

Preliminary Study on Electrochemical Ion Imprinted Polymeric Film in Sensor Development for Cd(II) Ions Determination in Water †

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Abstract: Preliminary results on an electrosynthesised ion imprinted polymeric film (IIP-film) for Cd(II) ions determination in sensor development are here reported. The sensor was prepared by electropolymerisation of 4-aminophenylacetic acid (4-APA) monomer in presence of Cd(II) ions, which acts as the template. The screen printed carbon electrodes (SPCE) were used as transducer during sensor development, whereas the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were selected as the electrochemical methods for the synthesis and Cd(II) ions sensing, respectively. The incubation of the developed sensor in NaOH 250 mM involved into remove the template and the formation of specific recognition cavities into the polymer. A multivariate optimisation based on central composite design (CCD) was employed to study the effect of three independent parameters on electrochemical performances of the sensor. The electrochemical characterisation of sensors was performed in ferrocyanide-ferricyanide redox couple and in KCl 0.1 M, the latter revealing redox properties from the polymeric film. The performances of sensors and the control (non-imprinted film, NIP) was observed in sodium acetate buffer (100 mM, pH = 5) over the Cd(II) concentration range 0.1–10 μ M.

Keywords: ion imprinted polymer; 4-APA; electrochemical sensor; Cd(II) ions; electropolymerisation

1. Introduction

Heavy metals pollution refers to global issue, due to the high toxicity and dangerous effects on environment and human health. Among heavy metals, cadmium is one of the most toxic. Main sources of this ion in environment is industrial wastewater, fertilizers and so on. Currently, the most analytical method for cadmium detection is represented by atomic adsorption spectroscopy (AAS), inductively coupled plasma-mass spectroscopy (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Those techniques are sensitive, accurate but also expensive, and on-site determination of targets is not so suitable. Because the complexity of those instrumentation, there is the need to point different methods to be available for on-site determination. Electrochemical methods can be used for that. Moreover, diverse electrochemical methods are today explored for the determination of heavy metals in water environment [1].

Imprinted polymers define robust and artificial materials able to mimic recognition processes of such analytes, such as proteins, small molecules, or ions [2]. The process results in the selective formation of ion-sized imprinted cavities, which are complementary to a specific template in terms

of its functional groups. These materials can be easily applied to identify, monitor and remove the target ions in water environment [3]. In this view, the ion imprinted polymers (IIPs) can be described. Their synthesis can be carried out both chemically and electrochemically. The latter leads to the preparation of imprinted films, which are compatible in conjunction with transducers in sensor development [4,5]. Very few works report the electrochemical synthesis of ion imprinted polymers and their application as sensors for metal ion detection [6,7]. With this regard, we propose the synthesis, optimisation, characterisation and subsequent application of an electrosynthesised IIPs for the electrochemical detection of cadmium (II) in water. The proposed sensor was prepared by electropolymerisation of 4-aminophenylacetic acid (4-APA) monomer in presence of Cd(II) ions, which acts as the template. The screen printed carbon electrodes (SPCE) were used as transducer during sensor development, whereas the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were selected as the electrochemical methods for the synthesis and Cd(II) ions sensing, respectively. The incubation of the developed sensor in NaOH 250 mM involved into remove the template and the formation of specific recognition cavities into the polymer. A multivariate optimisation was employed for studying the effect of three independent parameters on electrochemical performances of the sensor. The electrochemical characterisation of sensors was performed in ferrocyanide-ferricyanide redox couple and in KCl 0.1 M, the latter revealing redox properties from the polymeric film. The performances of sensors and the control (NIP) was observed in sodium acetate buffer (100 mM, pH = 5) over the Cd(II) concentration range of 0.1–10 μ M.

