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Improvement of surface plasmon resonance biosensor with magnetic beads via assembled polyelectrolyte layers

*Ying Sun, Daqian Song, Yuping Bai, Liying Wang, Yuan Tian, Hanqi Zhang**

College of Chemistry, Jilin University, Qian jin Street 2699, Changchun 130012, PR China

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ABSTRACT

The conjugates of magnetic beads coupled with an antibody can be trapped on the Au film firmly due to the magnetic force for the immunoassay of a surface plasmon resonance (SPR) biosensor. However, this approach exhibits significant limitations in robustness and sensitivity due to incomplete dissociation of magnetic beads from the Au film. The incorporation of a polyelectrolyte film on the Au surface can prevent the magnetic beads from the direct contact with the Au film. The layer-by-layer assembly of polyelectrolyte was used as spacer between the gold surface and the magnetic bead. Different layers of polyelectrolyte can be assembled onto the Au film based on an electrostatic force between polycations and polyanions. After the polyelectrolyte film was fabricated on the Au film, the deposition of the magnetic beads was maintained effectively on the film, which favors the sensitivity of the biosensor and the regeneration of the sensing membrane. When the polyelectrolyte layers of $(PAH/PSS)_3$ were constructed on the Au film, the SPR biosensor with magnetic beads exhibited a satisfactory response to human IgG in the concentration range from 0.25 to 30.00 $\mu g\,mL^{-1}$, and the determination limit obtained is eight times lower than that obtained with $(PAH/PSS)_1$ layer.

1. Introduction

Layer-by-layer (LBL) assembly is a promising procedure that uses electrostatic interaction of polyion complexes to fabricate polyelectrolyte multilayer on the substrates [1,2]. The electrostatic multilayer assembly is based on the alternate adsorption of oppositely charged polyelectrolytes from their dilute solutions and suitable for the production of high-quality films with a defined composition in the nanometer range [3]. The process can be achieved by the alternate immersion of certain substrates into oppositely charged water-soluble polymers. Other interactions, such as, hydrogen bonding [4], charge transfer [5], host–guest interactions [6], covalent bonds [7] and biospecific interactions between significant polymers have also been applied [8]. Up to now, the majority of studies employed the electrostatic self-assembly (ESA) method. The technique of electrostatic LBL assembly of oppositely charged polyions allows films to be built up on linear polyions, proteins and nanoparticles with precise localization of components in the multilayer [9]. Owing to its independence on the morphology and size of the substrate used, the LBL assembly process is an easy and powerful technique for tailoring the material surface and creates a monolayer of adsorbed polymer at each assembly step. These assembly-coated substrates have considerable potential in biomedical applications [9].

The fabrication of ultrathin polymer films on material surfaces is important for various scientific and biomedical fields in order to modify or improve the intact characteristics of these surfaces, which can be exposed to various environments, including biological systems. The effective coating of

* *Corresponding author*. Tel.: +86 431 85168399; fax: +86 431 85112355.
E-mail address: analchem@jlu.edu.cn (H. Zhang).
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biomedical materials with polymers thus results in a potentially drastic change in biological affinity [6]. Polyelectrolytes are a promising class of material, which can conveniently be assembled on the Au film based on electrostatic force between polycations and polyanions. Moreover, such polyelectrolyte multilayers have shown exceptional ability as selective membranes for controlling or preventing the transport of ion or molecules [10,11]. Such properties have been used to support the deposition of magnetic beads on the Au sensing film.

The surface plasmon resonance (SPR) technology has been used extensively as a platform for biochemistry and also developed into a commercial sensor system under intense development for a wide range of applications [12–14]. The wide applications of such equipment began with one protein immobilized on the substrates comprising a modified thin evaporated Au film. The binding event of protein–protein leads to changes in the refractive index of the substrates that could be detected by the shifts of resonant wavelength or angle. One of the potential applications of the SPR instrument is the immune sensor which is based on the analyte binding to corresponding antibody immobilized on the SPR sensing surface that could detect analyte in complex biological media with high specificity and sensitivity [15–19].

