Journal of Pharmaceutical and Biomedical Analysis xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor

Leila Abbasy[a,b,c,1], Arezoo Mohammadzadeh[d,e,f,g,1], Mohammad Hasanzadeh[h,*], Nasrin Razmi[i]

[a] Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Hematology-Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, 51664, Iran
[f] Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[g] Endocrinology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[h] Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[i] Department of Science and Technology, Physics, Electronics and Mathematics Linköping University, Sweden

ARTICLE INFO

Article history:
Received 13 April 2020
Received in revised form 21 June 2020
Accepted 23 June 2020
Available online xxx

Keywords:
Biomedical analysis
Biomacromolecules
Binding affinity
Biosensor
Encapsulation
Prostate cancer.

ABSTRACT

In this study, electrically-conducting poly [Toluidine Blue (PTB)] was applied as artificial receptor. It was organized by molecular imprinting approaches and *via* electrochemical technique for the sensitive monitoring of prostate-specific antigen (PSA). The protein-imprinted PTB was electropolymerized in a pre-formed glutaraldehyde-cysteamine (GA-Cys A) matrix on the surface of gold electrode, which significantly boosted the stability against degradation of the Molecular Imprinted Polymer (MIP) on the surface of pre-modified gold electrode. Moreover, the MIP bio-receptor ability towards protein recognition was explored by some electrochemical techniques. The binding affinity of MIP system was considerably upper than that of non-imprinted polymer (NIP) system, indicating the success of the method in generating imprinted materials that was specifically use to PSA protein. The incubation of the MIP modified electrode in various concentration of PSA (from 1–60 μg/L) resulted in the increase of the $Fe(CN)_6^{3-/4-}$ redox peak current. The bio-device also showed linear response from 1–60 μg/L and LLOQ of 1 μg/L by using DPV technique, leading to PSA monitoring in clinical samples. The proposed MIP-based biosensor was satisfactorily applied to the determination of PSA in human plasma samples. Therefore, the developed bio-device provides a new approach for sensitive, simple, rapid, and cost-effective monitoring of 1 μg/L of PSA. Notably, this approach could appear as an appropriate candidate for point-of-care (POC) use in clinical and biomedical analyses.

1. Introduction

Molecular imprinting technology refers to a technique for providing synthetic polymers in which specific artificial recognition sites are created in the polymer for specific purposes. The shape and size of the sites or cavities resemble the molecular structure of the target molecule, enhancing the selectivity and affinity [1]. Recently, MIPs have attracted a lot of attention and have been applied in many fields such as drug delivery [2], capillary electrophoresis [3], enzyme-like catalysis [4], solid-phase extraction [5], and chemical sensors [6]. MIPs have been popular since 1972 [7] and have been considered as a simple, and highly selective approach for sensing. Owing to their easy preparation process and their capability of being used in different conditions, *e.g.,* at different temperatures [8], organic-aqueous solutions and different pHs, they are highly qualified as a candidate to be applied in molecular sensing

* Corresponding author.
E-mail address: hasanzadehm@tbzmed.ac.ir (M. Hasanzadeh).
[1] Leila Abbasy and Arezoo Mohammadzadeh have Equal contribution and are co-first author.

https://doi.org/10.1016/j.jpba.2020.113447
0731-7085/

and monitoring area [9]. To prepare molecular imprinting polymer (MIP), cross-linker [10], functional monomer [11], as well as initiator and template are the essential components [12,13]. According to the template-monomer binding interaction, MIPs can be synthesized *via* three major molecular imprinting approaches, including covalent molecular imprinting [14], semi-covalently [15], and non-covalent molecular imprinting [16,17]. Non-covalent imprinting [18] is the most commonly used one because of some advantages, such as its access to a variety of functional monomers, an easily conducted procedure through synthesis, fast connection kinetics, and removal of template [19]. After initial design of molecular imprinted polymer in the presence of the desired molecule, the target molecules are eluted from the matrix of the polymer by various washing methods [20]. So, the recognition sites should be specific for their target and the pores [21] are proportional to confirmation of template molecules [22]. MIPs can also be prepared for any type of target and according to the published reports, the best results for molecules have been obtained for molecular weight ranging between -200 to 1200 decagrams [23,24]. On the other hand, biological macromolecules such as proteins owing a large structure have some drawbacks due to the poor rebinding efficiency and highly cross-linked polymer networks; thus, the template mobility is limited by imprinted cavities [25]. To overcome the mentioned problem, the surface molecular imprinting helps us by reducing the mass transfer resistance [26,27]. The surface molecular imprinting technique can further solve the issues occurring in traditional MIP and lead to fast mass transfer and binding kinetics in macromolecules with large size [28,29].

In this study, electrically conducting poly [Toluidine Blue (PTB)] was applied as artificial receptor, organized by Molecular Imprinting approaches and *via* electrochemical technique to the sensitive monitoring of prostate-specific antigen (PSA). The protein-imprinted PTB was electropolymerized in a pre-formed glutaraldehyde-cysteamine (GA-Cys A) on the surface of gold electrode, which significantly boosted the stability against degradation of the Molecular Imprinted Polymer (MIP) on the surface of pre-modified gold electrode. The MIP bioreceptor ability towards the protein recognition was studied by some electrochemical techniques. The binding affinity of MIP system was found considerably higher than that of non-Imprinted Polymer (NIP) system, indicating the achievement of the method in generating imprinted materials to PSA recognition. Furthermore, the incubation of the MIP modified electrode in various concentration of PSA (from 0.005–60 μg/L) resulted in increment of the $Fe(CN)_6^{3-/4-}$ redox peak current. The bio-device also showed linear response from 0.005–60 μg/L and LLOQ of 0.005 μg/L by using DPV technique, leading to monitoring of the PSA in clinical samples. The proposed MIP-based biosensor was applied to the determination of PSA in human plasma samples with acceptable results. The developed biodevice will provide a new approach for sensitive, simple, rapid, and cost-effective monitoring of PSA. Notably, the approach can appear an appropriate candidate for point-of-care (PoC) use in clinical and biomedical analyses. To the best of our acknowledge, this is the first report on the MIP-based biosensing of PSA using P(TB) modified electrode.

