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Enhancing sensitivity of surface plasmon resonance biosensor by Ag nanocubes/chitosan composite for the detection of mouse IgG

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ABSTRACT

A novel surface plasmon resonance (SPR) biosensor based on Ag nanocubes/chitosan composite was fabricated for mouse IgG detection. Ag nanocubes (AgNCs) were successfully synthesized with sulfide-mediated protocol. They were characterized with transmission electron microscopy (TEM) and UV–vis adsorption spectrum. AgNCs were etched by 3-Mercaptopropinic acid (MPA) and simply mixed with chitosan and glutaraldehyde. The Ag nanocubes/chitosan composite was deposited on Au film by the spin-coating. The electronic coupling between the AgNCs and the surface plasmon wave leads to the amplification of the SPR response, which results in the sensitivity enhancement of SPR biosensor. Moreover, antibody can be immobilized on Au film with aldehyde groups via Schiff alkali reaction. The traditional SPR biosensor based on MPA shows a response for mouse IgG in the concentration range of 2.50–40.00 μg mL^{-1}. The SPR biosensor based on AgNCs/chitosan composite shows a good response for mouse IgG in the concentration range of 0.60–40.00 μg mL^{-1}. The limit of quantification by the biosensor based on AgNCs/chitosan composite substrate is about 4 times lower than traditional biosensor, which has proved the SPR biosensor here proposed with more sensitivity and better performance than traditional SPR biosensor.

1. Introduction

In recent years, metal nanomaterials have been widely used for the fields of biological medicine, energy and environment, spaceflight and aviation, national defense, etc. Metal nanoparticles have excellent optical and physicochemical properties due to their small size distribution and surface effects. Noble metal nanoparticles, such as Au and Ag, can enhance the signal of SPR biosensor, because the electronic coupling between the localized surface plasmon of the nanoparticles and the plasmon wave associated with the surface of the biosensor leads to the enhanced shifts of the resonant angles [1]. Due to the stable chemical property, Au nanoparticles are always elected as sensing enhancers for signal amplification or the sensitivity enhancement. Compared to Au nanoparticles, Ag nanoparticles have less stable chemical property. But, Ag nanoparticles plasmon coupling has sharper angular resonance, yielding increased sensitivity [2,3]. Therefore, the studies of Ag nanoparticles were also gradually increasing. The morphologies of many metal nanoparticles have been studied including spheres [4], rods [5], bipyramids [6], triangular nanoplates [7], cubes [8], wires [9], etc. AgNCs have 8 vertices which act as "hot spots" and are very sensitive to bulk and local dielectric changes. Due to sharp corners/edges, uniform size and perfect physicochemical properties, AgNCs had been applied in localized surface plasmon resonance (LSPR) [10] and surface enhanced Raman scattering (SERS) [11]. However, there are few reports on the application of AgNCs in SPR biosensor.

Chitosan is a copolymer which can be obtained by deacetylation of chitin [12]. It has been known as a bioactive molecule due to its nontoxic, biodegradable, biocompatible natural polymer and antimicrobial activity [13,14]. Chitosan can slightly dissolve in acid solution. It can be covalent cross-linked with glutaraldehyde deposited on Au film surface to form a membrane of hydrogels. It also has good mechanical properties to overcome dissolution, and biomolecules can easily be immobilized on the surface for the permanent network structure.

In this paper, AgNCs were synthesized and separately characterized with TEM and UV–vis absorption spectrum. Then 3-Mercaptopropinic acid (MPA) was used to etch AgNCs. AgNCs modified with MPA were mixed with chitosan and glutaraldehyde. Chitosan polymer can not only protect AgNCs against being oxidized, but also link with MPA and glutaraldehyde. The wrapping AgNCs/chitosan provided an active substrate interface with sufficient aldehyde groups and antibody could be immobilized through Schiff alkali reaction with glutaraldehyde directly. The experiment result shows that AgNCs/chitosan biosensor has advantages of

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good sensitivity and simple operational process compared with the traditional SPR biosensor based on MPA.

