Sensors and Actuators B: Chemical

A hemin-based molecularly imprinted polymer (MIP) grafted onto a glassy carbon electrode as a selective sensor for 4-aminophenol amperometric

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ABSTRACT

Molecular imprinting technology is becoming a versatile tool for the preparation of tailor-made molecular recognition elements. This work investigates the performance of a hemin-modified molecularly imprinted polymer (MIP) used as an amperometric sensor for the detection of 4-aminophenol (4-APh). MIP particles were prepared by the precipitation polymerization method with hemin introduced as the catalytic center to mimic the active site of peroxidase. 4-APh was used as the template molecule, methacrylic acid (MAA) as the functional monomer, trimethylolpropane trimethacrylate (TRIM) as the cross-linker and 2,2′-azobisisobutyronitrile (AIBN) as the initiator. The synthesized polymer particles were characterized in terms of particle size, porosity and morphology. The amperometric sensor used for 4-APh detection was prepared by modifying a glassy carbon electrode surface with the hemin-based MIP. Under optimized operational conditions, a linear response was obtained in the range of 10.0–90.0 μmol L^{-1}, with a sensitivity of 5.5 nA L μmol^{-1} and a detection limit of 3.0 μmol L^{-1}. The sensor showed good repeatability (RSD = 2.7% for n = 7). It exhibited to be very selective for 4-APh even in the presence of structurally similar compounds (2-aminophenol, catechol, guaiachol, 2-cresol and chloroguaiachol). Recoveries in the range 93–111% were obtained using the sensor for the determinations of 4-APh in tap and river water samples.

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1. Introduction

Micro-organisms, enzymes, receptors and antibodies are often used as molecular recognition materials in biosensor technology [1]. However, there are intrinsic difficulties in the practical application of such devices, due to the instability of biomolecules. As a result, considerable efforts has been done to overcome these problems and to a novel promising technology, namely the molecular imprinting technique, which can be used to produce synthetic materials to mimic biological receptors [2]. The general principle of molecular imprinting is based on procedures where the functional and cross-linking monomers are copolymerized in the presence of a target analyte (the imprinting molecule), which acts as a molecular template. The polymerization process can be performed by reversible covalent bonding or non-covalent interactions between monomers and imprinting molecules.

Molecularly imprinted polymers (MIPs) have been successfully used in the solid phase extraction [3], biomimetic catalysis [4], binding assays [5] and sensor applications [6–11]. They are promising candidates for the replacement of biomolecules as the recognition element in chemical sensors [12]. Various signal transducers, including field effect transistors [6] as well as fluorescence [7], surface plasmon resonance [8], electrochemical [8–10] and piezoelectric [11] detectors have been employed in construction of MIP-based sensors. Nonetheless, despite an increased interest in these devices, the literature remains sparse, especially concerning electrochemical sensing. The integration of MIPs with sensor technology may offer considerable potential for the development of devices that offer significant advantages compared to the current methodologies.

In this sense, MIPs were recently synthesized in our laboratory for use as catalytic recognition centers [13–15] in the detection of phenolic compounds of biological and environmental interests. In order to improve the detection process, the preparation of an amperometric sensor for 4-aminophenol detection employing a

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hemin-based MIP grafted onto a glassy carbon (GC) electrode surface using Nafion® is proposed.

2. Experimental

2.1. Chemicals and solutions

4-Aminophenol (4-APh), iron protoporphyrin IX (hemin) methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM) and 2,2′-azo-bis-iso-butyronitrile (AIBN) were purchased from Sigma–Aldrich (Steinheim, Germany). All other reagents were of analytical grade and used without further purification.

All solutions were prepared using purified water (>18 MΩ cm) in a Milli-Q system (Millipore Inc.). The pH values of the buffer solutions were determined using a Corning Model 350 pH/Ion Analyser.