In our previous work [20], the SPR biosensor system with magnetic beads was developed to detect the combination of antigen with antibody. Magnetic beads have increasingly been used in the area of bioscience [21]. Magnetic microbeads have unique magnetic features which are not present in other materials and they can be applied to special medical techniques. Recently, magnetic particles have been used as labels for biosensing and researches in magnetic label-based bioassays have become an area of increasing interest. The magnetic properties of the beads are very stable over time, in particular, because this property is not affected by chemical reagent or subject to photo-bleaching [22]. The conjugates of antibodies immobilized on magnetic beads were used instead of the antibodies alone for the determination of the antigen. Magnetic beads were found to be ideal for use even in the flow cell. The introduction, trapping and release of the magnetic beads in the flow cell were controlled by magnetic pillar and the flow of the carrier solution. There was not a covalent link between the Au film and the antibody as traditional antibody immobilization on the sensing film in SPR biosensor. However, it is difficult to achieve complete dissociation of magnetic beads from the Au film due to the direct contact between magnetic beads and the Au film. In this study, to overcome this limitation, polyelectrolyte multilayer was constructed between Au thin film and magnetic beads to prevent the direct contact of the magnetic beads and the Au film, which results in the easy regeneration and the sensitivity enhancement of SPR biosensor.

2. Experimental

2.1. Materials

Poly(allylamine hydrochloride) (PAH), poly(styrenesulfonate) (PSS), 3-mercaptopropionic acid (MPA), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O > 99\%$) and ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O > 99\%$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dextran T40 was obtained from Pharmacia (Uppsala, Sweden). Human IgG, goat anti-human IgG, rabbit IgG and bovine serum albumin (BSA) were purchased from Ding Guo Biotech (Beijing, China). All the other chemicals were of analytical grade and the aqueous solutions were prepared with Milli-Q water (18.4 MΩ cm^{-1}).

Human IgG, rabbit IgG, goat anti-human IgG, and BSA were stored at −20 °C, and all other biological reagents were stored at 4 °C. PBS buffer (pH 7.4) was used as running buffer. Ethanol and acetone were used for cleaning the Au film.

2.2. Instrumentation

In this paper, the wavelength modulation SPR biosensor setup installed in our laboratory was used [15,17]. In this system, the light emitted from the lamp is polarized to give transverse magnetic (TM) polarized light, and two lenses were employed to make the light parallel. The parallel polychromatic light beam passes through an optical prism with a thin gold film on the bottom and excites SPR at the interface between the gold film and polyelectrolyte film. The output light from the prism is guided into the spectrophotometer. The incident angle was fixed to ensure that SPR occurs. A 90 μL of flow cell (18 mm × 5 mm × 1 mm) was used. A magnetic pillar was set above the prism to trap the conjugates of magnetic beads coupled with antibody on the sensing film. The diameter and height of the magnetic pillar are 25 and 12 mm, respectively. The sensor surface was at a distance of 13 mm from magnetic pillar and in the magnetic field of 900 G that detected with Tesla meter. The SPR biosensor system with magnetic beads and polyelectrolyte multilayer is illustrated in Fig. 1.

2.3. Procedures

2.3.1. Measurement of the shift in the resonant wavelength

The Kretschmann configuration was applied to achieve the resonant condition by attenuated total reflection (ATR) at the interface between a prism and a thin Au film (about 50 nm thick). This technique involves measuring the reflected intensity dip versus the change of refractive index (RI) over a range of incident wavelength. Interaction between the analyte in the solution and the biomolecule immobilized on the SPR biosensor produces a change in RI that is observed as a shift in the SPR resonant wavelength. The incident angle is fixed and the resonant wavelength at which the strongest coupling occurs is measured. Therefore, the surface plasmon resonance manifests itself on a characteristic dip in the wavelength dependence on the intensity of reflected light. The intensity of the reflected light is the minimum at the dip which is corresponding to the resonant wavelength. The amount of antibody immobilized on the surface was monitored as an increase of SPR signal ($\Delta\lambda$). The SPR wavelength response ($\Delta\lambda$) is directly related to the concentration of surface-bound ligands. So the adsorption of the antibody on the sensor surface and their binding to corresponding antigen were observed as a shift in the resonant wavelength. The minimum detectable human IgG concentration is the amount of analyte providing the signal of about 0.5 nm, which corresponds to the minimum shift

