Highly Sensitive Molecularly Imprinted Impedimetric Sensor Based on AuNPs-Modified Screen Printed Electrode for 17β-Estradiol Detection

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Received: November 01, 2016; accepted: June 9, 2017

Abstract

17β-Estradiol (E2) residues in food with concentration range even as low as 1-10 pM can cause serious diseases such as early puberty in children and can also increase the risk of ovarian and breast cancer in women. In this work, we report on the development and optimization of an impedimetric sensor for high sensitive detection of E2 based on Molecularly Imprinted Polymer (MIP). The MIP layer was synthesized via electro-polymerization by cyclic voltammetry onto gold nanoparticles (AuNPs)-modified screen printed carbon ink electrode (SPCE). The electrode provides a detection limit below 10 fM in aqueous buffer solutions with a linear relationship in concentration range from 100 fM to 10 nM. The new result is obtained by thorough optimization of the density of AuNPs and the thickness of p-ATP layer with Faradaic impedance spectroscopy as readout method. The sensor showed an excellent regeneration capability, a good stability with time, and a surprisingly low cross selectivity compared to other proteins.

Keywords: MIP, EIS, Estradiol, Screen-printed electrode, AuNPs

1. Introduction

17β-Estradiol (E2) is widely used in livestock and poultry industries to promote animal's growth potential, as well as to improve the ability of animal lactation for higher ratio of lean meat and milk. However, estradiol residues in food can cause serious diseases such as early puberty in children and can also increase the risk of ovarian and breast cancer in women and wildlife, bisexual animals, as well as reduction of fertility even with the E2 concentration in the range of as low as 1-10 pM.

For the purpose of E2 detection, biosensor is one of the practically applicable devices due to its capability to rapidly detect and quantify biological components with simple analyzing methods. However, the technique for bio-component immobilizing onto solid electrodes of biosensors still remains a challenge for manufacturers as well as scientists.

Recently, the technique of molecularly imprinted polymers (MIPs) has received much consideration in the development of diagnostic medical devices. An overview of the most recent developments of MIPs can be found in ref. [1]. MIP-type receptors are normally synthesized in situ on the surface of small electrodes. The resulting artificial receptors for target molecules inside a polymer matrix are assumed to display a comparable selectivity and specificity to natural receptors [2, 3]. The binding cavities formed inside and on top of the MIP film are obtained after removing template molecules from the polymer layer by an electroelution process. The specific properties of these receptors are determined by the size and shape of the template molecules and by the number and spatial arrangement of weak non-covalent interaction points of the template molecules with their surrounding polymer matrix [4, 5]. Therefore, the optimization of MIPs-film thickness is key factor for increasing the sensitivity of MIP-based sensors. Our optimized MIPs-film thickness provides the best condition for obtaining the largest number of recognition cavities as well as increasing binding capability of the target molecules in an analyte. Consequently, one should consider the micro- or nano scale morphology of the polymer membrane. Several surface-modification techniques have been reported in literature [6]. Most of these techniques focus on designing the bulk morphology of polymer membranes either by altering the structure of the template molecules or by creating multi-layers MIP based on composite materials [6, 7]. One should also consider the conductivity of the MIP layer for electrical signal readout. In this work we presented our solution using a novel screen-printed carbon electrodes modified by in situ synthesized gold nanoparticles for MIP synthesis.

Hereby, the AuNPs were prepared by electrodeposition on the SPCEs using the cyclic-voltammetry (CV) method developed earlier by Truong et al. [7]. On top of these AuNPs/SPCEs, ultrathin 4-aminothiophenol (p-ATP) layers were polymerized in order to generate MIP membrane with nano-scale morphology and high conductivity. The

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use of AuNPs on our electrodes makes the MIP membrane grow more homogeneously and also enlarges its active surface. The specific properties of our synthesized MIP membrane for detecting E2 are characterized and carefully discussed in considering the effects of morphology on the sensor's sensitivity.

2. Experimental

2.1 Reagents and apparatus

Reagents E2, $HAuCl_4$, 4-aminothiophenol (p-ATP) were purchased from Sigma Aldrich, Germany. All the reagents were of analytical grade or of the highest commercially available purity and used as supplied without further purification. All solutions were prepared with deionized water.

The SPCEs were from BioDevice Technology Ltd., Japan in the form of three-electrode sensor chips. The active surface area of the working electrode was 2.64 mm^2. All electrochemical measurements were performed with an electrochemical analyzer at room temperature. The impedance spectra were recorded in 0.1 M KCl solution containing 5 mM of each $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ within the frequency range from 50 mHz to 100 kHz. The amplitude of the AC excitation voltage was set to 10 mV between working electrode and Ag/AgCl electrode, and the DC amplitude was around the open-circuit potential.