2.2. Apparatus and procedure

Amperometric and cyclic voltammetric measurements were performed using a PGSTAT-30 potentiostat (Autolab Echo Chemie, Utrecht, The Netherlands), controlled by GPES 4.9 software.

All experiments employed an electrochemical cell containing 5.0 mL of electrolyte solution, with a Ag/AgCl (saturated KCl) reference electrode, a Pt wire auxiliary electrode and a modified glassy carbon (~3 mm diameter) working electrode. Oxygen was removed from the solution by purging with nitrogen gas.

The morphological characteristics of the MIPs were analyzed by scanning electron microscopy (SEM), using a JEOL JSM-6360 LV microscope. The pore parameters and the surface areas of the MIPs were measured with a Quantachrome Autosorb automated gas sorption instrument.

2.3. Precipitation polymerization procedure

Molecularly imprinted polymers were prepared by thermal radical polymerization [16]. 4 mmol of MAA, 4 mmol of cross-linker (TRIM) and 0.02 mmol of hemin were dissolved in a mixed solvent composed of dimethylsulfoxide (DMSO) and acetonitrile. 57.5 μmol of 4-APh (template) and 0.127 g of 2,2′-azobisisobutyronitrile initiator were added to the solution. To avoid the undesirable presence of oxygen, the solution was degassed by sonication for 10 min followed by purging nitrogen for 10 min. The beakers containing the solutions were then sealed with multiple layers of Parafilm®, and placed in a water bath at 60 °C for thermal polymerization. After 9 h of polymerization, the MIP particles were collected on a nylon filter (0.27 μm pore size) and washed with methanol and acetic acid (9:1, v/v) to remove the template.

A non-molecularly imprinted polymer (NIP) was also prepared by following the same procedures but without adding the 4-APh template.

2.4. Electrochemical sensor preparation

20 mg of the hemin-based MIP were dispersed in 1 mL of methanol, under sonication for 20 min. 10 μL drops of the suspension with 10 μL of Nafion® solution were then transferred onto the clean glassy carbon electrode surfaces, and dried at room temperature. Nafion® was used as a selected binder to improve the MIP fixation, based on its chemical, mechanical and thermal stability, as well as cation selectivity and high conductivity.

3. Results and discussion

3.1. Preparation and characterization of the hemin-based molecularly imprinted polymer

MIP was synthesized using a larger amount of solution than that used in the traditional polymerization [14], with hemin as the catalytic center to mimic the active site of peroxidase. The procedure was possible due to the unique structural features of the hemin molecule, as well as the controlled interactions between the functional monomer (MAA) and the template (4-APh). The most commonly used functional monomer is usually MAA, which can form hydrogen bonds with the template molecules prior to the polymerization. The resulting specific and precisely located interactions provided the selectivity of the MIP. The cross-linker TRIM, which possesses three allyl groups, produces the porous structure of polymers much more efficiently than does ethylene glycol dimethacrylate (EDMA), which possesses two allyl groups. It has also been shown that imprinted polymers prepared using the tri-functional crosslinker have higher load capacity [17]. In addition, a mixed solvent consisting of DMSO and acetonitrile (7:1, v/v) was chosen as the reaction medium and porogen, avoiding the hydrogen bonding interference of a polar solvent (such as acetone) while increasing the porosity of the polymers.

