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A novel immunosensor based on immobilization of hepatitis B surface antibody on platinum electrode modified colloidal gold and polyvinyl butyral as matrices *via* electrochemical impedance spectroscopy

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Abstract

Hepatitis B surface antibody (HBsAb) was immobilized to the surface of platinum electrode modified with colloidal gold and polyvinyl butyral (PVB) as matrices to detect hepatitis B surface antigen (HBsAg) via electrochemical impedance spectroscopy (EIS). The electrochemical measurements of cyclic voltammetry and impedance spectroscopy showed that $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ reactions on the platinum electrode surface were blocked due to the procedures of self-assembly of HBsAb-Au-PVB. The binding of a specific HBsAb to HBsAg recognition layer could be detected by measurements of the impedance change. A new strategy was introduced for improving the sensitivity of impedance measurements via the large specific surface area and high surface free energy of Au nanoparticles and the encapsulated effect of polyvinyl butyral. The results showed that this strategy caused dramatic improvement of the detection sensitivity of HBsAg and had good linear response to detect HBsAg in the range of 20–160 ng·ml^{-1} with a detection limit of 7.8 ng·ml^{-1}. Moreover, the studied immunosensor exhibited high sensitivity and long-term stability.

Keywords: Hepatitis B; Electrochemical impedance spectroscopy; Colloidal gold; Polyvinyl butyral

1. Introduction

The extremely high selectivity and affinity of antibody molecules to their corresponding antigens have widely been exploited for analytical purposes mainly as radioimmunoassays (RIAs) [1] or enzyme-linked immunosorbent assays (ELISAs) [2–8]. However, in immunosensors, which represent the logical further development of immunoassays, the required transduction of the biological recognition into a physical signal is in most cases achieved either optically [9] or electrochemically [10–12]. Immunosensors are of great interest because of potential utility as specific, simple, label-free and direct detection techniques and reductions in size, cost and time of analysis compared with conventional immunoassay techniques [13]. Recently the immunochemical interactions may be directly detected by a change in potentiometric difference [14], current [15], resistance [16], mass [17], and heat [18].

Among the various transduction techniques, electrochemical impedance spectroscopy (EIS) is an effective method to probe the interfacial properties of modified electrode and often used for understanding chemical transformations and processes associated with the conductive supports [19–21]. Frequency dependence of the impedance of the electrode double layer yields useful information about the adsorption kinetic and the dynamics of charge transfer at electrode interface are strongly influenced by the nature of the electrode surface and the structure of the electrical double layer [22,23]. The adsorption or desorption of insulating materials on conductive supports is anticipated to alter the interfacial electron-transfer features (capacitance and resistance) at the electrode surface.

In immunosensors, the redox couple ($Fe(CN)_6^{4-/3-}$) often serves as a probe for the insulating properties and the density of the adsorbed layer. In the presence of $Fe(CN)_6^{4-/3-}$,

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electron transfer is observed and Faradic impedance is measured. The formation of antigen–antibody will change the electrochemical impedance because the electrode is coated with a blocking layer. When antigens bind to the surface-immobilized antibodies, the access of the redox couple is hindered to a higher degree than in the absence of antigens. As the Faradic reaction of a redox couple becomes increasingly hindered, the electron transfer resistance will increase and the capacitance will decrease, accordingly.

However, the binding reaction of antigen at the antibody-immobilized surface is often insufficient to produce a large signal change for impedance measurements. To overcome this shortcoming, hepatitis B surface antibody (HBsAb) molecules were immobilized by the self-assembled technique on the platinum electrode modified colloidal gold and polyvinyl butyral (PVB) as matrices via the large specific surface area and high surface free energy of Au nanoparticles, and the entrapped effect of polyvinyl butyral in the present paper. The self-assembled process of the immunosensors can be characterized by cyclic voltammetry and electrochemical impedance spectroscopy. The results of our experiment indicated the novel immunosensors exhibited excellent electrochemical characteristics to hepatitis B surface antigen (HBsAg) with the substantial improvement in sensitivity, low detection limit, stability and selectivity. It is hoped that the attractive properties of the HBsAb-Au-PVB-modified electrodes would find various practical applications.

