Colorimetric immunosensor for determination of prostate specific antigen using surface plasmon resonance band of colloidal triangular shape gold nanoparticles

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Abstract

In this work, we demonstrated the development of a colorimetric immunosensor using surface plasmon resonance band of gold nanoparticles for the detection of prostate specific antigen (PSA). To develop this biosensing tool, triangular gold nanoparticles (AuNPs) were synthesized using Tween-20 as a nonionic surfactant and then, conjugated with PSA capture antibody (Ab1-AuNPs). When exposed to Ab1-AuNPs, PSA antigens were found to be successfully captured by nanosystem (PSA)-Ab1-AuNPs. Next, (PSA)-Ab1-AuNPs were incubated with second PSA antibody (2)-decorated magnetite (Fe3O4-Ab2) and separated by an external magnetic force to leave Ab1-AuNPs in the supernatant solution to be directly analyzed using UV–Vis spectroscopy. It was found that the absorption intensity was directly proportional to the PSA concentration. As a result, the linear range for PSA detection was found to be 0.01–20 ng mL−1 with a detection limit of 0.009 ng mL−1. Because of significant stability of the prepared Ab1-AuNPs and excellent selectivity to the PSA antigen, this simple and sensitive sensing system is proposed to be potentially effective in the fast and real-time analysis of clinical samples from prostate cancer patients. We believe that the simple platform of this immunosensor to be useful in the development of future point-of-care sensing tools, working on the quantification of biomarkers in a drop of blood.

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Keywords:
Colorimetric immunosensor
Sandwich assay
Surface plasmon resonance
Total prostate specific antigen
Magnetic nanoparticles

1. Introduction

Prostate specific antigen (PSA) is a glycoprotein that generally exists in healthy adult males below 4 ng mL−1, but clinically, abnormal levels of PSA is an important serum biomarker for early and differential diagnosis of benign prostatic hyperplasia (BPH) and prostate cancer and evaluation of patient’s response to chemotherapy [1,2]. In this way, it is really important to establish new sensing methods to approach simple, rapid and accurate detection of such biomarker for early-stage diagnosis of prostate cancer to avoid unpredictable metastasis.

Till now, a variety of assays such as chromatography [3], mass spectroscopy [4], fluorescence spectroscopy [5], nuclear magnetic resonance spectroscopy [6], white light reflectance spectroscopy [7], capillary electrophoresis [8], chemiluminescence [9], enzyme-linked immunosorbent assays (ELISA) [10], electrochemiluminescence [11,12], radioimmunoassay [13], time-resolved immunofluorometric assay [14], surface plasmon fluorescence immunoassay [15], bioluminescent immunooassay [16], electrochemical [17], surface-enhanced Raman scattering [18], and microcantilever method [19] have been developed for the determination of PSA. These methods unfortunately encounter with challenges for clinical applications, necessitating skilled personnel working with expensive, time-consuming and complex technology [20–22].

In attempt to removing such limitations, sandwich-type colorimetric immunoassays based on lateral surface plasmon resonance as a noninvasive strategy have represented capacity of simplicity, low-cost translation, and tailoring to point-of-care applications.

With introduction of nanomedicine, a convergence of nanoscience and medicine, nanomaterials have attracted great attention in all fields...
of science [23–28]. Of various nanoparticle systems exploited in the era of bioanalytical sciences, gold nanoparticles with different sizes and shapes have been successfully used as a sensing probe for sensitive analysis of cancer biomarkers [29,30]. Due to having unique and tunable surface plasmon resonance (SPR) properties, gold nanoparticles (AuNPs) have been the subject of intensive research on UV–Vis extinction bands. When the frequency of an incident photon with the conduction electrons of AuNPs is in proper resonance, SPR as wavelength-selective absorption with extremely large molar extinction coefficients can be induced [31].

Due to having a narrow plasmon peak, stability in the complex system of biological samples, and greater molar extinction coefficient, non-spherical gold nanoparticles especially triangular AuNPs are prone to improve sensitivity of detection and to lower the limit of detection. Triangular AuNPs significantly exhibit excellent stability at high salt concentrations (0.1 mol L\(^{-1}\)), and are laborious to separate [31]. To reach efficient separation, application of magnetic nanoparticles in connection with gold nanoparticles leads to approaching lower detection limits and fast responsive biosensors [32]. In the field of bio-separation, Magnetic nanoparticles are defined as core nanomaterials and magnetite (Fe\(_3\)O\(_4\)) nanoparticles are generally used to magnetically isolate proteins, DNA, viruses and even whole mammalian cells [33].

