

DESIGN OF AN ELECTROCHEMICAL POLYPYRROLE-IMPRINTED POLYMER FOR BISPHENOL A DETECTION IN AQUEOUS MEDIA

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Abstract:

This study concerns the design of an electrochemical sensor functionalized with a polypyrrole-molecularly imprinted polymer (PPy-MIP) for bisphenol A (BPA) detection. Here, N, N'-methylene-bis-acrylamide was chosen as the chelating agent due to its accessible carbonyl and amine groups. Extraction of BPA templates from the vicinity of the PPy-polymeric matrix was optimized by electrochemistry and Fourier Transform Infrared spectroscopy (FTIR). The limit of detection of the designed sensor was of order of 1 nM and the sensitivity was equal to $(9.66 \pm 0.86) \mu\text{A/M}$ respectively. The dissociation constant K_d , which provides information on the affinity between the templates and the cavities, was estimated from a combined one-site binding - Hill model. K_d was of order of $(2.05 \pm 0.18) 10^{-9}$ M.

Keywords: Bisphenol A, Polypyrrole, Molecularly imprinted polymer, Electrochemical sensor, FTIR characterization.

1. INTRODUCTION

Bisphenol A (BPA) was extensively used as an additive in plastic industries for producing epoxy resins, polycarbonate, and food and beverage containers [1-3]. BPA was also utilized in dental sealants and water pipes [4]. Several studies demonstrated that the extensive use of BPA is at the origin of several health concerns. BPA is in fact suspected to cause cancers [5-7], diabetes, obesity, cardiovascular diseases [8,9], and to perturb the endocrine system [7]. BPA is also suspected to cause environmental pollution [10]. Designing reliable, sensitive, robust and selective methods for BPA detection is thus of significance. Different analytical methods are used to detect BPA including high-performance liquid chromatography coupled to mass spectrometry [11], gas chromatography-mass spectrometry [12], fluorescence [13] and enzyme-linked immunosorbent assay [7].

Despite their sensitivity and accuracy, these techniques present some limitations, mainly the non-possibility of using them for routine and in-site analysis, high cost and the need for large volumes of samples. These drawbacks can be overcome by chemical sensors in general and electrochemical ones, in particular [14]. Several studies reported the design of electrochemical sensors for BPA detection. The main difference between them is related to the functionalization technique [15-18]. Among all these techniques, molecular imprinting is probably the most promising one as it offers good chemical, physical and thermal stability, high selectivity, excellent sensitivity and reusability [19-21].

In the present work, we have designed an electrochemical sensor functionalized with a polypyrrole-based MIP for the sensitive detection of BPA in aqueous media. Several reasons have motivated the choice of PPy as a polymeric matrix: chemical stability, good redox properties and relatively high electrical conductivity [22]. The PPy-MIP-BPA films were characterized by FTIR and the metrological performances of the sensors were determined in terms of sensitivity, limit of detection (LOD) and dissociation constant K_d .

2. EXPERIMENTAL

2.1. Reagents

Sulfuric acid (H_2SO_4 , 98%), hydrogen peroxide (H_2O_2 , 30%), methanol (CH_3OH), acetic acid (CH_3COOH), lithium perchlorate (LiClO_4), potassium ferricyanide (III) ($\text{K}_3\text{Fe}(\text{CN})_6$), potassium hexacyanoferrate(II) ($\text{K}_4\text{Fe}(\text{CN})_6$), N, N'-methylene-bis-acrylamide, pyrrole and bisphenol A were purchased from Sigma Aldrich (France) and used as received. Pyrrole (Py) was filtered, through a basic alumina column, and stored in dark at 4°C before its use for electropolymerization.

2.2. Electrochemical measurements

Chronoamperometry (CA), cyclic voltammetry (CV) and square wave voltammetry (SWV) measurements were performed at room temperature with a CHI 500E potentiostat and three-electrodes: gold, platinum and Ag/AgCl as the working, auxiliary and reference electrodes respectively. All measurements were made in triplicate.

2.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR measurements were carried out under ambient conditions with a Perkin Elmer "Spectrum two" spectrometer equipped with an attenuated total reflectance (ATR) cell with a resolution of 2 cm⁻¹. A background spectrum was recorded before each measurement, and the "background corrections" were then operated.

2.4. MIP design

Before each electrochemical measurement, gold surfaces were cleaned with acetone for 10 min and then with ethanol (10 min). A 50 µl-drop of a piranha solution (98% H₂SO₄ / 30% H₂O₂ 3:1 v/v) was then deposited, for 3 min, to clean and activate the Au substrates. The electrodes were then rinsed copiously with deionised double distilled water, before being dried under an ambient atmosphere.