2. Materials and Methods

2.1. Materials

Acetic acid, 4-Aminophenylacetic acid (4-APA, 98%), sodium acetate trihydrate, cadmium nitrate tetrahydrate (98%), ethylenediaminetetraacetic acid (99%) were purchased from Sigma-Aldrich (Italy). Sulphuric acid and sodium hydroxide solutions were commercially available as analytical reagent grade. All reagents were used without further purification. MilliQ water was used for washing the polymeric film after the preparation. Sodium acetate buffer (100 mM, pH = 5).

2.2. Apparatus

CV and DPV measurements were performed using a PalmSens potentiostat equipped with a cable connector (DropSens, Italy) for screen-printed electrodes. PSTrace was the software to control the instrument and data acquisition. The polymeric film was deposited on screen-printed carbon electrode (SPCE). The SPCEs were composed of three-electrode configuration on a planar ceramic support (3.3×1 cm) and they consisted of a carbon disk-shaped working electrode (4 mm diameter), a platinum electrode as counter electrode and a pseudo Ag/AgCl paste electrode as reference electrode. SPCE were commercially available (Metrohm, Italy).

2.3. Preparation of Electrosynthesised Ion Imprinted Polymer and Non-Imprinted Polymer

The preparation of ion imprinted polymer (IIP) based on poly-4-aminophenylacetic (poly-4-APA) films was performed by cyclic voltammetry (CV) in a potential range between -0.2 and 1.2 V vs. pseudo Ag/AgCl, at a scan rate of 50 mV s^{-1} for 40 cycles in a solution of H_2SO_4 0.5 M containing 1 mM of Cd^{2+} ions. The porogen was chosen based on previous works about the electrosynthesis of poly-4-APA on SPE [8]. After the electropolymerisation, the electrode was rinsed with MilliQ water and incubated in different solvent (EDTA 100 mM and 250 mM, H_2SO_4 500 mM, NaOH 100 mM and 250 mM) to remove the target. The preparation of the control (non-imprinted polymer, NIP) was obtained with the same protocol, but without adding the template into the polymerisation mixture. The treatment in NaOH 250 mM was also performed on NIP. All prepared sensors were taken in air when not in use.

2.4. Cd^{2+} Ion Sensing

The electrochemical responses of IIP and NIP films towards Cd²⁺ ions were recorded using DPV measurements in the potential range of -0.2 to + 0.4 V, modulation amplitude of 50 mV, step potential of 4.95 mV, and equilibration time of 2 s. Cd²⁺ ions interacted with the imprinted film by drop-casting on the electrode surface an appropriate amount (100 µL) of a solution of sodium acetate buffer (100 mM, pH = 5) containing different concentration of Cd²⁺ ions (0.1–10 µM), by leaving the drop on the electrode for 10 min. After each measurement, the electrode surface was gently washed with sodium acetate buffer for 2 min.

2.5. Experimental Design in Optimisation Studies

Multivariate optimisation was conducted with the light to optimise the development of IIPs and NIP. The selected optimisation model was the central composite design (CCD), which allowed the selection of main three factors affecting the development of the sensors, such as (i) the monomer concentration, (ii) the rate between template-monomer (mainly affecting the number of cavities on the polymeric network) and (iii) the number of CV cycles during the electrosynthesis. MODDE (version 12) was used for design, mathematical modelling and optimisation. The levels of studied independent variables are listed in Table 1.

Table 1. Levels of independent variables considered in this work.

Variable	Low	High
Monomer concentration (X ₁)	0.5	5
Rate Cd ²⁺ /monomer (X ₂)	1	3
Number of CV cycles (X ₃)	10	40

The response was the difference of current (Δi , µA) recorded in ferrocyanide-ferricyanide redox probe before and after the electropolymerisation of the different imprinted sensors. Based on CCD principle, the design consisted of 2^k fractional factorial points plus 2^k axial points and 1 center point, where k defines the number of central points (in this case, k =3). 18 experiments run were conducted, and the second order polynomial equation consisted of linear, quadratic and first order interaction terms is shown below (Equation (1)):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j(\neq i)}^k \beta_{ij} X_i X_j + \varepsilon, \tag{1}$$

where Y is the response variables, X_i represent the dependent variables, β₀, β_i, β_{ii}, β_{ij} were the regression coefficient for intercept, linear, quadratic and interaction terms, respectively.

