Biosensors and Bioelectronics 26 (2011) 4177–4183

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Development of urinary albumin immunosensor based on colloidal AuNP and PVA

Kobra Omidfar*, Ahmad Dehdast, Hajar Zarei, Behnoush Khorsand Sourkohi, Bagher Larijani

Endocrine and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran

ARTICLE INFO

Article history:
Received 10 January 2011
Received in revised form 11 April 2011
Accepted 11 April 2011
Available online 16 April 2011

Keywords:
HSA immunonosensor
Poly vinyl alcohol
Conjugate gold nanoparticle- monoclonal Ab
Differential pulse voltammetry
Square wave voltammetry

ABSTRACT

The development of immunosensors with high sensitivity and specificity in detecting the pathogenic or physiologically relevant molecules in the body, offers a powerful opportunity in early diagnosis and treatment of diseases. In this study, we developed a new competitive immunosensor with employing antibody (Ab) labeled AuNP (Ab-AuNP) and PVA modified screen-printed carbon electrode (SPCE) surface to detect the urine albumin. Field emission scanning electron microscopy (FE-SEM) of modified electrode showed a suitable and stable attachment between HSA antigen- mAb and AuNP. Cyclic voltammetric (CV) method demonstrated that modification process was well performed. Electrochemical measurements including differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were employed for quantitative antigen detection. The electrochemical measurements performed with other proteins mixed with samples demonstrated a high specificity and selectivity for this biosensor in detecting the HSA. In optimal conditions, the immunosensor could detect HSA in a high linear range (from 2.5 to 200 μg/mL) with a low detection limit of 25 ng/mL. This new strategy could be improved and applied to detect the other antigen.

1. Introduction

Biosensors are the devices that combine a biological component with a physicochemical detector in order to detect an analyte. They have been the subject of great interest and progress in recent years, mostly because finding an effective mean of rapid and accurate analysis of biological samples for diagnosis of disease, is crucial. Immunosensors, as the biosensors designed based on the specific interaction between antigen and antibody, having practical benefits in the fields of medicine and basic sciences (Wei et al., 2010; Yang et al., 2010).

The serum albumin is a protein with molecular weight of 67,000 Da. This protein, which is the most abundant plasma protein in mammalian, is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. It also acts as a plasma carrier for transport of various molecules such as hormones, fatty acids, drugs, bilirubin and vitamins (Butrtis and Ashwood, 1999; Datta and Dasgupta, 2009).

In a healthy person, only a small proportion of the total protein excreted in the urine is albumin (up to 30 mg/day), while in many diseases, an increased rate of excretion of albumin by kidneys leads to the higher levels of presence of this protein in urine, a condition called albuminuria (Datta and Dasgupta, 2009). Albuminuria is an indicator of glomerular damage and is known as an independent risk factor for both renal and cardiovascular disease in diabetic and the general population. Hypertension, dyslipidemia, several immune disorders, vigorous exercise, hematuria, urinary tract infection, dehydration, and certain drugs, are associate with albuminuria (Matheson et al., 2010; Romundstad et al., 2002).

In clinic, the most common methods for measurement of urinary albumin are based on immunoassays techniques. The well established methods for quantitative measurement of the urine albumin are immunoturbidimetry (IT), fluoroimmunoassay (FIA), enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA) (Comper et al., 2004; Contois et al., 2006; Hoffmann et al., 2007; Matheson et al., 2010; Romundstad et al., 2002; Shaikh et al., 2008; Townsend, 1986).

However, these methods often require a long reaction time, special equipment and reagents multiple processing steps and professional staff. On the other hand, high-performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC–Mass) are among measurement techniques with high sensitivity for detection of urinary albumin. However, complicated and time-consuming sample pretreatment and sophisticated instrument requirement make them difficult to perform (Brinkman

* Corresponding author at: Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, P.O. Box 14395/1179, Tehran, Islamic Republic of Iran. Tel.: +98 21 88220037 38; fax: +98 21 88220052.
E-mail address: omidfar@tums.ac.ir (K. Omidfar).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.04.022

et al., 2004; Contois et al., 2006; Hoffmann et al., 2007; Shaikh et al., 2008).

In emergency cases, when the availability of specialized staff and instrumentation is limited, the biosensors could be an ideal and alternative tool for measuring the analyte in samples. Instrument independence, fast response, high sensitivity and specificity, low cost, and easiness of use and interpretation are among the benefits of biosensors (Piro et al., 2010; Wei et al., 2010; Yang et al., 2010).

Up to now, several array biosensors have been developed for detection of albumin in urine and blood samples. However, most of them are preliminary studies (Lowe, 1979; Muratsugu et al., 1993; Navratilova et al., 2001).

Recently, to increase the sensitivity of bioelectronic devices the metallic nanoparticles, in particular gold nanoparticles (AuNPs), have been introduced (Ho et al., 2009; Idegami et al., 2008; Leng et al., 2010; Wang et al., 2008).

In this study, we present a simple, yet novel, electrochemical immunosensor, constructed by antibody (Ab) labeled AuNP (Ab-AuNP) and poly vinyl alcohol (PVA) modified electrode for detecting urinary albumin at trace level. The variable properties of AuNPs make them versatile materials for development of electrochemical sensors in conjunction with disposable screen-printed carbon electrodes (SPCE).