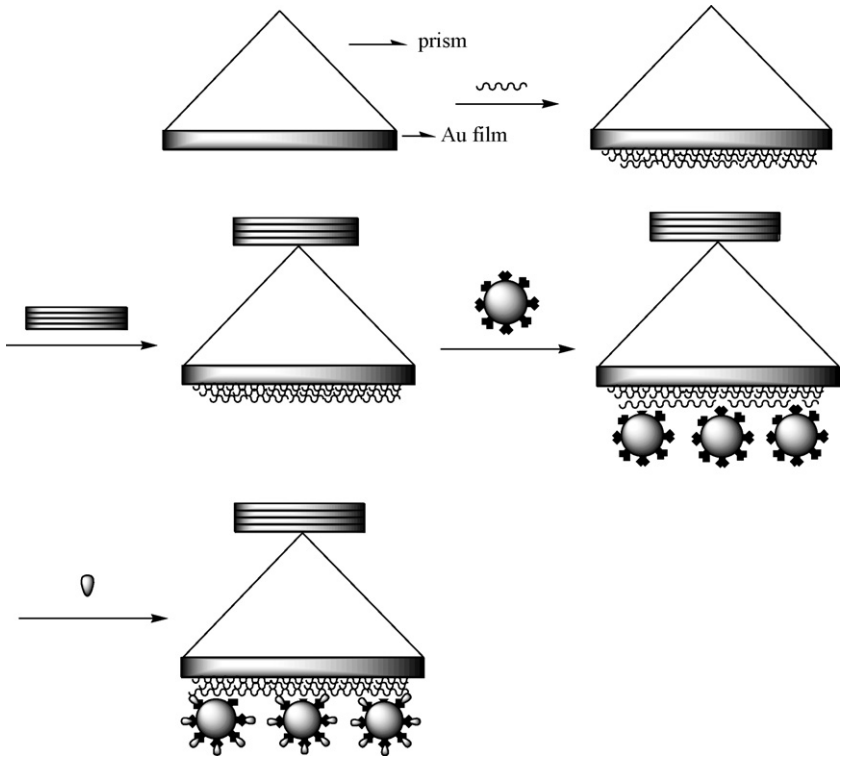

Fig. 1 – The scheme of the presented immunoassays. (∿∿) Polyelectrolyte, (▬) magnetic pillar, (●) magnetic bead, (Y) antibody, and (▼) antigen.

of the resonant wavelength that can be measured in the SPR spectrum.

2.3.2. *Modification of the Au film*

The Au film was cleaned with H_2SO_4/H_2O_2 (volume ration of 7:3) solutions for 1 min, followed by a thorough rinsing with water and drying with N_2. Then, the LBL assembly polyelectrolyte multilayers were constructed on the cleaned Au film [23]. The overall process of LBL assembly consists of a cyclic repetition of the following steps: (1) The cleaned Au film was immersed into 10 mmol L^{-1} MPA/ethanol solution for 24 h, (2) immersed in 3 mg mL^{-1} PAH solution (pH 8.0) for 5 min, (3) rinsed with water and dried under a stream of dry nitrogen, (4) followed by exposing to 3 mg mL^{-1} PSS solution (1 mol L^{-1} $MnCl_2$, 0.01 mol L^{-1} HCl, pH 2.0) for 3 min, and (5) rinsed with water and dried under a stream of dry nitrogen. PAH and PSS were electrostatic adsorbed on the Au film, alternately. The repetition of the procedure allowed us to produce multilayer of different polyelectrolyte layers (PAH/PSS) deposited on the Au film. After the polyelectrolyte multilayers were prepared, a magnetic pillar was set above the prism to trap the conjugates of magnetic beads coupled with goat anti-human IgG.

2.3.3. *Preparation of the conjugates*

$FeCl_3{\cdot}6H_2O$ and $FeCl_2{\cdot}4H_2O$ was added dropwise into the dextran T40 solution. The mixture was heated and titrated to alkaline by adding ammonia water. The resulting black suspension was neutralized with acetic acid and centrifuged. Unbound dextran T40 was removed by washing in a high-gradient magnetic field (HGMF). The magnetic particles were eluted with sodium acetate solution and their diameter is about 12 nm determined by atomic force microscopy (AFM).

