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Journal of Applied Chemical Research, 17, 3, 41-51 (2023)

Electroanalytical Sensor Based on Molecularly Imprinted Polymer-Modified Screen- Printed Carbon Electrode for the Determination of Thyroxine

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(Received 24 Nov. 2022; Final revision received 14 Feb. 2023)

Abstract

A susceptible voltammetric sensor for thyroxine is designed and generated by electro-polymerization of molecularly imprinted polymer (MIP). Thyroxine selective molecularly imprinted polymer is synthesized by electropolymerization of the p-phenylenediamine–resorcinol mixture directly on the screen-printed carbon electrode (SPCE) surface in the presence of the thyroxine molecules. Electropolymerization of monomers deposited a film on the electrode surface which blocked ferricyanide reduction. Elimination of the Thyroxine forms the cavities which caused a remarkably improved ferricyanide signal which was again blocked after the rebinding of thyroxine. The decrease of the ferricyanide peak of the MIP electrode depended linearly on the thyroxine concentration. Various factors affecting the current response of the electrode were studied and optimized. This sensor shows a linear response range of 1×10^{-8}- 2×10^{-6}M and a lower detection limit of 5×10^{-9} M. The sensor was successfully applied to the detection of thyroxine in human plasma samples.

Keywords: molecularly imprinted polymers; electrochemical sensors; screen-printed carbon electrode; thyroxine.

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Introduction

Thyroxine (T4) is an important biological hormone of iodoamino acid derivative of thyronine, produced in the follicular cells of the thyroid gland. It plays a central role in various functions such as carbohydrate metabolism, oxygen consumption, protein synthesis, and fetal neuro-development [1–6]. The detection of T4 is of immense clinical significance due to its ability to control the secretion of thyroid-stimulating hormone (TSH) and the diagnosis of hyperthyroidism and hypothyroidism.

Among various analytical methods for detection of T4, the following should be mentioned: immunoassay [7–12], chemiluminescence [13], time-resolved fluorescence [14], high-performance liquid chromatography [15], and mass spectrometry [16]. However, these methods require elaborate pre-treatment and they are cumbersome; hence alternative simpler strategies are desirable. In this context, electroanalytical techniques are facile and easy to automate.

The electrochemical response of T4 has been investigated earlier using the hanging mercury drop electrode (HMDE) [17], silver electrode [18], chemically modified carbon paste electrode (CPE), single-walled carbon nanotubes (SWNTs), multi-walled carbon nanotubes (MWNTs) modified glassy carbon electrodes [19–23]. Alternately, modified carbon paste electrodes have also been studied using cetyltrimethyl ammonium bromide (CTAB) [24] and phenylhydrazine [25]. Recently the bare Edge plane pyrolytic graphite (EPPG) electrode has also been investigated to determine T4 in biological and clinical samples [26].

Screen-printed carbon electrodes (SPCEs) are gaining wide acceptance as electrochemical sensors in clinical and environmental fields on account of their reproducibility and scalability [27–30]. In addition, the composed material of SPCEs, such as carbon inks and polymeric binders, are particularly attractive since they are inexpensive with low background currents and wide potential windows exhibiting diverse electroanalytical behavior [31]. In this study, the researcher demonstrated the applicability of the SPCE for the detection of T4 in the neutral phosphate buffer electrolyte (PBS) and plasma samples using sensitive square wave voltammetry (SWV) and Cyclic voltammetry (CV) techniques.

Experimental

Reagents

P-Phenylenediamine dihydrochloride (p-PD), resorcinol (Res) and thyroxine were supplied by Sigma-Aldrich (Munich, Germany). Sodium acetate, hydrochloric acid, methanol, ethanol,

phosphoric acid and acetic acid were provided from Merck (Schuchardt, Germany). Solvents were HPLC grade and all of the analytes were analytical grade.

Instruments

Electrochemical data were gained by using a potentioastat with a three-electrode system sama500. DropSens 110 SPCEs (3.4 cm length × 1.0 cm width × 0.05 cm height) were purchased from DropSens. These SPCEs are a three-electrode system with a 4 mm diameter disk carbon working electrode, silver reference electrode, and a carbon counter electrode.

