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Computational design and synthesis of a high selective molecularly imprinted polymer for voltammetric sensing of propazine in food samples

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ABSTRACT

Using a molecularly imprinted polymer (MIP) as a recognition element, the design and construction of a high selective voltammetric sensor for propazine were introduced. A computational approach was developed to study the intermolecular interactions in the pre-polymerization mixture and to find a suitable functional monomer in MIP preparation. Having confirmed the results of computational method, three MIPs were synthesized with functional monomers methacrylic acid (MAA), acrylamide (AAM) and 4-vinylpyridine (4-VP). The results indicated that to prepare MIPs, acrylamide (AAM) is the best candidate. A propazine selective MIP and a nonimprinted polymer (NIP) were synthesized and then incorporated in the carbon paste electrodes (CPEs). The sensor was applied for propazine determination through the cathodic stripping voltammetric method. Two dynamic linear ranges of 0.01–1.0 μmol L^{-1} and 1.0–55.0 μmol L^{-1} were obtained. The detection limit of the sensor was calculated as 0.001 μmol L^{-1}. This sensor was used successfully for propazine determination in food samples.

1. Introduction

The extended utilization of s-triazine herbicides in agriculture makes their access to the environment possible. Propazine (2-chloro-4,6-diisopropylamino-1,3,5-triazine) is a triazine herbicide used to control broadleaf weeds and annual grasses during the production of milo grain sorghum [1,2]. This compound provides post-emergent protection by interfering with photosynthetic electron transport of target weeds (photosystem II inhibitor). Compared with other chlorinated and/or organophosphorus-containing pesticides, its toxicological effect on humans is less. Nevertheless, an important environmental problem can arise from their persistence, due in part to their low solubilities in water, and in part to their strong retention by sorption on carbonaceous materials [3] and clays, and consequently in soils and hydric sediments.

Although chromatographic techniques after rather involved and costly separation processes have been used for monitoring of s-triazines such as propazine in the environment. In the recent decade, there have been several reports on the electroanalytical determination of s-triazines in soils and waters. They were based on the reduction of these molecules on mercury electrodes using different experimental techniques after much simpler pre-treatment of the samples [4–8].

Molecular imprinting technology [9,10] gets increasingly interesting for the preparation of useful materials with predetermined selectivity for application in several areas of analytical chemistry [11]. Molecular imprinted polymers (MIPs) are cross-linked synthetic polymers obtained by copolymerizing a monomer with a cross-linker in the presence of a template molecule (print molecule). The polymer, with its template being washed away, contains recognition sites that are complementary in size, shape and chemical functionality to the template molecules. The produced imprinted polymer is able to rebind selectively with the template (analyte) and its analogs structures. The highly selective recognition characteristics of molecular imprinted polymer are comparable to those of the natural biological species such as receptors and antibodies. However, MIPs possess several advantages over their biological counterparts including low cost, ease of preparation, and good physical and chemical stability over a wide range of experimental conditions and solvents. Many publications have dealt with the use of MIPs for specific purposes, *e.g.* stationary phases for chromatography [12], capillary electrochromatography [13], electrochemical sensors [14], quartz crystal microbalance [15], biomimetic sensors [16], solid phase extraction [17,18], and membrane separation [19]. Imprinted polymers have already been developed for several triazines [20–27]. In most of

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them, methacrylic acid was used as a functional monomer in the construction of triazines MIPs. The literature survey showed there are no reports on the study of the effects of other monomers on construction of triazine MIPs. Computer aided-molecular design was used to achieve the best monomer for making propazine MIP.

MIPs are promising materials continually being used in sensors as recognition elements or modifying agents (instead of other commonly used modifiers). The application of MIPs in electrochemistry is rather recent and is directed to combine their intrinsic properties to selected electrochemical reactions in order to improve the response of the electrode [25–27].

Recently the combinatorial and computational methods have been considered as alternative approaches for the rational design of MIPs [28,29]. The characterization of molecular complexes formed between templates and monomers, with the aim of achieving a clearer picture of the interactions that are the basis of MIP technology, has been the goal of numerous theoretical studies [30]. Over the past few years, a number of studies have been reported describing the application of *ab initio* and density functional theory (DFT) computational methods to the rational design of molecularly imprinted polymers [29].

Here a DFT-based computational approach was used as the rational design of MIPs for propazine as template molecule. The MIPs were then synthesized as sensing materials to develop a modified carbon paste electrode to determine of propazine 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 is based on the reduction of propazine after its selective extraction in the carbon paste electrode. In general, the measurements with MIP modified sensors have been carried out based on a three-step methodology, namely analyte extraction in the electrode, electrode washing, and electrochemical measurement. This procedure, however, is time consuming. In order to reduce the analysis time, the stripping voltammetric (SV) method was used, in which the electrode washing step was omitted and the two other steps (analyte extraction and electrochemical measurements) took place in one step. It is the first time that such a procedure is used. The developed sensor has been successfully applied to determine propazine in food samples. Based on our knowledge this is the first sensor for PZ monitoring.