The organic matrixes such as PVA with high biocompatibility and rigidity properties are suitable for immobilization of proteins. (Tsai et al., 2007; Yu et al., 2008).

In this study PVA was used as a sensor substrate for immobilization of albumin on the SPCE surface and the resulted modified electrode was studied electrochemically. The immobilized protein displayed good stability and electrocatalytic activity.

The process was followed by conjugation of a home-made mouse mAb against human serum albumin (HSA) to AuNPs. The detection process was based on the reaction of analyte with specific conjugated Ab to AuNPs. The electrochemical signal from the bound Ab-AuNP would be obtained after oxidization in 1 M HCl at 1.25 V for 40 s, followed by the reduction of $AuCl_4^-$ in differential pulse voltammetry (DPV) and square wave voltammetry (SWV) modes.

Using the oxidoreduction properties of the AuNPs in acidic medium, makes it possible to quantify the nanoparticles and in turn to quantify the analyte in the sample (Ho et al., 2010; Idegami et al., 2008).

2. Materials and methods

2.1. Reagents

$HAuCl_4$, trisodium citrate, sodium azide, RPMI 1640, fetal calf serum (FCS), streptomycin, penicillin, bovine serum albumin (BSA), human serum albumin (HSA), hemoglobin, protein G column and vinyl alcohol were all purchased from Sigma–Aldrich (St. Louis, MO, USA). The phosphate buffer solution (PBS) was consisted of 0.01 M phosphate buffered saline, 0.137 M NaCl, and 0.003 M KCl (pH 7.5). The analytical grade HCl was purchased from Merck (Merck, Spain). All the solutions were prepared in distilled water.

2.2. Apparatus

All the electrochemical measurements were performed by potentiostat with a connector for screen printed electrodes and SPCEs. A round-shaped graphite working electrode with 3 mm diameter, an Ag/AgCl reference electrode, and a graphite auxiliary electrode (Drop Sens, Spain) were included. In order to limit the area of interaction, we designed a single Teflon-based electrochemical cell with 5 mm diameter (50 μL volume) in the sensing area (Scheme S1, supplementary material). All the centrifugations were carried out by using the centrifuge instrument (Beckman, Avanti J-25I, England). Scanning electron microscope (SEM) image was applied for Ab-AuNP and electrode modification characterization using a JEOL JSM-7000F thermal-type field emission scanning electron microscope (Hitachi, Tokyo, Japan).

2.3. Production and purification of monoclonal antibody

Albumin mAb producing hybridoma cell lines were established from the fusion of mouse spleen lymphocyte and Sp2/0 myeloma cells as was explained previously (Omidfar et al., 2007).

The hybridoma cells were cultured in 50 mL flask containing 3% FCS in RPMI. The antibodies were purified from the culture supernatants via affinity chromatography on HiTrap protein G columns (Sigma–Aldrich, St. Louis, MO). The eluted antibodies were dialyzed against PBS and concentrated in Ulterafree-15 centrifugal filter units (Millipore, Billerica, MA).

2.4. Synthesis of Au NPs

Metal nanoparticle can be chemically synthesized by the reduction of a metal salt through a strong reducing agent in solution. In this study, 20 nm AuNPs were synthesized by reducing the tetrachloroauric acid in presence of reducing agent of trisodium citrate. In brief, 100 mL tetrachloroauric acid (0.01%, w/v $HAuCl_4$) was brought to boil and then a solution of 1% trisodium citrate was added with constant stirring. When the color of solution changed from yellow to wine red, (approximately after 8 min), it was cooled down to the room temperature. The pH was adjusted to 8.5 with concentrated sodium hydroxide and then sodium azide 0.01%, w/v was added. The obtained AuNP solution was stored at 4 °C in a dark glass bottle (Omidfar et al., 2010).

2.5. Preparation of Ab-AuNPs

For conjugation, 300 μg of HSA mAb (30 μg/mL, in PBS, pH 7.5) was added to 10 mL pH-adjusted colloid gold solution at room temperature. The solution remained under continuous overnight stirring at 4 °C. In order to block the residual surface of the colloidal AuNPs the BSA solution (10%) was added. The solution was stirred for 5 min at room temperature and then centrifuged (15,000 × *g*, 45 min, 4 °C). The pellet was separated and suspended into 10 mL PBS containing BSA (1%, w/v) and 0.05% sodium azide. Centrifugation was repeated and the pellet was finally re-suspended in 10 mL PBS and stored at 4 °C (Omidfar et al., 2010).

2.6. Fabrication of the immunosensors

Vinyl alcohol was dissolved in acetate buffer at 70 °C for 30 min to give different percents (wt.%) of homogeneous vinyl alcohol solutions. The process was followed by mixing the HSA with vinyl alcohol to obtain a homogeneous solution. In the next step, 5 μL of 2% vinyl alcohol containing 250 ng HSA was dropped on the electrode surface and then under UV condition (20 min, 220–250 nm) the vinyl alcohol was polymerized to give the PVA. This protocol allowed the HSA to be immobilized by physical entrapment in PVA pores at the electrode surface. To block the nonspecific-binding sites, the PVA-HSAs complex was incubated with PB (0.1 M, pH 7.0) containing 1% BSA and 0.05% tween 20 for 15 min at 37 °C. Then the modified working electrodes were rinsed by PBS and 0.05% tween 20 to remove the unbounded BSA from the electrode surface. Subsequently, 25 μL of Ab-AuNPs solution was spread on the electrodes and incubated for another 30 min at 37 °C. After the incubation, the unbound Ab-AuNPs were rinsed three times with PBS containing 0.05% tween 20.

