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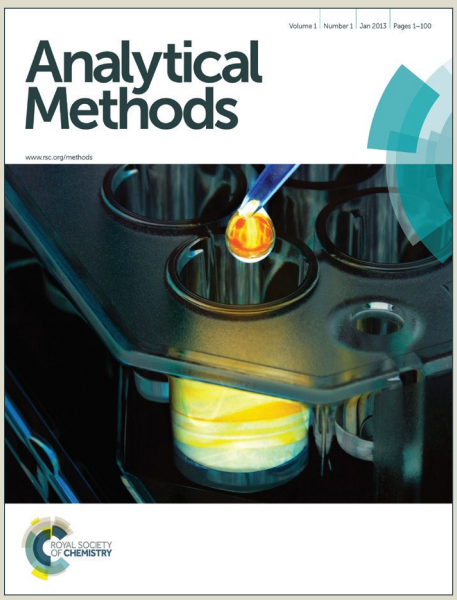

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Cu_2O rhombic dodecahedra as superexcellent electroactive substance for ultrasensitive electrochemical immunosensor

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Abstract

A novel biomolecular immobilization strategy based on Cu_2O rhombic dodecahedra–polydopamine–gold nanoparticles nanocomposite was used for the detection of alpha-fetoprotein (AFP). Cu_2O rhombic dodecahedra as a new kind of Cu_2O nanocrystals having excellent catalytic properties were coated with bio-functional polydopamine (PDA) by a simple self-polymerization in mild basic solution. Then, highly loaded and uniformly dispersed gold nanoparticles (Au NPs) were deposited on Cu_2O-PDA through in situ deposition. Under optimal conditions, a linear relationship between the CV peak current and the logarithm of AFP concentration was obtained from 0.001 to 100 ng mL^{-1} with a detection limit of 0.6 pg mL^{-1} at the signal-to-noise ratio of 3. The high sensitivity and stability, low cost and simplicity in fabrication procedures of the proposed immunosensor provides potential applications for clinical immunoassays.

Keywords: Cu_2O rhombic dodecahedra, cuprous oxide, polydopamine, immunosensor, alpha-fetoprotein

1. Introduction

Simply and rapidly detecting the concentration of alpha-fetoprotein (AFP), one of the specific tumor markers of Hepatocellular Carcinoma, has vital significance for the early diagnosis of liver cancer.[1,2] Immunoassays have widespread applications in the detection of this tumor markers due to its biological specifity.[3] Compared with other immunoassays such as enzyme-linked immunosorbent assays and single radial immunodiffusion assays, electrochemical immunoassays have many advantages such as high sensitivity, low cast, fast

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analysis and simplicity in fabrication procedures.[4,5] Nanoparticles (NPs) have received great attention on catalysis and electrocatalysis in the past few years because of their unique physical and chemical properties.[6] This is attributed mainly to its high surface area to mass ratio and excellent biocompatibility.[7]

Recently, some studies about Cu_2O-based nanocomposites have been reported and applied in biosensing devices.[8-10] Cu_2O is a p-type semiconductor with a direct band gap of 2.1 eV and has important applications in superconductor, hydro-gen production and negative electrode material.[11] In addition, Cu_2O nanoparticles has a potential application in electrochemical analyses due to its unique electro-catalytic activity, high stability and low cost.[12] Some research groups have done plenty of studies on the controllable synthesis and electro-catalytic activity of different polyhedral Cu_2O nanocrystals. Polyhedral Cu_2O nanocrystals which not only have high surface area and oriented attachment but also can supply more efficient active sites have a better ability to charge the transfer than the conventional nanoparticles.[13] Liu et al. used graphene-wrapped Cu_2O nanocubes as electrocatalyst for glucose sensor with higher sensitivity and lower detection limit.[9] Zhang et al. prepared a highly uniform porous Cu_2O octahedra,[14] which has stronger ability to promote electron transfer than both Cu_2O octahedra and Cu_2O nanoparticles. Huang et al. synthesized a new series of Cu_2O nanocrystals with systematic shape evolution from cubic to rhombic dodecahedral structures.[15] Interestingly, they found the rhombic dodecahedral structures have the best catalytic activity due to its high number density of surface copper atoms. Therefore, it has become a potential material for electrochemical analysis. However, heretofore no reports are available on the biosensor based on Cu_2O rhombic dodecahedra.