2. Experimental

2.1. Reagent and materials

Hepatitis B surface antibody (HBsAb) and hepatitis B surface antigen (HBsAg) (E.C 1.1.3.4, 1.28 $\mu g \cdot ml^{-1}$) were purchased from Kehua Bioengineering Company (Shanghai China). Bovine serum albumin (BSA, 96–99%), gold chloride and tannic acid were obtained from Sigma Chemical (St. Louis, MO, USA). Polyvinyl butyral (PVB) was bought from Shanghai Chemical Reagent (China). All platinum electrodes were purchased from Yatong Chem. (Chongqing, China) with 10-mm length and 1-mm diameter. All chemicals and solvents used were of analytical grade and were used as received. Double distilled water was used throughout this study. The standard HBsAg stock solutions were prepared with phosphate buffer solution (PBS, pH 7.4) and stored at 4 °C. The HBsAb was stored in the frozen state, and its standard solutions were prepared daily with PBS solution as in use. The preparation of phosphate buffer solution of pH 7.4: KH_2PO_4 0.2 g, $Na_2HPO_4 \cdot 12H_2O$ 2.9 g, NaCl 8.0 g and KCl 0.2 g was dissolved in 1000-ml double distilled water.

2.2. Apparatus

Voltanmmetric measurements were carried out with a CHI 660A electrochemistry work station (Shanghai CH Instruments, China). A three-compartment electrochemical cell contained a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and HBsAb-Au-PVB-modified platinum electrode ($\Phi = 1$ mm) as working electrode. The size of Au colloid was estimated from transmission electron microscopy (TEM) (H600, Hitachi Instrument, Japan). The pH measurements were made with a pH meter (MP 230, Mettler-Toledo Switzerland) and a digital ion analyzer (Model PHS-3C, Dazhong Instruments, Shanghai, China). The AC impedance of the immunoelectrode membrane was measured with a Model IM6e (ZAHNER Elektrick, Germany).

2.3. Preparation of colloidal gold

All glassware used in the following procedures was cleaned in a bath of $K_2Cr_2O_7$–H_2SO_4 (*caution*: Piranha solution reacts violently with almost any organic materials and should be handled with extreme care!), rinsed thoroughly in double distilled water and dried in air. Gold colloids were prepared according to the literature [24]. Solution A: 1 ml of 1% $HAuCl_4$ solution was added to 99 ml water. Solution B: 4 ml of 1% trisodium citrate solution. The two solutions were heated up to 60 °C, respectively. During mixing, solution B was added to solution A quickly. The mixture was heated for 35 min subsequently. The solution color was claret. The size of the prepared Au colloid was about 16 nm, which was estimated from transmission electron microscopy (TEM) (Fig. 1).

2.4. Antibody immobilization

2.4.1. Preparation of the PVB-Au-HBsAb-modified immunosensor

The working electrode was first polished with 0.6-μm gold sand paper, rinsed thoroughly twice with water, boiled in nitric acid (1:1) for 10 min, ultrasonicated in acetone and

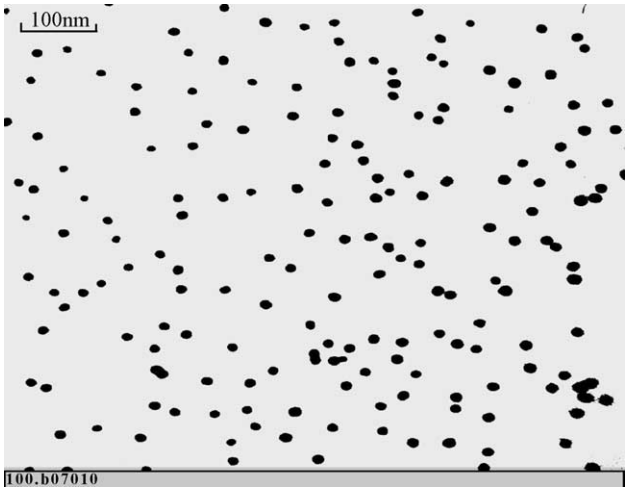

Fig. 1. The TEM images of Au colloid particles. The size of the Au colloid particles is about 16 nm.

washed in water two times, respectively, then dried in air before use. A sol–gel method was adopted to prepare the electrode. An appropriate amount (unless otherwise specified, 150 μl was used) of hepatitis B surface antibody solution (1.28 μg ml^{-1}) was mixed with 0.3-ml colloidal gold into a beaker in ice water. Ten minutes later, 3 ml of polyvinyl butyral ethanol solution (2%, v/v) was added to the beaker quickly. The mixture was stirred adequately and deposited upon the surface of the platinum electrode. The platinum electrode was dipped into the homogeneous mixing solution to a depth of 10 mm for 10 min and then removed. After being stored for about 24 h at 4 °C, the modified immunosensor was incubated in 0.25 wt.% BSA for 60 min at 37 °C in order to block the remaining active groups. Then the finished immunosensor was stored at 4 °C when not in use. The schematic diagram of the immunosensors and the structure of the electrode coating are shown in Fig. 2.