2.2 Synthesis of AuNPs

The working electrode of SPCE was first modified by electrodeposition of AuNPs using cyclic voltammetry method in order to create a monolayer of in situ AuNPs on the surface. In this process, $HAuCl_4$ was dissolved and diluted in 100 mM PBS to a final concentration of 100 µM. After that, 35 µL of this solution was dropped onto the chip surface covering all three electrodes. Then, during cyclic voltammetry a potential from - 600 to + 500 mV vs. Ag/AgCl was cycled for 10, 15 and 20 times at 50 mV/s scan rate. The cyclic voltammogram obtained during the electrodeposition showed that there is only one reduction peak of Au^{3+} to Au^0 was noticed at -0.4 V, inducing that AuNPs were deposited on electrode surface.

2.3 Synthesis of E2-imprinted MIP and non-imprinted NIP films

First, the AuNPs/SPCs were immersed into 25 mM of p-ATP ethanol solution and left in the dark at room temperature over night. In this step, a monomerlayer of p-ATP was self-assembled on the surface of the AuNPs/SPCE. This self-assembled monolayer of p-ATP is based on the strong Au-S bonds between the –SH (thiol) group of the p-ATP molecules and the Au surface. Next, E2 molecules were grafted on the surface of the electrode by immersing p-ATP self-assembled/AuNPs/SPCE into E2 solution at concentration of 1 mM for 6 h. Then, the E2 grafted/p-ATP self-assembled/AuNPs/SPCE was immersed into polymerization solution containing 1.25 mM E2, 6.25 mM p-ATP and 100 mM KCl and scanned by cyclic voltammetry (10, 15, 17 and 20 cycles) in the potential range from - 200 mV to + 600 mV vs. Ag/AgCl at a scan rate of 50 mV/s. The gradual formation of E2-imprinted polymer film on the surface of the working electrode was observed after each scanning cycle based on the obtained repretitive voltammograms. Finally, the E2 molecules were removed from the polymer matrix of E2-imprinted MIP film by electroelution. In this final step, 35 µL of HCl 1M was dropped onto the surface of E2-MIP/AuNPs/SPCE and applied a constant potential of +600 mV vs. Ag/AgCl for 600 s. During this condition, the positive constant potential breaks the hydrogen bonds between the protonated E2 molecules and the polymer matrix. Hence, E2 specific cavities forms in the polymer matrix that is complementary to the E2 templates in shape, size, and non-covalent binding functionalities [8].

Before using our fabricated E2 removal-MIP/AuNPs/SPCEs for E2 detection, the electrodes were stored in PBS 100 mM at 4 °C. For reference purpose, non-imprinted polymer (NIP)-modified electrodes were prepared in the same described process except the omission of E2 molecules in the initial electro-grafting and subsequent electro-polymerization steps.

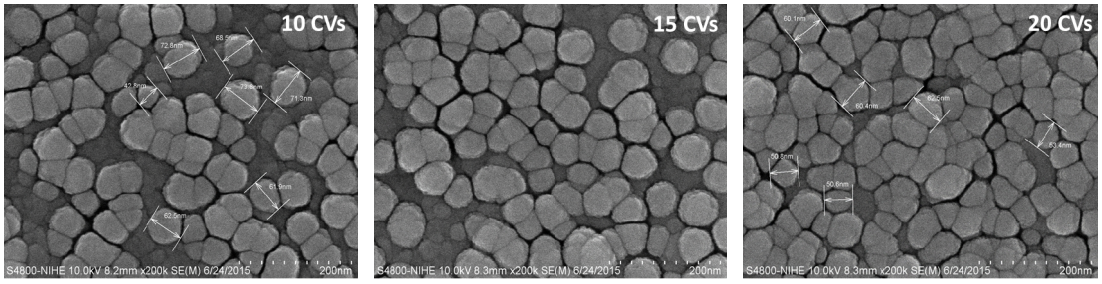

Fig. 1. Scanning electron microscopy (SEM) images of in situ AuNPs on SPCE formed using a cyclic voltammetry (CV) method for 10, 15 and 20 cycles.