Nevertheless, one important issue we have to consider is that the small molecules like Cu_2O nanoparticle modified electrochemical biosensor usually show poor stability and are easy to fall off. To solve this problem, some research groups wrapped some biomaterials[16] or filming materials[17] around the small molecules, and achieved initial success. Lee et al. reported a very good film-forming biomaterial dopamine.[18] It is an important catecholamine neurotransmitter with excellent self-polymerizing ability[19] and biocompatibility.[20] Recent research shows that the stable polydopamine (PDA) film is formed by covalent polymerization and non-covalent self-assembly dopamine.[21] After the polymerization reaction, the PDA film is

easily adapted for a variety of materials such as biological molecules and metallic nanoparticles[22,23] without surface pretreatment because of its good chemical stability[24], excellent biocompatibility[20] and hydrophilic[25].

As an important nonenzymatic tags, gold nanoparticles (Au NPs) have been widely used in immunosensors because of its superior properties such as the large surface area, good biocompatibility and easy preparation.[26,27] Due to its good conductivity, Au NPs can accelerate the electron transfer through the electrode surface.[28,29] In addition, it can firmly adsorb proteins via the high-surface free energy and be simply linked with amino group or hydrosulfide group, etc. For example, the surface of PDA film.[30] Moreover, as a good biocompatible material, Au NPs can maintain the bioactivity of proteins.[31] Therefore, it showed great potential application in sensitive electrochemical biosensors.

In this study, we prepared an ultrasensitive AFP immunosensor based on in situ deposition of Au NPs on the PDA functionalized Cu_2O rhombic dodecahedra nanoparticles. Here, a uniform Cu_2O rhombic dodecahedra was synthesized by mixing an aqueous solution of $CuCl_2$, sodium dodecyl sulfate (SDS) surfactant, NaOH and $NH_2OH{\cdot}HCl$ reductant.[15] Then, the Cu_2O nanoparticles were covered in PDA film by self-polymerization of DA. With the residual catechol groups of the PDA film, Au NPs could be linked simply and steadily on the nanocomposite. By taking advantages of the excellent biocompatibility, film forming ability of PDA, and high electrocatalytic activity of Cu_2O rhombic dodecahedra nanoparticles as well as the large surface area and high biocompatibility of Au NPs, the fabricated Cu_2O-PDA-Au nanocomposite exhibited excellent performance as immunosensors.

2. Experimental

2.1. Chemicals and materials

Anti-AFP and AFP were purchased from Biocell Co. (Zhengzhou, China) while dopamine hydrochloride was from Alfa Aesar. Bovine serum albumin (BSA), anhydrous copper (II) chloride ($CuCl_2$; 97%), sodium dodecyl sulfate (SDS; 100%) and hydroxylamine hydrochloride ($NH_2OH_3{\cdot}HCl$; 99%) were bought from Sigma. Sodium hydroxide (98.2%) was acquired from Chemical Reagent Company of Shanghai (Shanghai, China). Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) was purchased from Aladdin Chemistry Co. Ltd.. All other chemicals were of analytical grade and used as received without further purification. Phosphate buffer solution

(PBS) of various pH values were prepared by mixing the 1/15 mol L^{-1} stock solutions of KH_2PO_4 and Na_2HPO_4 at specific ratios. All solutions were established with ultrapure water (resistivity > 18 MΩ cm^{-1}).