2.4.2. Preparation of the Au-HBsAb-modified immunosensor

The bare platinum electrode was first made anodic by using cyclic voltammetry. The clean platinum electrode is held at a potential of +1.5 V in a 0.1 M NaOH stirred solution for 2 min followed by + 0.5 V (vs. SCE) for 45 s in the same medium in order to make it charged positively. Then the positively charged platinum electrode was dipped into the homogeneous mixing solution containing HBsAb and gold nanoparticles via the self-assembly and the opposite-charged adsorption techniques. The immobilized method may be explained by the fact that the gold nanopartilces are a highly negatively charged species as a result of the adsorption of citrate in the fabrication and HBsAb can be firmly immobilized on the gold nanoparticles surface because of the strong interactions between gold nanoparticles and biological macromolecules.

2.4.3. Preparation of the PVB-HBsAb-modified immunosensor

An appropriate amount (unless otherwise specified, 150 μl was used) of hepatitis B surface antibody solution (1.28 μg ml^{-1}) was mixed with 3 ml of polyvinyl butyral ethanol solution (2%, v/v) in a beaker. The mixture was stirred by the bare platinum electrode adequately and then dipped into the homogeneous mixing solution for 10 min, and then removed to store at 4 °C.

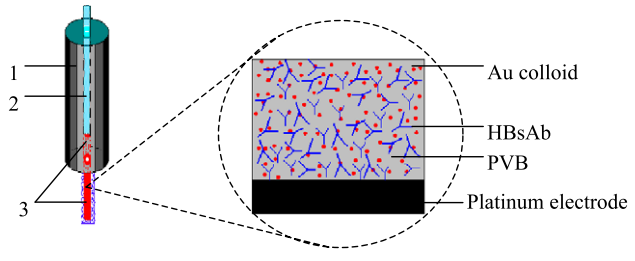

Fig. 2. A schematic diagram of the immunosensor showing: (1) PVC tube, (2) cuprum wire, (3) platinum electrode modified with HBsAb-Au-PVB.

2.5. Electrochemical measurements

The electrochemical characteristics of the modified electrode were characterized by using cyclic voltammetry during the self-assembled process. Electrochemical experiments were performed in a conventional electrochemical cell containing a three-electrode system and swept the potential between −0.3 and +0.7 V (vs. SCE) with a sweeping rate of 0.1 $V \cdot s^{-1}$. Impedance measurements and cyclic voltammetry measurements were performed in the presence of a 2.5 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe in PBS (containing 0.1 M KCl, pH 7.4). Impedance measurements were performed at the frequency range from 10^{-2} to 10^{6} Hz at the formal potential of 220 mV, using alternating voltage of 10 mV. The change of electron-transfer resistance is calculated as following the equation:

$$\Delta R_{et} = R_{Ab-Ag} - R_{Ab}$$

where $R_{Ab\text{-}Ag}$ is the value of electron transfer resistance after HBsAg binding to HBsAb, R_{Ab} is the value of the immobilized HBsAb.

3. Results and discussion

3.1. Electrochemical characteristics on electrode surface

Electrochemical impedance spectroscopy (EIS) is an effective method for probing the feature surface-modified electrode. Fig. 3 shows the results of Faradic impedance spectroscopy on a bare platinum electrode (curve a), Au-modified platinum electrode (curve b), Au-PVB-modified platinum electrode (curve c) and the HBsAb-Au-PVB-modified platinum electrode (curve d) in the presence of redox probe $Fe(CN)_6^{4-/3-}$ measured at the formal potential. It can be seen that the bare platinum electrode exhibits an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. When the bare platinum electrode was dipped into the colloidal gold solution, we were surprised to find that the EIS of the Au-modified electrode was similar to that of the bare platinum electrode. This implied that the conductivity of the Au-modified platinum electrode was essentially equivalent to a bulk platinum electrode. After being modified with Au colloids and PVB, the EIS of the Au-PVB-modified electrode shows a higher interfacial eT resistance, indicating that the PVB molecules obstructed eT of the electrochemical probe (Fig. 3c). When the HBsAb-Au-PVB-modified platinum electrode was finally obtained, the interfacial resistance increased again (Fig. 4d). The impedance change of the modified process also showed the HBsAb had attached to the platinum electrode surface.