To prevent an eventual anchoring of BPA on gold surfaces, we have electrodeposited by chronoamperometry (during 2 s) a thin blocking layer of PPy at an applied potential of 1.05 V/SCE [23-24]. A pre-polymerization complex was then formed, by dissolving in an aqueous solution: 10⁻³ M of BPA (template), 10⁻² M of pyrrole (functional monomer), 10⁻⁴ M of N, N'-methylene-bis-acrylamide (chelating agent), and 10⁻¹ M of lithium perchlorate. After 15 mn, we operated an electropolymerization by CA for 10 s. This technique provides in fact a better control of the film's thickness and morphology compared to chemical oxidation [23-24]. BPA templates were then removed from the vicinity of the polymeric matrix by immersing the substrates in a protic solution (methanol/acetic acid, MeOH/AA, 4/1, v/v) for 30 min. This step was followed by a copious rinse with ultra-pure water during 30 min.

The corresponding non-imprinted polymer (NIP) was designed in the same conditions in the absence of BPA molecules.

3. RESULTS

3.1. Electrochemical characterization of the designed MIP

Cyclic voltammetry (CV) was conducted to control the functionalization and extraction steps. All measurements were done in an aqueous solution containing 5 mM of [Fe(CN)₆]^{3-/4-} probes and 0.1 M KCl at a scan rate of 100 mV/s. Results, presented in Figure. 1, highlight a significant current decrease after the electropolymerization process which reflects the formation of a homogenous BPA-PPy film on the gold surface. The further current increase after BPA extraction indicates that the electrochemical system of charge transfer becomes more reversible, confirming thus that the protic solution was able to extract the BPA templates from the vicinity of the PPy polymeric matrix.

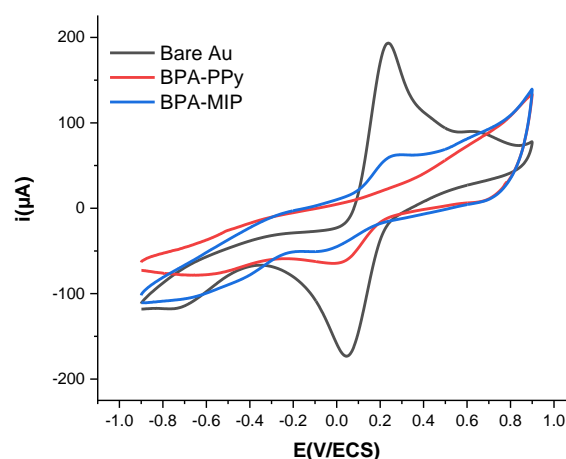


Figure 1: Cyclic voltammograms of bare gold, BPA-PPy and MIP films. Measurements were done in 5 mM of [Fe(CN)₆]^{3-/4-} and 0.1 M of KCl at a scan rate of 100mV/s.

3.2. Fourier Transform Infrared Spectroscopy (FTIR) characterization

FTIR analysis were investigated, in the range of 500 to 4000 cm⁻¹, for physicochemical characterization of the NIP, the PPy-BPA and BPA-MIP films. As it can be seen in Figure. 2, the FTIR spectra of the three films are rather similar as they have the same backbone. The peaks at 1220 cm⁻¹ and 2965 cm⁻¹ are attributed to N-H stretching and alkene C-H stretching bonds of polypyrrole, while those at 1588 cm⁻¹ and 1702 cm⁻¹ are assigned to the amide N-H and C=O stretching vibrations, of N, N'-methylene-bis-acrylamide. Vibrational bands at 693 cm⁻¹, 1102 cm⁻¹ and 2968 cm⁻¹, characteristic of BPA molecules are only present in BPA-PPy films.

They were assigned to C-H benzene derivative, C-O stretching tertiary alcohol and alkene C-H stretching, respectively.

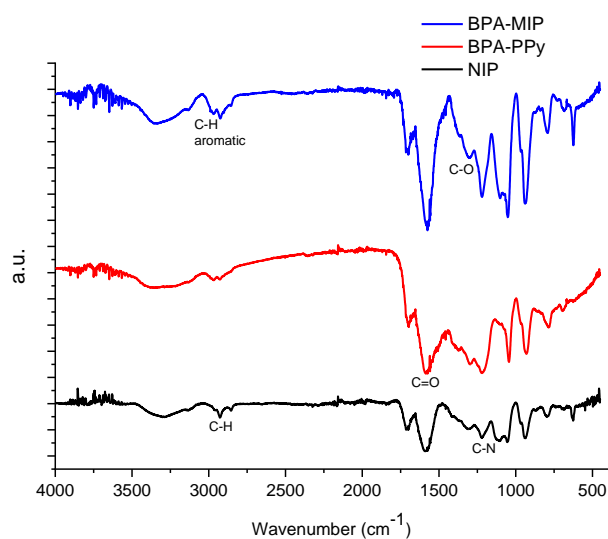


Figure. 2: FTIR spectra of the NIP, BPA-PPy and MIP films.