3. Results and Discussions

The electropolymerisation of 4-APA in presence of Cd²⁺ produced a sensitive polymeric imprinted film for that template, showing superior characteristics against its control. The optimal condition of synthesis was established by employing a multivariate experimental design, which approach is currently gained interest by scientists to optimised sensors and biomimetic sensors. The advantage of using the produced IIPs consisted on revealing a redox property of the polymer, directly addresses on the interaction between imprinted cavities and template. The interaction was visible close to + 0 V (see related DPV measurements), which potential is quite high than normally observed for the electroactivity of Cd²⁺ in solution.

3.1. Preparation of Electrosynthesised IIP and NIP Films

Figure 1a presented a typical cyclic voltammetry recorded during the electropolymerisation of 2.1 mM 4-APA in presence of 2.1 mM Cd^{2+} ions in 0.5 M H_2SO_4 on screen printed carbon electrode. Figure 1b shows the electropolymerisation of 4-APA on SPCE, without the template (NIP).

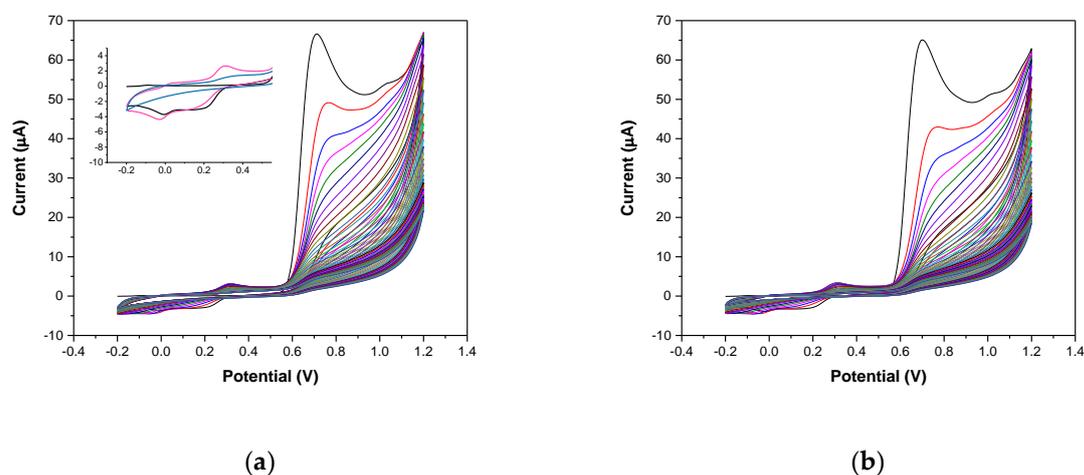


Figure 1. (a) The electropolymerisation of 2.1 mM 4-APA in presence of 2.1 mM Cd^{2+} in 0.5 M H_2SO_4 . Inset: Focused CV for (black) 1th, (pink) 2nd, (blue) 17th cycle; (b) The electropolymerisation of 2.1 mM 4-APA in 0.5 M H_2SO_4 . Voltammetric condition: (i) potential range: -0.2 to $+1.2$ V; (ii) scan rate: 50 mV s^{-1} ; (iii) CV cycles: 40.

During the CV, the first peak at $+0.70$ V indicated the formation of cation radicals that promoted the polymerisation process, once to the oxidation of 4-APA. Further peaks at $+0.19$ V and at -0.025 V are related to the reduction of the polymer film on the SPCE surface. Following the second potential cycle, two oxidation waves appeared at the potentials of $+0.040$ and $+0.305$ V, corresponding to the oxidation of the formed polymeric film. After around 17 cycles of polymerisation, it was notable a decrease in the anodic peaks current, indicating the subsequent formation of the polymer film (see Inset of Figure 1a). Finally, the formation of the film produced a partial blockage of the electrode surface. The electropolymerisation of NIP (Figure 1b) followed the same interpretation of the process, with differences in terms of current appeared along the second cycle of CV.