2. Materials and methods

2.1. Chemical and reagents

Toluidine blue (C.I. 52040) was obtained from Merck Company (Germany). Potassium ferrocyanide $K_4Fe(CN)_6$, potassium ferricyanide $K_3Fe(CN)_6$, and KCl were purchased from Sigma-Aldrich (Ontario, Canada). HNO_3, HAS, and Hydrochloric acid (HCL) were also purchased from Scharlau Chemie S.A. Phosphate buffer saline (PBS) solution (0.1 M) was prepared by Na_2HPO_4 (0.1 M) and NaH_2PO_4 (0.1 M) in deionized water. We also acquired Human plasma samples from the Iranian Blood Transfusion Research Center (Tabriz, Iran). Biotinylated antibody, PSA, standard buffer, and dilute solutions were obtained from CanAg Diagnostic AB Technology Co., (Gothenburg, Sweden). Human CA 15-3 ELISA kit (CanAg/CA15-3 EIA) including CA 15-3 antigen, and Human Cancer Antigen CEA ELISA Kit including CEA antigen were purchased from CanAg Diagnostic AB technology Co., (Gothenburg, Sweden).

2.2. Apparatus

Electrochemical measurements were made by means of a computer-controlled electrochemical system comprising of AUTOLAB system with PGSTAT302 N (Eco Chemie, Utrecht, The Netherlands) by using NOVA 1.11 software. The conventional three-electrode electrochemical cell was used by gold electrode, Ag/AgCl, and a platinum wire as the working, reference, and counter electrodes, respectively. Electrochemical characterization of bio-imprinted polymer was carried out by CV technique using $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ (1 mM) in a potential range from -0.5 to 0.7 V, at a scan rate of 40 mV s^{-1}. The morphology of the electrode surface was characterized by a high-resolution field emission scanning electron microscope (FE-SEM, Hitachi-SU8020, Czech), with an operating voltage of 3 kV.

2.3. Electropolymerization of TB on the surface of gold electrode

The gold electrode surface was first cleaned by H_2SO_4 solution (0.1 M) and through applying cyclic voltammetry method in the potential range of 0–1.5 V, at scan rate of 100 mV/s using scan number of 15. Then, the electrode was incubated in a cysteamine solution (10 mM) for one hour. After drying, the Au electrode was incubated again with glutaraldehyde for another one hour. Next, the Au electrode was polymerized by using TBs in PBS solution (pH = 7.4) and *via* CV technique in the potential range of -0.5 to 0.7 V, at scan rate of 40 mV/s using scan number of 60 (Fig. 1). Finally, the Au electrode was polymerized once again by using KNO_3 (1 M) in PBS solution (pH = 7.4), with the aid of CV technique in the potential range of -0.3 to 0.5 V, at the scan rate of 40 mV/s, using scan number of 60.

2.4. Electropolymerization of TB in the presence of PSA antigen and fabrication of biosensors

The surface-related parameters, such as the dens of polymer film, amount of the used monomer/template, *etc*., were adjusted with the aim of increasing the analytical performance of the MIP-based biosensor for the selective and sensitive monitoring of PSA in the standard and human plasma samples. Regarding surface imprinting, the dens of polymer film should match the symmetrical size of anchored biological element (template). Due to large dimensions and high MW (≈34 kDa) of PSA, the electroconductive film was grown until extreme electro-catalytic behaviour appeared without decrease in site accessibility and bimolecular template extraction during re-binding step.

In this study, gold electrode surface was cleaned by H_2SO_4 solution (0.1 M) through employing cyclic voltammetry technique in the potential range of 0–1.5 V, at scan rate of 100 mV/s using the scan number of 15. Then, the electrode was incubated in cysteamine solution (10 mM) for one hour. After drying, the Au electrode was laid in glutaraldehyde for another one hour. Afterwards, the Au electrode was polymerized by using TBs in PBS solution (pH = 7.4), in the presence of PSA antigen *via* CV technique in the potential range of -0.5 to 0.7 V, at scan rate of 40 mV/s using scan number of 60. Later, the Au electrode was polymerized again by using KNO_3 (1 M) in PBS solution (pH = 7.4), and through CV technique in the

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447

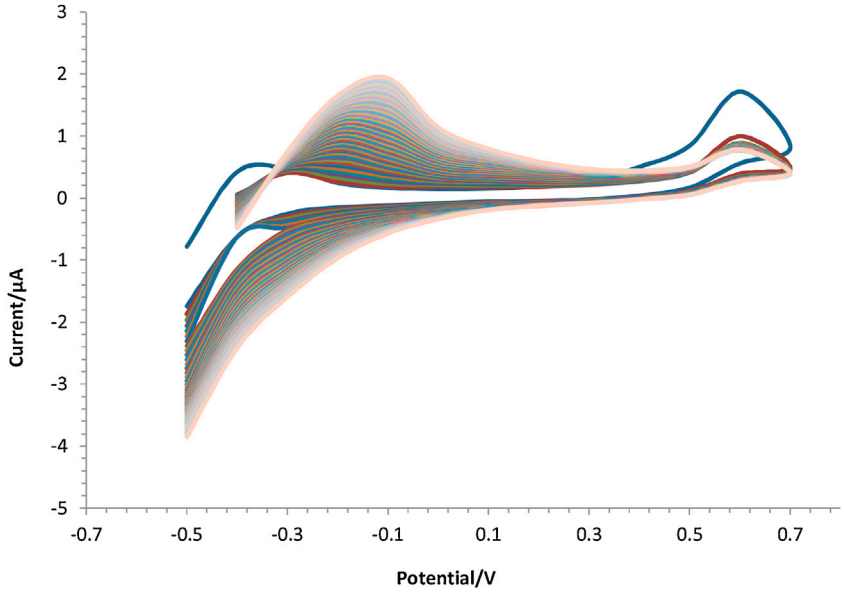

Fig. 1. Cyclic voltammogram of electropolymerization of TB on the surface of the gold electrode in the presence of 0.5 mM of TB in 0.1 M PBS (pH 7.4) at 40 mV/s^{-1} with 60 number of cycle.