Electrochemical and FTIR results confirm thus the success of both BPA imprinting and templates' extraction strategies.

3.3. BPA detection

3.3.1. Follow-up of BPA detection

The BPA extraction step was followed by incubation in an aqueous solution of increasing concentrations of BPA. The optimum incubation duration was equal to 20 min (results not shown here). Square wave voltammetry (SWV) was then performed to follow up the electrochemical response of the designed sensor for BPA molecules detection (in the range 10^{-9} to 10^{-5} M).

Results, reported in Figure. 3, show an increase in the current intensity of the redox probe's oxidation peak with BPA concentrations. This can be attributed to the fact that the aromatic fragments of the BPA molecule serve as an electronic relay to the electrodes' surface [7]. These results confirm the good recognition of the analytes by the cavities formed in the polymeric matrix.

3.3.2. Sensors metrological performances

The calibration curve (redox probe's peak current variations versus cumulative BPA concentration) is plotted in Figure.4a. The limit of detection, defined as the lowest detected BPA concentration, was equal to 1 nM.

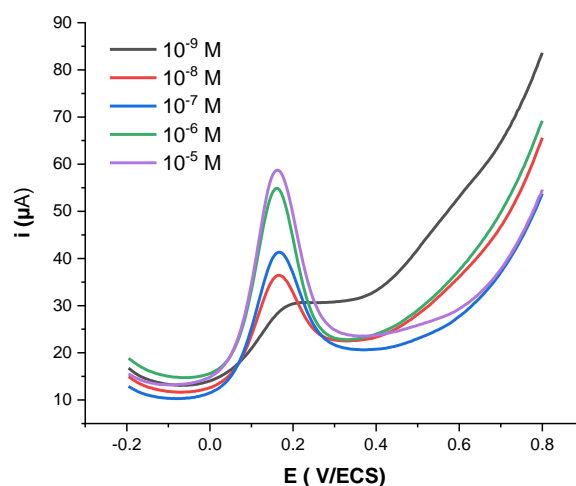


Figure.3: Square wave voltammograms after incubation in increasing concentrations of BPA. Measurements were made in an aqueous solution, containing 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KCl.

The sensitivity, estimated from the slope at the origin of the calibration curve in a semi-logarithmic scale (Figure 4. b), was of order of $(9.66 \pm 0.86) \mu\text{A}/\text{M}$.

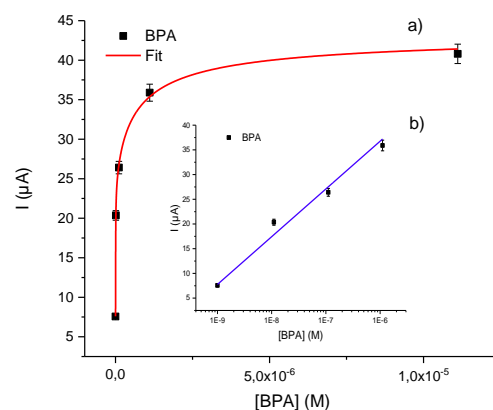


Figure.4. a) Calibration curve of the designed BPA-MIP electrochemical sensor, b) Slope at the origin in semi-logarithmic scale.

The dissociation constant, which provides an idea on the affinity between BPA molecules and their imprints in the PPy matrix, was estimated from the fitting of the calibration curve with a combined (one site binding/Hill) model:

$$y(C) = \frac{A_1 \times C}{K_{d1} + C} + \frac{A_2 \times C^\alpha}{K_{d2}^\alpha + C^\alpha}$$

In which $y(C)$ is the output sensor's signal, for a given BPA concentration C , K_{d1} is the dissociation constant, A_1 and A_2 are empiric constants, α is the Hill coefficient and K_{d2} is a pseudo-dissociation

constant associated with the polymeric structural rearrangement during binding/rebinding processes.

K_{d1} and K_{d2} constants were of order of $(2.05 \pm 0.18) \times 10^{-9}$ M and $(6.04 \pm 2.81) \times 10^{-7}$ M respectively. The low value of K_{d1} reveals a strong affinity between the MIP cavities and BPA molecules. Hill coefficient α was of order of 0.75, indicating negative cooperative interactions between the binding sites and BPA molecules [25].

4. CONCLUSION

A polypyrrole-molecularly imprinted polymer sensor was designed for BPA detection. Fourier-transformed infrared spectroscopy and electrochemistry measurements were first carried out to characterize the NIP, the BPA-PPy and BPA-MIP films. The designed sensors exhibit encouraging analytical performances: a LOD of 1 nM, a sensitivity of $(9.66 \pm 0.86) \mu\text{A/M}$ and a dissociation constant, of order of $(2.05 \pm 0.18) 10^{-9}$ M, permitting to consider its further use for BPA detection in real media.

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