The physical configurations of MIPs vary according to the employed polymerization method. The morphologies of the MIP and NIP formed by the precipitation polymerization procedure are shown in Fig. 1. It can be seen from the SEM micrographs that the technique produced uniform microspherical particles, as a result of the control of the separation point during the polymerization
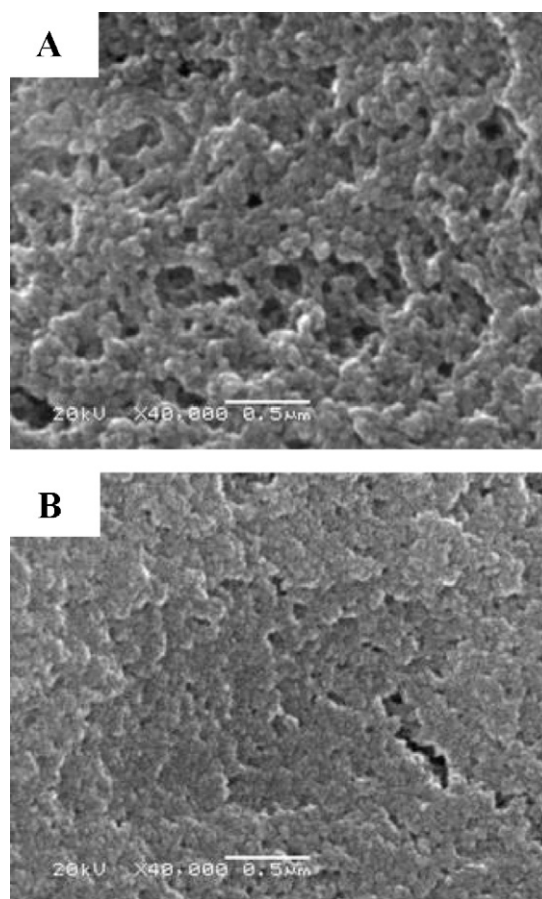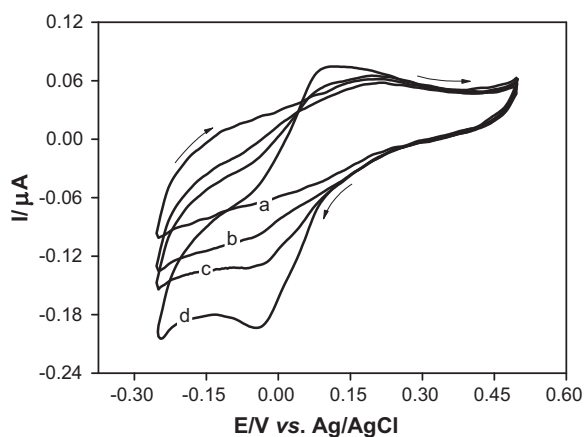

Fig. 1. Scanning electron micrographs (40,000×) of the 4-APh-imprinted polymer synthesized for precipitation methods: (A) MIP and (B) NIP.

Fig. 2. Cyclic voltammograms obtained uing the GC electrode modified with hemin-MIP, in (a) the absence of H_2O_2, (b) the presence of 200 μmol L^{-1} H_2O_2, (c) the presence of 10 μmol L^{-1} 4-APh, and (d) 20 μmol L^{-1} 4-APh. Electrolyte: 0.05 mol L^{-1} TRIS buffer solution (pH 7.0). Scan rate: 0.01 V s^{-1}.

achieved using a dilute monomer solution. The MIP and NIP particles produced in this study were uniform microspheres, which possessed higher surface areas and more complementary sites than particles produced by bulk polymerization. The pore size distributions and surface areas measured using the BET method showed that surface area (2.3 m^2 g^{-1}) and porosity (0.01 cm^3 g^{-1}) of NIP were much smaller than the corresponding values obtained for MIP (12.7 m^2 g^{-1} and 2.34 cm^3 g^{-1}, respectively).

3.2. Electrochemical behavior of the hemin-MIP modified electrode

Assays were performed to evaluate the catalytic activity of the modified glassy carbon electrode during 4-APh detection. Fig. 2 shows the electrochemical behavior in TRIS buffer solution (pH 7.0), which was similar to those observed with enzyme modified electrode by cyclic voltammetry, using a cathodic scan, with $E_{initial} = 0.5$ V and $E_\lambda = -0.25$ V, in (a) the absence or (b) the presence of 200 μmol L^{-1} of peroxide, and in the presence of 10 μmol L^{-1} (c) or 20 μmol L^{-1} (d) of 4-APh. An increase of the reduction current was observed after adding peroxide (Fig. 2b), with a decrease of the anodic current, suggesting that hemin catalyzed peroxide reduction in the same way as peroxidase-based biosensors. After adding 4-APh to the solution (Fig. 2c and d), a significant new increase occurred at a potential of -0.07 V (vs. Ag/AgCl), as is found for peroxidase-based biosensors, where the electron transfer is mediated by the phenolic compounds. This behavior suggests that the hemin-based MIP acts as an efficient electrocatalyst, with an action similar to that of peroxidase.