Construction of working Electrode

Initially, 5 mM p-PD, 5 mM resorcinol and 0.4 mM Thyroxine mixture in 70% methanol containing 100 mM acetate buffer, pH 6 were prepared. Then, in order to do electropolymerization of monomers and formation of nonconductor film on the SPCE surface, 30 μL of the prepared solution was added to the surface of SPCE and cyclic voltammetry in the range of 0 and 0.8 V (8 scans) at a scan rate of 50 mV/s was applied. The non-imprinted electrode was prepared similarly in the absence of Thyroxine. Template molecules were removed by using acetonitrile: 0.3 M Hydrochloric acid (10:1 v/v) mixture.

Electrochemical experiments

The electrochemical analysis of Thyroxine was performed according to the following process: Extraction step: 50 μL of the thyroxine solution was placed onto the thyroxine-imprinted SPCE surface and incubated for 15 min at room temperature.

Washing step: To remove unbounded Thyroxine, the SPCE surface was washed three times with 100 μL of the washing solution (water/acetonitrile (98:2) solution).

Analyzing step: The 50 μL of 10 mM ferricyanide solution (in 100 mM KCl) was dropped on MIP- modified SPCE, and cyclic voltammetry was applied by sweeping between −0.2 and 0.6 V at a scan rate of 50 mV/s and decrease in redox current response of ferricyanide solution was recorded.

Quantification of Thyroxine in plasma samples

In blood, thyroxine is mainly bound to iodothyronine-binding serum proteins because of its strong hydrophobicity [18]. To dissociate thyroxine from iodothyronine-binding serum

proteins and precipitate proteins, to 150 µL of plasma sample, 150 µL of 0.1 mol/L NaOH was added before 500µL of 100% methanol was added for proteins precipitation. The supernatant was diluted with 5 mL buffer solution at pH 3 after centrifugation. After incubation for 15 min, the electrode was washed by 50 µL of the washing solution for three times. Then, 50 µL of 10 mM ferricyanide solution (in 100 mM KCl) was placed on the MIP-modified SPCE surface. For determination of the calibration curve and limit of detection, pool Serum samples were spiked with thyroxine at different concentration levels between 10 – 200 nM. The recoveries and reproducibility of the method were considered and summarized in Table 1. According to the result, the average recovery of the proposed method was 94% at the studied levels. These results demonstrated that the MIP-based sensor had a good sensitivity.

Results and discussion

Preparation of MIP-modified electrode

An electrochemical biosensor was designed for the detection and quantification of Thyroxine based on MIP- modified SPCE. Firstly, non-conductive polymer film deposited on the surface of SPCE by electropolymerization in the presence of p-Phenylenediamine and resorcinol as monomers and Thyroxine as a template. In the next step, by removing Thyroxine from the polymer, complementary binding sites formed on the working electrode surface. Therefore, the Electro-polymerization of p-Phenylenediamine - resorcinol mixture on a SPCE in the presence of 0.4 mM Thyroxine was examined. Figure 1 shows CVs during the electro-polymerization, the current decreased with the subsequent sweeps and approached zero, indicating the construction of a non-conducting film on the electrode surface. thyroxine is not electroactive in 0 - 0.8 V. Therefore, similar CVs were obtained in the presence and absence of thyroxine.

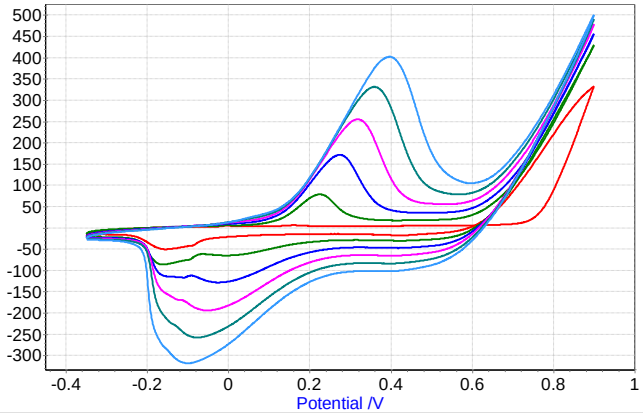

Figure 1. CVs during the electro-polymerisation showing the construction of a MIP film on the electrode surface.

The construction of molecularly imprinted film on SPCE was shown by SEM image of modified electrode surface in Figure 2.