potential range of -0.3 to 0.5 V, at scan rate of 40 mV/s using scan number of 60. In the end, the surface of the modified electrode was washed using 0.1 M NaOH solution and by CV technique in the potential range of -0.3 to 0.5 V, at scan rate of 50 mV/s using scan number of 100.

As shown in Fig. 2, the surface of the Au electrode is modified with cysteamine and glutaraldehyde as a cross-linker. Then, the surface of the electrode is modified by PTB in the presence of template (PSA). The PSA molecules are trapped into the PTBs matrix by an interaction between the C—OOH groups of the PSA and the $-NH_2$ groups of the PTBs film. TB is a small redox-active phenazine material which convey a positive charge with methyl or amine functional groups involved in the benzene rings and N substituted by S in the azine ring. Electrostatic interaction and numerous H-bonds between N—H/SH groups of TB and the OH/NH—— groups existing at PSA protein (antigen) might be responsible for the creation of the protein-polymer layer. At the final step, the biosensor is placed in 0.1 M NaOH solution for removal of the molecular template and preparation of MIP.

The obtained results showed the inhibition of P(TB) growth on the surface of gold electrode in the presence of biomacromolecule PSA, when compared with the electropolymerization of TB in the absence of template agent. Due to entrapment within the polymeric matrix of the non-conductive protein, a barrier might be created.

3. Results and discussion

3.1. Morphology and elementary analysis of bio-imprinted sensors

FE-SEM analysis was done to detect the morphology of PTBs/Au, PSA/PTBs/Au, and MIP/PTBs/Au electrodes.

As shown in Fig. S1 (see supporting information), the polymerization of TB leads to construction of a dendrite morphology on the surface of Au electrode, which provides highly dense PTBs for subsequent imprinting of the target molecule. The weight percentages of Elements, including carbon, nitrogen, and oxygen were obtained 4.77, 2.26, and 92.97, respectively by the EDX spectrum. The results showed that the considerable weight percentages of carbon, nitrogen, and oxygen were obtained as a result of the PTBs formed on the surface of gold electrode.

Fig. S2 (see supporting information) shows FESEM images of the modified electrode after the polymerization of PTB in the presence of PSA (template) on the surface of Au electrode. As shown in Fig. S2 (see supporting information), the variation of the morphology compared with the previous modification indicated a successful tramping of the biological template (PSA) on the surface of gold electrode in the electropolymerization process of TB. The result of FE-SEM was confirmed by EDS images.

On the other hand, Fig. S3 (see supporting information) shows that when the template is removed from the matrix of polymer, a film of PTBs with hollow cavities is appeared on the surface of the gold electrode, which demonstrates the successful formation of MIP-based biosensor.

3.2. Electrochemical characterization

The structural differences between the polymerized films either in the absence or presence of the template protein can be evaluated by using the blocking effect of the redox probe ($[Fe(CN)_6]^{3-/4}$).

According to Fig. 3, a high electrochemical blocking effect on the redox process of $Fe(CN)_6]^{3-/4-}$ was observed for both MIP and NIP modified electrodes. As can be seen, bare gold electrode has an oxidation peak in 0.1 V with the intensity of 17 μA. But, after preparation of MIP and NIP, the peak current decreased significantly and confirmed hindrance behavior of the surface-blocking redox process of Fe (III)/Fe (IV)]. Therefore, successful entrapment of protein within the polymeric matrix during electropolymerization is confirmed. It is also important to point out that the MIP hinderance is more operative than the NIP, because it combines both of the earlier effects.

Furthermore, the washing conditions applied for template (PSA) elimination were optimized in order to preserve the reliability of the imprinted sites and polymeric network, while eliminating as much template as possible. Here, we well-removed template from the MIP polymeric matrix after continuous potential cycling of the electrode surface between -0.3 and 0.5 V, at 100 mV.s^{-1}, in 0.1 M NaOH, over 100 cycles. Then, the electrode was washed with pure water. DPVs measurements in the presence of Fe $(CN)_6$ $^{3-/4-}$