2.7. Electrochemical measurements

A schematic representation of the operation of the electrochemical immunosensor for detection of HSA is provided in Scheme 1. The modified electrode was placed in electrochemical cell, and 50 μL of 1 M HCl solution was applied to the SPCE surface for electrochemical study. By employing the CV, DPV and SWV, the analytic signals were recorded. The basic study of electrode modification was carried out using CV, at the potential range of −0.7 to +1.1 V and the scan rate of 50 mV/s. The biosensor investigation was carried out by DPV and SWV methods. The preoxidation of AuNPs was performed at the constant potential of +1.25 V for 40 s for both DPV and SWV. The DPV measurements were done in scanning of potential range from 0.1 to 0.4 V with a step potential of 4 mV and pulse amplitude of 50 mV and pulse time 20 ms. The SWV measurements were performed in scanning of potential range from 0.0 to 0.4 V with a step potential of 4 mV and a pulse amplitude of 25 mV at a frequency of 15 Hz. The generated cathodic current was recorded as the analytical signals of AuNP oxidoreduction in presence of HCl. All the electrochemical measurements were performed at room temperature.

2.8. Intra- and inter-assay imprecision

In order to investigate the reproducibility of assay, three samples of known concentration (low and moderate) were tested for HSA in eight independent analytical runs.

3. Results and discussion

The purpose of this research was to combine the useful properties of nanomaterial and nonconductive polymer for development of protein electrochemical immunosensor. In this investigation PVA polymer and AuNPs were used to design a disposable electrochemical immunosensor with high sensitivity which can be offered for urinary albumin detection.

3.1. Surface characteristics of the PVA-modified SPCEs

To confer the electrode surface with desired biocompatibility and also to minimize the non specific adsorption, PVA was selected for immobilization of HSA onto the electrode due to its high biocompatibility and rigidity.

PVA is a non-toxic biocompatible synthetic polymer with exclusive features including flexible molecular chains, excellent adhesion to electrodes, and ductile nature. Indeed, PVA presents an unusual property through extremely mild freezing and thawing conditions. After the freeze–thaw process, the PVA chains would be physically cross-linked by semi permanent entanglement, molecular association, or crystallization and produce a suitable three-dimensional structure for immobilization of biological molecules. Therefore presentation of biological molecules, in this step to the PVA, would result to entrapment of molecules. Following physical entrapment and immobilization of biological molecules in pores of stable organic matrix, the phase weak interactions such as hydrogen bonding, Vander Wals, or electrostatic forces would occur between them (Tsai et al., 2007; Yu et al., 2008). Therefore, we selected PVA as a layer for improving the immobilized functional antigen molecules on the electrode surface.

For this purpose, we assessed the optimal concentration of PVA for stabilizing and also immobilizing the antigen on the electrode surface. The obtained cyclic voltamograms of the modified SPCEs with different concentrations of PVA are shown in Fig. 1. The results showed that the current response of PVA 1% was greater than 2%, 3%, 6% and 9% (Fig. 1). It decreases dramatically with increase in PVA

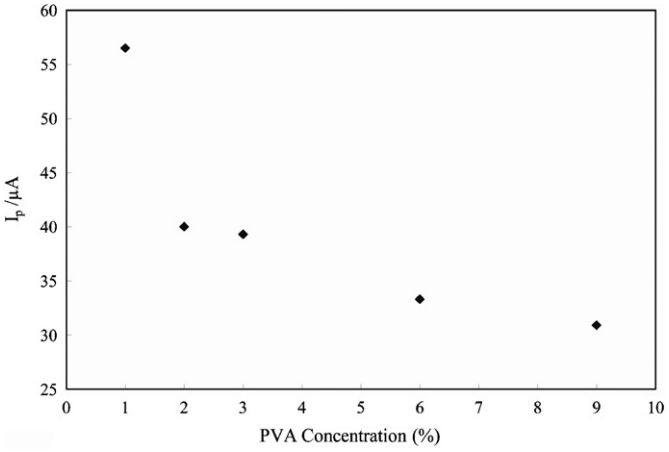


Fig. 1. Effect of the different concentrations of PVA 1%, 2%, 3%, 6% and 9% in the presence of HSA on the response of the immunosensor.

concentration due to the insulating properties of PVA. Subsequently a progressive decrease in signal intensity was observed in presence of PVA 6% and 9%, because of self-limiting electrodeposition of nonconducting polymers. These results indicate that high concentration of PVA is not suitable for modification of the electrode surface.

On the other hand, the current response difference between PVA 2% and 3% was negligible probably because of similarity in their intensity of current. However the surface coverage, HSA holding and stability of PVA 2% was better than PVA 3%. Based on these observations, the PVA 2% was selected for HSA immobilization and biosensor fabrication. All the CV measurements were carried out in 1 M HCl at scan rate of 50 mV/s.