2. Experimental

2.1. Instruments and reagents

Electrochemical data were obtained with a three-electrode system using an Autolab potentioastat/galvanostat model PGSTAT302. The differently prepared MIP or non-imprinted polymer (NIP) involving sensors (modified and non-modified electrodes) was used as working electrodes. A platinum wire and an Ag/AgCl (saturated KCL) electrode were used as the counter and reference electrodes, respectively. A Metrohm pH-meter (model 691) was also applied for the pH measurements. Methacrylic acid (MAA), acrylamide (AAM) and 4-vinylpyridine (4-VP) obtained from Sigma–Aldrich (Munich, Germany), were purified by passing through a short column of neutral alumina, followed by distillation under reduced pressure. Ethylene glycol dimethacrylate (EGDMA), obtained from Fluka (Buchs, Switzerland), was distilled under reduced pressure in the presence of a hydroquinone inhibitor, and stored at 4 °C until use. 2,2-Azobisisobutyronitrile (AIBN) were supplied by Sigma–Aldrich (Munich, Germany), and used as received. Graphite powder was purchased from Fluka (Buchs, Switzerland). Propazine (PZ) and cyanazine (CZ) were obtained from Sigma–Aldrich (Munich, Germany). 1×10^{-3} mol L^{-1} stock solution PZ was prepared in mixed solvents (50% (v/v) of distilled water and methanol). All other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Computational approach

In order to understand the properties of MIP at molecular level, the model of the template–monomer complexes were set up. The structure of template, monomers and all template–monomer complexes were drawn in the Gaussview 4.1 software. Then all calculations were carried out using the Gaussian 03 [31] program. Using the popular hybrid functional B3LYP, electronic energies were calculated through the density functional theory. Geometry optimization was performed at the B3LYP/6-31G(d) level. The binding energy of template–monomer complexes, ΔE, were calculated through the following equation:

$$\Delta E = E(\text{template–monomer complex}) - E(\text{template}) - E(\text{monomer}) \quad (1)$$

A well-known problem in the theory of intermolecular interactions is the occurrence of the so-called basis set superposition error (BSSE) [32]. As the two molecules approach each other, the energy of the system falls not only because of the favorable intermolecular interactions but also because the basis functions on each molecule provide a better description of the electronic structure around the other molecule. Despite the well-known existence of BSSE and the means to correct it, only few reports of computationally assisted design of MIPs refer the correction of this error [27,33]. An approximate way of assessing BSSE is the counterpoise (CP) correction [34], which is recommended for the accurate computation of molecular interaction energies by *ab initio* and DFT methods [35,36].

Because polymerization occurred in solution, we must take the effect of solvent, or salvation, in energy calculations into account because it leads to changes in energy and stability of the template–monomer complexes. Methods for evaluating the solvent effect may broadly be divided into two types: those describing the individual solvent molecules and those that treat the solvent as a continuous medium [37,38]. Continuum models [39], which are more popular, consider the solvent as a uniform polarizable medium with a dielectric constant of ε, while the solute is placed in a suitably shaped cavity in the medium [40]. In this section to study the effect of solvent in energy calculations, the Polarizable Continuum Model (PCM) developed by Tomasi and coworkers [41,42] was used.

In this study, electronic energies were first calculated using the PM3 method. The most stable structures from this step were further optimized through the DFT method at B3LYP/6-31G(d) level. The BSSE error was corrected using the counterpoise (CP) correction. The polarizable continuum model was used to introduce the effect of solvent in energy calculations.

2.3. Synthesis of polymers

The general procedure to prepare imprinted polymer was just like the previous report [20]: the template molecule (1 mmol) was mixed for 5 min with the selected functional monomer (4 mmol) in a 10.0 mL screw capped glass vial followed by the addition of 30 mL of toluene as polymerization solvent. The cross-linker EGDMA (20 mmol) and the initiator AIBN (0.2 mmol) were then added to the above solution. To remove dissolved oxygen, the solution was purged with high purity nitrogen (99.999%) for 5 min. Finally, the test tube was sealed under the nitrogen atmosphere and was then placed in a water bath at 60 °C for 16 h with stirring at 350 rpm. A steel sieve was employed to select particles with size of 200 μm and the template was removed by soxhlet extraction with a mixture of methanol and acetic acid (9:1, v/v) for 8 h. The