3. Results and disscussion

3.1 Optimization of the preparation of AuNPs/SPC electrodes

It is well-known that substrates have strong impact on the forming of MIP films in their growth rate and thickness. For example, with the gold electrodes, a vast amount of p-ATP initiators can be activated to form oligomeric chains during the initiation step. This could be explained due to the large and high conductivity of Au surface. Therefore, in the case of AuNPs/SPCE, the chain propagation is expected to occur slowly. In other words, the performance of MIP/AuNPs/SPCE sensors will strongly depend on the initial in situ synthesis of the AuNPs on the electrode. In this process, the size and density of AuNPs are decisive for the morphology of the MIP membrane, affecting in turn the sensitivity of the sensor.

We investigated the size and density of AuNPs by changing the numbers of scanning cycles and by identifying the optimal concentration of $HAuCl_4$ [7, 9]. After 20 scanning cycles in 100 µM $HAuCl_4$ solution, the resulted SEM image of AuNPs showed an average diameter of 55 ± 7 nm and maximal areal density of AuNPs with clear inter-particle separation (see Figure 1).

To evaluate the operation of the sensors, faradaic impedance measurements were performed with AuNPs/SPCEs under 10, 15, and 20 cycles. The MIP coating on these AuNPs/SPCEs was uniformly prepared with 17 electro-polymerization cycles based on our optimization analysis. The impedance spectra in Fig. 2 showed that the percentage change of charge transfer resistance R_{CT} (% ΔR_{CT}) upon E2 rebinding was at the highest value of 93% for the AuNPs/SPCE at 20 cycles. Hence, the 20 cycles scanning was selected in our further experiments as the optimal value.

3.2 Optimization of the preparation of MIP film on AuNPs/SPCE

As discussed in Sec. 3.1, the uniform deposition of AuNPs was the precondition to form a MIP membrane on the electrode. Here, one has to search for a compromise in the sense that an ultrathin membrane can host only a limited number of binding sites while too thick MIP layers make it difficult to remove all template molecules. The layer thickness can be readily and stepwise controlled by the number of electro-polymerization scanning cycles. To study the effect of the number of cycles on the sensitivity of resulting sensor, four electrodes with AuNPs coverage at 20 cycles were used to form MIP layers under 10, 15, 17, and 20 electro-polymerization cycles. Fig. 3 showed the highest %ΔR_{CT} at 84.2% at 17 cycles after E2-removal/MIP/AuNPs/SPCE exposure to our selected E2 concentration of 100 fM. Our result showed that the MIP membrane reached its highest sensitivity at 17 polymerization cycles and this optimal cycle number can be used as the first good guess for sensor fabrication.

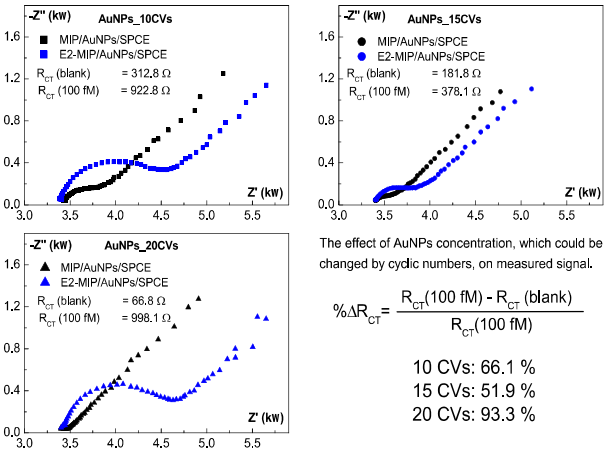

Fig. 2: Electrochemical impedance spectra obtained before (blank sample) and after E2 rebinding onto the surface of the E2 removal-MIP/AuNPs/SPCE sensors. In situ AuNPs were deposited on surface of SPCE using cyclic voltammetry at various cycle numbers (10, 15 and 20).

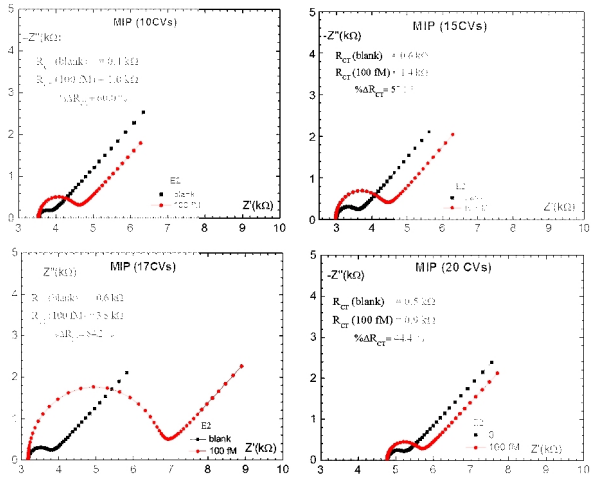

Fig. 3: Electrochemical impedance spectra obtained before and after E2-rebinding that occurred on surface of E2 removal-MIP/AuNPs/SPCE for the case of using AuNPs-modified electrode with 20 cycles. The MIP films were electropolymerized at various cycles (10, 15, 17 and 20).