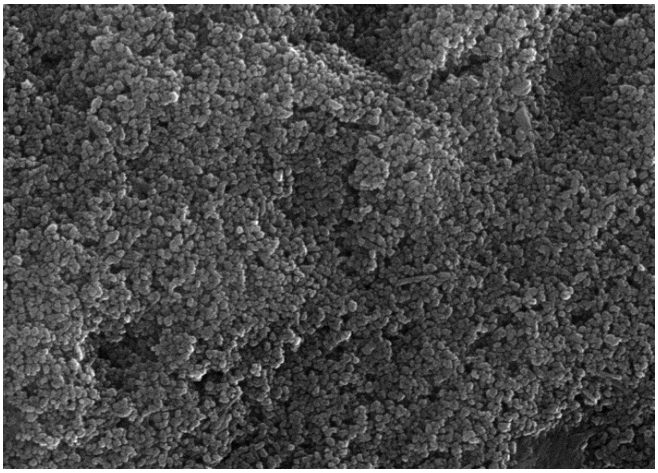

Figure 2. FESEM image of MIP- modified SPCE surface.

After removal of the template, the electrode was incubated for 15 min with a solution containing 100 μM Thyroxine. After the washing step, 50 μL of Ferricyanide solution was added to the SPCE surface. Ferricyanide was used as a redox probe to detect permeability after electro-polymerization, template removal, and rebinding. Figure 3 shows the cyclic voltammograms of these steps. Bare SPCE presented the highest response. Afterward, electro-polymerization, the current for ferricyanide redox peak was suppressed entirely. After removal of the template, in the MIP-modified electrode, ferricyanide oxidation and reduction signals appeared. This signal was again suppressed after the target rebinding. The relative current decrease depends linearly on the thyroxine concentration from 1 to 100 nM (Figure 5). These results demonstrated that the electrochemical sensor offered excellent template recognition ability and sensitivity.

B. Optimization of the sensor composition

To achieve the optimum recognition ability of MIP modified-SPCE, the quantity of electro-polymerization mixture components, including monomer ratios, thyroxine concentration, and the number of scans, was altered in the constant settings of voltammetry assay. The resulting electrode at each condition was applied to detect thyroxine in the presence and absence of thyroxine, and the difference in the response current (ΔI) was used for comparison. Herein, $\Delta I = I0 - Ic$, where I0 and Ic are the currents at 0.3 V, when the quantity of thyroxin is 0 and 100 μM, respectively.