3.1. Optimisation of Sensor Performances by Experimental Design

The optimisation of performances was possible by a multivariate approach, that considered all factors together, including linear, quadratic and interaction terms in the model. All the selected factors were related on the electrosynthesis process. Among them, with the emphasis to develop imprinted materials, the relationship between all reagent should be described. Preliminary results shown the factor's importance on responses were the initial concentration of functional monomer (X_1) and the number of CV cycles during the electrosynthesis (X_3). Figure 2 shows the significant coefficients related to factors.

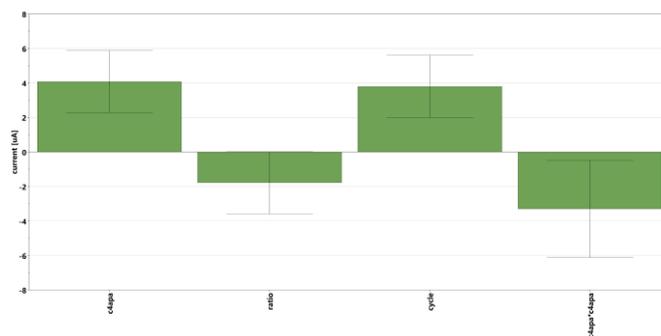


Figure 2. Plot of the significant coefficients obtained from model.

The regression equation for the achieved responses, including significant factors, is reported (Equation (2)).

$$Y = 14.86 + 4.08X_1 - 1.78X_2 + 3.80X_3 - 3.30X_1X_1, \tag{2}$$

After evaluation of the Equation (2), it appears as the monomer concentration is in strong correlation with the other factors. In particular the ratio of monomer/ Cd^{2+} should be regulated to assume a correct orientation of cavities on the polymer network. The factor related on the grown of the electrosynthesised imprinted film was also significant, confirming as the deposition of the film on electrode surface is involved in the difference of currents recorded by the electrochemical probe. With the light to maximise the responses, the experimental conditions used for further measurements were (i) 2.1 mM 4-APA, (ii) 2.1 mM Cd^{2+} (ratio 1:1), and (iii) 40 CV cycles during the electroynthesis.

3.2. Electrochemical Characterisation of IIP and NIP Films

The prepared sensors were first subjected to electrochemical characterisation in ferrocyanide-ferricyanide redox probe and in KCl 0.1 M, by applying a CV measurement for bare SPCE, IIP film and NIP film after the electrodeposition (Figure 3a,b).