2.2. Apparatus

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were performed using a Potentiostat/Galvanostat Model 283 electrochemical workstation (Ametek, USA). The three-electrode system consisted of a bare or modified glassy carbon electrode (GCE) which was used as a working electrode, a saturated calomel electrode (SCE) as a reference electrode and a Pt wire counter electrode. The transmission electron microscope (TEM) images were obtained with a H600 transmission electron microscope (Hitachi Instruments, Japan).

2.3. Synthesis of Cu_2O rhombic dodecahedra

Cu_2O rhombic dodecahedra were synthesized according to the previously reported procedure [15] with slight modifications. In brief, 7.5 mL of 0.1 mol L^{-1} $CuCl_2$ solution was added into a sample vial. And it was placed in a water bath set at 32-34 °C. Then 1.3 g of SDS powder was added to the vial with vigorous magnetic stirring. After SDS powder was completely dissolved, 2.7 mL of 1.0 mol L^{-1} NaOH solution was introduced rapidly and the color of the solution changed into deep blue immediately. Next, 36 mL of 0.1 mol L^{-1} $NH_2OH_3 \cdot HCl$ was quickly injected into the vial in 5 seconds. Finally, the system was stirred for another 60 min for nanocrystal growth and centrifuged at 3000 rpm for 5 min. After the top solution was decanted, the precipitate was washed twice with 35 mL of a 1:1 volume ratio of water and ethanol. The precipitate was centrifuged and washed again with anhydrous ethanol, and then dispersed in 5 mL of ethanol, at last was stored in the refrigerator for use.

2.4. Synthesis of Cu_2O-PDA-Au nanocomposites

The 1 mL prepared Cu_2O rhombic dodecahedra solution was taken into 10 mL of pH 7.0 Tris-HCl. The mixture was first to be ultrasonically treated for 10 min to ensure that Cu_2O nanoparticles dispersed in the buffer solution. Then 20 mL of 1.5 mg mL^{-1} fresh dopamine solution was added. Subsequently, the solution was violently stirred in ice-water bath for 6 h. The next step is to wash with ultrapure water and then it was dispersed in 2 mL of ethanol. The polydopamine-coated Cu_2O rhombic dodecahedra (Cu_2O-PDA, 25 mg mL^{-1}) was obtained.

The Au NPs functionalized Cu_2O-PDA was synthesized by a typical method [32] named in situ deposition with slight modifications. Briefly, the 1 mL obtained Cu_2O-PDA solution was dispersed in 1.0 mL of 0.1% (w/v) $HAuCl_4$ and then the appropriate sodium citrate solution was injected and stirred for 1.5 h. After centrifugation and washing with ultrapure water and dispersed in 10 mL of ethanol, Cu_2O-PDA-Au nanocomposite (2.5 mg mL^{-1}) was obtained.

2.5. Fabrication of the immunosensor

Prior to the modification, the bare GCE (4 mm in diameter) was polished to a mirror-like surface with 1.0 and 0.3 μm alumina slurry and then it was successively ultra-sonicated in ultrapure water and ethanol for 3 min, at last it was dried with N_2. Afterwards, 10 μL of the Cu_2O-PDA-Au suspension (2.5 mg mL^{-1}) was carefully dipped onto the cleaned bare GCE surface to dry at 4 °C for 6 h. After the Cu_2O-PDA-Au NPs modified electrode was dried, the resulting electrode was incubated in 1.0 mL anti-AFP solution (10 ng mL^{-1}) for 12 h at 4 °C. In order to block the possible remaining active sites the modified electrode was immersed in 1% BSA solution for 2 h at 4 °C after rinsed with 1/15 mol L^{-1} pH 7.0 PBS. Finally, the functionalized electrode was carefully rinsed again with ultrapure water to remove the physically absorbed proteins. The schematic representation of the preparation process of Cu_2O-PDA-Au nanocomposite and AFP immunosensor is shown in Scheme 1.