In this work, we demonstrate the development of a colorimetric sandwich-type immunosensor for detection of PSA using lateral surface plasmon resonance of triangular AuNPs and magnetic force separation of Fe\(_3\)O\(_4\) NPs decorated with PSA-specific antibodies (Ab\(_1\)-AuNPs and Fe\(_3\)O\(_4\)-Ab\(_2\)).

2. Materials and methods

2.1. Chemicals

TWEEN-20, NaOH, HCl, KH\(_2\)PO\(_4\), and KH\(_2\)PO\(_4\) were purchased from Merck (Darmstadt, Germany). 3-(Trimethoxyethyl)propylamine (APTS), acetic acid, hydrogen tetrachloroaurate (HAuCl\(_4\)3H\(_2\)O, 99.5%), magnetite (Fe\(_3\)O\(_4\)) and hexane were obtained from Sigma-Aldrich company. (St. Louis, Missouri, United States). Monoclonal anti-PSA antibodies (Ab\(_2\) (1H12, epitope 4) and Ab\(_2\) (5A6, epitope 6)) were obtained from HyTest (Turku, Finland). PSA ELISA kit was purchased from (R&D Systems, Minneapolis, MN, USA).

In our work, we demonstrate the development of a colorimetric sandwich-type immunosensor for detection of PSA using lateral surface plasmon resonance of triangular AuNPs and magnetic force separation of Fe\(_3\)O\(_4\) NPs decorated with PSA-specific antibodies (Ab\(_1\)-AuNPs and Fe\(_3\)O\(_4\)-Ab\(_2\)).

2.2. Apparatus

UV–Vis spectroscopy was performed on a Perkin–Elmer Lambda 25 spectrophotometer with the use of semi-micro spectrophotometric cuvettes. Transmission electron microscopy (TEM) images were recorded using a Philips CM10 TEM (USA) at an accelerating voltage of 80 kV. The size distributions of the nanoparticles were obtained using Nanotrac Wave™ (Microtrac, San Diego, CA, USA). The size of nanoparticles was calculated by fitting the data to a polydisperse model using the Dynamics software version 5.26 (Microtrac, San Diego, CA, USA).

2.3. Preparation of Ab\(_1\)-AuNPs

The AuNPs were prepared based on our previously reported method [31]. Briefly, 4 mL of Tween-20 solution 20% (v/v) in PBS (pH = 7.0) was added to 1 mL of HAuCl\(_4\) (10 mmol L\(^{-1}\)). Carboxylic functional groups of Tween-20 were activated using a solution containing EDC (20 mg mL\(^{-1}\)) and NHS (10 mg mL\(^{-1}\)) in borate buffer (pH 9) under shaking (60 rpm) at room temperature and dark for 30 min.

To covalently attach AuNPs with Ab\(_1\), the activated nanoparticles were incubated with Ab\(_1\) (50 μg mL\(^{-1}\)) solution at 8 °C and dark under shaking (60 rpm) for 24 h. After washing with Tween-20 solution 20% (v/v) to remove unreacted Ab\(_1\), the antibody-conjugated Au NPs (Ab\(_1\)-AuNPs) were centrifuged at 4000 × g for 5 min, and finally the precipitation was finally dispersed and stored in a solution containing 0.1% sodium azide at 4 °C for next applications.

2.4. Preparation of Ab\(_2\)-Fe\(_3\)O\(_4\)

The Fe\(_3\)O\(_4\) NPs were prepared by the silanization method [34]. Initially, in a glass container under ambient conditions, 0.5% (v/v) APTS solution was added to a dispersion of Fe\(_3\)O\(_4\) solution (0.5 mg mL\(^{-1}\)) in hexane containing 0.01% (v/v) acetic acid. The mixture was shaken for 72 h during which the nanoparticles were precipitated. The APTS-modified Fe\(_3\)O\(_4\) NPs (APTS-Fe\(_3\)O\(_4\)) were separated using an external magnet and then washed with hexane three times to remove all unreacted silanes. The product was finally redispersed in deionized (DI) water.

Carboxylic functional groups of APTS-Fe\(_3\)O\(_4\) were activated using a solution containing EDC (20 mg mL\(^{-1}\)) and NHS (10 mg mL\(^{-1}\)) in borate buffer (pH 9) under shaking (60 rpm) at room temperature and dark for 30 min.