The performance of MIP as a recognizer for 4-APh was evaluated in amperometric experiments performed at -100 mV vs. Ag/AgCl in 0.05 mol L^{-1} TRIS buffer solution (pH 7.0) containing 100 μmol L^{-1} of H_2O_2. These experiments were performed using the GC elecrode (1) alone (Fig. 3a), (2) modified with Nafion® (Fig. 3b), (3) modified with Nafion® and NIP (Fig. 3c), and (4) modified with Nafion® and MIP (Fig. 3d). It can be seen that practically no responses were detected with the unmodified (Fig. 3a) or Nafion®-modified (Fig. 3b) GC electrodes, although a small response was observed with the NIP electrode (Fig. 3c) for the first addition. The low signal for the NIP electrode can be attributed to the lack of molecular recognition since NIP does not possess the cavities that would provide selective active sites. Fig. 3d shows that the GC electrode modified with MIP provided good reduction currents, indicating that MIP behaves as a catalyst in a similar way as a peroxidase system.
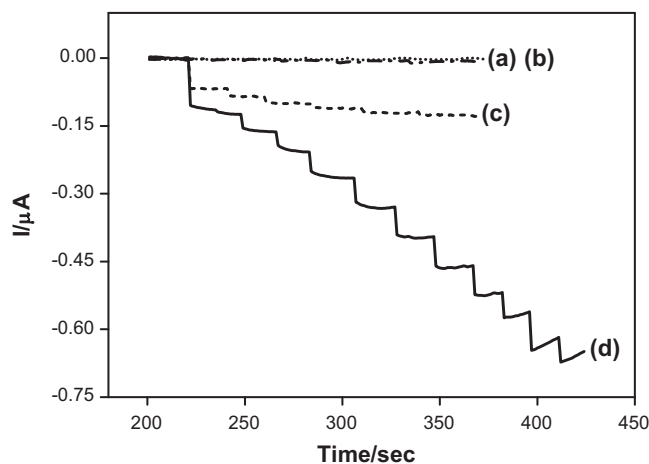
Fig. 3. Amperometric responses of 4-APh obtained with (a) GC electrode, (b) a GC electrode covered with a Nafion® membrane; (c) a GC electrode modified with NIP and covered with Nafion® membrane and (d) a GC electrode modified with MIP and covered with Nafion® membrane. Electrolyte: 0.05 mol L^{-1} TRIS buffer (pH 7.0) containing 100 μmol L^{-1} of H_2O_2. Applied potential: -0.1 V vs. Ag/AgCl.

3.3. Influence of hydrogen peroxide

Hydrogen peroxide plays a key role in the catalytic action of peroxidase enzymes, with high concentrations inhibiting catalysis [18]. Amperometric curves recorded in the absence (Fig. 4A(a)) or presence (Fig. 4A(b)) of 50 μmol L^{-1} hydrogen peroxide, with successive additions of 4-APh into the electrochemical cell containing phosphate buffer (0.1 mol L^{-1}, pH 7.0) clearly showed the important role of hydrogen peroxide in the catalytic process.

Fig. 4B shows the dependence of the sensor response on the H_2O_2 concentration in the range 10–200 μmol L^{-1}, using a fixed amount of 4-APh in solution. The current increased with the H_2O_2 concentration, and eached a maximum at 100 μmol L^{-1}. This concentration was chosen for subsequent expriments, based both on these results and on the fact that higher concentrations result in lower stability of peroxidase systems as well as electrodes [19].