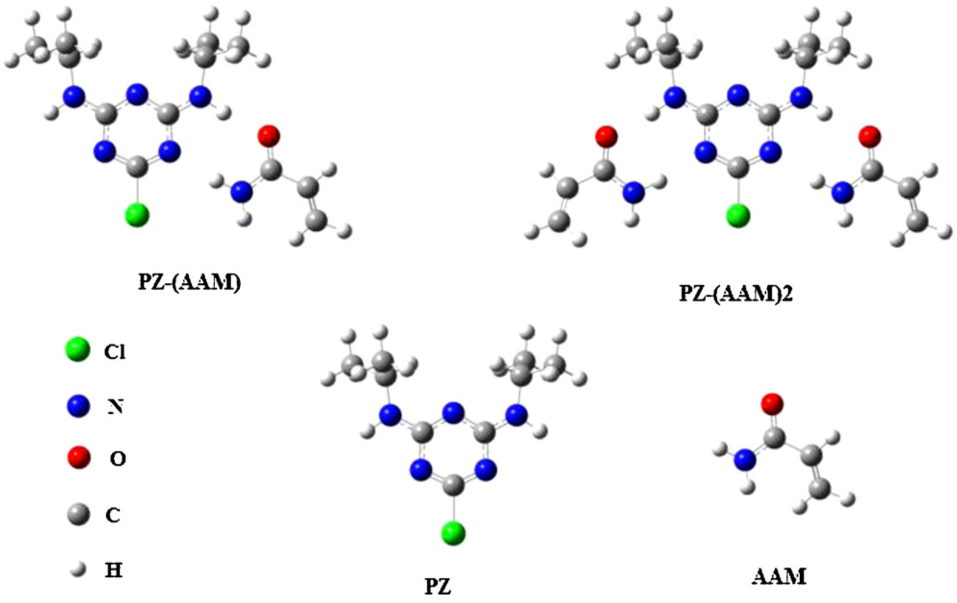


Fig. 1. Optimized conformations of the most stable 1:1 and 1:2 complexes of PZ with acrylamide (AAM) derived by Hortree Fock (HF) method with 6-31G(d) basis set.

reference non-imprinted polymers (NIPs) were prepared using the same procedure in the absence of template molecule.

2.4. Preparation of the sensors

The bare carbon paste electrode (CPE) was prepared by thoroughly mixing analytical grade graphite and paraffin oil in a 65:35 (w/w, %) ratio. The PZ modified CPE was prepared through mixing different percentages of graphite powder, paraffin oil, and MIP (or NIP). To become homogeneous, each mixture was mixed in a mortar for at least 10 min. The obtained paste was packed into one end of a Teflon holder in which electrical contact was made with a copper rod that run through the center of the electrode body. The electrode surface was polished using a butter paper to produce a reproducible working surface. Using the cyclic voltammetric technique, the electrochemical behavior of PZ at the surface of each electrode was studied. Best results were obtained at 66.7:20.0:13.1 (w/w, %) ratio of graphite powder, paraffin oil, and MIP (or NIP). This optimized electrode composition was then used for the voltammetric determination of PZ.

2.5. General analytical procedure

The electrochemical measurement of PZ was carried out according to the following procedure.

A 10 ml volume of 0.1 mol L^{-1} HCl solution adjusted to pH 3 was introduced into the electrochemical cell and deaerated with pure nitrogen for 10 min. While the solution was stirred at 400 rpm, an accumulation potential of −0.3 V was applied to the electrode for 3 min. The stirring was then stopped, and after 10 s rest period, differential pulse voltammograms in the range of −0.3 to −0.8 V was recorded and used for background correction. Then, an appropriate volume of sample solution was added to the voltammetric cell. The solution was purged for 5 min and after 3 min accumulation at −0.3 V, its voltammogram was recorded. All measurements were carried out at room temperature (25 ± 1 °C).

2.6. Preparation of samples

The onion, tomato and/or lettuce samples were weighted and crushed with a juicer to produce a liquid including juice and scum. This liquid was spiked with two different amounts of PZ to reach a final concentration of 0.01 and 0.05 mg/kg of each compound. Each spiked sample was stirred in an ultrasonic bath for 10 min and centerfused. The solution was separated and its propazine content was determined using the proposed procedure.

The rice seeds were dried at room temperature. The polished rice was prepared by removing bran, which was also kept for analysis. The samples of rice and bran were dried and powdered. Prepared sample solutions were spiked with appropriate amounts of PZ and were stirred in an ultrasonic bath for 10 min and then were filtered. To the filtrates 10 ml of methanol were added and diluted to 25.0 ml by the supporting electrolyte to reach final concentrations of 0.01 and 0.05 mg/kg of this compound.

3. Results and discussion

3.1. Theoretical study of template–monomer interactions

In a typical computational approach, a virtual library of functional monomers were created and screened for all possible interactions between monomers and the template molecule. Monomers with the highest binding scores were subsequently selected to produce full scale MIPs with hopefully superior recognition properties. In this work, seven functional monomers, *i.e.*

Table 1
Calculated interaction energies (E, kJ mol^{-1}) for 1:1 and 1:2 template–monomer complexes with and without BSSE correction in the gas-phase.

Complexes	$\Delta E_{\text{non corr.}}$	$\Delta E_{\text{corr.}}$
PZ–(MAA)	−78.015	−62.769
PZ–(TFMAA)	−75.939	−61.415
PZ–(AA)	−72.693	−57.345
PZ–(AAM)	−68.325	−53.466
PZ–(4-VP)	−36.806	−26.732
PZ–(MMA)	−34.884	−22.908
PZ–(ACN)	−31.470	−22.805
PZ–(MAA)2	−154.216	−123.870
PZ–(TFMAA)2	−148.677	−119.713
PZ–(AA)2	−143.598	−113.060
PZ–(AAM)2	−134.712	−105.055
PZ–(4-VP)2	−72.660	−52.143
PZ–(MMA)2	−68.839	−45.242
PZ–(ACN)2	−61.604	−44.364

Table 2
Calculated interaction energies (E, kJ mol^{-1}) for 1:2 template–monomer complexes in different solvents.