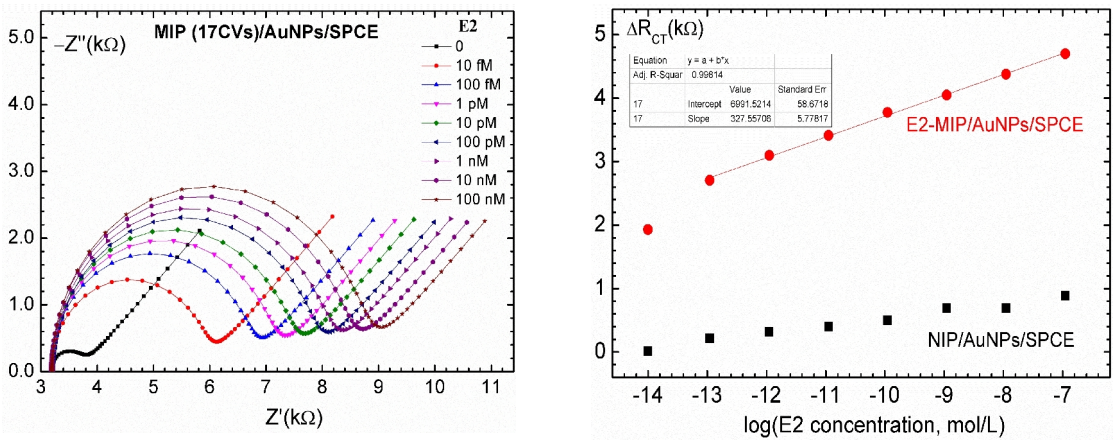

Fig. 4. Impedance spectra of a) MIP/AuNPs-modified electrode exposed to difference concentration of E2; and (b) the changes of the electron transfer resistance, R_{CT}, derived from the respective faradaic impedance spectra upon the sensing of E2 rebinding on the synthesized polymer (MIP and NIP) surfaces.

3.3 Reproducibility and characterization of MIP- and NIP dose-response curves

To evaluate the performance of the MIP-modified electrodes, the sensors were exposed to various concentrations of E2 (from 10 fM to 100 nM). The corresponding Nyquist plots of the impedance spectra were shown in Fig. 4. We observed that the semicircle diameter in the Nyquist plots (equal to the R_{CT}) increases significantly with increasing E2 concentration. This can be understood in a way that the rebinding of E2 molecules in the recognition cavities of the MIP film hinders the interfacial electron transfer, resulting correspondingly in an increased electron-transfer resistance. A dose-response curve was obtained by plotting the increase of R_{CT} as a function of the logarithm of the E2 concentration (Fig. 4). The linear range in this lin-log representation spans approximately two orders of magnitude (from 100 fM to 100 nM) and the calibration curve can therefore be described with the following empirical formula: ΔR_{CT} (kΩ) = 0.70 + 0.33* log(C) (mol/L) with high R^2 coefficient of 0.99814. The limit of quantification (LoQ) of this sensor for E2 detection was estimated to be 10 fM with a sensitive electrode area of 2.64 mm^2. In case of the non-imprinted reference chip (NIP), the R_{CT} increase was negligibly small (see Figure 4) since the NIP cannot serve for any specific recognition.

3.4 Cross selectivity towards potential competitor molecules

In order to evaluate the selectivity for E2 detection of the developed sensor, reference measurements were performed with estriol, testosterone, stigmasterol and cholesterol. Because their molecular structures and weights are very close to E2, they are ideal for stringent cross-selectivity tests. As showed in the Fig. 5, E2-removal/MIP/AuNPs/SPCE sensor had highest adsorption capability for E2 compared to the others in the cross-selectivity tests at 1 pM concentration. This is due to E2 specific cavities in the polymer matrix. The selectivity coefficients for E2 vs. the other are in the range from 8.4 to 11.5.