A possible mechanism for the sensor response is illustrated in Fig. 5. This mechanism is similar to those proposed for determination of phenolic compounds using biomimetic catalysts of dopamine β-monooxygenase, peroxidase and tyrosinase enzymes [20–22], where the most important stage for phenolic quantification is the electrochemical reduction of quinone species on the electrode surface, recycling the substrate, and consequently resulting in signal amplification and reduction of the detection potential of the phenolic compound.

### 3.4. Optimization of the parameters for the amperometric detection of 4-aminophenol

The sensor response was influenced by the applied potential, as shown in Fig. 6A. The optimum sensor response was obtained at -100 mV vs. Ag/AgCl. This value is similar to the reduction potential of 1,2-quinone to catechol [23], which also reinforces the notion that the phenolic compound was electrochemically regenerated and produced a pseudo-bioelectrocatalytic amplification cycle [24,25], as shown schematically in Fig. 5.

The influence of pH on the sensor response was examined using experiments carried out in 0.1 mol L^{-1} phosphate buffer solution, with the pH varying from 6.0 to 8.0. The best result was obtained at pH 7.0 (Fig. 6B).

Five different 0.1 mol L^{-1} buffer solutions (HEPES, phosphate, TRIS, PIPES and McIlvaine) were then tested, with best response (data not shown) obtained using the Tris buffer. Fur-