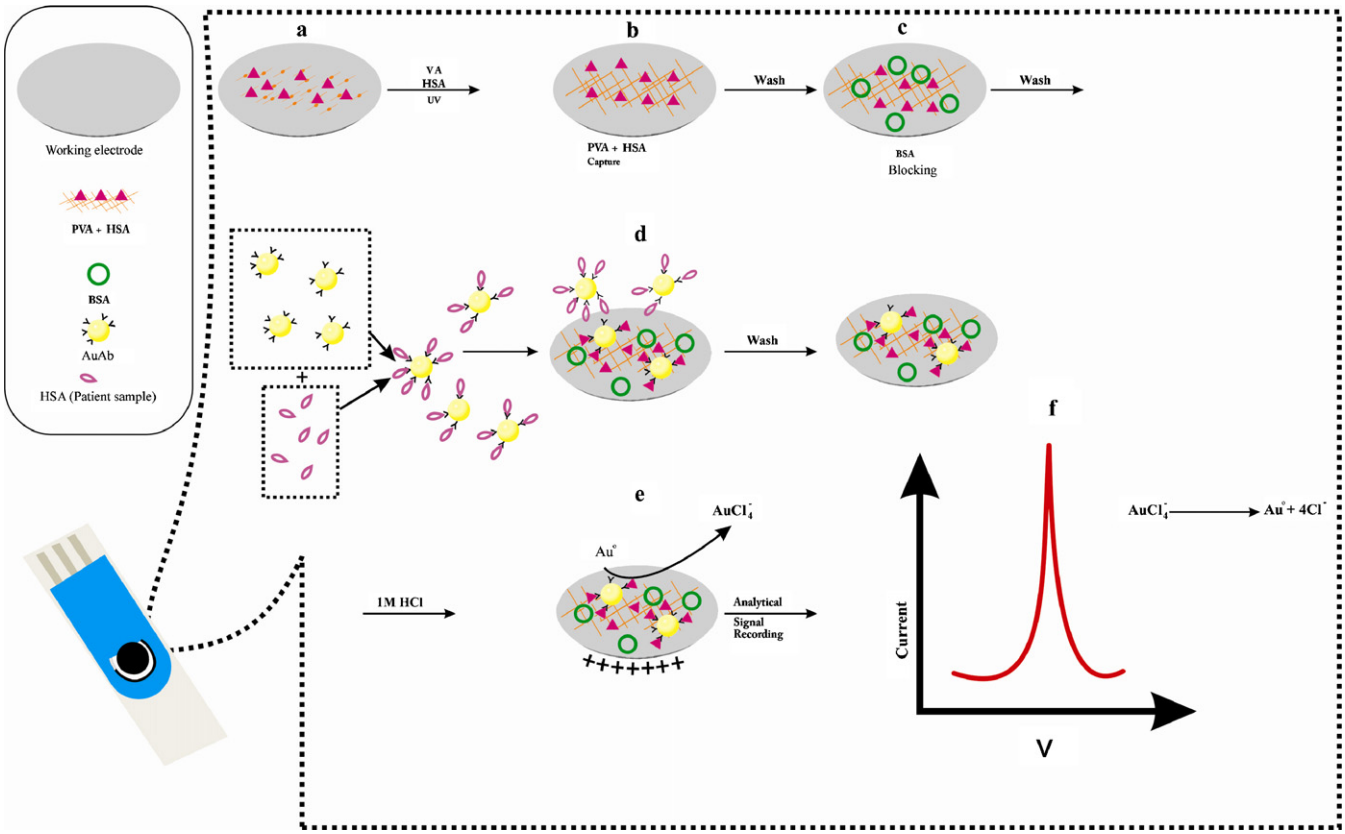
The protocol of Omidfar et al. (2002) was followed to test the over-time stability of the PVA containing HSA immobilized on the electrode surface. The results showed that the modified PVA-HSA electrode preserved its detective capacity after three months storage at 37 °C.

Furthermore, the efficiency of immobilization of antigen onto the electrode surface in the presence and absence of PVA were separately examined. We found that the antigen immobilization efficiency was approximately 18% greater than the bare electrode. This approach provides better stability and efficiency than those obtained by Ho et al. (2010) using PEG modified electrode. This finding implies that PVA layer on the electrode surface not only improves the stability and repeatability of modified electrode, but also increases the efficiency of immobilized antigen at the electrode surface, and leads to a greater number of functional epitopes available for recognition of the Ab-AuNP.

3.2. SEM imaging of immunosensor surface

The morphology of AuNPs, Ab-AuNPs complex surface, and modified electrode biocomposite surface film were investigated by electron microscopy. TEM observation of synthesized nanoparticle showed the spherical particles, indicating that AuNPs was formed during the chemical reduction process. The achieved average diameter of the colloidal gold particles obtained by UV–vis measurements and examination under the transmission electron microscope was 18–20 nm (Omidfar et al., 2010).