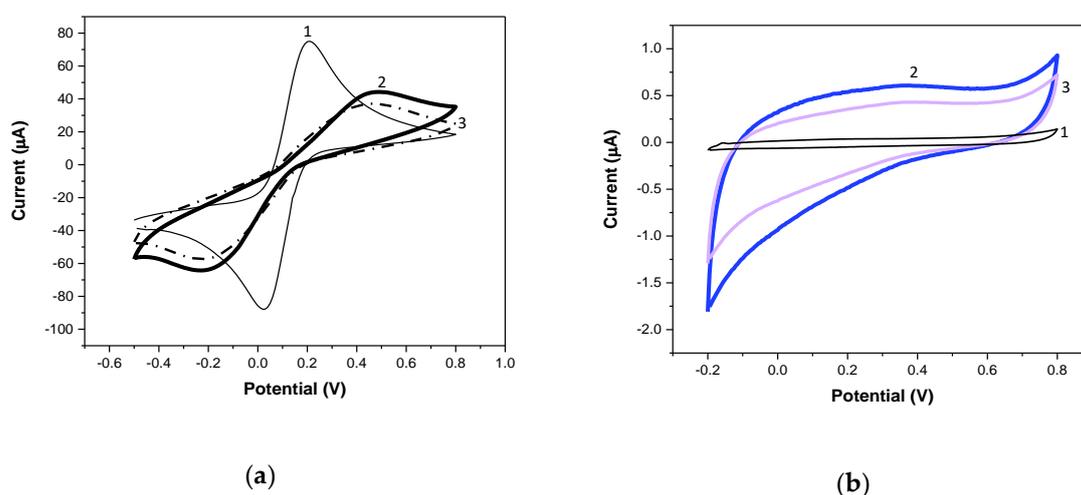


Figure 3. (a) Electrochemical characterisation by CV (5th cycle) in ferrocyanide-ferricyanide redox probe for (1) bare screen-printed carbon electrode; (2) Cd^{2+} -IIP film/SPCE and (3) NIP film/SPCE after polymerisation. Voltammetric condition: (i) potential range: -0.5 to $+0.8$ V; (ii) scan rate: 50 $mV s^{-1}$; (iii) CV cycles: 5; (b) Electrochemical characterisation by CV (5th cycle) in 0.1 M KCl for (1) bare screen printed carbon electrode; (2) Cd^{2+} -IIP film/SPCE and (3) NIP film/SPCE after polymerisation. Voltammetric condition: (i) potential range: -0.2 to $+0.8$ V; (ii) scan rate: 50 $mV s^{-1}$; (iii) CV cycles: 5.

Both electrochemical characterisation revealed higher electroactivity of the imprinted polymer when compared to NIP film. In addition, as shown in Figure 2b, no signals were obtained for bare SPCE. The electroactivity of IIP film than NIP suggest the imprinting effect of the polymer, where possibly Cd^{2+} ions is able to enhance the overall electrochemical process during polymerisation.

The removal of the template ion—to obtain the imprinted cavities—was carried out by exposure of the sensor to different solutions, such as EDTA 100 mM and 250 mM, H_2SO_4 500 mM, NaOH 100 mM and 250 mM. In all cases, different times of elution were tested, in a range between 1 and 15 min (1, 3, 5, 10, 15 min, respectively). As the most effective method, NaOH 250 mM incubated for 3 min was used. CV markable differences recorded in KCl 0.1 M for NIP and IIPs treated with NaOH 250 mM were visible (Figure 4), confirming the elution of Cd^{2+} ions from the imprinted cavities.

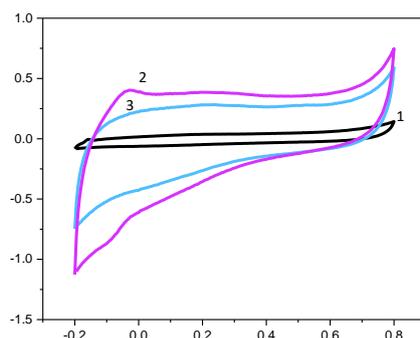


Figure 4. Electrochemical characterisation by CV (5th cycle) in 0.1 M KCl for (1) bare screen printed carbon electrode; (2) Cd^{2+} -IIP film/SPCE and (3) NIP film/SPCE after treatment in 250 mM NaOH for 3 min.. Voltammetric condition: (i) potential range: -0.2 to $+0.8$ V; (ii) scan rate: 50 mV s^{-1} ; (iii) CV cycles: 5.

3.3. Electrochemical Performances of IIP and NIP Film

The electrochemical sensing of Cd^{2+} ions was performed by DPV measurements on NIP and Cd^{2+} -IIP film. 100 mM sodium acetate buffer (pH = 5) was selected as the electrolyte solution for the determination of Cu^{2+} ions. DPV measurements recorded for Cd^{2+} -IIP film are shown in Figure 5, and related calibration curves are also reported (Figure 5b).