The solution of the oxidizing agent sodium metaperiodate ($NaIO_4$) was added dropwise into the solution containing dextran–Fe_3O_4 magnetic bead to oxidize the hydroxyl of the magnetic particles surface to aldehyde group partially. The mixture was purified by HGMF method. After that, the goat anti-human IgG was added into the magnetic bead solution. In this way the antibody was immobilized on the surface of magnetic bead via Schiff alkali reaction. BSA solution was added into the solution above to block the nonreactive group. Then the solution of potassium borohydride (KBH_4) was added into the mixture to deoxidize the double chemical bond between carbon and nitrogen to produce single chemical bond. Last, the mixture was magnetically purified and the magnetic bead–antibody conjugates were dispersed in pH 6.0 acetic acid buffer as the stock solution. The different dilution ratios of conjugates solutions were prepared by diluting the stock solution with PBS buffer.

2.3.4. *Immunoassay*

Conjugates of magnetic beads coupled with the goat anti-human IgG at dilution ratio of 1:10 (v/v) were introduced into the flow cell equipped with a magnetic pillar above the prism and a sensing film of $(PAH/PSS)_3$ polyelectrolyte layers on the Au film, followed by an introduction of a solution containing human IgG with a 90 μL volume. The concentra-

tions of the human IgG are 0.25, 0.50, 1.00, 2.00, 5.00, 10.00, 20.00 and 30.00 $\mu g\,mL^{-1}$, respectively. The introductions of the analytes were all by hand. Then, shifts of the resonant wavelength ($\Delta\lambda$) were measured in real-time. After incubation for 20 min, the immunoassay was stable and the maximal shift of the resonant wavelength ($\Delta\lambda_{eq}$) due to antibody–antigen immunoassay was measured. Then PBS buffer was introduced into the flow cell and another sample solution was introduced into the flow cell.

To evaluate the selective binding of human IgG, rabbit IgG was also determined by the same procedure as used to determine human IgG. All measurements using this system were carried out at room temperature under atmospheric pressure.

2.3.5. *Regeneration*

After a series of human IgG immunoassays were performed, the sensing membrane of the SPR biosensor was regenerated. The magnetic pillar was taken away from the prism and Milli-Q water was introduced into the flow cell to discard the conjugates of magnetic beads coupled with goat anti-human IgG. After 20 min, the conjugates were removed from the Au film and the resonant wavelength returned to the primary wavelength. Then new conjugates were introduced into the flow cell for the next measurement.

3. Results and discussion

3.1. *The polyelectrolyte layers on the Au film*

Traditionally, molecular self-assembly was used to form a sensing layer on the metal film substrate, but the SPR biosensor surface needs to be activated before immobilization and the antibody has to bind specifically on the Au film functional with many different kinds of reagents as the link. When the magnetic pillar was set above the prism, the conjugates of magnetic beads coupled with antibody can be immobilized on the bare Au film, which greatly predigested the operation [20]. However, the magnetic beads could be deposited on the Au film surface in the process of the antibody immobilization, resulting in the background and the difficulty in the biosensor regeneration. In the previous work [20], tetramethyl ammonium hydroxide (TMAH) was used to regenerate the biosensor. Because the TMAH solution is alkaline, the activity of the sensing film could be affected. When the sensor was regenerated with TMAH (pH 12), the Au film without modification of polyelectrolyte layers can be used to prepare the conjugates sensing layer for about five times. The sensor surface stabilized around 80% initial activities after regeneration for the fifth time [20]. However, in this paper, when the sensor was regenerated with water (pH 7), the same Au film modified with polyelectrolyte layers can be used to prepare the conjugate sensing layer for about eight times. The sensor surface stabilized around 82% initial activities after regeneration for the eighth time.