Fig. 2a and c showed the characteristics of modified SPCEs with PVA, PVA-HSA, and PVA-HSA-AuNPs-Ab, respectively using the SEM images. A dense electrode structure was observed



Scheme 1. Schematic illustration of a direct competitive electrochemical immunosensor fabrication process: (a) VA-HSA was dropped on the working electrode of SPCE, (b) photopolymerized VA under UV exposure (20 min, 220–250 nm), (c) BSA blocking, (d) incubation of the patient/standard samples with Ab-AuNPs, (e) the preoxidation of AuNPs was performed at the constant potential of +1.25 V in 1 M HCl, (f) and then, the current signal was recorded by the voltammetric modes.

in the absence of PVA (Fig. 2a). When HSA was introduced a rough structure, as an indicator of protein adsorption, was appeared (Fig. 2b). In addition, a suitable tree-dimensional structure was revealed for both PVA and PVA-HSA electrode (Fig. 2a and b). A rough structure with an array composed of radius and globular conjugated Ab-nanoparticle was observed on the modified PVA-HSA-AuNPs-Ab electrode (Fig. 2c), whereas the surface of the PVA-HSA and PVA modified SPCE did not show such a structure. Increasing the size (60–80 nm) of synthesized AuNPs after Ab conjugation, obtained by SEM image, confirmed the successful conjugation of mAb to AuNPs surfaces (Fig. 2d).

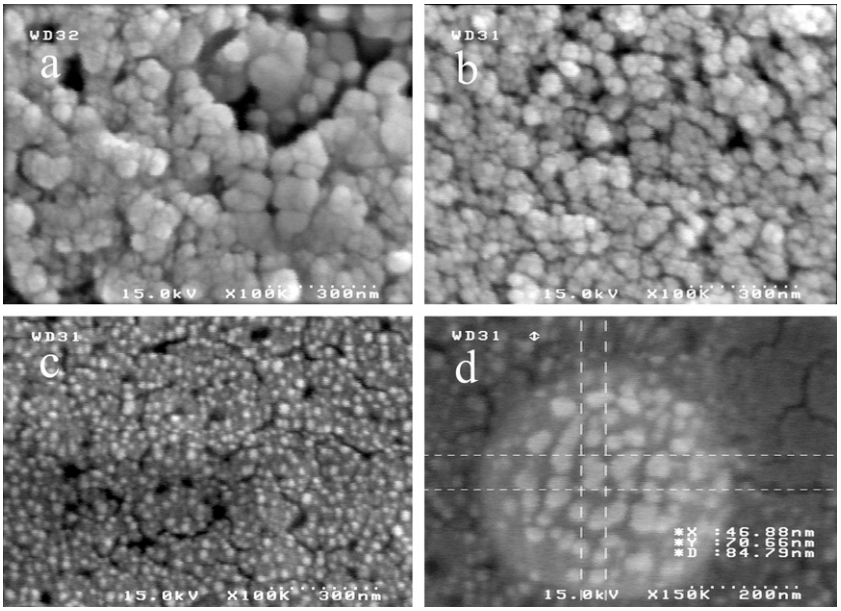


Fig. 2. SEM images of immuonosensor, (a) PVA, (b) PVA-HSA, (c) PVA-HSA-Ab-AuNPs modified electrode, and (d) size distribution of the AuNPs after the formation of the Ab-AuNPs.

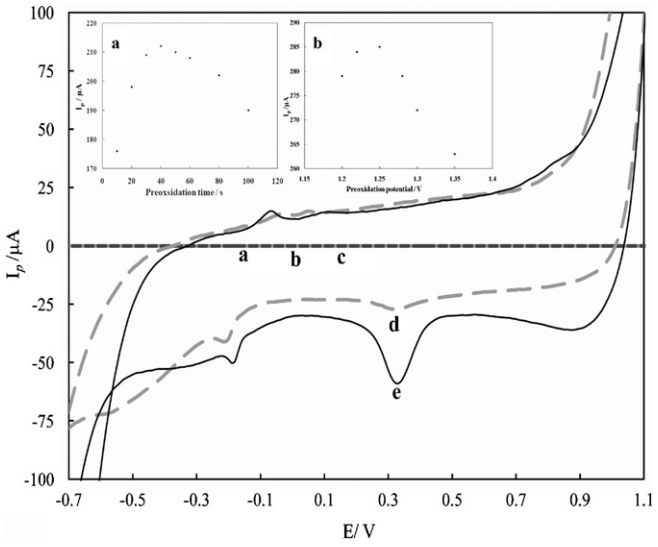


Fig. 3. Cyclic voltammograms of the different modified electrode measured in 1 M HCl at the potential range of −0.7 to +1.1 V and the scan rate of 50 mV/s: (a) bare electrode, (b) PVA, (c) PVA-HSA, (d) PVA-HSA-AuNP and (e) PVA-HSA-Ab-AuNP. Inset: effects of preoxidation time (10, 20, 30, 40, 50, 60, 70, 80, 90 s) (a) and potential (1.2, 1.22, 1.25, 1.27, 1.30, 1.35, 1.40 V) (b) on DPV response to 250 ng/mL HSA at the potential range.

3.3. Electrochemical optimization of the immunosensor

Fig. 3 displays the cyclic voltammograms of bare electrode and various modified SPCEs.

No signal was observed in the cyclic voltammograms of bare and PVA modified electrodes, mainly because PVA is a nonconductive polymer and no oxidoreduction reaction in the electrode surface would occur. To measure unspecific adsorption, 25 μL of AuNPs solution was dropped onto the modified PVA-HSA electrode and current response was recorded. We observed a weak signal from the immunosensor for PVA-HSA-AuNPs SPCE. This may be due to the non specific interaction between AuNP and PVA. As indicated in Fig. 3, a stronger signal was produced at 0.3 V in the presence of Ab-AuNP on the electrode surface which was generated by preoxidation of bound AuNP and subsequent reduction of $AuCl_4^-$ in electrochemical methods.