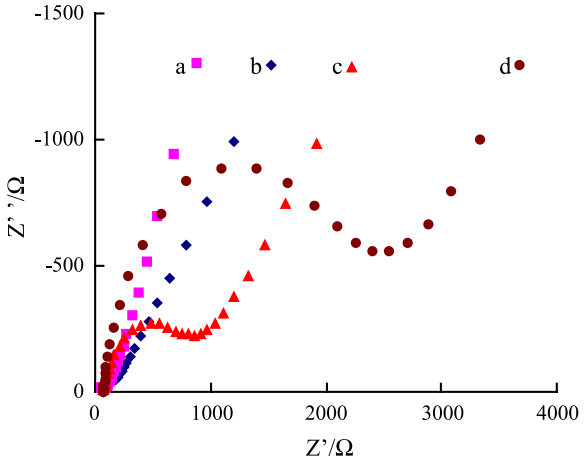

Fig. 3. Electrochemical impedance spectroscopy (EIS) in PBS (pH 7.4)+0.1 M KCl+2.5 mM $Fe(CN)_6^{4-/3-}$ solution at, (a) a bare platinum electrode; (b) Au-modified platinum electrode; (c) Au-PVB-modified platinum electrode; (d) HBsAb-Au-PVB-modified platinum electrode. The frequency range is at $1\times10^{-2}\sim1\times10^{6}$ Hz at 20 °C (Z' vs. Z'' at 220 mV vs. SCE).

Cyclic voltammograms (CVs) of the ferricyanide are valuable and convenient tools to monitor the barrier of the modified electrode, because the electron transfer between the solution species and the electrode must occur by tunneling either through the barrier or through the defects in the barrier. Therefore, it was chosen as a marker to investigate the changes of electrode behavior after each assembly step. Fig. 4 shows cyclic voltammograms of different modified electrodes in 2.5 mM ferricyanide solution. When electrode surface has been modified by some materials, the electron transfer kinetics of $Fe(CN)_6^{4-/3-}$ is perturbed. Well-defined CVs, characteristic of a diffusion-limited redox process, are observed at the bare platinum electrode (Fig. 4a). After the bare platinum electrode was modified with HBsAb-Au-PVB, an obvious decrease in the anodic and cathodic peaks was observed (Fig. 4b). The reason is that the immobilization of HBsAb-Au-PVB insulates the electrode and perturbs the interfacial electron transfer considerably. After the HBsAb-Au-PVB-modified electrode was obscured with BSA, the amperometric response of the electrode was decreased and increased in the peak-to-peak separation between the cathodic and anodic waves of the redox probe (Fig. 4c). This is consistent with the enhanced electron transfer barriers introduced upon the assembly of these layers. Especially, after the HBsAg molecule is coupled onto the antibody molecule, an obvious disappearance of the anodic and cathodic peaks was obtained (Fig. 4d). The reason is that the antigen–antibody complex acts as the inert electron and mass transfer blocking layer, and it hinders the diffusion of ferricyanide toward the electrode surface.

In order to further demonstrate whether hepatitis B surface antibody had been immobilized on the platinum electrode, hepatitis B surface antibody labeled by horseradish peroxidase (HRP) was used in the preparation of the immunoelectrode via ELISA. The HBsAb-HRP-Au-PVB-modified platinum electrode prepared in the same way was scanned at the potential between −0.3 and +0.7 V (vs. SCE) with a scanning rate of 0.1 $V\cdot s^{-1}$ to the different concentrations of H_2O_2 from 20 $mmol\cdot l^{-1}$ to 240 $mmol\cdot l^{-1}$ in a phosphate buffer solution, pH 7.4. As shown in Fig. 5, the peak current increased with the increment of H_2O_2 concentration and started to level off when the concentration of H_2O_2 was more than 160 $mmol\cdot l^{-1}$. This indicated that HBsAb-HRP have been immobilized on the electrode surface. On the basis of the CV, ELISA and EIS results, we can conclude that HBsAb is successfully immobilized on the platinum electrode via colloidal gold and polyvinyl butyral as matrices.