Complexes	Water	DMSO	MeCN	MeOH	Acetone	Toluene
PZ–(AAM)2	−49.520	−50.162	−50.808	−51.475	−53.651	−103.386
PZ–(AA)2	−48.009	−49.420	−49.562	−50.109	−53.304	−101.337
PZ–(MAA)2	−46.080	−46.083	−46.618	−47.673	−50.120	−99.126
PZ–(TFMAA)2	−45.786	−44.982	−45.587	−46.730	−49.478	−96.182
PZ–(4-VP)2	−21.875	−22.219	−21.789	−22.279	−23.798	−49.782
PZ–(MMA)2	−16.739	−17.865	−17.374	−18.063	−19.495	−45.784
PZ–(ACN)2	−0.837	−1.777	−1.300	−1.673	−3.613	−33.670

acrylamide (AAM), 4-vinylpyridine (4-VP), methacrylic acid (MAA), acrylic acid (AA), 2-(trifluoromethyl)-acrylic acid (TFMAA), acrylonitrile (ACN) and methyl methacrylate (MMA) were theoretically selected as possible functional monomers. The conformation of the template, functional monomers and template–monomer complexes were optimized to the lowest energy using the DFT method at B3LYP/6-31G(d) level. As an example, Fig. 1 shows the optimized geometries of 1:1 and 1:2 template–monomer complexes between PZ and AAM.

Table 1 summarizes the calculated interaction energies for all complexes formed between PZ and functional monomers before and after BSSE correction in the gas-phase.

As can be seen, BSSE correction influences the values of binding energy, but the stability order is maintained. The obtained results reveal that the 1:2 complexes are more stable than 1:1 complexes formed between the template and all the monomers tested. Furthermore, the data indicate that there are no significant difference between interaction energies of the template and AAM, MAA, AA and TFMAA monomers. In the solution, however, the stability and order of binding energy are quite different.

The interaction energies of 1:2 complexes are obtained in different solvents and the results are summarized in Table 2. As can be seen, a significant decrease in the binding energy of each case was observed relative to that of the gas-phase. This makes sense since solvation of a species involves intermolecular interactions of the same nature as monomer–template; so the solvent acts as a competitor. These results indicate that PZ interacts most strongly with AAM and most weakly with ACN. Regardless of salvation, there are no significant difference between interaction energies of the template and AAM, MAA, AA and TFMAA monomers. Therefore, each of these monomers can be used for construction of PZ-MIP. In spite of this fact, the data show that the most stable complex can be formed when AAM and toluene were selected as functional monomer and polymerization solvent.

To examine the accuracy of the theoretical calculations, three polymers for PZ were prepared by precipitation polymerization using AAM (MIP1), MAA (MIP2) and 4-VP (MIP3) as functional monomers, EGDMA as cross-linker and toluene as polymerization solvent based on previous reports [20]. The prepared MIPs were used as mediators for construction of carbon paste modified electrodes. These sensors were applied for monitoring PZ using cathodic stripping voltammetric technique. The most sensitivity was achieved when AAM was used as a functional monomer (Fig. 2). This confirmed the results of the theoretical study. Therefore, MIP1 was selected for preparation of the PZ sensor.

3.2. Electrochemical behavior of propazine

PZ is electrochemically active under acidic conditions. Electrochemical reduction pathways for this herbicide have been proposed based on differential pulse polarography (DPP) studies [43]. Theoretical deconvolutions of the experimental polarograms were consistent with a 2-electron cleavage of the C—Cl bond via a mechanism involving a protonation step between the two electron transfers. For the process, this intervening protonation step was considered to be the rate determining step (r.d.s.). This was followed by chloride elimination to produce an intermediate that was further reduced through an irreversible 2-electron reduction of the ring to yield the final product. An approximation for the ring pK_a value (1.7) was determined by graphing pH *vs.* peak potential. With increasing pH, the peak height decreased and was no longer detected for pH > 4 solutions. This was consistent with the notion that a protonated species undergoes reduction [44].

In primary experiments, the electroreduction of PZ in HCl solution was investigated at the surface of CP, NIP-CP and MIP-CP electrodes using the cyclic voltammetric technique. The resulting voltammograms (Fig. 3) revealed an electrochemically irreversible system that confirmed the previous report [44]. The enhancement in the peak current of MIP-CP electrode in comparison with other tested electrodes indicates the role of MIP as a suitable recognition element in construction of a sensor for determination of PZ.