On the other hand, augmentation of AuNP concentrations on the electrode surface led to an increase in cathodic peak current in the CV method. The explanation would be that AuNPs with high conductivity could enhance the transfer of electron from surface to the electrode. Indeed, the electron transfer in electrochemical cell is caused by oxidoreduction property of AuNPs in presence of HCl. Taking together, these results showed that recorded current of our immuonosensor, might be greatly depend on the concentrations of immobilized AuNPs on electrode surface.

To optimize the condition time and potential, the following parameters were tested. At first, a constant potential (1.3 V) was selected according to the literature (Idegami et al., 2008). Second, the DPV measurements were carried out in deferent times ranging from 20 s to 100 s (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 s). Finally the optimal time was chosen based on the cathodic current peak and potentials of biosensor response (Inset (a) to Fig. 3). To select the optimized conditions potentials, the immunosernor was tested in various condition potentials ranging from 1.2 V to 1.4 V (1.2, 1.22, 1.25, 1.27, 1.30, 1.35, 1.40 V) and 1.25 V potential was chosen as optimized conditions potentials for DPV investigation of immunosensor (Inset (b) to Fig. 3).

In order to obtain the maximum electrochemical sensitivity, the optimal concentration of coating antigen (HSA) and Ab-AuNPs are needed. In this study, we used the direct competition ELISA rules, with the checkerboard titrations, to obtain this optimal concentration.

Therefore, the appropriate concentration of HSA and Ab-AuNPs were prepared. First, different concentrations of HSA (50, 100, 200, 250 and 300 ng) were immobilized in PVA 2% and dropped on the electrode surface. The proper amount of the antigen for the assay was determined as 250 ng. We then examined different volumes of Ab-AuNPs for preparing the immunosensor. The results suggested that the optimal amount of Ab-AuNPs was 20 μL.

The optimum reagent concentrations were defined as the concentration giving maximum current signal around 200–220 μA using the DPV mode in the absence of analyte with minimum reagent expenses. Different incubation times (20–60 min) were assessed in order to obtain the optimal time. The maximum generated currents achieved at 30 min incubation at 37 °C. In the literature, several combinations of immunoreagents are evaluated to select the most sensitive assay. In our study, the total time taken to complete the assay was approximately 80 min whereas the other approaches need longer time (Bonel et al., 2010; Ho et al., 2010; Idegami et al., 2008).

3.4. Analytical performance for the detection of HSA

The HSA immunosensor response is based on the conjugated Ab-AuNP and antigen reaction. The intensity of current response would decrease with increasing possibility of interaction between free HSA in high concentrations of standard and real samples and in conjugated Ab-AuNP compared with immobilized antigen and vice versa.

Here, the oxidation of AuNPs at high potential and the denaturation of the biomolecules in highly acidic condition were carried out simultaneously. Thus, the detachment of the possible biocomposite surface provided a large electroactive area for the oxidized Au ions to reduce back efficiently during the DPV and SWV modes. Additionally, the loss of the oxidized Au ions by diffusion could be avoided due to the negative charge of the chelated compounds with the high concentration of chloride ions in the acidic electrolyte. Finally, negatively charged Au chelates were attracted by constant application of the highly positive voltage, which resulted in promotion of electrodeposition on the PVA modified electrode.

Qualitative analyses were carried out by taking advantage of the DPV and SWV modes with different concentrations of HSA. The standard solution was prepared by diluting the albumin stock solution (1 mg/mL) with the normal urine samples to obtain the final concentrations of 0. 25, 0.50, 2.5, 10, 15, 20, 65, 80, 150, 180, and 200 μg/mL. The following experiments were obtained by placing a mixture of 5 μL of serial concentrations of standard solution and 20 μL of the Ab-AuNPs solution onto the surface of the electrodes and subsequently, holding the working electrode in condition potentials of +1.25 V for 40 s and then the DPV measurements carried out between +0.4 V and 0.1 V with scan rate of 50 mV/s (Fig. 4). The generated cathodic current was recorded as an electrochemical response. An increase in the peak current of DPV with diminution of HSA concentrations in standard samples was observed. The current response was increased from 0.025 to 200 μg/mL with detection limit of 0.025 μg/mL (Inset to Fig. 4).