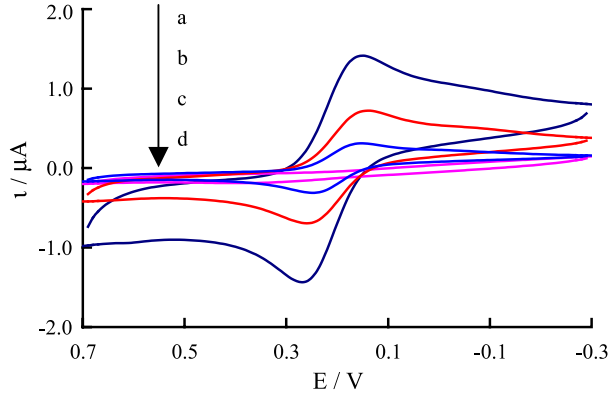

Fig. 4. Cyclic voltammograms (CVs) of the different electrodes in a 2.5 mM $Fe(CN)_6^{4-/3-}$ solution (10 mM PBS+0.1 M KCl, pH 7.4), (a) bare platinum electrode; (b) HBsAb-Au-PVB-modified platinum electrode; (c) HBsAb-Au-PVB-modified platinum electrode incubated with BSA; (d) HBsAb-Au-PVB-modified platinum electrode combined with HBsAg. The scan rate was 100 $mV\cdot s^{-1}$.

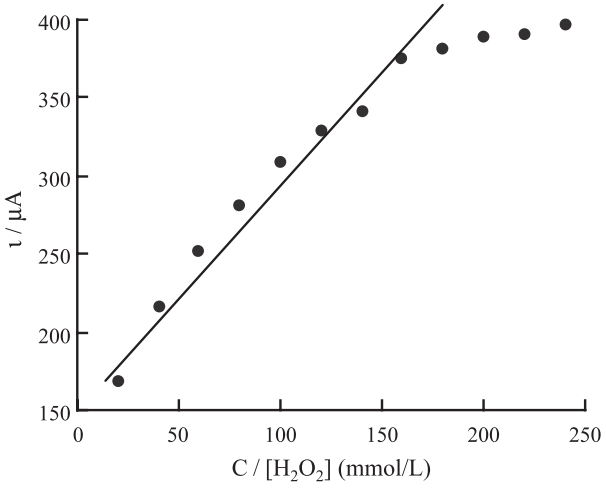

Fig. 5. Cyclic voltammograms of the HBsAb-Au-PVB-modified immunosensor, in which HBsAb was labeled by horseradish peroxidase, to different H_2O_2 concentrations in a phosphate buffer solution, pH 7.4 at room temperature under N_2 protected conditions. Scan rate, 50 $mV\cdot s^{-1}$.

3.2. Optimization of analytical conditions

Fig. 6a shows the effect of temperature on AC impedance (R_{et}) for 60 ng·ml^{-1} HBsAg at the range from 10 to 50 °C in a 2.5 mM $Fe(CN)_6^{4-/3-}$ solution (10 mM PBS + 0.1 M KCl, pH 7.4). Various incubation temperatures were reported in the different literatures, ranging from 25 to 37 °C. As shown in Fig. 6a, the eT value increased with the increasing temperature up to 35 °C, and then it decreased at higher temperature. However, temperatures over 40 °C caused irreversible behavior involved in the process. As is well known, an optimal temperature of immunoreaction would be 37 °C, which supports this observation at this temperature, so the temperature of 35 °C was selected as a compromise.

The effect of pH on AC impedance R_{et} was shown in Fig. 6b. The influence of pH was studied between 5.5 and 8.5 for the same concentration of HBsAg in a 2.5 mM $Fe(CN)_6^{4-/3-}$ solution (10 mM PBS + 0.1 M KCl). The immunosensor response decreases with increasing pH value from 5.5 to 7.5 and then increases as the pH increases further. It is well known that it is facile to electron transfer at the optimal pH value, so the electron transfer resistance is the smallest. The experimental results show that most immunoreactions exhibit optimal binding at this pH [25]. Therefore, a pH 7.4 of PBS was used as the medium for the immunoreaction.