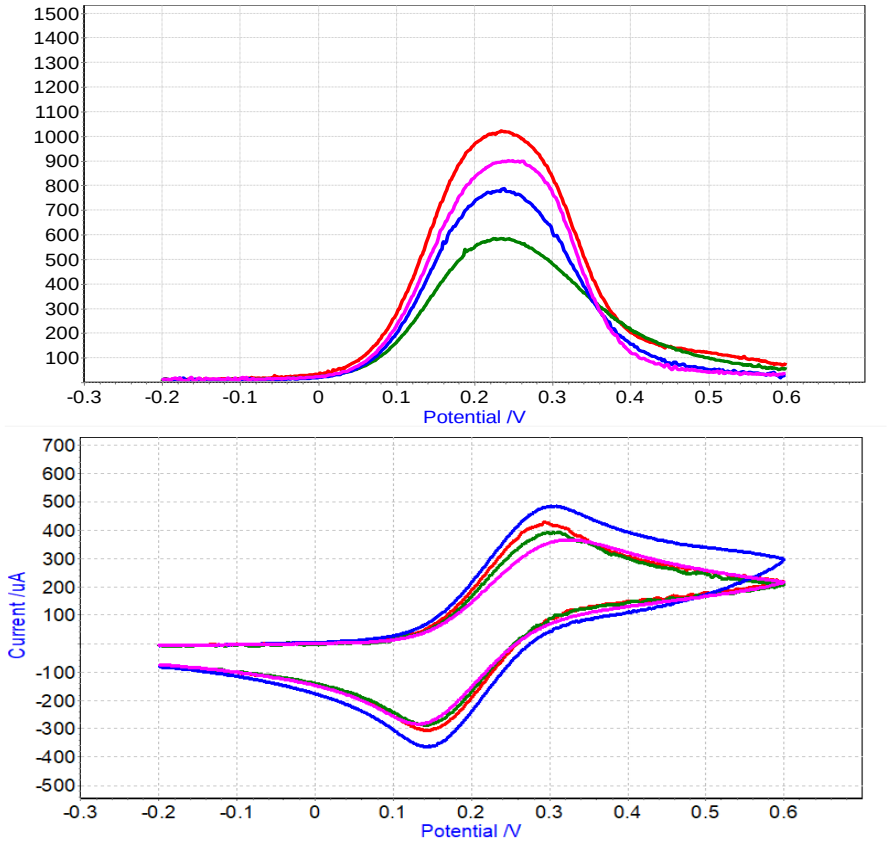

Figure 3. The cyclic voltammograms and square wave voltammetry of Bare SPCE (red), SPCE after electro-polymerization (green), MIP-SPCE after template removal (pink), MIP-SPCE after rebinding of 50 nM thyroxine (blue) in 10 mM ferricyanide at a scan rate of 50 mV/s.

For primary optimization purposes, the MIP-modified electrode was prepared with different combinations of p-PD to Res. Extraction and voltametric detection of thyroxine was carried out for each prepared electrode. The attained results were displayed in Figure 4a. The result demonstrates that the best response for the prepared sensor is in the mass ratio of 1:1 (p-PD: Res).

As shown in Figure 4b, an increase in the thyroxin concentration in the polymerization mixture leads to an increase in the response current due to an increase in the conductivity of the electrode surface by more binding cavities. Thus, 0.6 mM of thyroxine is the optimum amount for the maximum response current, according to Fig. 4b. Higher values will cause the polymer film not to have sufficient strength at the electrode surface. The best number of scans for electropolymerization was 7 cycles, because more than this value, the thickness of the film was increased and removal of templates trapped in the inner layers became impossible.

C. Optimization of thyroxine extraction conditions

The effect of the solution pH on the thyroxine extraction in the electrode was studied. To this aim, 20 µl of thyroxine solutions with different pH values dropped on modified SPCE for 15 min. Then, the electrodes were transferred into the solution of the electrochemical cell after the washing step. The result of these experiments confirmed, pH = 3 is favorable for the thyroxine extraction on the MIP modified SPCE. It is clear that at the under and above pH 3, ΔI is decreased due to the decrease in thyroxine extraction on the electrode surface. The effect of incubation time on the thyroxine extraction and the electrode signal was also considered. It is clear that the increase in the incubation time provides a serious growth in the thyroxine extraction amount till about 15 min, and after that, the response of the electrode does not grow further by increasing the incubation time. Therefore, 15 min was chosen for extraction time to decrease the analyzing time of thyroxin as far as possible.

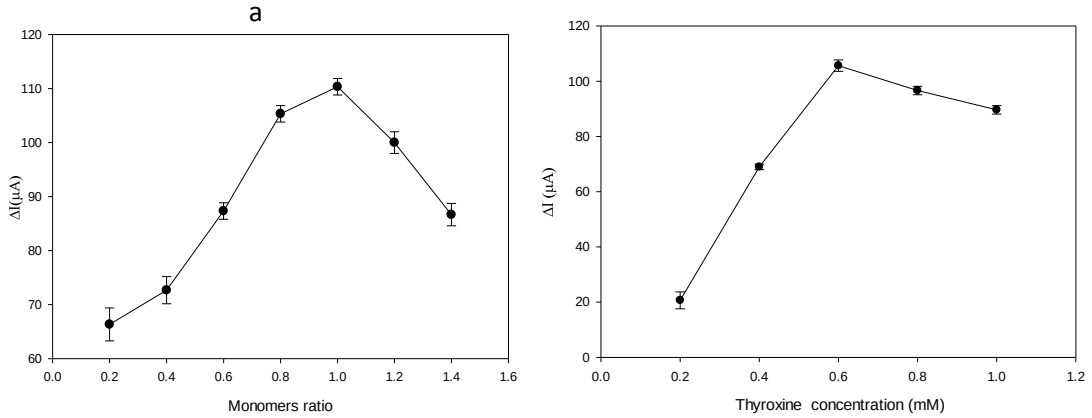

Figure 4. Optimization of composition of the MIP-SPCE and incubation time. Variation of the electrode response for thyroxin with changing amounts of (a) Monomers ratio, (b) Thyroxine amount.