In order to prevent magnetic beads from direct contact with the Au surface of the biosensor regeneration, the surface was modified with the LBL assembly film of polyelectrolyte layers. The adsorption of polyelectrolytes to charged surfaces can be applied to build up polyelectrolytes multilayers of alternating charge by LBL assembly. By alternating exposure of a charged substrate to solutions of cationic and anionic polyelectrolytes, respectively, multilayers of polyelectrolytes are formed. The alternating electrostatic adsorption of cationic and anionic polyelectrolytes at charged surfaces has proven to be an easy method for preparation of ultrathin organized polymer films. The polyelectrolyte layers of $(PAH/PSS)_3$ were first constructed by LBL assembly and the shifts of the resonant wavelength were measured. An obvious red shift of resonant wavelength (λ) was observed, which is due to the construction of every polyelectrolyte layer on the Au film. The maximum shift of the resonant wavelength caused by forming these polyelectrolyte layers of $(PAH/PSS)_3$ is 52.81 nm. Then after the Milli-Q water was introduced into the flow cell to rinse the polyelectrolyte layers time after time, there was no obvious shift of resonant wavelength. This observation indicated that the $(PAH/PSS)_3$ polyelectrolyte layer is stable. In addition, the surface of the Au film modified with $(PAH/PSS)_3$ was measured by atomic force microscope (Fig. 2). The results showed that the average thickness of the polyelectrolyte layers was about 9.61 nm, which demonstrated that almost all Au surface was effectively covered by $(PAH/PSS)_3$ multilayers [23].

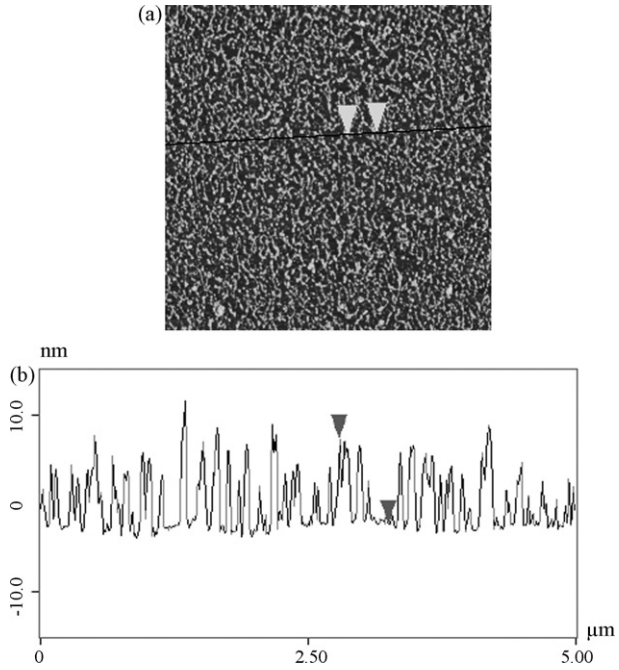

Fig. 2 – (a) AFM image of $(PAH/PSS)_3$ on the Au film and (b) cross-section analysis showing dimensions of the surface features along the line indicated in the AFM image.

The polyelectrolyte layers of $(PAH/PSS)_2$ and $(PAH/PSS)_1$ were also constructed on the Au film and the maximum shift of the resonant wavelength caused by forming these polyelectrolyte layers were observed. With the increasing layers of the polyelectrolyte, the resonant wavelength moves to the longer wavelength. The shifts of resonant wavelength due to LBL assembly of $(PAH/PSS)_2$ and $(PAH/PSS)_1$ are 33.61 and 15.45 nm, respectively. After rinsing with water again and again, the SPR spectrum could not return to the primary form and the shifts of the resonant wavelength were almost unchanged, which

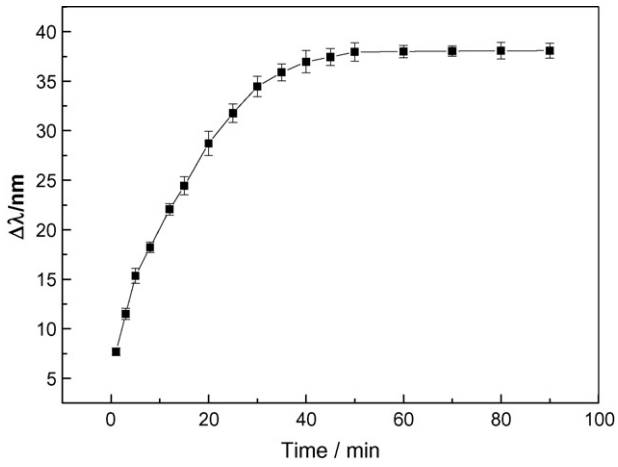

Fig. 3 – The kinetic adsorption curves of magnetic beads coupled with antibody conjugates on the Au film modified with polyelectrolyte layers.

indicated that the polyelectrolyte layers formed on the Au film are robust.