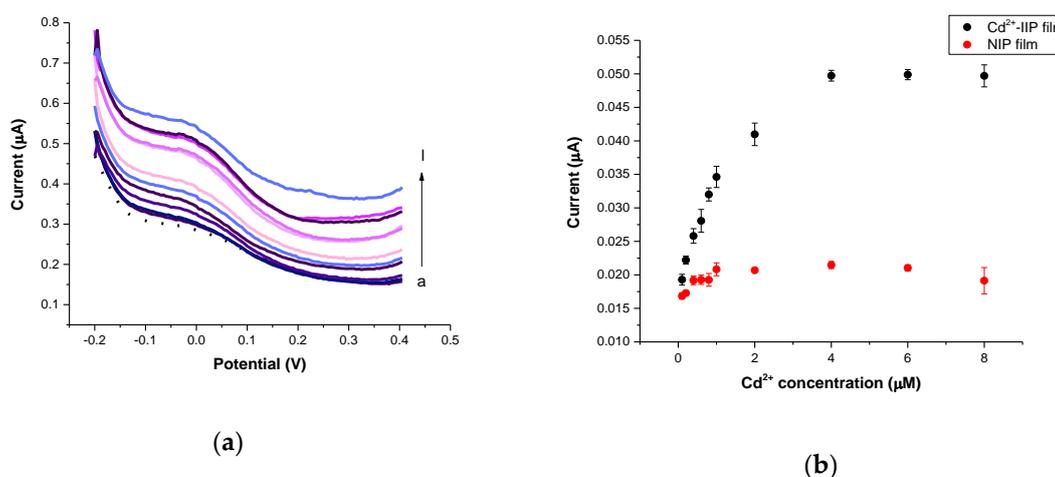


Figure 5. (a) Differential pulse voltammograms recorded for Cd^{2+} -IIP film after the exposure to (a) blank (sodium acetate buffer), (b) 0.1, (c) 0.2, (d) 0.4, (e) 0.6, (f) 0.8, (g) 1.0, (h) 2.0, (i) 4.0, (j) 6.0, (k) 8.0, (l) $10 \mu\text{M}$ of Cd^{2+} ions in presence of sodium acetate buffer; (b) Comparison of the electrochemical responses between Cd^{2+} -IIP and NIP films along all tested Cd^{2+} concentration; (c) Comparison

between responses from Cd²⁺-IIP and NIP film in the linear range revealed between 0.1 and 1 μM Cd²⁺ ions.

As shown from Figure 5a, the current responses value increased within the tested Cd²⁺ ion concentration. However, saturation reached value upper than 2 μM, due to the occupancy of cavities. Notably, the imprinted sensor shows high affinity and specificity towards Cd²⁺ ions than that obtained for NIP films, confirming the imprinting effect on this polymer. The linear regression was established between 0.1 and 1 μM, with a sensitivity of 0.0163 μA μM⁻¹. In addition, it was possible to evaluate the imprinted factor as 6.86, highly indicating the specific recognition of template from imprinted cavities on Cd²⁺-IIP films.

The proposed imprinted sensor shows high sensitivity and possesses superior specific properties towards Cd²⁺ ions. This preliminary results are currently encouraged us to perform further experiments in regard to selectivity properties of the imprinted polymer against NIP and its application to real water matrices, which discussion will be presented soon.

4. Conclusions

Preliminary study on electrosynthesis of ion imprinted polymeric sensor on SPCE transducer for Cd²⁺ ion determination in water is here reported. The electrosynthesis of the imprinted cavities revealed the newly approach to produce highly sensitive films towards environmental targets. In this light, the developed imprinted polymeric film shows greater sensitivity than NIP film, with an imprinting factor of 6.86. Those achieved preliminary results open the possibility to employ this sensor for quantitative determination of Cd²⁺ ions in water. Further experiments to evaluate more properties of the sensor are currently under study.

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Conflicts of Interest: The authors declare no conflict of interest.

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