The influence of the immunochemical incubation (i.e. when the antigen–antibody reaction occurs) time on response signals was also investigated. The HBsAb-modified electrode was used to study the effect on the sensitivity of the immunosensor to HBsAg. In the incubating solution, when the analyte antigens reach the antibodies at the electrode surface of the immunosensor, it takes some time for the contacting species to form immunocomplexes. Fig. 6c displays the effect of incubation time on the immunoassay. The incubation times were 10, 20, 30, 40 and 50 min, using the same HBsAg concentrations. The eT value obtained in this study increases with the incubation time rapidly up to 30 min and after that the variation leaves off. So 30 min was chosen for the subsequent study to evaluate the analytical performance of the immunosensor.

3.3. Amplified immunoassay of HBsAg by AC impedance spectroscopy

Although AC impedance could change upon interaction of HBsAb with HBsAg, the signal is small in some extent. In this paper, a new amplification strategy was introduced for improving the sensitivity of impedance measurements using colloidal gold and polyvinyl butyral as matrices. The reason is that the gold nanoparticles are a negatively charged species as a result of the adsorption of citrate in the fabrication process, which can enhance the connection between gold nanoparticles and HBsAb, and at the same time the network complex of polyvinyl butyral is introduced to enhance the stability of immobilized HBsAb. A substan-

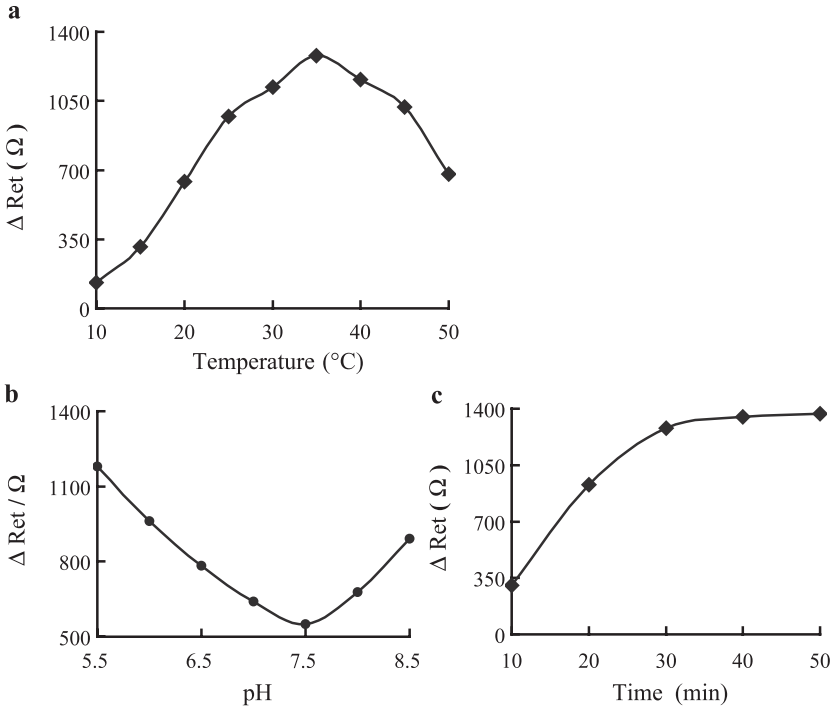

Fig. 6. The influence of (a) temperature; (b) pH value, and (c) incubation time on the impedance response of the immunosensor in a 2.5 mM $Fe(CN)_6^{4-/3-}$ solution at $1 \times 10^{-2} \sim 1 \times 10^{6}$ Hz at 25 °C (Z' vs. Z'' at 220 mV vs. SCE).

tial increase in the diameter of the semicircle could be observed when the HBsAb-Au-PVB-modified platinum electrode was interacted with HBsAg. In this method, a higher electron transfer resistance was achieved, which meant that probe accessed to the surface of electrode very difficultly.

In EIS, the total impedance was determined by several parameters: (1) electrolyte resistance, R_s; (2) the lipid bilayer capacitance, C_{dl}; (3) charge transfer resistance, R_{et}; (4) Warburg element, Z_w. The complex impedance can be presented as the sum of the real, Z_{re}, and imaginary, Z_{im} components that originate mainly from the resistance and capacitance of the cell. For the sake of giving more detailed information about the impedance of the modified electrode, a modified Randles equivalent circuit (Fig. 7) was chosen to fit the measured results. The two components of the scheme, R_s and Z_w, represent bulk properties of the electrolyte solution and diffusion of the applied redox probe, respectively. Thus, they are not affected by chemical transformations occurring at the electrode interface. The other two components of the circuit, C_{dl} and R_{et}, depend on the dielectric and insulating features at the electrode/electrolyte interface. In electrochemical impedance spectroscopy (EIS), the semicircle diameter of EIS equals the electron transfer resistance, R_{et}. This resistance controls the electron transfer kinetics of the redox-probe at electrode interface, which is relative to the concentration of antigen. In order to view the procedure of antibody immobilization and amplified immunoassay clearly, we only considered the relation between R_{et} and the concentration of HBsAg.