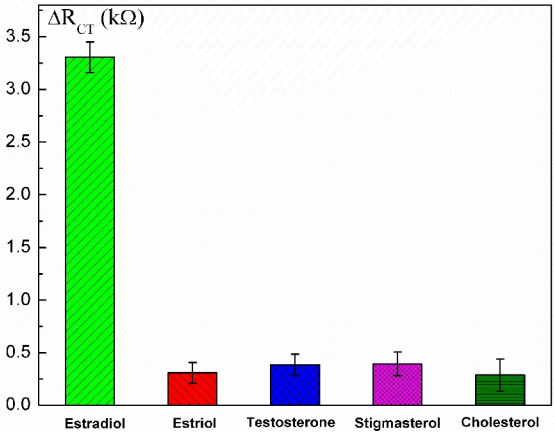

Fig. 5. Selectivity of the fabricated biosensor with an E2-imprinted film for different analytes including Estriol, Testosterone, Stigmasterol and Cholesterol.

4. Conclusions

In this study, we have reported on the development of a new sensor platform for the detection of E2. The resulted receptors for E2 were based on molecular imprinting with the electro-polymerization of p-ATP onto AuNPs/SPCE electrodes. After the investigation of the input parameters ($HAuCl_4$ concentration, scanning rate, and voltage regime), we found that the 20 cycles scanning

is the best observed value to obtain an almost coalesced AuNPs layer in which the Au particles are still clearly distinguishable with homogeneous size and shape. Our AuNPs-modified carbon electrodes were then used for the electro-polymerization of the imprinted p-ATP layer. We also showed that 17 scanning cycles is optimal to obtain a maximal signal sensor response, which creates a compromise MIP film thickness to obtain a sufficient number of receptor sites while still allowing for an efficient extraction of the template molecules.

The fabricated sensor showed the capability of detection limit below 10 fM in aqueous buffer solutions with a linear relationship in concentration range from 100 fM to 10 nM. The sensor has the advantages of low cost (cost of carbon ink printed electrode but can use as gold ink printed electrode), detection platform simplicity, and high reproducibility. Our study also found that EIS is a practical and applicable method for monitoring the rebinding of target molecules with their surrounding polymer matrix on the electrode surface. The sensor platform could be easily applied for detection of other small proteins, with selection of the detection platform based on the desired sensitivity.

Acknowledgments

This research is funded by Hanoi University of Science and Technology (HUST) under grant number T2015-267.

References

[1] M. Peeters, K. Eersels, T. Junkers, P. Wagner, Molecularly imprinted polymers: Synthetic receptors for diagnostic medical devices, in: S. Li, S.A. S. Cao, Piletsky, A.P.F. Turner (Eds.), Mol. Imprinted Catal. – Princ. Synth. Appl., Elsevier, Amsterdam (2016) 253-271.

[2] O. Brüggemann, Molecularly Imprinted Polymers: A New Dimension in Analytical Bioseparation, in: Synth. Polym. Biotechnol. Med., (2003) 134-161.

[3] A. Poma, A.P.F. Turner, S. a. Piletsky, Advances in the manufacture of MIP nanoparticles, Trends Biotechnol. 28 (2010) 629-637.

[4] G. Vasapollo, R. Del Sole, L. Mergola, M.R. Lazzoi, A. Scardino, S. Scorrano, et al., Molecularly Imprinted Polymers: Present and Future Prospective, Int. J. Mol. Sci. 12 (2011) 5908-5945.

[5] Y. Fuchiwaki, I. Kubo, Biomimetics Learning from Nature, InTech, 2010. doi:10.5772/198.

[6] X. Zhang, Y. Peng, J. Bai, B. Ning, S. Sun, X. Hong, et al., A novel electrochemical sensor based on electropolymerized molecularly imprinted polymer and gold nanomaterials amplification for estradiol detection, Sensors Actuators, B Chem. 200 (2014) 69-75.

[7] T.N.T. Lien, T.T. Nguyen, A.L.T. Luu, Y. Ukita, Y. Takamura, No Title, Proceeding 16th Int. Conf. Miniaturized Syst. Chem. Life Sci. Okinawa, Japan. (2012) 1912-1914.

[8] M.-F. Pan, G.-Z. Fang, B. Liu, K. Qian, S. Wang, Novel amperometric sensor using metolcarb-imprinted film as the recognition element on a gold electrode and its application., Anal. Chim. Acta. 690 (2011) 175-181.

[9] T.T.N. Lien, Y. Takamura, E. Tamiya, M.C. Vestergaard, Modified screen printed electrode for development of a highly sensitive label-free impedimetric immunosensor to detect amyloid beta peptides, Anal. Chim. Acta. 892 (2015) 69-76.