To covalently attach APTS-Fe\(_3\)O\(_4\) (0.2 mg mL\(^{-1}\)) NPs with Ab\(_2\), the activated NPs were incubated with Ab\(_2\) (50 μg mL\(^{-1}\)) solution at 8 °C and dark under shaking (60 rpm) for 24 h. The prepared Fe\(_3\)O\(_4\)-Ab\(_2\) NPs were separated using an external magnet and then washed with DI water three times to remove all unconjugated Ab\(_2\) molecules. The final product was dispersed in PBS buffer (10 mmol L\(^{-1}\)) containing 0.1% sodium azide and stored at 4 °C for next applications.

2.5. Determination of PSA

In order to analyze PSA, 1.5 mL of standard solutions (1 mmol L\(^{-1}\) PBS, pH = 7.4) containing distinctive content of PSA were incubated with 50 μL of Ab\(_1\)-AuNPs (0.1 mg mL\(^{-1}\)). The shaker incubator with a rotation speed of 200 rpm at 25 °C for 70 min was used for the completion of antigen–antibody reactions.

Afterward, 50 μL of Fe\(_3\)O\(_4\)-Ab\(_2\) suspension was added and incubated with a rotation speed of 200 rpm on a shaker at 25 °C for 60 min. After magnetic separation (MS) of the formed immune-complexes (Fe\(_3\)O\(_4\)-Ab\(_2\)-(PSA)-Ab\(_1\)-AuNPs), a portion of the supernatant was directly transferred into semi-micro spectrophotometer cuvettes for measurement of absorption.

The absorptions were recorded at 525 nm as the λ\(_{\text{max}}\) of Au NPs SPR. Final absorption was calculated by subtracting the absorption of the corresponding blank samples. In order to confirm the reproducibility, each immunoassay had to be completed in triplicate. Scheme 1 illustrates the principle of the immunosensor development.

2.6. Serum sample analysis

The potential basic and clinical applicability of the immunosensor was tested for the determination PSA in blood serum samples obtained from healthy and patient volunteers. The results of the immunosensor were compared with those of ELISA method. The samples were diluted 2-fold by 1 mmol L\(^{-1}\) PBS (pH 7.0) before analysis.

3. Results and discussion

3.1. Characteristics of immunosensor

3.1.1. UV-Vis spectroscopy analysis

The engineered immunosensor was first characterized by UV–Vis spectroscopy. As shown in Fig. 1, Panel A, the absorption of bare Fe\(_3\)O\(_4\) (APTS-Fe304), Fe304-Ab\(_2\), and Fe304-Ab\(_2\)-(PSA) nanoparticles were the measurement in the range of 200–900 nm. After immobilization of Ab\(_2\) on Fe304, an obvious absorption peak appeared at 300 nm.
(curve b), which can be an indicator of Ab₂ immobilization on the surface of Fe₃O₄ NPs. After the interaction of PSA and Fe₃O₄-Ab₂ (curve c), the slight bathochromic shift in plasmon band position confirms the formation of immuno-complex.

As shown in Fig. 1, Panel B, the bare AuNPs exhibit an absorption peak at 515 nm, ascribing to SPR of AuNPs (curve j). After being coated with antibodies (Ab₁-AuNPs) (curve k), an obvious absorption peak appeared at 300 nm, indicating the successful immobilization of Ab₁ on the surface of AuNPs and red-shifting of SPR peak from 515 to 525 nm due to the increase in the hydrodynamic diameter of AuNPs. Moreover, the results clearly indicated immune-complexation of PSA and Ab₁-AuNPs (Fig. 1, Panel C, curve e) resulted in an obvious absorption peak at 620 nm, which can be due to the aggregation of Ab₁-AuNPs. After interaction of Fe₃O₄-Ab₂ and (PSA)-Ab₁-AuNPs, the SPR peak shifted from 620 nm to 725 nm, indicating to the significant increase in the diameter of the formed nano-complex (Ab₁-AuNPs-Fe₃O₄-Ab₂) (Fig. 1, Panel C, curve f). When magnetic separation was applied on Fe₃O₄-Ab₂-(PSA)-Ab₁-AuNPs, the UV–Vis absorption of Ab₁-AuNPs decreased significantly (Fig. 1, Panel C, curve g) because the formation of complexation between (PSA)-Ab₁-AuNPs and Fe₃O₄-Ab₂ led to the dropping of Ab₁-AuNPs concentration in the supernatant. Fig. 1, Panel C, curve h indicates that the absorption peak of Ab₁-AuNPs is not affected by Fe₃O₄-Ab₂ NPs.