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447

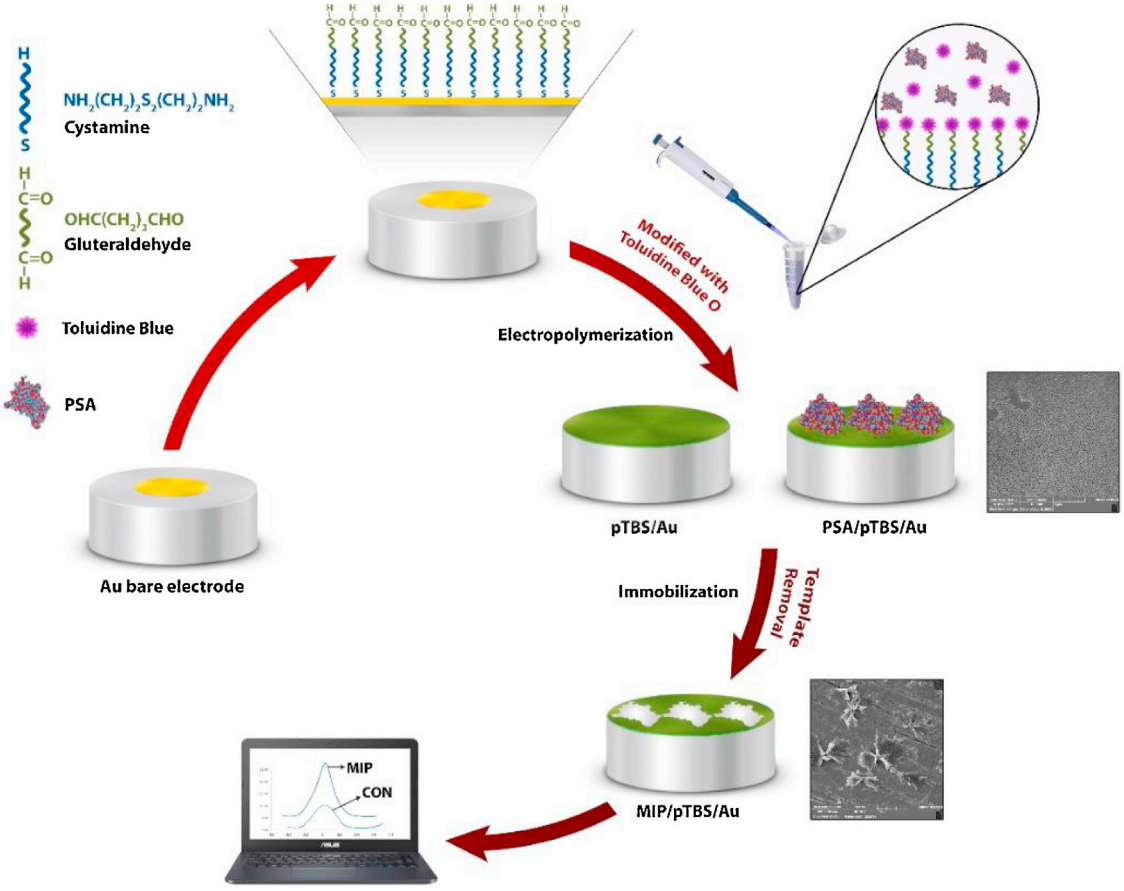

Fig. 2. The Steps to build a bio-imprinted electrochemical immunosensor for the recognition of PSA.

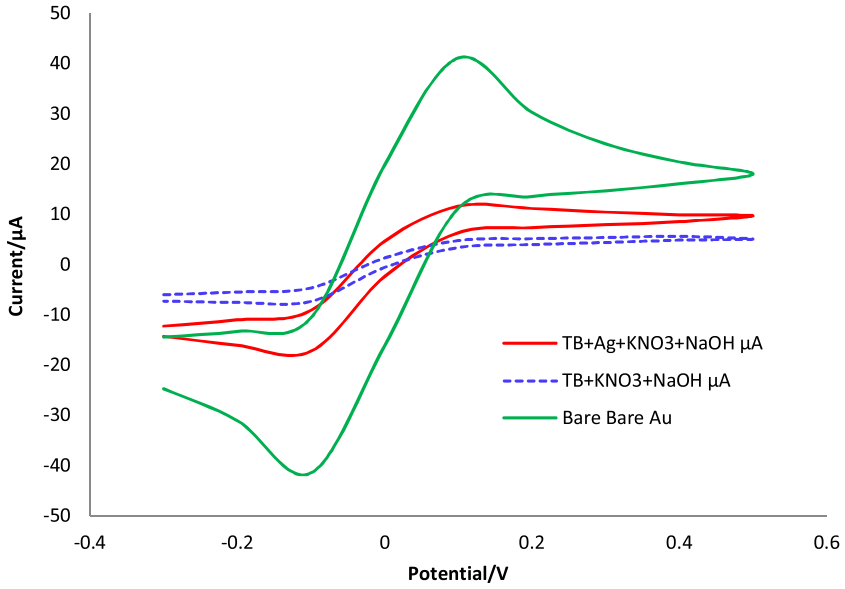

Fig. 3. Compares the CV of the bare gold electrode with gold electrode modified with 0.5 mM of TB and gold electrode modified with TB + PSA antigen. Potential Range = 0.5 to -0.3. Supporting media is 0. 1 M $[Fe(CN)_6]^{3-/4-}$ containing KCl.

(redox probe) were made at the MIP surface exhibited a significant increase of the peak current after template elimination from the imprinted surface (Fig. S4 (see supporting information)). Also, DPVs of NIP (Fig.7) revealed only a slight decrease of peak current regarding the $Fe(CN)_6^{3-/4-}$ after surface treatment with the 0.1 M NaOH, as extraction solution. According to the results, no serious damage in the polymer network was observed after the washing procedure.

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447

3.3. Electroanalytical characteristic of the PSA/PTBs/ gold electrode

DPV and CV techniques were applied to monitor the response of $Fe(CN)_6^{3-/4-}$ after binding of PSA to the receptor surface. As shown in Fig. 4, DPV responses in different concentrations of PSA (0.001–60 μg l^{-1}) is linear. According to Fig. 4, peak currents amplified with the increase of PSA concentration, which indicated the bio-electrocatalytical behaviour of MIP polymer.

Besides, the linear range was acquired as 0.001–60 μg.l^{-1} by DPV. Also, regression equation is as follows:

$$I(\mu A) = 0.2622C_{PSA} + 4.959 R^2 = 0.9805$$

The lower limit of quantification (LLOQ) for the detection of PSA using MIP-based biosensor was obtained as 0.001 μg l^{-1}.