2. Materials and methods

2.1. Reagents and materials

Goat anti-mouse IgG, mouse IgG, bovine IgG and human IgG were purchased from Beijing Biosynthesis Biotechnology Company. Polyvinylpyrrolidone (PVP, weight-average molecular weight M_w=29,000 g mol^{-1}), 3-Mercaptopropinic acid (MPA), 2-aminoethanethiol (MEA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Jkchemical. Hydrogen tetrachloroauratehydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Acros. Ethylene glycol (EG) was purchased from Beijing Chemical Works. Chitosan (molecular weight=1,000,000; degree of deacetylation $\geq$95%) was purchased from Aladdin. All other chemicals were of analytical reagent grade. All the solutions were prepared with ultrapure water, and all the glassware was cleaned with aqua regia before the experiments.

Goat anti-mouse IgG, mouse IgG, bovine IgG and human IgG were stored at −20 °C and all other biological reagents were stored at 4 °C. Sodium phosphate buffered saline (PBS, 0.01 mol L^{-1}, pH 7.4) was used as running buffer.

2.2. Equipment

The wavelength modulation SPR biosensor was installed in our laboratory [15,16]. An iodine–tungsten lamp is used as the excitation light source. A glass slide with Au film is put on base of a prism (K9 glass) using suitable index matching oil. In the sensing unit, Kretschmann configuration is applied to achieve the surface plasmon resonance through attenuated total reflection (ATR) at the interface between a prism and a thin metal layer. When the light passes through a polarizer and two lenses, it becomes TM-polarized parallel light. At the interface between the Au film and the sample solution, the parallel polychromatic light beam passes through the optical prism and excites surface plasmon. Then the output light is guided into the optical fiber and enters the spectrophotometer (HR2000+, Ocean Optics Inc., USA). The spectrometer employed in this study, which is equipped with a peltier and a fan for temperature control, provides an optical wavelength resolution (FWHM) less than 0.20 nm. So, the thermal drifts occurred by the experiments and the apparatus were not obvious.

2.3. Procedures

2.3.1. Synthesis of Ag nanocubes

Ag nanocubes were prepared by Xia's method [17]. AgNCs were synthesized with sulfide-mediated protocol. When the sand bath was heated to 180 °C and reached stable, 30 mL of ethylene glycol was added into 100 mL round bottom flask. Then 400 μL of Na_2S solution (3 mmol L^{-1} in ethylene glycol) was added into the round bottom flask under vigorous stirring for 8 min. Then 7.5 mL of PVP solution (0.02 g mL^{-1} in ethylene glycol) was added into the mixed solution, and immediately 2.5 mL $AgNO_3$ (0.048 g mL^{-1} in ethylene glycol) was added into this solution, then heated for 12 min. After heating, the solution was centrifuged and washed with acetone and deionized water several times to remove and discard the supernatant. AgNCs were diluted to 20 mL with ultrapure water. UV–vis absorption spectrum and TEM were used to characterize the AgNCs. Next, 10 μL of MPA (10 mmol L^{-1} in water) was added to 500 μL prepared AgNCs solution and sonicated for 30 min. Then the solution was centrifuged at 9000 g for 10 min to remove excess MPA, and transferred in 400 μL ultrapure water.

2.3.2. Preparation of sensing substrate based on AgNCs/chitosan composite

Here cross-linking and spin-coating were applied for the immobilization of AgNCs/chitosan composite. The procedure was as follow: 400 μL of AgNCs modified with MPA was mixed with 1.5 mL of chitosan solution (0.16 wt%) and 100 μL of glutaraldehyde aqueous solution (1.5 wt%). Then the obtained solution was mixed totally by a vortex for 60 min. Next, the 50 nm layer of Au film dipped with MEA overnight was washed by ethanol and dried with gaseous nitrogen. 200 μL of AgNCs/chitosan composite was deposited on the modified Au film, and the spin-coating was performed in a two-step procedure: (1) 500 rpm for 5 s; (2) 3500 rpm for 30 s. Finally, Au film was dried in air at room temperature for 12 h to fabricate the substrate based on AgNCs/chitosan composite.

2.3.3. Antibody immobilization

In the assay, PBS buffer was injected into the flow cell to make the baseline keep constant. Then the goat anti-mouse IgG was injected and immobilized on the substrate of AgNCs/chitosan composite in the flow cell for 12 h. During this process, antibody was attached to the aldehyde-activated by covalently bonds on the surfaces of substrate. Before the analysis, 1 mol L^{-1} ethanolamine hydrochloride (pH 8.0) was used to block the non-specific binding sites on the biosensor surface.