Table S1 (supplementary material) summarizes the detection limit and the linear response range obtained by current study and previously reported methods. In comparison with some other analytical methods of urine albumin detection, such as HPLC (Shaikh et al., 2008), immunoassay (Shaikh et al., 2008), IT (Datta and Dasgupta, 2009), and piezoelectric immunosensor (Navratilova

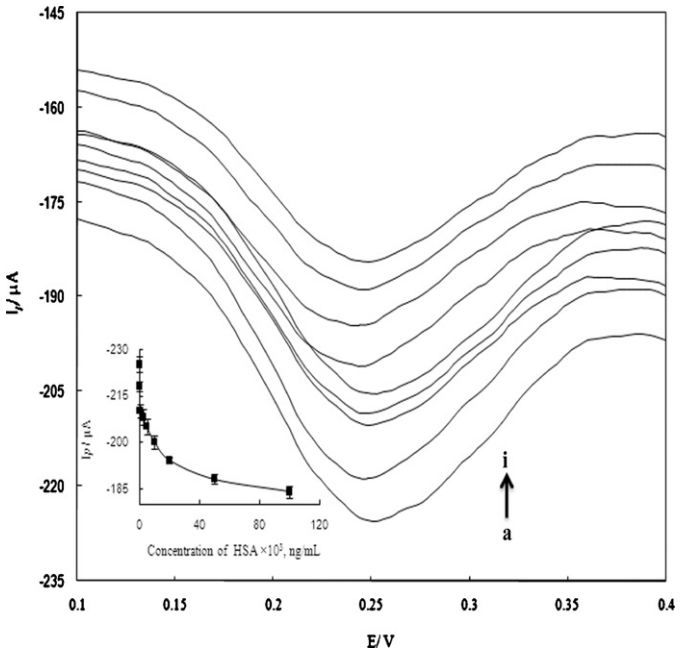


Fig. 4. Differential pulse voltammograms for the electrochemical detection of HSA upon serial dilutions of antigen ((a) 0. 25, (b) 0.50, (c) 2.5, (d) 10, (e) 20, (f) 80, (g) 150, (h) 180, and (i) 200 μg/mL) in 1 M HCl at scan rate of 50 mV/s. Inset: dose/response curve of HSA in urine using the PVA modified SPCEs. Each data are represents the mean of three replicates.

et al., 2001), our immunosensor shows lower detection limit and higher sensitivity.

The linear range of our immunosensor was between 2.5 and 200 μg/mL which compared to other electrochemical immunosensors is a wide linear range (Bonel et al., 2010; Ho et al., 2010).

The measurement was repeated with SWV method and similarity of the result obtained from SWV to DPV (Fig. S1 and Inset, supplementary material) confirmed that our designed HSA immuonosensor has a stable response and condition for detection of antigen with various electrochemical methods.

The measured reproducibility intra-assay CV (coefficient of variation) for low and moderate ranges of HSA were 3.0 and 3.5%, respectively, and the inter-assay reproducibility were 4.0 and 5.0%, respectively. Our goal was to describe an immunosensor with a detection range of ng and μg/mL. The PVA-modified electrode with Ab-AuNP assay can promise a biosensor with trace detection limit.

3.5. Specificity of the immuonosensor

The specificity of the immunosensor plays an important role in analyzing biologicals samples. Therefore the effects of possible interferences that might interfere with the determination of target analytes and affects the specificity of our immunosensor were investigated. The immunosensor was separately incubated with all the following substances at a concentration well above the maximum levels encountered in the clinical samples.

A solution of HSA (30 μg/mL) containing urea, creatinine, human hemoglobin, human immunoglobulin, glucose, tetracycline, ascorbic acid, cysteine and acetaminophen were prepared. Then the immunosensor was separately incubated with 30 μg/mL HSA solution, with and without interference. The comparison results showed no obvious peak currents changes (Table S2 and Fig. S2, supplementary material). This suggests that the immunosensor would display good specificity for determination of HSA.

Table 1
Comparison of urine albumin determination by immunosensor, IT, and CLIA methods. Data are presented as the mean of three replicates.

Samples	Methods			RSD(%)
	Immunosensor (μg/mL)	IT (μg/mL)	CLIA (μg/mL)	
1	9.5	10	10	5.2
2	2.5	2.65	2.75	6.6
3	5	5.25	5.15	5.5
4	10	10.5	10	5
6	33.1	35	34.2	5.7
7	20	20.6	21	3
8	50	52.2	51.2	4.4
9	180	200	189	5.5
10	37	35	35	5.4
13	63.4	58.2	60.2	8.2
14	55.6	51.2	53.2	7.9
15	97.2	87	92	9
16	121.6	124	114	1.9
17	150.1	141.3	145.3	6

IT, immunoturbidimetry; CLIA, chemiluminometric immunoassay, albumin concentrations < 30 μg/mL, normal; albumin between 30 and 299 μg/mL, microalbuminuria; albumin > 300 μg/mL, macroalbuminuria or clinical albuminuria. Urine samples with concentrations equal or higher than 180 μg/mL are diluted.

3.6. Response characteristics of the immunosensor

The reliability of our immunosensor was also examined by performing the test in 24 h urine samples collection and comparing the results with those obtained via IT and a home-made CLIA (chemiluminometric immunoassay) method (Table 1). In this study, we used the immunosensor, IT and CLIA to test 17 diabetic and control urine samples. An increase in the peak current of DPV with diminution of HSA concentrations in both real samples of normal and diabetic persons was observed (Table 1).

The relative standard deviations (RSDs) of the determination estimated from three repeated measurements for diabetic and control samples are shown in Table 1.

The results revealed that our proposed immunosensor had significant concordance with the two other methods in diagnostic judgment.

The excellent agreement from the obtained results within the uncertainty of the albumin demonstrates the suitability of the immunosensor for the screening of real samples.