3.2. *Immobilization of the conjugates*

Due to the magnetic pillar above the prism, the conjugates of magnetic beads coupled with antibody can be immobilized on the modified Au film. Because the magnetic bead covered with dextran T40 is easily trapped by the magnet, the sensing film of the conjugates was structured on the gold surface due to the magnetic force. The force exerted on a magnetic particle is the product of the magnetic field and the gradient of the field. The conjugates of magnetic beads coupled with antibody were injected into the flow cell for 90 min and the shifts of the resonant wavelength were measured over time. It can be seen from Fig. 3 that with the conjugates constructed on the Au film, an obvious resonance wavelength red shift was observed. The red shifts of resonant wavelength ($\Delta\lambda$) in 50 min reached about 99% of the maximum shifts of the resonant wavelength which is equal to 38.18 nm. These results indicated that the immobilization was completed and the sensing layer film was formed well.

3.3. *Determination of human IgG*

To examine the effect of polyelectrolyte layers on the performance of the sensor, the polyelectrolyte layers of $(PAH/PSS)_1$, $(PAH/PSS)_2$ and $(PAH/PSS)_3$ were separately constructed on the Au film to study the antigen–antibody immunoreaction. When the polyelectrolyte layer of $(PAH/PSS)_1$ was constructed on the Au film, the shift of the resonant wavelength was 36.54 nm after the immobilization of conjugates, and human IgG was determined in the concentration range of 2.00–30.00 $\mu g\,mL^{-1}$. When the polyelectrolyte layers of $(PAH/PSS)_2$ were constructed on the Au film, the shift of the resonant wavelength was 37.90 nm after the conjugate immobilization, and human IgG was determined in the concentration range of 0.50–30.00 $\mu g\,mL^{-1}$. When the polyelectrolyte layers of $(PAH/PSS)_3$ were constructed on the Au film, the shift of the resonant wavelength was 38.18 nm after the conjugate immobilization, and human IgG was determined in the concentration range of 0.25–30.00 $\mu g\,mL^{-1}$. The sensing film with $(PAH/PSS)_3$ layer exhibits a larger dynamic range since it results in up to about eight times lower determination limit than that obtained with $(PAH/PSS)_1$ layer. The association constant of human IgG with antibody is $3.195 \times 10^7\,L\,mol^{-1}$. The limit of detection (LOD) was calculated using signal-to-noise ratio of 3. Here the obtained LOD of human IgG is 0.078 $\mu g\,mL^{-1}$. The immunoassay of conjugates binding to human IgG with the concentration 5.0 $\mu g\,mL^{-1}$ was performed 11 times and the relative standard deviation (R.S.D.) is 2.2%. Fig. 4 shows the relationships between shifts of resonant wavelength and concentrations of human IgG. In additional, the assay of human IgG with standard SPR sensors by traditional method without magnetic beads was also performed, and the SPR biosensor yields response to human IgG in the concentration range of 2.00–30.00 $\mu g\,mL^{-1}$.

Based on theoretical analysis, the sensitivity of the wavelength modulation SPR sensor is higher at long wavelength than that at short wavelength, and the sensitivity of the wavelength modulation SPR sensor increases rapidly with increasing wavelength towards longer wavelength [24]. It can be seen clearly from Fig. 4 that with the increase of thickness for the polyelectrolyte layers, the distance between the sensing layer and the Au film surface is farther, followed with the resonant wavelength moving to longer wavelength. This observation is consistent with the notion that the space of the surface was expanded producing more binding points and enhancing sensitivity of the wavelength modulation SPR biosensor. In addition, the polyelectrolyte layers with appropriate thickness could effectively prevent the magnetic beads from depositing on the Au film and increase the life expectancy of this biosensor. However, with the shift of the resonance wavelength towards long wavelength, the curve of the SPR spectra will broaden. Due to the limitation of instrumental wavelength resolution, the thickness of polyelectrolyte