As a contrast, the traditional biosensor sensing chip was prepared by injecting 10 mmol L^{-1} of MPA into the reactor and incubated for 3 h. Then Au film modified with –COOH was activated with NHS and EDC (30 mg L^{-1}, respectively) for 20 min. The goat anti-mouse IgG was injected into the reactor for the antibody immobilization and blocked the non-specific binding sites with 1 mol L^{-1} ethanolamine hydrochloride.

2.3.4. Immunoassay

All SPR experiments were conducted at room temperature. After the immobilization of goat anti-mouse IgG, different concentrations of mouse IgG was separately injected into flow cell. In the reactor, the specific interaction between mouse IgG and goat anti-mouse IgG can be completed within 40 min. Before injected into the next sample, PBS was injected to dissociate antigen from antibody immobilized on the surface of the chip. In order to ensure the reliability of the assays, all experiments were conducted in triplicate. Schematic illustration of the assay procedure is shown in Fig. 1. The selective binding of rabbit IgG was tested via determining bovine IgG and human IgG by the same procedure that used for mouse IgG determination.

3. Results and discussions

3.1. Synthesis and characterization of Ag nanocubes

In the synthesis of AgNCs, Na_2S was used as sulfide-mediated protocol. Ethylene glycol (EG) was served as solvent. When $AgNO_3$ was added, Ag_2S nanocrystallites were formed. The formed Ag_2S nanocrystallites could catalyze the reduction of $AgNO_3$ [17]. Meanwhile, Ag_2S nanocrystallites could limit the formation of twinned Ag seeds and effectively accelerate the formation of Ag cube [18]. In the process of the whole synthesis, this reaction mechanism is as follow [19,20]:

$$2HOCH_2CH_2OH \rightarrow 2CH_3CHO + 2H_2$$

$$2Ag^+ + 2CH_3CHO \rightarrow CH_3CO\text{-}OCCH_3 + 2Ag + 2H^+$$

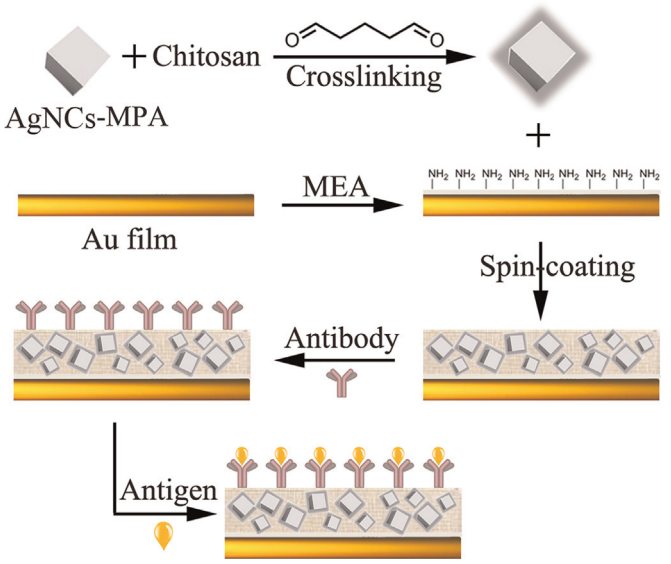

Fig. 1. A schematic diagram of experimental procedure. ( ) AgNCs modified with MPA, ( ) AgNCs/chitosan composite, ( ) antibody, ( ) antigen.

In addition, PVP was added as the stabilizer and could bind selectively to the {100} facets of Ag, which facilitated further formation of the cubic shape. UV–vis adsorption spectrum was applied to characterize AgNCs. Fig. 2a shows UV–vis adsorption spectrum of AgNCs in the solution. It can be seen from Fig. 2a that the two major adsorption peaks are located at 347 nm and 457 nm corresponding to plane dipole and plane quadrupole resonances. Then AgNCs were characterized by TEM. The TEM image in the Fig. 3 shows that the AgNCs have good monodispersion and the edge lengths of those cubes are 50 ± 5 nm. The UV–vis adsorption spectrum and TEM image illustrated that the AgNCs are synthesized successfully.