3.1.2. TEM and DLS analysis

The size and shape of gold nanoparticles strongly influence their chemical and other properties. The triangular shaped nanoparticles show attractive optical properties in comparison to spherical one [35,36]. So, in the present work, triangular-shaped gold nanoparticles were used instead of conventional citrate-capped gold nanoparticles for constructing colorimetric immunoassay. The prepared NPs were characterized by TEM and DLS, as shown in Fig. 1. As expected, the Fe₃O₄-Ab₂ NPs were spherical with a mean diameter of 34.5 nm (Panels D₁ and D₂). As shown in Fig. 1, Panels E₁ and E₂, Ab₁-AuNPs had a truncated triangular shape with an average edge length of 28.4 nm. The TEM and DLS images of the Fe₃O₄-Ab₂ and Ab₁-AuNPs aggregation in the presence of PSA antigen resulted in the mean diameter of 88.2 nm (Fig. 1, Panels F₁ and F₂), representing that Fe₃O₄-Ab₂ and Ab₁-AuNPs have the capability of forming a sandwich structure Fe₃O₄-Ab₂-(PSA)-Ab₁-AuNPs.

3.2. Optimization of the experimental parameters

3.2.1. Interaction of PSA with Ab₁-AuNPs

To find the optimum incubation time for completion of complex formation between PSA and Ab₁-AuNPs, UV–Vis spectra were recorded at different time points. The results, as shown in Fig. 2, Panel A, indicated...
with the increase of incubation time (until 70 min), the absorption intensity at 525 nm was declined and intensity at 620 nm increased, showing Ab1-AuNPs are gradually bound with PSA antigen molecules during 70 min. After 70 min, due to saturation of occupation sites, no significant changes were observed in absorption (Fig. 2, Panel AII). So the optimum incubation time was found to be 70 min.

The formation of the complex between the PSA and Ab1-AuNPs was studied at four temperatures. As can be seen in Fig. 2, Panel B, with an increase in temperature from 25 °C to 37 °C, absorption at 525 nm was gradually decreased until reaching to its minimum value at 37 °C, suggesting the minimum interaction between PSA and Ab1-AuNPs. However, the optimum temperature for the interaction of PSA with Ab1-AuNPs was found to be 25 °C.

3.2.2. Optimization Fe3O4-Ab2 interaction with (PSA)-Ab1-AuNPs

To find the optimum incubation time for completion of complex formation between Fe3O4-Ab2 with (PSA)-Ab1-AuNPs, UV-Vis spectra were recorded at different time points. The results, shown in Fig. 2, Panel CI indicated SPR peak decreased at 525 nm and 620 nm and increased at 725 nm until 60 min. After 60 min, no significant changes were observed in absorption spectra (Fig. 2, Panel CII). So, the optimum incubation time needed for the Fe3O4-Ab2-(PSA)-Ab1-AuNPs complex formation was found to be 60 min.

The complex formation between the (PSA)-Ab1-AuNPs and Fe3O4-Ab2 was studied at four different temperatures. As can be seen in Fig. 2, Panel D, with the increase in temperature from 25 °C to 37 °C, absorption at 525 nm was decreased to its minimum value, representing that the good interaction between the (PSA)-Ab1-AuNPs and Fe3O4-Ab2 occurs. Based on the results, the optimum temperature for maximum interaction of (PSA)-Ab1-AuNPs with Fe3O4-Ab2 was found to be 25 °C.

3.2.3. Optimization of pH

The performance of the immunosensor and net charges of biomolecules (PSA and antibodies) can be greatly affected by pH adjustment. So the influence of pH on immunosensor performance was investigated in more detail. As nonionic surfactant, Tween-20 was selected as AuNPs stabilizer instead of citrate in this study, steric repulsion between NPs...
resulted from formed layers of tween-20 molecules led to the steric stabilization of the prepared Au NPs and prevent to aggregation of nanoparticles in high ionic strength solutions.