Thyroxine determination

Voltammetric determination of thyroxine was carried out under optimized conditions employing cyclic voltammetry at the MIP-SPCE for different concentrations of thyroxine. The redox peak current of ferricyanide decreased with the increase in thyroxine concentration due to the occupation of imprinted cavities by thyroxine and gradually reached a steady state when the imprinted sites were saturated. The calibration graph of the prepared MIP-based sensor is presented in Figure 5.

The synthesized sensor shows a linear relationship over thyroxin concentration in the range of 1×10^{-8}- 2×10^{-6}M and a lower detection limit of 5×10^{-9} M. Each point of the calibration graph is the mean of three replications. The linear calibration curve of ΔI versus concentrations

(c) can be described by the following equation: y = 0.1119x+6.659. The correlation coefficient is calculated to be 0.990.

The stability of MIP-SPCE

To consider of imprinting factor of MIP-SPCE toward thyroxine, the response of non-imprinted modified electrode on the redox peak of ferricyanide was investigated. The template removing step was done for NIP-SPCE, but no response signal was observed. For stability assay, the modified SPCE was stored in air at room temperature for 40 days and used. It reserved 91.5 % of its primary response, demonstrating good storage stability.

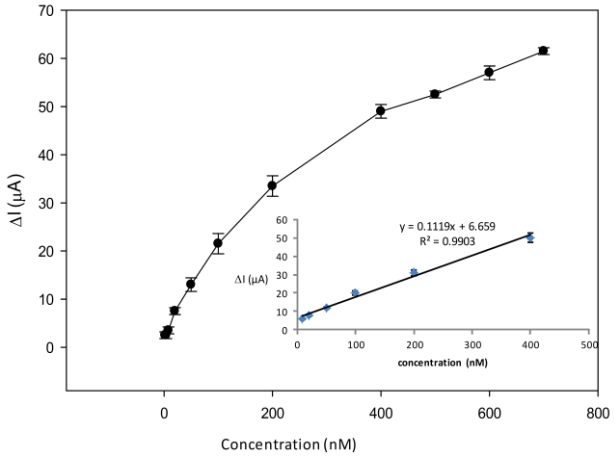

Figure 5. Calibration curve obtained for the presented sensors at the optimized conditions; (inset: calibration curve at the linear range).

Thyroxine Detection in real samples

In order to consider the ability of the sensor for thyroxine detection in practical application, it was used for thyroxine determination in human plasma. A series of sample solutions were prepared by adding a certain volume of thyroxine to 150 µL of human plasma. After sample preparation as mentioned in section 2.5, the electrochemical measurement was carried out.

Acceptable results and recoveries were obtained via the proposed method, as shown in Table 1. The suitable results obtained show that the presented sensors can be applied to real sample assays.

A highly sensitive voltammetric sensor for thyroxine detection at low concentrations was offered. A conductive imprinted polymer was designed and synthesized for thyroxine recognition. The offered sensor was applied successfully for thyroxine determination in plasma samples.

Table 1. Determination of thyroxin in plasma samples.

sample	Spiked concentration (nM)	Average concentration found (nM)	Average recovery(%)	RSD
1	10	9	91	8.33
2	50	45	90	4.26
3	100	95	95	4.22
4	150	135	103.3	3
5	200	190	95	2.56

Conclusion

The prepared electrochemical sensor has high selectivity and high sensitivity for Thyroxine molecules. In summary, the sensor was made by electro-polymerization of p-phenylenediamine–resorcinol mixture directly on the screen-printed carbon electrode (SPCE) surface in the presence of the Thyroxine molecule. The MIP/SPCE linear response was determined for Thyroxine molecules in the range of 1×10^{-8} - 2×10^{-6}M in the buffer solution with a limit of detection of 5×10^{-9} M. Based on the data obtained in this study, it could be concluded that the MIP based sensor made for Thyroxine has quite a high sensitivity and stability, reasonable low cost, short response time and excellent reproducibility. These advancements will also allow for developing of a fast, inexpensive, and transportable device for thyroxin in situ detection, which might be applied in interesting clinical, pharmacological, and biomedical investigations.

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