3.4. Real sample analysis

PSA monitoring was carried out in the presence of potential biological-interfering agents to evaluate the ability of the engineered bio-MIP sensor. Human plasma sample was used for this purpose. Redox behaviour of $Fe(CN)_6^{3-/4-}$ was monitored by DPV upon binding of PSA to the imprinted surface. The prepared MIP-based biosensor was exploited for measuring PSA in human plasma by using CV and DPV techniques (Fig. 5).

Additionally, to evaluate the performance of bio-imprinted polymer, different concentrations of PSA antibody were spiked into human plasma samples and assessed by CVs and DPVs techniques. As shown in Fig. S5 (see supporting information), the designed MIP-based biosensor is able to determine PSA in the concentration range of one to 60 μg.L^{-1}. The calibration curve is also as follows:

$I(\mu A) = 0.1377\ C_{PSA} + 6.1528$, $R^2 = 0.9865$ by DPV

Moreover, the appropriate LLOQ was related to the best recognition ability of the imprinted sensor and the significant biocompatibility and electrocatalytical nature of P(TB). In this work, the LLOQ was lower than those previously reported methods, such as fluorometry based on microfluidic paper-based devices, spectrophotometric methods, and flow injection analysis (Table S1). The most important advantage of MIP is their tendency to bond with the pattern molecule. Other advantages of these polymers include:

I The created three-dimensional network, which causes the target molecule to be trapped. By washing the polymer, the target molecule is removed and cavity of the same size and shape are formed with the functional groups of the target molecule.
II Preparation of these polymers is very simple and low-cost.
III Surface imprinting with thin polymers is more efficient, with more improved mass transfer and overall greater sensitivity than conventional MIP systems.

Among the most commonly used MIP preparation procedures, such as drop-coating, composite making or spin-coating, *etc*., MIP films prepared by electrochemical polymerization have unique advantages, such as (I) simple and fast preparation, (II) high adherence to the electrode surface, (III) easy control of the film thickness and morphology, and (IV) high reproducibility in different conductive materials. Once polymerization is complete, the template molecule is subsequently extracted from the polymeric matrix using a suitable approach leaving specific recognition sites available for (re)binding.

Despite their electrocatalytic features and subsequent ability to form polymers under electropolymerization, phenazines have not been previously employed in the preparation of imprinted materials for proteins. These redox polymers are conductive since they retain monomer-type redox activity after polymerization, which makes them highly attractive in electroanalysis. On the other hand, toluidine blue, a member of the water-soluble azine-type redox dye family, has unique chemical and electrochemical properties that lead to its vast use, as an electronic mediator, in the construction of enzymatic electrodes for biosensing applications. At a sufficiently high electrode potential, electropolymerization of the dye on electrode surface occurs giving origin to effective 3D mediator structures with improved electrocatalytic activity, already tested in electroanalytical context towards some common redox bioactive compounds, such as NADH, nitric oxide, H_2O_2 and glucose. Although electropolymerization of phenazines can occur directly on bare electrode surfaces, sometimes, it is difficult to form polymer films with a good stability and space filling on metal surfaces due to the poor adhesion between the growing phase and the substrate, which inhibits the nucleation and the growth of film along the substrate. Alternatively, the polymer can be grown from well-organized self-assembled monolayers improving the adhesion of the polymer film to the electrode surface. The novelty of this work arises from the use of TB for the first time as the monomer to fabricate an electrically conductive MIP-based electrochemical sensor for detection of PSA. Fabrication of a disposable biosensing device is technically simple and time as well as cost of the analysis considerably reduce compared to the previously reported immunoassays used for PSA detection, suggesting the possibility of biomarker screening in PoC with good sensitivity and selectivity. In this work, the electroactive poly (TB) film was tethered to the Au surface *via* Au–S bonds due to the cross-linking procedure, which greatly enhances the stability of the polymer film at the surface of Au as suitable substrate.

3.5. Selectivity study

The most important and the main feature of a biosensor is its capability of detecting the molecular targets in the presence of other interfering species. The selectivity of the proposed MIP-based bio-sensor was investigated in the presence of CEA and HSA as interfering species. As shown in Fig. S7 (see supporting information), the intensity of peak currents of the MIP/PSAPTBs/Gold electrode is tested by CV and DPV techniques in the potential range of −0.3 to 0.5 V in $Fe(CN)_6^{3-/4-}$ containing KCl (0.1 M) solutions. The results showed that the special recognition of PSA antibody was higher than that of CEA and HSA as inference substances. Fig. S6 (see supporting information) also shows that the bio imprinted polymer has a high selectivity towards PSA due to the distinct molecular structure of imprinted cavities, which is proportional to the size and shape of the PSA antibody. However, before removal of template (PSA), there is no current for the target biomacromolecules [Fig. S7 (see supporting information)].

3.6. Stability study of PSA imprinted polymer

Stability of an ideal sensor is of great importance though its relevance certainly depends on the application. The most important features of MIPs are definitely their stability and in case of dealing with sensing assays, their capability of being repeatedly used for a longer period of time. The above mentioned properties of MIPs make them promising platform for the construction of biosensors. Furthermore, in this study, the stability of engineered bio-imprinted sensor was examined by DPV technique in 0.1 M of Fe $(CN)_6^{3-/4-}$ containing 0.1 M KCl. The signal variation result of the peak current of the bio-imprinted sensor after 24 h of being stored at 4 °C is depicted in Fig. S8 (see supporting information). A slight fall in the peak currents of the response was observed after 24 h, suggesting acceptable stability of the fabricated bioassay.