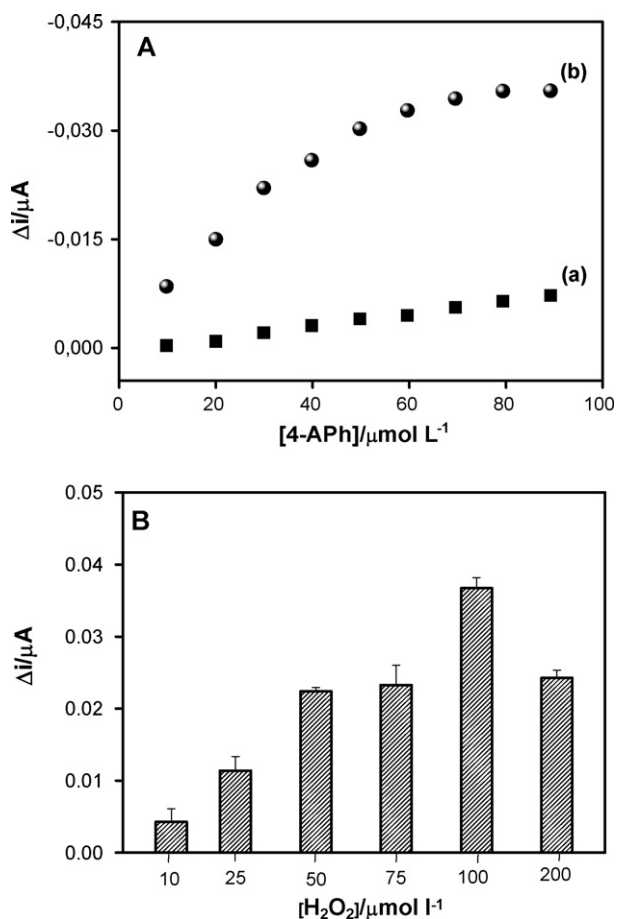
Fig. 4. (A) Signals obtained with the proposed amperometric sensor (a) in the absence and (b) in the presence of 50 μmol L^{-1} of H$_2$O$_2$. Applied potential: −0.1 V vs. Ag/AgCl. Electrolyte: 0.1 mol L^{-1} phosphate buffer (pH 7.0). (B) Dependence of H$_2$O$_2$ concentration on the current variation (Δi) using the proposed sensor. Electrolyte: 0.1 mol L^{-1} phosphate buffer (pH 7.0) containing 30 μmol L^{-1} of 4-APh. Applied potential: −0.1 V vs. Ag/AgCl.
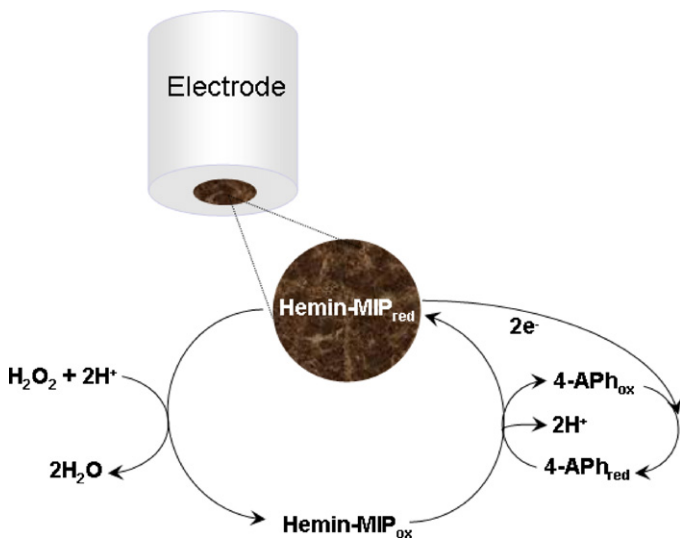
Fig. 5. Proposed mechanism for 4-APh detection using the proposed sensor. The molecularly imprinted polymer (MIP) with hemin is represented by Hemin-MIP$_{red}$ is the reduced MIP, Hemin-MIP$_{ox}$ is the oxidized MIP, and 4-APh$_{red}$ and 4-APh$_{ox}$ are the reduced and oxidized phenol species, respectively.
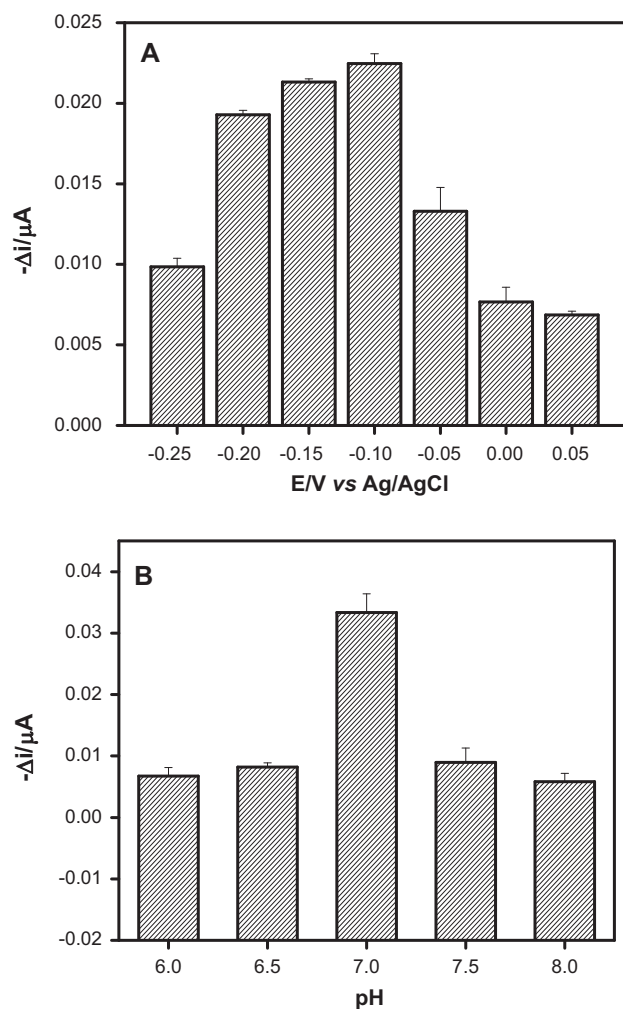
Fig. 6. (A) Influence of the applied potential on the sensor response. Measurements were carried out in 0.1 mol L^{-1} phosphate buffer (pH 7.0) containing 30 μmol L^{-1} of 4-APh and 50 μmol L^{-1} of H$_2$O$_2$. (B) Response profiles for the sensor in phosphate buffer solutions at different pHs. Electrolyte: 0.1 mol L^{-1} phosphate buffer containing 30 μmol L^{-1} of 4-APh and 100 μmol L^{-1} of H$_2$O$_2$. Applied potential: −0.1 V vs. Ag/AgCl.