4. Conclusion

In the present study, we made a new electrochemical immunosensor based on PVA and conjugated AuNP-Ab for quantitative measurement of urinary albumin. This one-step assay is based on the competitive immunochemical reaction between the free albumin in urine samples and immobilized albumin into the working electrode surface for the limited antibody sites.

High reproducibility, as well as high sensitivity and specificity of the test, was confirmed using samples from various patients as well as healthy controls. The detection limit of the test was approximately 25 ng/mL. This HSA immunosensor showed no cross-reactivity with other human proteins at the biological ranges. Furthermore, no interference in detection of albumin was observed in the presence of various human proteins and common medications, and also other substances that are likely to be present in the urine. These results suggest that the HSA immunosensor is user friendly, reliable, and highly specific for the detection of urinary albumin at the range of MAU. This model of immunosensor has a great potential for disease diagnostics and other biosensor applications.

Acknowledgment

This research was supported by a grant from Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences, Tehran, I.R. Iran.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.04.022.

References

Bonel, L., Vidal, J.C., Duato, P., Castillo, J.R., 2010. Anal. Methods 2, 335–341.
Brinkman, J.W., Bakker, S.J., Gansevoort, R.T., Hillege, H.L., Kema, I.P., Gans, R.O., de Jong, P.E., de Zeeuw, D., 2004. Kidney Int. (Suppl 92), S69–75.
Butrtis, C.A., Ashwood, E.R., 1999. Tietz Textbook of Clinical Chemistry, third ed. Sunders Company, Philadelphia, PA.
Contois, J.H., Hartigan, C.V., Rao, L., Snyder, L.M., Thompson, M.J., 2006. Clin. Chim. Acta 367, 150–155.
Comper, W.D., Osicka, T.M., Clark, M., Macisaac, R.J., Jerums, G., 2004. Kidney Int. 65, 1850–1855.
Datta, P., Dasgupta, A., 2009. J. Clin. Lab. Anal. 23, 314–318.
Hoffmann, M.M., Bugert, P., Seelhorst, U., Wellnitz, B., Winkelmann, B.R., Boehm, O., Ma¨rz, B.W., 2007. Clin. Chem. 53, 540–542.
Ho, J.A., Chang, H.C., Shih, N.Y., Wu, L.C., Chang, Y.F., Chen, C.C., Chou, C., 2010. Anal. Chem. 82, 5944–5950.
Ho, J.A., Chiu, J.K., Hong, J.C., Lin, C.C., Hwang, K.C., Reuben Hwu, J.R., 2009. J. Nanosci. Nanotechnol. 9, 2324–2329.
Idegami, K., Chikae, M., Kerman, K., Nagatani, N., Yuhi, T., Endo, T., Tamiyae, E., 2008. Electroanalysis 20, 14–21.
Leng, C., Lai, G., Yanb, F., Jua, H., 2010. Anal. Chim. Acta 666, 97–101.
Lowe, C.R., 1979. FEBS Lett. 106, 405–408.
Matheson, A.D.P., Willcox, M., Flanagan, J.J., Walsh, B., 2010. Diab. Metab. Res. Rev. 26, 150–171.
Muratsugu, M., Ohta, F., Miya, Y., Hosokawa, T., Kurosawa, S., Kamo, N., Ikeda, H., 1993. Anal. Chem. 65, 2933–2937.
Navratilova, I., Skladal, P., Viklicky, V., 2001. Talanta 55, 831–839.
Omidfar,.K., Kia, S., Kashania, S., Paknejad, M., Besharatie, A., Kashanian, S., Larijani, B., 2010. Appl. Biochem. Biotechnol. 160 (3), 843–855.
Omidfar, K., Kashanian, S., Paknejad, M., Kashanian, S., Larijani, B., Roshanfekr, H., 2007. Hybridoma 26, 217–222.
Omidfar, k., Rasaee, M.J., Zaraee, A.B., Amir, M.P., Rahbarizadeh, F., 2002. J. Immunoassay Immunochem. 23, 385–398.
Piro, B., Zhang, Q.D., Reisberg, S., Novel, V., Dang, L.A., Duc, H.T., Pham, M.C., 2010. Talanta 82, 608–612.
Romundstad, S., Holmen, J., Hallan, H., Kvenild, K., Kruger, Q., Midthjell, K., 2002. J. Intern. Med. 252, 164–172.
Shaikh, A., Seegmiller, C.J., Borland, M.T., Burns, E.B., Ladwig, M.P., Singh, J.R., Kumar, R., Larson, T.S., Lieske, J.C., 2008. Clin. Chem. 54, 1504–1510.
Townsend, J.C., 1986. Clin. Chem. 32, 1372–1374.
Tsai, Y.C., Huang, J.D., Chiu, C.C., 2007. Biosens. Bioelectron. 22, 3051–3056.
Wang, S., Zhang, X., Mao, X., Zeng, Q., Xu, H., Lin, Y., Chen, W., Liu, G., 2008. Nanotechnology 19, 435–501 (6 pp.).
Wei, Q., Li, T., Wang, G., Li, H., Qian, Z., Yang, M., 2010. Biomaterials 31, 7332–7339.
Yang, M., Li, H., Javadi, A., Gong, S., 2010. Biomaterials 31, 3281–3286.
Yu, J.J., Zhao, F.Q., Zeng, B.Z., 2008. J Solid State Electrochem. 12, 1167–1172.