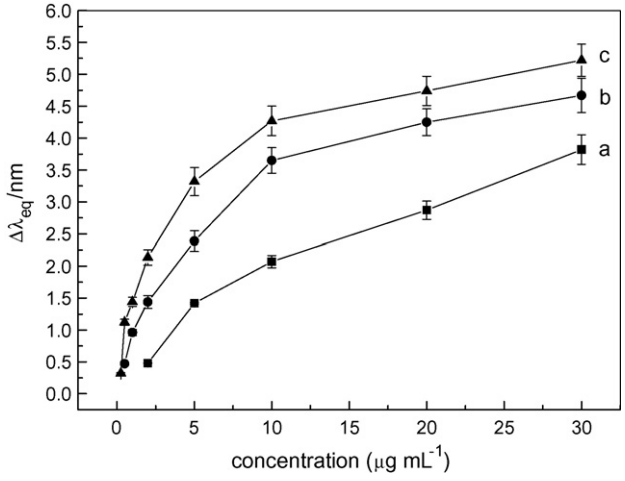

Fig. 4 – The relationship between the concentration of human IgG and the shift of resonant wavelength $\Delta\lambda_{eq}$ for different polyelectrolyte layers: (a) $(PAH/PSS)_1$, (b) $(PAH/PSS)_2$, and (c) $(PAH/PSS)_3$.

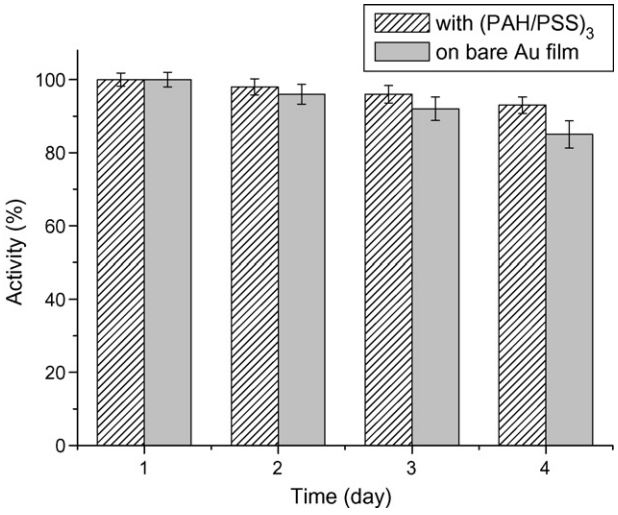

Fig. 5 – The surface capacity and stability of antibody immobilization on the polyelectrolyte layer of $(PAH/PSS)_3$ and bare Au film.

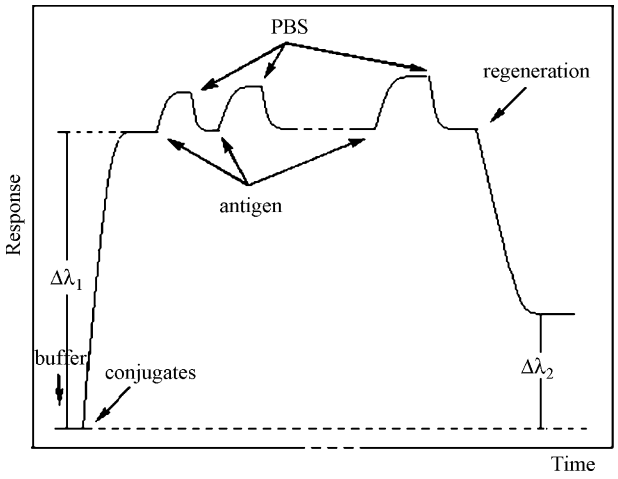

Fig. 6 – The SPR response curve of the whole cycle.

layers is limited and too many polyelectrolyte layers are not suitable.

The evaluation of the selective binding of human IgG was also performed by detecting rabbit IgG. After magnetic beads coupled with goat anti-human IgG conjugates were immobilized with the magnetic pillar for 90 min, rabbit IgG at the concentrations of 5 and 10 $\mu g\,mL^{-1}$ were separately injected into the flow cell for 20 min. There were no observable shifts of the resonant wavelength, which indicated the selective binding of human IgG.