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447

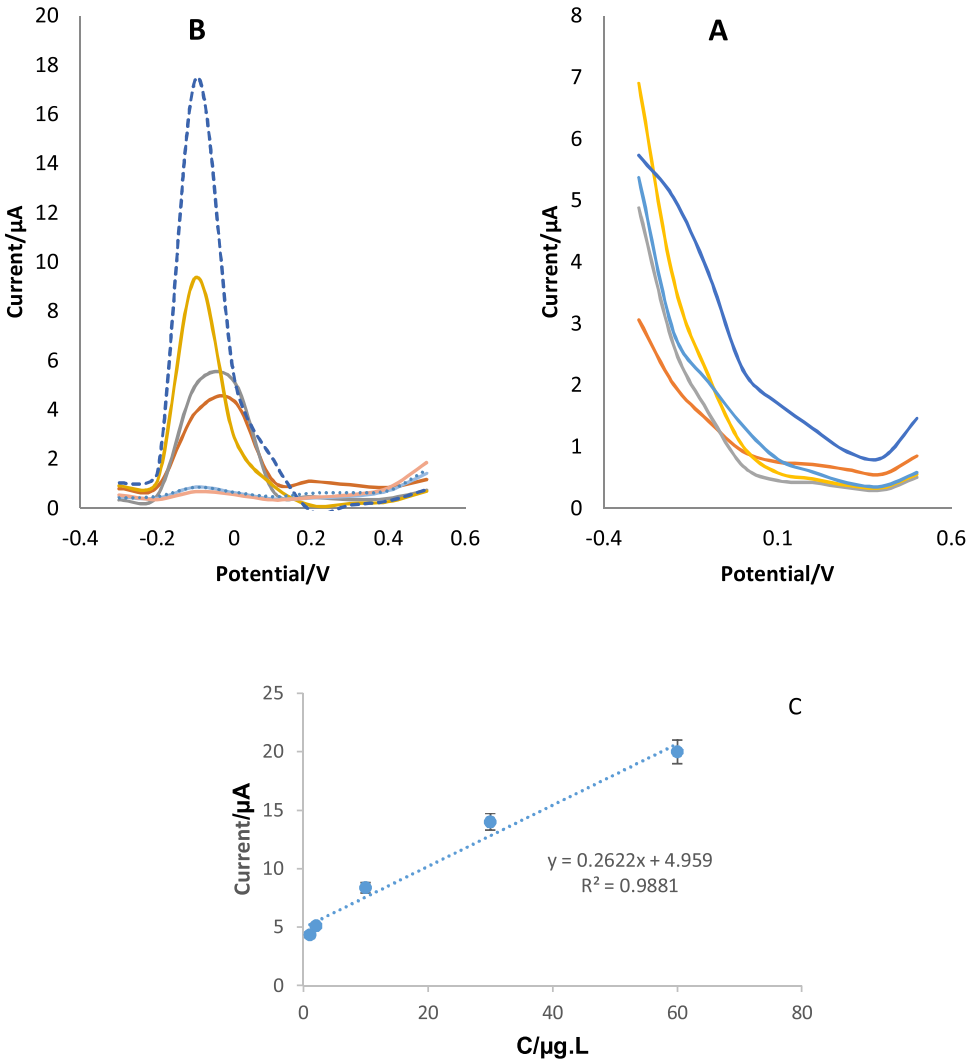

Fig. 4. DPVs of PSA/PTBs/gold electrode in different concentration of PSA (1,2,10,30,60 µg/mL) after (A) and before (B) elution in the solution 0.1 M $[Fe(CN)_6]^{3-/4-}$ containing KCl. Potential Range = 0.5 to -0.3. Scan Rate: 50 mV/s. Inset is calibration curve. (n = 3, SD = 1.78).

3.7. Reproducibility, precision, stability and regeneration of the immunosensor

The reproducibility of the immunosensor was assessed by fabricating five modified electrodes independently under the same experimental condition, and using them for detection of 1–60 µg/L of PSA. The relative standard deviations were 1.86 % and 1.94 % for detection of 1–60 µg/L of PSA, respectively. These results suggested the satisfactory fabrication reproducibility and precision of the immunosensor. The long-time stability of the immunosensor was also investigated by storing it at 4 °C in refrigerator. The response of the immunosensor for 60 µg/L of PSA after 14 days was about 96 % of its initial response. For 1 µg/L of PSA, the same stability was observed and after one week the immunosensor response was about 97.1 % of its initial response. Regeneration is a key factor for developing a practical immunosensor. After the immunosensor was used to detect PSA, the modified electrode was treated with 3 M of urea solution for 30 min to detach the antigen-antibody linkage. After rinsing with distilled water, we used the regenerated immunosensors to detect the same PSA concentration. The RSD of 1.22 % was obtained for four regenerated immunosensors in the PSA concentration of 60 µg/L. The effect of PSA concentration on reproducibility of the system was also investigated. In the presence of 1–60 µg/L of PSA for four regenerated immunosensors, the RSD were 2.11 % and 2.0 % respectively. This result indicated that the antibody has the same activity in a wide concentration range of PSA.

Table 1
PSA detection in plasma samples with the proposed immunosensor and ELISA as a reference method.

Sample	Suggested method/ µg/L	Reference Method (ELISA)/ µg/L
1	1.11	1
2	2.46	2
3	10.3	10
4	30.69	30
5	60.5	60

3.8. Application of Immunosensor for PSA Detection in Clinical plasma and Biopsy Samples (Accuracy of the proposed method)

The immunosensor was used for detection of PSA in five human plasma samples containing different concentrations of PSA using the standard addition method. The amount of PSA was examined by the proposed immunosensor and ELISA method (Table 1). As indicated, a favorable accuracy is observed between ELISA method and the proposed immunosensor.