Moreover, the Ab1-AuNPs, shown in Fig. S1, Panel A (see Supplementary Material), represented remarkable stability over a wide range of pH values (5–9). Fig. S1, Panel B (see Supplementary Material) shows the result of the study on absorption of (PSA)-Ab1-AuNPs at pH values ranging from 5 to 9. With the increase of pH, the SPR peaks shifted to the red region, and the size of Au NPs increased in contrast to the unreacted NPs. In pH values above 9, (PSA)-Ab1-AuNPs were completely aggregated (Fig. S1, Panel D, curve a, see Supplementary Material) (Fig. S1, Panel D, curve b, see Supplementary Material). Fig. S1, Panel C (see Supplementary Material) shows the absorption of Ab1-AuNPs after applying an external magnetic field for separation of Fe3O4-Ab2-(PSA)-Ab1-AuNPs at the measured pH values. The tighter the interaction of Ab1-AuNPs with Fe3O4-Ab2 via PSA molecules, the lower the Ab1-AuNPs in the supernatant and the shorter absorption peak. The greater absorption peaks were observed at pH of 5, 6 and 9, which can be due to the dissociation of Fe3O4-Ab2-(PSA)-Ab1-AuNPs complex and release of Ab1-AuNPs. However, based on absorption data, it can be concluded that the most stable complexes are formed at physiological pH.

In addition, the effect of pH on the sensitivity of the immuno sensor showed that maximum sensitivity in the performance of the immuno sensor is observed in physiological pH values (Fig. S2, see Supplementary Material).

3.3. Analytical features

3.3.1. Determination of PSA

Under the optimized conditions, UV–Vis absorption spectra of the Ab1-AuNPs after incubation with Fe3O4-Ab2 in the presence of standard concentrations of PSA were recorded, and the results showed that after applying external magnetic field to isolate Fe3O4-Ab2-(PSA)-Ab1-AuNPs, the intensity of plasmon absorption peak of Ab1-AuNPs is inversely proportional to the concentration of PSA (Fig. 3, Panel A). For
this analysis, linear dynamic range (LDR) and limit of detection (LOD) were found to be 0.01–20 ng mL$^{-1}$ and 0.009 ng mL$^{-1}$ with an equation of $\Delta A = 0.1075 \pm 0.0032$ [PSA](ng mL$^{-1}$)$^{-1}$ $0.0878 \pm 0.0064$ (Fig. 3, Panel B). Table 1 represents the main characteristics of the sensing tools and comparison of the immunosensor with this PSA detection methods. This comparison confirms that the sensing tool in this work as a simple and rapid colorimetric method can rival in the sensitivity and limit of detection.

3.3.2. Interference study

For the assessment of immunosensor specificity, assortment of biomarkers including PSA, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), cortisol (COR), myoglobin (Myo), carcinoembryonic antigen (AgCE) and sodium (Na$^+$), potassium (K$^+$), calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$) were investigated for interfering signals. As shown in Fig. 3, Panel C, the maximum change in absorption intensity were observed in the presence of at least 10-folds higher concentrations of the other tested proteins. The result is indicative of high selectivity of the method, which can be attributed to the specificity of the antibodies modified nanoparticles toward PSA.

3.3.3. Accuracy, repeatability, stability and reversibility of immunosensor

To demonstrate the viability of the immunosensor in clinical assays, content of PSA in serum samples of healthy donor and prostate cancer patient were analyzed and revalidated by standard ELISA technique. It was observed that there was no significant difference between the results from the immunosensor and ELISA results ($p < 0.05$), confirming the satisfactory accuracy and precision of the immunosensor. In comparison with some parameters, immunosensor represented advantages over ELISA method (Table 2). Moreover, the presence of proteins other than PSA in the serum samples showed no remarkable effect(s) on the sensitivity and specificity of the sensing tool. Since the differences of absorption values was used in the calculations, the effect of background absorption (at 525 nm) in the serum samples was removed in the assays.

For the reversibility of engineered immunosensors, we utilized glycine-HCl (0.15 mol L$^{-1}$, pH 2.0) in two regeneration cycles for

**Table 1**

Comparison of the immunosensor with published biosensors for detection of PSA.