3.4. *The stability of the conjugates trapped on the Au film*

In order to test the stability of the sensing layer modified with the polyelectrolyte layers of $(PAH/PSS)_3$ and immobilized with the conjugates, the immune binding assay for human IgG and goat anti-human IgG was continued for 4 days. The change in surface binding activity is determined by the change of the $\Delta\lambda_{eq}$. Within 24 h, the sensing layer lost approximately 2% activity. After 4 days, the surface stabilized around 94% initial activity. The reason of the loss of binding capacity may be that running buffer strips the antibody immobilized. The activity would decline a little with more times, whereas it is still favorable for the measurement of immunoreaction. The stability assay of the sensing layer without LBL assembly was also taken and the surface stabilized around 85% initial activity on the fourth day. Though the operation of LBL assembly was relatively complicated, the bare Au film was protected by the polyelectrolyte layers that were favorable for the stability of the conjugates trapped on the Au film and the determination of the human IgG. There was improvement in the stability of this substrate compared to conjugates immobilization without LBL assembly of polyelectrolyte multilayer (Fig. 5).

3.5. *Effects of different polyelectrolyte layers on the regeneration of the biosensor*

In order to prevent the direct contact between magnetic beads and the Au film, the Au film was modified with the LBL assembly film of different (PAH/PSS) layers. After the measurement of antigen–antibody immunoassay, the magnetic pillar was taken away from the prism and Milli-Q water was introduced into the flow cell to discard the conjugates. Fig. 6 shows the SPR response curve of the whole cycle, including the status of buffer only, the immobilization of conjugates sensing layer, the binding of antigen at different concentrations and the regeneration of the biosensor. The sensing response $\Delta\lambda_1$ and $\Delta\lambda_2$ due to both the conjugate immobilization and the sensor regeneration are shown in Table 1. It can be seen clearly from Table 1 that due to the polyelectrolyte layers modified on the Au film, the regeneration of the SPR biosensor was improved gradually. For the polyelectrolyte layers of $(PAH/PSS)_3$, the shift of resonant wavelength ($\Delta\lambda_1$) was 38.18 nm after conjugate immobilization, and the shift of resonant wavelength ($\Delta\lambda_2$) is 3.20 nm after regeneration, which indicates that most of magnetic beads on the sensing film were discarded and the regeneration was complete. The polyelectrolyte layers of $(PAH/PSS)_3$ could effectively prevent the direct contact between magnetic beads and the Au film, which could reduce the background enhancement caused by the deposition of magnetic beads on the Au film.

Table 1 – The shifts of resonant wavelength due to the immobilization of conjugates ($\Delta\lambda_1$) and the regeneration of the biosensor ($\Delta\lambda_2$) on different (PAH/PSS) layers

Sensor surface	$\Delta\lambda_1$ (nm)	$\Delta\lambda_2$ (nm)
Without (PAH/PSS) layer	35.26 ± 1.68	28.56 ± 1.13
$(PAH/PSS)_1$	36.54 ± 1.96	20.02 ± 0.98
$(PAH/PSS)_2$	37.90 ± 2.10	12.31 ± 0.63
$(PAH/PSS)_3$	38.18 ± 2.12	3.20 ± 0.16

4. Conclusion

A surface LBL assembly was employed to prepare polyelectrolyte layers on the Au film as a potential sensing film for monitoring of antigen–antibody immunoreaction using SPR biosensor. Compared to other technologies, SPR biosensors have several advantages such as real-time monitoring, label-free detection and minimal reagents consumption. The reproducibility of results is relative favor for the measurement. Due to the limitation of the homebuilt SPR biosensor, the sensitivity of this sensor was no better than those obtained by other methods, while the sensitivity and reproducibility of result were better compared with those obtained by the biosensor without the modification of polyelectrolyte multilayer on the Au film.

Such polyelectrolyte multilayer was simple to prepare, and did not require special instruments and operation. The polyelectrolyte film of $(PAH/PSS)_3$ could prevent the Au film from contact with magnetic beads and was found to improve the determination of human IgG. Though the operation of the assay was relatively complicated that compared to the assay of magnetic beads directly immobilized on the bare Au film, a substantial improvement was achieved in the sensitivity enhancement, stability and regeneration of sensing film. This LBL assembly approach allows the control of dielectric films according to the demand of the assay. This general method can be used to modify the surfaces of Au film with the inhibition of metal particles, oxide monolayer and multilayer species deposition.

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