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447

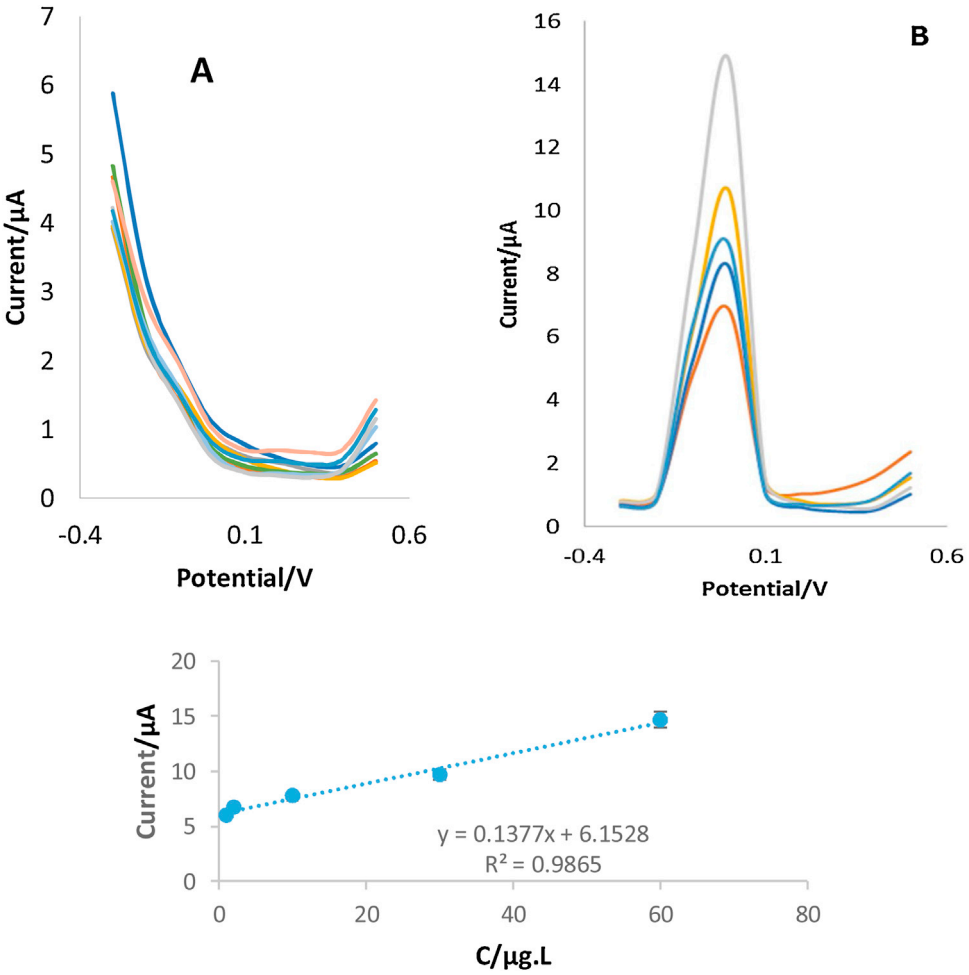

Fig. 5. DPVs of PSA/PTBs/gold electrode in different concentration of PSA (0.1,1,2,10,30,60 µg/mL) in human plasma samples after (A) and before (B) elution in the solution 0.1 M [Fe $(CN)_6$] $^{3-/4-}$ containing KCl. Potential Range = 0.5 to -0.3. Scan Rate: 50 mV/s. Inset is the calibration curve. C) Calibration curve. (n = 3, SD = 0.86).

A good agreement between immunosensor results and the obtained values by ELISA methods indicated the applicability of the proposed PSA immunosensor for the analysis of real biological samples. Furthermore, the immunosensor was used to determine secreted levels of PSA *in vitro* cell preparations. To do so, the extracted PSA from cancer cell biopsies was applied as real sample and the normal prostate tissue was used as control signal. Also, T-PER containing extracted PSA from approximately 3000 cancer cells of biopsy prostate cancer four different patients with varying PSA content were tested. The results showed that for prostate cancer samples the immunosensor response increased in comparison with normal human prostate cells, suggesting that the proposed immunodevice is suitable for determining of PSA in different cancer biopsies samples. So, the proposed immunosensor can be applied as a useful assay format for cancer diagnostics. In general, the developed immunodevices can provide a new strategy for label-free, rapid, simple and cost-effective screening of PSA compared to other reports. Furthermore, the inherent characteristics of the rigid used polymer may lead to robustness, long-term storage stability and potential re-usability of the prepared MIP biosensor. Overall, this approach seems to be suitable for point-of-care use in clinical context.

4. Conclusions

In this work, sensitive monitoring of prostate-specific antigen (PSA) was reported by applying the electrically conducting poly (Toluidine Blue (PTB)) as an artificial receptor, in combination of molecular Imprinting approaches and *via* electrochemical technique. The electropolymerization of the protein-imprinted PTB in a pre-formed glutaraldehyde on the gold electrode surface leaded to increase the stability against the degradation of the molecular imprinted polymer (MIP). Electrochemical techniques were also carried out to assess the ability of the MIP bioreceptor towards the protein recognition. Compared with non- Imprinted Polymer (NIP) system, the binding affinity of MIP system was remarkably higher. Greater binding affinity of the MIP assay made it more successful in generating imprinted materials that was specifically used to PSA protein. Different concentration of PSA (from 1–60 µg/L) were prepared for incubation of the MIP modified electrode and the results showed an increase in the $Fe(CN)_6$ $^{3-/4-}$ redox peak current. The results further revealed a wide linear range from 1–60 µg/L and LLOQ of 1 µg/L with a high affinity, selectivity and acceptable stability, suggesting the monitoring of the PSA in clinical samples. Acceptable results obtained by applying the bioassay scheme for PSA detection in human plasma samples. The developed biodevice is also likely to provide a new approach for sensitive, simple, rapid, and cost-effective monitoring of 1 µg/L of PSA. Moreover, the approach appears to be appropriate for point-of-care (POC) use in clinical and biomedical analysis. Also, changing the template molecules in MIPs could present a novel methodology for fabricating new biosensors to detect other target molecules.