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) was used for further investigation. Fig. 4 represents the recorded differential pulse stripping (DPS) voltammograms of 1.0 μmol L^{-1} PZ at the surface of MIP-CP, NIP-CP and at bare CP electrodes. As it is seen, under identical conditions, PZ yields a very well defined reduction peak (at −0.56 V) at MIP-CP electrode compared with those obtained with other electrodes. In addition, when three different electrodes are

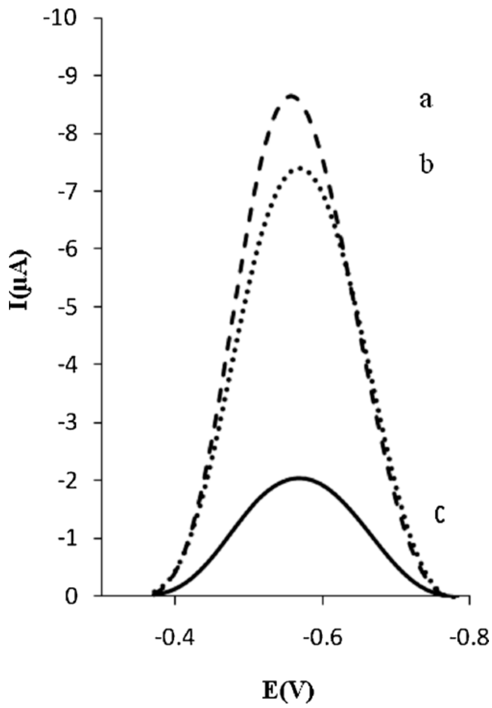


Fig. 2. CS voltammograms 1.0 μmol L^{-1} of PZ at the surface electrode modified with (a) MIP1, (b) MIP2 and (c) MIP3. Measurement conditions: pH 3, E_{ac} = −0.3 V, t_{ac} = 3 min, scan rate = 80 mV s^{-1}.

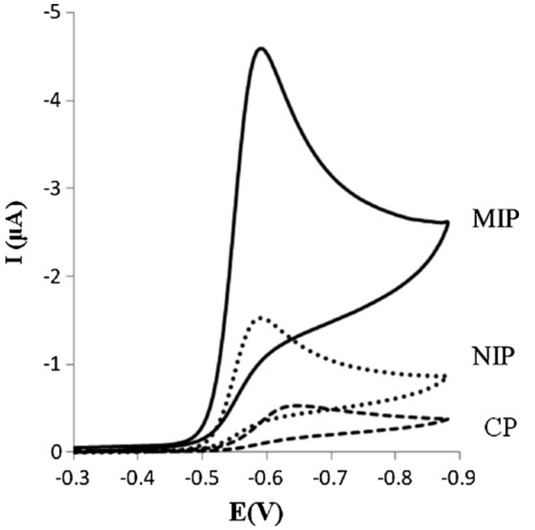


Fig. 3. Cyclic voltammograms of 5×10^{-4} mol L^{-1} of PZ at (a) MIP-CP (b) NIP-CP and (c) CP electrode. Measurement conditions: pH 3, scan rate = 80 mV s^{-1}.

used, no shift in the reduction peak potential of PZ is observed. The reduction peak current was used for monitoring the herbicide by the proposed modified electrode.

As mentioned before, the measurement of an analyte with MIP modified sensors was carried out via a three-step procedure including analyte extraction in the electrode, electrode washing and finally electrochemical measurement. Therefore, the response of the proposed electrode for determination of 1.0 μmol L^{-1} of PZ was compared when a lengthy three-step procedure and fast cathodic stripping voltammetric (CSV) procedures were used. As seen from Fig. 5, using the CSV technique not only increased the sensitivity of the sensor but also decreased the analysis time (omitting washing step and occurring of two other steps in one step). Thus stripping voltammetry technique was selected for further uses 1.0 μmol L^{-1} of PZ.

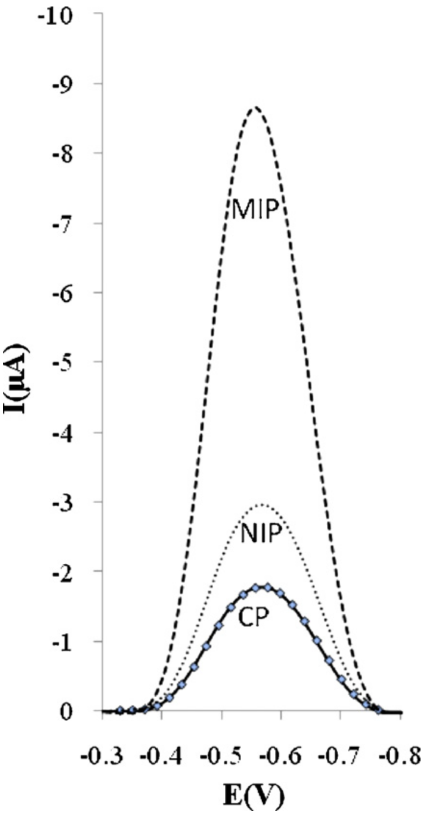


Fig. 4. CS voltammograms 1.0 μmol L^{-1} of PZ at the surface of (a) MIP-CP, (b) NIP-CP and (c) CP electrode. Measurement conditions: pH 3, $E_{ac} = -0.3$ V, t_{ac} = 3 min, scan rate = 80 mV s^{-1}.