<table>
<thead>
<tr>
<th>Biosensor materials</th>
<th>Technique</th>
<th>LDR (ng mL$^{-1}$)</th>
<th>LOD (pg mL$^{-1}$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$_3$O$_4$-TMb-H$_2$O$_2$</td>
<td>Photothermometry</td>
<td>1–64</td>
<td>1000</td>
<td>[37]</td>
</tr>
<tr>
<td>DNA-AuNPs</td>
<td>Colorimetry</td>
<td>0.5–8</td>
<td>330</td>
<td>[38]</td>
</tr>
<tr>
<td>PA-Cu-MoG</td>
<td>Fluorescence</td>
<td>0.0005–5</td>
<td>0.17</td>
<td>[40]</td>
</tr>
<tr>
<td>GCE-GO@AuNPs-GOD-SA-biotin-DNA</td>
<td>ECL</td>
<td>1–100</td>
<td>680</td>
<td>[41]</td>
</tr>
<tr>
<td>Ab$_2$-(PSA)-Ab$_1$-AuNPs-Cu</td>
<td>Colorimetry</td>
<td>1–10,000</td>
<td>72</td>
<td>[42]</td>
</tr>
<tr>
<td>PGE-PANI-AuNPs-PNT-ab$_2$-Ab$_1$-HRP</td>
<td>Electrochemistry</td>
<td>0.001–160</td>
<td>0.11</td>
<td>[43]</td>
</tr>
<tr>
<td>Ab-Au-Bi2Se3 nanosheets</td>
<td>Colorimetry</td>
<td>0–0.8</td>
<td>20</td>
<td>[44]</td>
</tr>
<tr>
<td>Ab$_1$-(PSA)-Ab$_2$-HRP-PpyNPs</td>
<td>Colorimetry</td>
<td>0.8</td>
<td>0.7</td>
<td>[45]</td>
</tr>
<tr>
<td>GCE-AuNPs-Ni(OH)$_2$-NGQDs-Ab$_1$-(PSA)-Ab$_2$-Fe$_3$O$_4$@MnO$_2$</td>
<td>Electrochemistry</td>
<td>0.000001–10</td>
<td>0.0000005</td>
<td>[46]</td>
</tr>
<tr>
<td>AuE-GRP-PS$<em>2$-b-PAA$</em>{27}$</td>
<td>Electrochemistry</td>
<td>0.0001–100</td>
<td>0.04</td>
<td>[47]</td>
</tr>
<tr>
<td>GCE-AuNP-Nc3-Ab</td>
<td>Electrochemistry</td>
<td>0.1–50</td>
<td>18</td>
<td>[48]</td>
</tr>
<tr>
<td>Peptide-Fc@AuSiO$_2$-Au</td>
<td>Fluorescence</td>
<td>0.001–1</td>
<td>0.3</td>
<td>[49]</td>
</tr>
<tr>
<td>AuE-MSTF-Apt</td>
<td>Electrochemistry</td>
<td>1–300</td>
<td>280</td>
<td>[50]</td>
</tr>
<tr>
<td>Fe$_3$O$_4$-Ab$_1$-(PSA)-Ab$_2$-AuNPs</td>
<td>Colorimetry</td>
<td>0.01–20</td>
<td>9</td>
<td>This work</td>
</tr>
</tbody>
</table>

3 min. The immunosensor displayed reversibility efficiency of 95.1% ± 1.2 (for five replication) up to 5 cycles with an acceptable reproducibility and precision. In our study, the engineered immunosensors were regenerated in the fixed concentration of PSA (i.e., 10 ng mL⁻¹). Additionally, the engineered Ab₁-AuNPs and Fe₃O₄-Ab₂ were found to be extremely stable at room temperature during 45 and 30 days post-fabrication with coefficient variation percentage (CV%) -5.4% and 5.2% respectively. The reagents of immunoassay were found to be stable up to 90 days at refrigerator temperature (4 °C) and after this period of time, dramatic decrease was observed in the performance of immunosensor.

4. Conclusions

In summary, we have demonstrated the feasibility of a colorimetric immunoassay for determination of PSA using SPR band of colloidal AuNPs. In this work, antibody-conjugated AuNPs were exposed to the immunoassay for determination of PSA using SPR band of colloidal gold nanorods. This incubation time permits complete antigen-antibody complexation reaction. This incubation time can limit instant analysis of PSA protein. In conclusion, we envisioned that the simple but reliable and efficient platform of this immunosensor to be useful in the development of future point-of-care sensing tools, working on the quantification of biomarkers in a drop of blood.

Author contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Acknowledgment

The authors would like to thank National Institute for Medical Research Development (NIMAD) for the financial support this work (Grant No. 957699).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2019.117218.

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