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447

CRediT authorship contribution statement

Leila Abbasy: Writing - original draft. **Arezoo Mohammadzadeh:** Writing - original draft. **Mohammad Hasanzadeh:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

All the authors (L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh and N. Razmi) have read, approved and made substantial contributions for the manuscript. None of the original material contained in this manuscript has been previously published nor is currently under review for publication elsewhere. Besides, authors declare no conflict of interests and/or commercial products or companies.

Acknowledgements

We thank Tabriz University of Medical Sciences for instrumental supporting of this research.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2020.113447.

References

[1] S. Sardaremelli, H. Razmi, M. Hasanzadeh, N. Shadjou, Int. J. Biological Macromol. 145 (2020) 311–324.
[2] D. Schär, C.A. Ramseier, S. Eick, N.B. Arweiler, A. Sculean, G.E. Salvi, Clin. Oral Implants Res. 24 (2013) 104–110.
[3] E. Turiel, A. Martin-Esteban, Anal. Bioanal. Chem. 378 (2004) 1876–1886.
[4] K. Lettau, A. Warsinke, M. Katterle, B. Danielsson, F.W. Scheller, Angew. Chemie Int. Ed. 45 (2006) 6986–6990.
[5] I. Chianella, S. Piletsky, I. Tothill, B. Chen, A. Turner, Biosens. Bioelectron. 18 (2003) 119–127.
[6] L. Chen, S. Xu, J. Li, Chem. Soc. Rev. 40 (2011) 2922–2942.
[7] G.Z. Kyzas, D.N. Bikiaris, N.K. Lazaridis, Chem. Eng. J. 149 (2009) 263–272.
[8] J. Costanza, K. Pennell, J. Rossabi, B. Riha, Remediation of Chlorinated and Recalcitrant Compounds-2002, Paper 1F-08, 2002.
[9] H. Momeneh, M.B. Gholivand, Anal. Biochem. 557 (2018) 97–103.
[10] T. Muhammad, Z. Nur, E.V. Piletska, O. Yimit, S.A. Piletsky, Analyst 137 (2012) 2623–2628.
[11] K. Sreenivasan, J. Appl. Polym. Sci. 68 (1998) 1863–1866.
[12] O. Ramström, K. Mosbach, Curr. Opin. Chem. Biol. 3 (1999) 759–764.
[13] K. Flavin, M. Resmini, Anal. Bioanal. Chem. 393 (2009) 437–444.
[14] F.L. Dickert, O. Hayden, Trends Analyt. Chem. 18 (1999) 192–199.
[15] M. Cieplak, K. Szwabinska, M. Sosnowska, B.K. Chandra, P. Borowicz, K. Noworyta, F. D'Souza, W. Kutner, Biosens. Bioelectron. 74 (2015) 960–966.
[16] R. Schirhagl, Anal. Chem. 86 (2014) 250–261.
[17] M.J. Whitcombe, I. Chianella, L. Larcombe, S.A. Piletsky, J. Noble, R. Porter, A. Horgan, Chem. Soc. Rev. 40 (2011) 1547–1571.
[18] E. Caro, N. Masqué, R.M. Marcé, F. Borrull, P.A. Cormack, D.C. Sherrington, J. Chromatogr. A 963 (2002) 169–178.
[19] H. Yan, K.H. Row, Int. J. Mol. Sci. 7 (2006) 155–178.
[20] S. Pardeshi, R. Dhodapkar, A. Kumar, Food Chem. 146 (2014) 385–393.
[21] Q. Zhao, H. Li, Y. Xu, F. Zhang, J. Zhao, L. Wang, J. Hou, H. Ding, Y. Li, H. Jin, J. Chromatogr. A 1376 (2015) 26–34.
[22] G. Vasapollo, R.D. Sole, L. Mergola, M.R. Lazzoi, A. Scardino, S. Scorrano, G. Mele, Int. J. Mol. Sci. 12 (2011) 5908–5945.
[23] B. Mattiasson, L. Ye, Molecularly Imprinted Polymers in Biotechnology, Springer, 2015.
[24] C. Álvarez-Lorenzo, A. Concheiro, Handbook of Molecularly Imprinted Polymers, 2013, pp. 309–349.
[25] N.M. Maier, W. Lindner, Anal. Bioanal. Chem. 389 (2007) 377–397.
[26] H. Duan, X. Wang, Y. Wang, J. Li, Y. Sun, C. Luo, Sens. Actuators B Chem. 236 (2016) 44–51.
[27] Y. Liu, B. Cao, P. Jia, J. An, C. Luo, L. Ma, J. Chang, K. Pan, J. Phys. Chem. A 119 (2015) 6661–6667.
[28] Y. Tian, Y. Wang, S. Wu, Z. Sun, B. Gong, Int. J. Anal. Chem. 2018 (2018).
[29] Z. Wang, F. Li, J. Xia, L. Xia, F. Zhang, S. Bi, G. Shi, Y. Xia, J. Liu, Y. Li, Biosens. Bioelectron. 61 (2014) 391–396.

Please cite this article as: L. Abbasy, A. Mohammadzadeh, M. Hasanzadeh et al., Development of a reliable bioanalytical method based on prostate specific antigen trapping on the cavity of molecular imprinted polymer towards sensing of PSA using binding affinity of PSA-MIP receptor: A novel biosensor, J. Pharm. Biomed. Anal., https://doi.org/10.1016/j.jpba.2020.113447