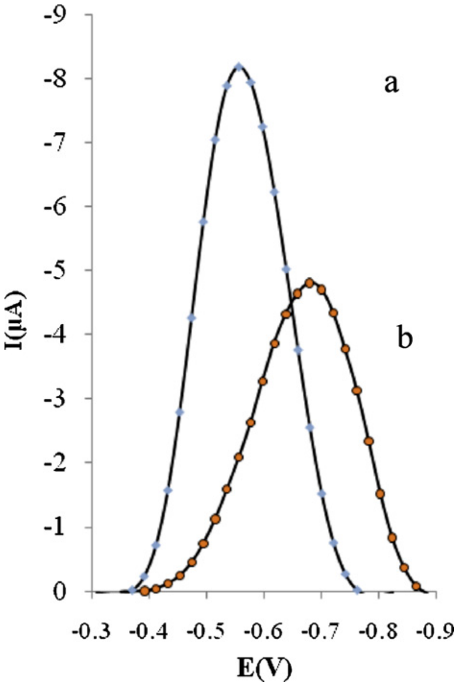


Fig. 5. Comparison of the response of MIP-CP electrode in 1.0 μmol L^{-1} PZ solution (a) with CSV procedure (pH 3, t_{ac} = 3 min, $E_{ac} = -0.3$ V, stirring rate = 400 rpm, scan rate = 80 mV s) and (b) with three steps procedure (extraction conditions: pH 4, extraction time = 10 min, stirring rate = 400 rpm. Electrochemical measurement conditions: pH 3, scan rate = 80 mV s^{-1}).

3.3. *Optimization of parameters for propazine detection*

3.3.1. *MIP-CP composition optimization*

In order to find the best composition for MIP-CP electrodes, the amount of different ingredients of the electrode including MIP, carbon, and paraffin oil were changed in fixed conditions of extraction and voltammetric determination. For initial optimization purposes, the MIP-CP electrodes were prepared with fixed amounts of carbon and paraffin oil and different amounts of MIP. The resulted electrodes at each case were used for propazine extraction and determination. The results showed that increasing the amount of MIP increased the electrode response, and the maximum signal for the proposed sensor appeared in the MIP amount of 0.005 g. Higher amounts of MIP in the MIP-CP electrode can increase the sensor response due to providing more recognition sites on the electrode surface. However, at the amount of MIP more than a 0.005 g, the sensor responses decreased. It may be due to electrode surface conductivity reduction.

Similar experiments were also carried out in order to investigate the effect of carbon and paraffin oil amounts on the electrode response. From the obtained results, the optimum amounts of carbon and paraffin oil were found to be 0.025 and 0.0075 g, respectively. Increasing the carbon content of MIP-CP electrode leads to an increase in the corresponding electrode response because of electron transferring capability enhancement of the electrode in the presence of higher carbon content. However, after a certain point (0.025 g), further increase in carbon content results in lowering the corresponding signal that may be attributed to this fact that larger

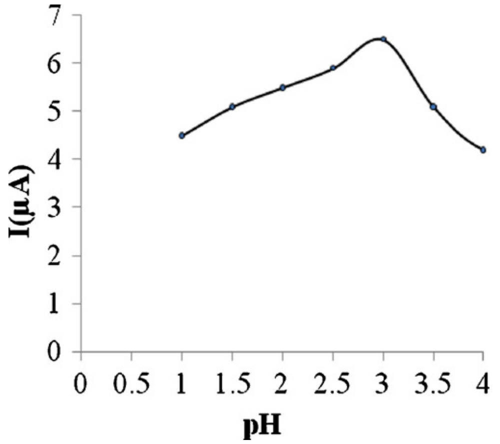


Fig. 6. Optimization of pH for PZ extraction in the MIP-CP electrode. Measurement conditions: propazine (1.0 μmol L^{-1}), E_{ac} = −0.3 V, t_{ac} = 3 min, scan rate = 80 mV s^{-1}.

carbon amount on the electrode surface leads to decreasing MIP content of the electrode surface. Also the amounts of binder (paraffin oil) in the paste were optimized and found to be 0.0075 g. Due to the insulating effect of the binder and reducing the electrode surface conductivity, further increase in binder content results in lowering the corresponding signal. Therefore, the paste containing 13.3:66.6:20% of mediator, carbon and binder was used for construction of the electrode.

3.3.2. The effect of pH

As mentioned before, the electrochemical behavior of PZ and its adsorption on the MIP sorbent are pH dependent. Due to protonation of PZ at low pH values, its extraction is low. Therefore, the interaction between this compound and the binding sites of the MIP will be favored at a pH greater than the pK_a value. On the other hand, triazines are electrochemically active under acidic conditions. The peak height decreased with increasing pH and was no longer detected for pH > 4 solutions. Thus, the effect of pH on the extraction and determination of the analyte by electrochemical method was studied through varying the pH in the range of 1–4 (Fig. 6). The pH of the sample solution was adjusted to the given value by HCl or by NaOH solution. The best results were obtained at a pH around 3. This pH was selected for further uses.