ther expriments resulted in an optimized TRIS concentration of 0.05 mol L^{-1}.

3.5. Sensor characteristics

Under optimized conditions, the proposed sensor showed a linear response in the range 9.8–79.4 μmol L^{-1} (Fig. 7), described by the following equation:

$$\Delta i(nA) = -1.40\,(\pm0.37) + 5.50\,(\pm0.06)\,[\text{4-APh}]\;(r = 0.9995, n = 8)\quad(1)$$

The detection and quantification limits, calculated according to IUPAC recommendations [26], were 3.0 and 10.0 μmol L^{-1}, respectively. The response time of the sensor, considering the time required to reach 100% signal, was approximately 1 s. Compared with other sensors and biosensors for phenol described in the literature [27–29], the proposed sensor provided a lower detection limit, with similar sensitivity and linear range. The sensitivity to phenol was at last thirteen times greater than that obtained using solid electrodes [30].

The stability of the MIP sensor was determined by amperometric measurements in 0.05 mol L^{-1} TRIS buffer solution (pH 7.0) with successive aditions of 4-APh (equivalent to 30 μmol L^{-1}) into the electrochemical cell and recording the current (Δi) associated with
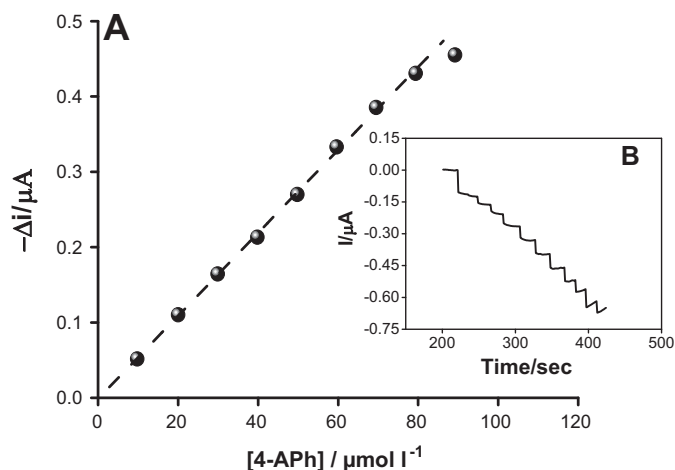
Fig. 7. (A) Typical analytical curve and (B) amperometric measurements for 4-APh detection uing the proposed sensor in 0.05 mol L⁻¹ TRIS buffer solution (pH 7.0) containing 100 µmol L⁻¹ of H_2O_2. Applied potential: −0.1 V vs. Ag/AgCl.

the sensor response to the analyte. There was no significant change in the response after 80 measurements. The sensor showed good reproducibility, with a relative standard deviation (RSD) of 2.7% obtained for ten determinations of 50 µmol L⁻¹ 4-APh. A RSD lower than 5% was obtained for the responses obtained using a series of five sensors, prepared in the same manner and tested in TRIS buffer solution (pH 7.0) containing 50 µmol L⁻¹ 4-APh. This good reproducibility reflects the ability of the catalytic sites and polymer cavities to recognize 4-APh.