Fig. 8 shows the derived calibration plot that corresponds to the electron transfer resistance (R_{et}) at the HBsAb-Au-modified platinum electrode (curve a), the HBsAb-PVB-modified platinum electrode (curve b) and the HBsAb-Au-PVB-modified platinum electrode (curve c) with different concentrations of the analyte HBsAg. From the experimental data the modified electrode with gold colloids and PVB took on the most sensitive change to R_{et} value and the immunosensor calibration curves are sigmoidal with HBsAg concentration, however, the immunosensor exhibits a linear dependence on the standard positive serum concentration ranging from 20 to 160 ng·ml^{-1} with a detection limit of 6.3 ng·ml^{-1}. The linear regression equation was $\Delta R_{et} = 360.7 + 14.9$ [HBsAg], with a correlation coefficient of 0.9962 (Fig. 8c). According to the linear regression equation, we can detect HBsAg concentration in the sample quantitatively.

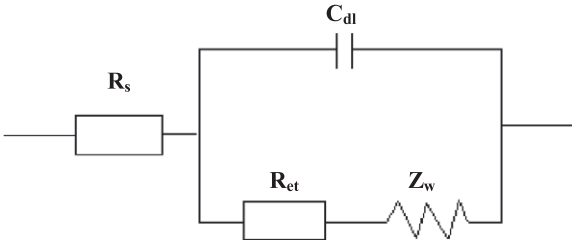

Fig. 7. Equivalent circuit used to model impedance data in a 2.5 mM $Fe(CN)_6^{4-/3-}$ solution (10 mM PBS + 0.1 M KCl, pH 7.4).

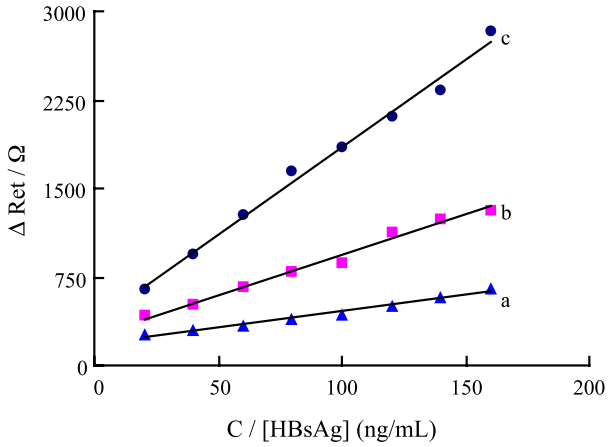

Fig. 8. The changes of electron transfer resistance (ΔR_{et}) of the different immunosensors after reaction with the different concentrations of HBsAg in a 2.5 mM $Fe(CN)_6^{4-/3-}$ solution (10 mM PBS + 0.1 M KCl, pH 7.4): (a) HBsAb-Au-modified platinum electrode; (b) HBsAb-PVB-modified platinum electrode; (c) HBsAb-Au-PVB-modified platinum electrode.

3.4. Regeneration and reproducibility of the immunosensors

Regeneration of immunosensors is of interest to the immunoanalysts. Although the antibody–antigen linkage can be broken under drastic conditions (e.g. in alkalinic or acidic solutions or with chaotropic agents), the immobilized immunoreagents could also suffer from the functional damage or even be released from the immunosorbents [26]. In this experiment, 0.2 M, glycine-hydrochloric acid (Gly-HCl) buffer solution (pH 2.8) was chosen to break the binding between antibody and antigen. After detecting 60 ng·ml^{-1} HBsAg, the immunosensor was immersed in a stirring 0.2 M, pH 2.8 glycine-hydrochloric acid (Gly-HCl) buffer solution for 5 min and removed to detect the same HBsAg concentration, repeated 20 times continuously. As shown in Fig. 9a, the immunosensor kept 93.1% of the original potentiometric value in the initial 13 times, the relative standard deviation (RSD) was 3.1% for 13 successive assays, because the extreme conditions degraded or chemically deactivated the antigen surface [27]. Good reproducibility may be explained by the fact that Au nanoparticles and PVB have little effect on immunoprotein activity and HBsAb-Au-PVB is firmly attached on the surface of the platinum electrode. The electrode-to-electrode reproducibility was estimated from the response to 60 ng·ml^{-1} HBsAg at eight different immunosensors. The results showed the AC impedance characteristics of eight different immunosensors which were prepared in the different times and batches in the presence of 60 ng·ml^{-1} HBsAg. The relative standard deviation (RSD) was between 3.15% and 2.12%.