3.3.3. The 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 PZ was examined over the potential range of 0.2 to −1 V. The plot of stripping peak current as a function of preconcentration potential indicated that the maximum peak current took placed at −0.3 V. Thus, accumulation potential of −0.3 V was chosen for subsequent uses.

The influence of accumulation time on the stripping peak current of 1.0 μmol L^{-1} PZ was also investigated (Fig. 7). Variation of the accumulation time showed that the peak current of PZ increased with increasing the accumulation time, gradually leveling off at periods longer than 3 min, presumably due to saturation of the electrode surface. Thus deposition time of 3 min was used throughout, as it combines good sensitivity and relatively short analysis time.

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 scan rates higher than 80 mV s^{-1}. Therefore, a scan rate of 80 mV s^{-1} and an accumulation time of 3 min were selected for further studies.

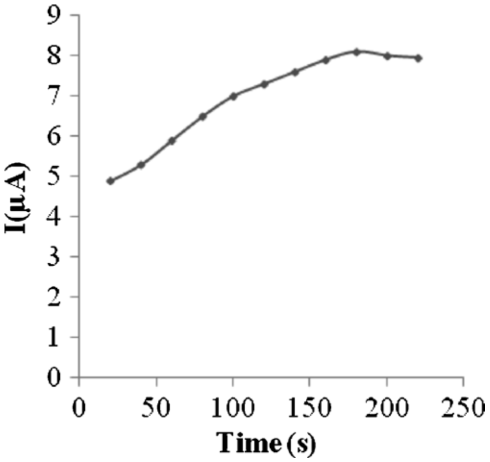


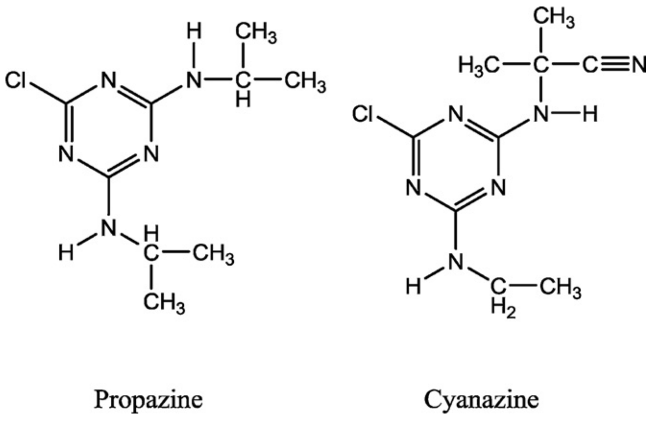
Fig. 7. Optimization of accumulation time for PZ extraction in the MIP-CP electrode. Measurement conditions: PZ (1.0 μmol L^{-1}), pH 3, E_{ac} = −0.3 V, t_{ac} = 3 min, scan rate = 80 mV s^{-1}.

3.4. MIP-CP electrode selectivity

CZ (Scheme 1) as an analog and potential interfering substance of PZ was used to examine the selectivity of the imprinted sensor. Stock solution of the analog was prepared in a 0.001 mol L^{-1} HCl (pH 3.0) and the amounts of its binding to PZ–MIP sensor were compared with PZ bound to MIP and NIP sensors. The amount of PZ bound to PZ-MIP was defined as 100%. As shown in Fig. 8, PZ-MIP bound to PZ is more than CZ bound to MIP and PZ bound to the NIP sensor. In this study the selective binding of PZ to PZ-MIP was 40% more than CZ bound to PZ-MIP and 70% more than PZ bound to NIP. Based on these results, it was concluded that the PZ-MIP prepared in this study was selective to PZ.

3.5. Analytical characterization

The obtained optimal conditions and parameters were used for plotting calibration curves. Differential pulse cathodic stripping voltammetry with an accumulation potential (E_{ac}) of −0.3 V with subsequent stripping step from −0.3 to −0.8 V was applied. The voltammetric responses of the MIP-electrode were obtained. After 3 min accumulation (t_{ac}), the proposed electrode exhibits two



Scheme 1. Structure of CZ and PZ.

Table 3
Recoveries and R.S.D. of propazine for spiked onion, tomato, lettuce and rice samples ($n = 3$).

Sample	Added (mg/kg)	Recovery (%)	R.S.D. (%)	LOD (mg/kg)	LOQ (mg/kg)
Onion	0.01	96.78	4.5	33.1×10^{-6}	110.3×10^{-6}
	0.05	98.95	3.9		
Tomato	0.01	95.30	4.1	48.5×10^{-6}	161.8×10^{-6}
	0.05	96.56	4.4		
Lettuce	0.01	89.04	3.3	38.9×10^{-6}	129.7×10^{-6}
	0.05	91.95	3.7		
Rice	0.01	92.69	5.1	43.0×10^{-6}	145.0×10^{-6}
	0.05	93.82	4.9		

workings concentration ranges of 0.01–1.0 and 1.0–55.0 μ mol L^{-1}. The linear regression equations are:

$$I_p \quad (\mu A) = 0.883 + 1.418 \quad C_{\text{Propazine}} \quad (r^2 = 0.999) \tag{2}$$

$$I_p \quad (\mu A) = 2.134 + 0.127 \quad C_{\text{Propazine}} \quad (r^2 = 0.999) \tag{3}$$

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 representing the standard deviation of the peak currents of the blank ($n = 12$) and m representing the slope of the first calibration curve for PZ. Both LOD and LOQ values are found to be 0.001 μmol L^{-1} and 0.003 μmol L^{-1}, respectively, which indicate the sensitivity of the proposed method.