3.2. Preparation of AgNCs/chitosan composite

Due to sharp corners/edges and uniform size, AgNCs own excellent optical properties which could cause surface plasmon resonance strongly. However, sharp corners/edges can be readily oxidized and excellent properties fade away, which limited their application. In this paper, thiol-frozen was used to stabilize high-energy structure of AgNCs. Ag–S bond can prevent AgNCs from the oxidation. Here, MPA was chosen as thiol reagent. When the

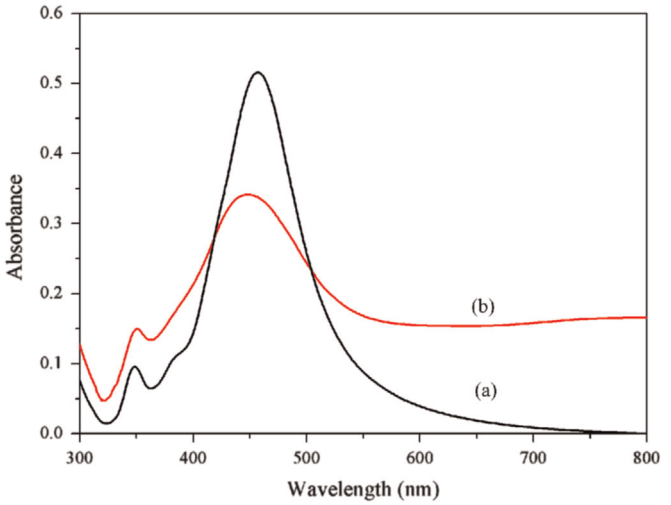

Fig. 2. UV–vis absorption spectrum of AgNCs (a) and AgNCs modified with MPA (b).

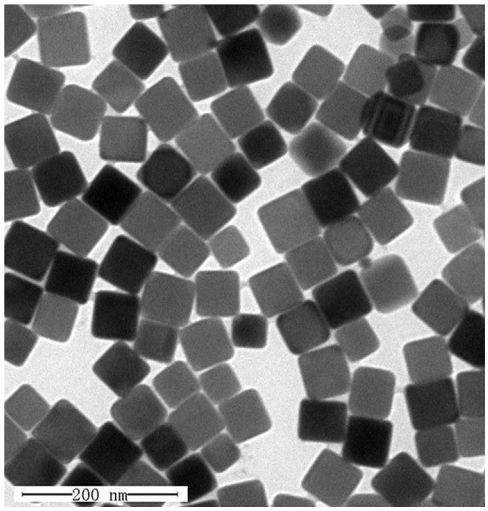

Fig. 3. Transmission electron microscope (TEM) image of AgNCs.

reaction was occurred between Ag nanoparticles and sulfhydryl compound, sulfhydryl compound can be self-assembled on the surface of Ag nanoparticles forming Ag–S bond, which changing the LSPR of Ag nanoparticles. And UV–vis absorption spectrum could accurately reflect the LSPR change of nanoparticles [21–23]. The UV–vis absorption spectrums of AgNCs and AgNCs modified with MPA were measured and shown in Fig. 2, the strong adsorption peak at 457 nm shifted 7 nm after modified with MPA. The weak adsorption peak at 347 nm moved to length wavelength. UV–vis absorption spectrum provides evidence that AgNCs modified with MPA was synthesized successfully.

Covalent crosslinking for chitosan can form a membrane of hydrogels. It has good mechanical properties and can overcome dissolution. In this assay, AgNCs modified with MPA and chitosan were mixed simply. They are linked through chemical covalent interaction.

3.3. Preparation of SPR biosensor substrate based on AgNCs/chitosan composite

Chitosan has the property of good bioactivity. Here, AgNCs modified with MPA, can connect with chitosan by chemical bond. In most of the SPR assay, the thiols were used to link the biomolecules on the SPR substrate. In this paper, Au film was firstly dipped into MEA ethanol solution. Due to the strong interaction between gold and thiol group, MEA can be assembled on the Au film following with $-NH_2$ exposed on surface of sensing film. Here, spin-coating was applied to obtain a thin layer. Chitosan was covalently cross-linked on the surface of modified Au film by glutaraldehyde. The Au film of amino group and aldehyde group are linked through chemical covalent interaction. If the membrane of hydrogel is too thick to light, the change of resonant wavelength could not be observed. When the substrate was used after modified, a red-shift of SPR curves could be observed.