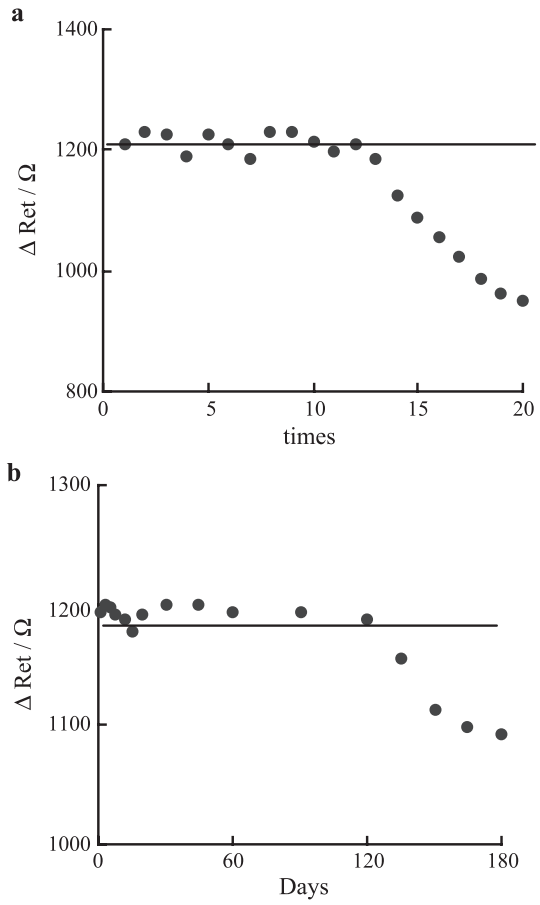

Fig. 9. The reproducibility (a) and lifetime (b) of the immunosensor to 60 ng·ml^{-1} HBsAg.

3.5. Selectivity and lifetime of the immunosensor

The effect of substances that might interfere with the response of the immunosensor was studied. The relative standard deviation (RSD) of inhibition impedance obtained for each interfering substance such as diphtheria antigen and bovine serum albumin was between 2.31% and 3.57%.

The long-time stability of the immunosensor was investigated on a 180-day period. When the immunosensor was stored dry at 4 °C and measured intermittently (every 3–5 days), no apparent change in the same HBsAg concentration was found over 120 days (Fig. 9b). However, when the Au nanoparticles or PVB was absent, the immunosensor retained ~ 27.6% of its initial sensitivity to HBsAg after 3 weeks. Good long-term stability can be attributed to the strong interactions between the Au nanoparticles and HBsAb.

3.6. Application of the immunosensors in human serum

Eighty shares college students' serum samples from our university hospital were determined by using the immunosensors that had been calibrated. The serum samples were diluted to different concentrations with phosphate buffer solution (pH 7.4), then R_{et} value was determined by EIS. The results for human serum samples obtained from the immunosensors were compared with those from an established ELISA technique. More than 93.3% of the results of the human serum samples obtained by this method were in agreement with those obtained by enzyme-linked immunosorbent assays (ELISAs). A good correlation was found between the results for the two methods.

4. Conclusion

EIS was applied to investigate the immune reaction in the presence of redox couple $Fe(CN)_6^{4-/3-}$. In order to enhance the response signal of antibody–antigen interaction, a new method was developed to amplify the response of impedance via the large specific surface area and high surface free energy of Au nanoparticles. The values of electron-transfer resistance obtained by impedance spectroscopy confirmed the procedure of this amplified immunoassay. The method can be generally applicable to amplified assay of other biomolecules and other transduction means, such as potentiometric or amperometric immunosensors.

Acknowledgements

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