and smooth electrode surface.

After 5 months, there was no significant variation (confidence level= 95%) in the response of the nano-MIP based electrode, compared to the initial responses of the electrodes, prepared with the same MIP. This indicates that the prepared MIP material maintains its initial affinity and adsorption capacity for the target molecule even after 5 months.

3.7. Application of the sensor for real sample analysis

The proposed sensor was applied for the determination of PMZ in spiked human serum samples. For this purpose, different amounts of PMZ solution were injected into the aimed serum samples, adjusting the PMZ content of the solutions equal to 10.0×10^{-10}, 1.0×10^{-10} and 0.1×10^{-10} M. Then, the sensor was applied for the determination of the mentioned spiked solutions. The PMZ concentrations of the described spiked solutions were found to be $10.05(\pm 0.03)\times10^{-10}$, $0.97(\pm0.02)\times10^{-10}$ and $0.11(\pm 0.01)\times10^{-10}$ M, respectively. The corresponding recoveries of these determinations were 105, 97 and 110%, respectively. These proper and acceptable percent recoveries certify the applicability of this electrode for real sample analysis when it contains very trace amount of the analyte.

The proposed determination method was statistically verified, by the application of a direct plasma injection HPLC method [30] to the samples, previously analyzed by the MIP(nano)-CP electrode. There was no significant difference (confidence level=95%) between the developed method and the used reference method, indicating the validity of this method for promethazine determination.

4. CONCLUSIONS

An interesting way for the fabrication of a high selective, extra sensitive and precise electrochemical PMZ sensor was introduced. The nano-sized imprinted polymer was used as a recognition element of an electrochemical sensor. It was shown that the alteration of MIP particles size from micro-scale to nano-scale in the electrode led to excellent analytical characteristics such as lower analyzing time, lower detection limit, higher sensitivity, more precision and etc. These advantages are attributed to more affinity of the selective sites of the MIP nanoparticles and good accessibility of them, compared to those of the bulky MIP particles. Electrochemical impedance spectroscopy studies indicated that the charge transfer rate is slightly higher in the case of MIP nanoparticles based electrode. Besides, the bulk resistance of the MIP(nano)-CP electrode was found to be lower than that of MIP(micro)-CP electrode.

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