3.4. Immobilization of goat anti-mouse IgG

When the substrate was prepared, the antibody can be directly immobilized on aldehyde groups modified Au film via Schiff alkali reaction. 100 $\mu g\ mL^{-1}$ of goat anti-mouse IgG was injected into flow cell and reach saturation. As result shows, the goat anti-mouse IgG can be effectively immobilized on the substrate through covalent attachment.

For evaluating the effects of AgNCs/chitosan composite on performance improvement for SPR biosensor, traditional SPR

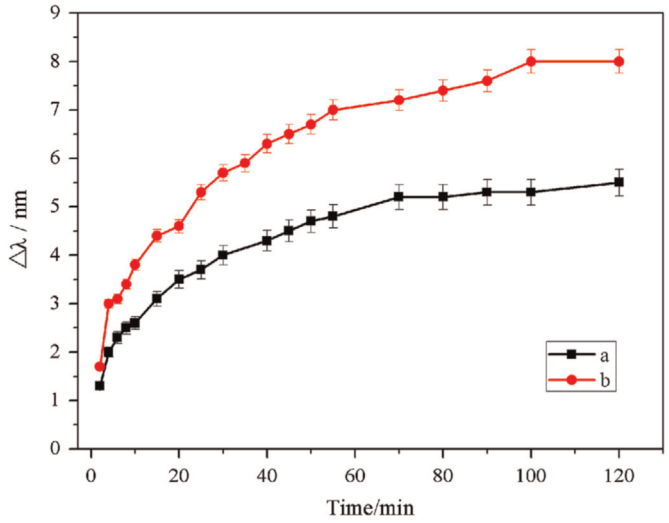

Fig. 4. The kinetic adsorption curves of immobilization of goat anti-mouse IgG at the concentration of 100 μg mL^{-1}: MPA modified Au film (a) and AgNCs/chitosan composite modified Au film (b).

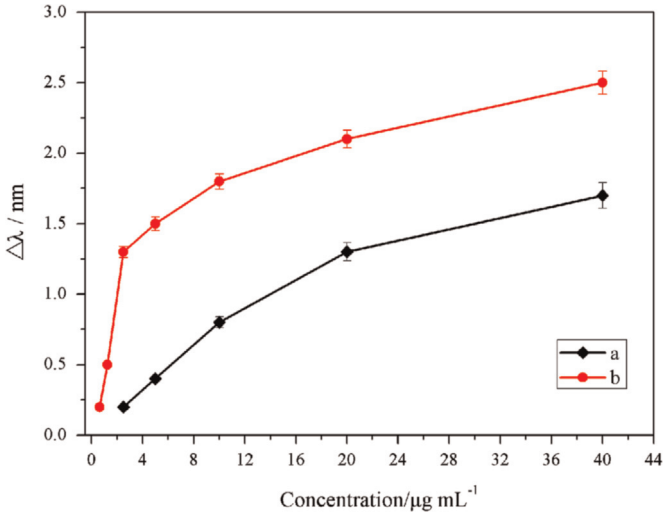

Fig. 5. The relationship between the shifts of the resonant wavelength and different concentration of mouse IgG: biosensor based on Au film (a); biosensor based on AgNCs/chitosan composite (b).

biosensor based on Au film without the modification of AgNCs was also discussed. Fig. 4 shows the kinetic adsorption curves of goat anti-mouse IgG (100 μg mL^{-1}) immobilized on the AgNCs/chitosan composite substrate and traditional Au film substrate. It can be seen from Fig. 4 that the resonant wavelength shows an obvious shift at first. With the time passed, the resonant wavelength increases slowly. When the time reaches 100 min, the resonant wavelength tends to be stable. This illustrates that the goat anti-mouse IgG can be immobilized firmly within the 100 min.

Meanwhile, it also can be seen from Fig. 4 that biosensor based on AgNCs/chitosan composite has more obvious resonant wavelength shift compared with traditional SPR biosensor. The maximum shift of the resonant wavelength is 8.0 nm for substrate based on AgNCs/chitosan composite, while 5.3 nm for substrate based on Au film.

In general, the SPR biosensor surface needs to be activated before antibody immobilization and molecular self-assembly was used to form a sensing layer on the Au film substrate. However, in this sensor, the antibody can be immobilized directly via Schiff alkali reaction. The aldehyde groups can more rapidly combine with goat anti-mouse IgG without activation. This manipulation is simple and time-saving for antibody immobilization.