3.6. Selectivity of the MIP-sensor

After removing the template molecules by extraction, a molecularly imprinted polymer is expected to present a high selectivity for the imprinted molecules. To confirm that the sensor was selective to 4-APh, the sensor response was investigated using phenolic compounds possesing analogous structures, as potential interferents. The reduction current increased sharply after adding 20.0 µmol L⁻¹ 4-APh (Fig. 8). Further sequential additions of 20.0 µmol L⁻¹ 2-APh, 20.0 µmol L⁻¹ catechol, 20.0 µmol L⁻¹ guaiachol, 20.0 µmol L⁻¹ 2-cresol and 20.0 µmol L⁻¹ chloroguaiachol into the electrolyte did not show any response in the amperogram. A further addition of 20.0 µmol L⁻¹ 4-APh sharply increased the reduction current again.
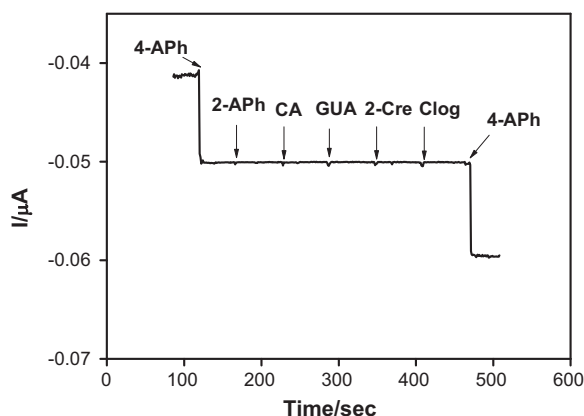
Fig. 8. Current vs. time profiles for successive additions of (a, c) 20 µmol L⁻¹ 4-APh, (b) 20 µmol L⁻¹ 2-APh, 20 µmol L⁻¹ catechol, 20 µmol L⁻¹ guaiacol, 20 µmol L⁻¹ 2-cresol and 20 µmol L⁻¹ chloroguaiacol, in a 0.05 mol L⁻¹ TRIS buffer solution (pH 7.0) containing 100 µmol L⁻¹ of H_2O_2. Working electrode: hemin-based MIP sensor. Applied potential: −0.1 V vs. Ag/AgCl.

Table 1
4-APh recovery data for water samples using the proposed sensor.

Samples	4-APh added (µmol L⁻¹)	4-APh found[a] (µmol L⁻¹)	Recovery[b] (%)
Tap water	–	<QL	–
1	10.0	9.30 (±0.06)	93 (±7)
2	12.0	13.4 (±0.09)	111 (±3)
River water	–	<QL	–
1	10.0	9.70 (±0.02)	97 (±5)
2	12.0	12.6 (±0.05)	105 (±2)

[a] The results are expressed as mean value ± SD based on 3 replicates. Confidence interval of 95%. LQ, limit of quantification.
[b] Recovery obtained from spiked samples.

Hence, at concentrations tested, the interferents did not affect the steady state current of 4-APh. The high selectivty of the sensor is due to the specific recognition sites, which reflect the template in terms of size, shape and arrangement of the functional group.

3.7. Application of the sensor

The hemin-based MIP sensor was tested by determination of 4-APh in two tap water and two river water samples. Prior to the analyses, the river water samples were filtered under vacuum through 0.45 µm cellulose acetate membranes. The accuracy of the method was evaluated by performing recovery tests after spiking the samples, as shown in Table 1. The recovery values obtained were in the range of 93–111%, which demonstrates the viability in using the modified electrode as a highly selective amperometric sensor for 4-APh in these matrices.

4. Conclusions

A new approach using a molecularly imprinted polymer for the amperometric detection of 4-aminophenol was investigated. A hemin-based MIP was used as an active catalytic site to mimic peroxidase enzymes for the determination of 4-APh. The sensor was highly selective and the preparation procedures were simple, fast and reproducible. Compared to peroxidase-based biosensors, the amperometric electrode modified with hemin-based MIP is more selective and stable showing a great potential for practical use.

Acknowledgements

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