When the concentration of PZ was controlled at 1.0 μmol L^{-1}, good repeatability was observed with a relative standard deviation (R.S.D.) of 4.2% for seven parallel detections. The sensor retained a response of 96% of the initial current after 7 days stored in ethanol at room temperature, and it showed no obvious decline after use for 20 times. Reproducibility of the proposed electrode was investigated using CSV. Five freshly packed electrodes were prepared on five consecutive days and the peak current values of a solution containing 1.0 μ mol L^{-1} of PZ were measured for each electrode.

The obtained result of five replicate measurements shows a relative standard deviation of R.S.D. < 5% for cathodic stripping voltammogram's currents.

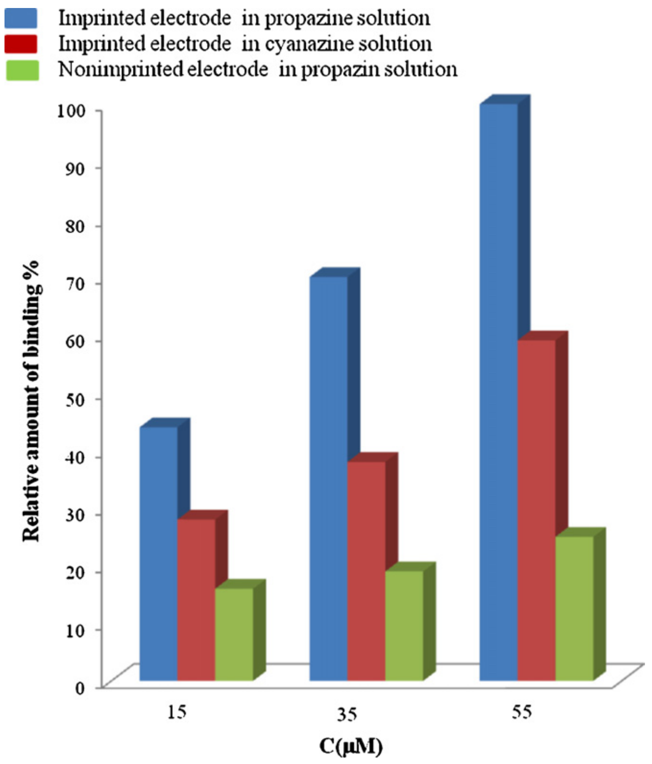


Fig. 8. Comparison of the imprinted sensor in CZ, PZ and nonimprinted sensor in various concentrations of PZ, pH 3, $E_{ac} = -0.3$ V, $t_{ac} = 3$ min, scan rate = 80 mV s^{-1}.

Table 4
Determination of propazine in water samples ($n = 3$).

Sample	Added (μM)	Found (μM)	Recovery (%)	R.S.D. (%)
Tap water	0.5	0.48	96.0	3.1
	1.0	0.97	97.0	2.9
River water	0.5	0.46	92.0	2.5
	1.0	1.03	103.0	3.8
Ground water	0.5	0.53	106.0	4.1
	1.0	0.95	95.0	3.5

3.6. Real sample analysis

By means of CSV, the applicability of the proposed sensor was examined to determine PZ in the spiked samples such as onion, tomato, lettuce and rice at two certain concentrations. The obtained results are summarized in Table 3. As it is obvious, the recovery of PZ was found to be between 89.0% and 99.0% using voltammetric methods. Also the relative standard deviation of the proposed method is less than 5.1%, which indicates the acceptable precision of the voltammetric determination of PZ using the modified MIP-CP electrode. Also the proposed sensor was used for monitoring the amounts of propazine in spiked natural water samples. The results are summarized in Table 4. As it is shown, the recoveries were obtained between 92.0% and 106.0%. This means that the proposed procedure should be applicable to the analysis of real samples with different matrices.

4. Conclusions

The results of the present study indicate that the computer-assisted design of MIPs based on DFT can be used as a powerful tool to screen functional monomers for a specified template molecule. The DFT calculations predict that AAM/toluene is the best combination of functional monomer/solvent which leads to the most stable prepolymerization complexes with PZ as template. The new electrode incorporating the MIP as a recognition element in carbon paste showed high selectivity and sensitivity toward PZ. The method presented is simple, sensitive and cost-effective. Also, the proposed sensor was used successfully for PZ determination in real samples. To the best of our knowledge, this sensor is the first report for monitoring PZ through a modified electrode.

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