3.5. Determination of mouse IgG

Before the determination of mouse IgG, ethanolamine hydrochloride was used to block the non-specific binding sites on the biosensor surface, and the biosensor surface was rinsed with PBS buffer until the resonant wavelength kept constant. Then the mouse IgG at different concentrations was separately injected into the flow cell at room temperature for the detection of immunoassay. The shifts of resonant wavelength caused by antibody–antigen interaction were monitored in real time. Fig. 5 shows the relationship between the shifts in the resonant wavelength and the concentration of mouse IgG. It can be seen from Fig. 5a that the traditional SPR biosensor based on MPA shows a response to mouse IgG in the concentration range of 2.50–40.00 μg mL^{-1}. The shift of resonant wavelength is 0.20 nm at 2.50 μg mL^{-1} and 1.70 nm at 40.00 μg mL^{-1}. Fig. 5b shows that the SPR biosensor based on AgNCs/chitosan composite sensing substrate exhibits a good response to mouse IgG in the concentration range of 0.60–40.00 μg mL^{-1}. The minimum shift of resonant wavelength is 0.20 nm at 0.60 μg mL^{-1} and the maximum of resonant wavelength is 2.50 nm at 40.00 μg mL^{-1}. The limit of quantification (LOQ) is defined as the lowest concentration of an analyte that can be quantitatively determined by the present method. The LOQ by the biosensor based on AgNCs/chitosan composite substrate is about 4 times lower than that obtained from the biosensor based on MPA. The experiment result shows that AgNCs/chitosan composite as sensing chip has better response of signal than traditional SPR biosensor. AgNCs have unique anisotropic nature and favor polarization of surface charge and intensity enhancement of local electromagnetic field. The electronic coupling between the AgNCs and the surface plasmon wave leads to the amplification of the SPR response, which results in the sensitivity enhancement of wavelength-modulation SPR biosensor.

The evaluation of the specificity was also performed by detecting human IgG and bovine IgG. After the goat anti-mouse IgG was immobilized on the surface of the biosensor, human IgG and bovine IgG at the concentrations of 10.00 μg mL^{-1} were separately injected into the flow cell for 40 min. As shown in Fig. 6, there are no observable shifts of the resonant wavelength, which indicate the selective binding of mouse IgG.

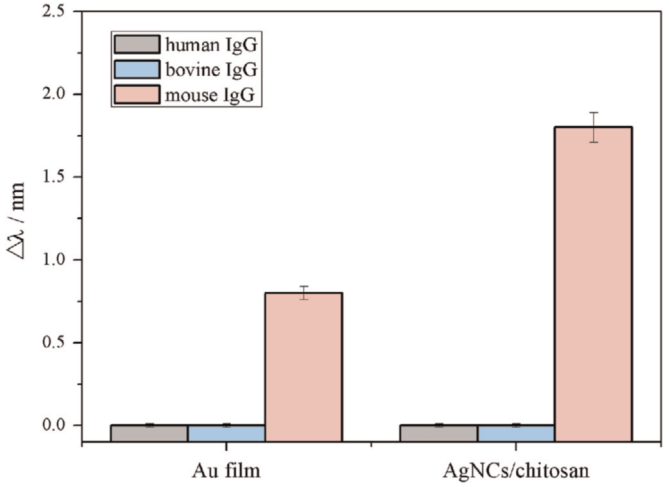

Fig. 6. The shifts of resonant wavelength measured with different antigens (mouse IgG, bovine IgG and human IgG) based on different sensing substrate.

4. Conclusions

In this paper, a novel surface plasmon resonance biosensor substrate was prepared based on AgNCs. AgNCs were synthesized successfully and characterized with TEM and UV–vis absorption spectrum. AgNCs/chitosan composite can effectively prevent AgNCs from being oxidized, and the antibody could be immobilized on the substrate via the aldehyde groups on the surface of AgNCs/chitosan composite, which simplifies the antibody immobilization. The result shows that SPR biosensor based on AgNCs/chitosan composite as sensing enhancers exhibits a satisfactory response to mouse IgG in the concentration range of 0.60–40.00 μg mL^{-1}. The lowest concentration that could be detected based on AgNCs/chitosan composite is 4 times lower than traditional SPR biosensor based on MPA. And AgNCs/chitosan composite can be proved to enhance sensitivity and improve performance of the SPR biosensor effectively.

Acknowledgments

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