3. Results and discussion

3.1. Characterization of Cu_2O-PDA-Au NPs

During the process of the work, we used a simple procedure synthesized Cu_2O rhombic dodecahedra nanoparticles. The morphology and size of Cu_2O nanoparticles were studied by SEM and TEM. Figure 1A and B give the images of Cu_2O rhombic dodecahedra. It can be seen that the diameter of them was about 60-90 nm. With the abundant amine groups and residual catechol groups of the PDA film, Au NPs could be linked simply and steadily on the nanocomposite by in situ reduction. As shown in Figure 1C, a large amount of Au NPs were uniformly distributed on the Cu_2O-PDA surface, which could lead a great increase of proteins binding sites due to its good biocompatibility and large surface area.

3.2. Electrochemical characteristics of different modified electrodes

The electrochemical characteristics of the different modified electrodes were investigated by cyclic voltammetry (CVs) which was carried out in pH 6.5 PBS, and the results are shown

in Figure 2. From Figure 2a, it is clearly showed that no obvious redox peaks were observed about bare GCE. After the electrode was modified with the nanocomposite, there was an apparent pair of stable redox peaks (Figure 2b), which should be caused by the strong electrocatalytic activity of Cu_2O rhombic dodecahedra and the large specific surface as well as high loading of Au NPs. This, in turn, indicated that Cu_2O-PDA-Au nanocomposite had been successfully immobilized on the surface of the electrode. Compared with Figure 2b, after the immobilization of anti-AFP, the peaks current decreased obviously (Figure 2c), which was mainly from the weak conductivity of macromolecular protein. Similarly, there was a further decrease with the adsorption of BSA (Figure 2d).

To further confirm the interface conductivity of the immunosensor surface in the process of electrodes modification, electrochemical impedance spectroscopy (EIS) has also been used. The semicircle diameter of EIS spectrum equals to the electron-transfer resistance (Ret). Figure 3 exhibits the impedance of different modified electrodes in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ solution. As shown in Figure 3, compared with bare GCE (Figure 3a), Cu_2O-PDA-Au nanocomposite modified electrode showed larger Ret (Figure 3b). The reason might be that the electrical conductivity of PDA biofilm is relatively weak. From Figure 3c, it shows that the Ret has a further increase, which might be due to the weak conductivity of macromolecular protein. Therefore, after the BSA was blocked onto the GCE/Cu_2O-PDA-Au/anti-AFP surface, the Ret was further increased (Figure 3d).

The CVs of GCE/Cu_2O-PDA-Au/anti-AFP/BSA in pH 6.5 PBS at different scan rates are shown in Figure 4. As can be seen from the CVs, the oxidation peak currents decreased linearly with the square of root of scan rate plots from 20 to 300 mV s^{-1} (shown in the inset), which suggested that the control type of the reactions on the modified electrode is diffusion control.

3.3. Optimization of experimental parameters

The performance of the immunosensor is usually related to the factors such as the pH of detection solution, the incubation temperature and incubation time. They have been investigated as follows.

The pH of the detection solution has great influence on the stability of the electronic mediator Cu_2O and the activity of the protein molecule. The current response of

GCE/Cu_2O-PDA-Au/anti-AFP/BSA in PBS at various pHs range from 5.0 to 8.0 has been examined (Figure 5A). It was found that the peak currents showed the maximum in pH 6.5, which suggested that Cu_2O nanoparticles may dissolve under strong acidic environment. So, we select pH 6.5 as the best pH of detection solution in the further study.

The incubation temperature could directly influence integrality and stability of the immunoreaction between anti-AFP and AFP. GCE/Cu_2O-PDA-Au/anti-AFP/BSA was incubated with 10 ng mL^{-1} AFP under a range of temperature from 20 to 46 °C. From Figure 5B, we can see the oxidation peak currents before 38 °C has a slow decrease with the temperature increase and the electrochemical response has a rapid increase as temperature over 38 °C. It was due to that the antigen-antibody complex increased the hindrance to electron transfer of the Cu_2O mediator and the low temperature inhibited the proteins activity while the high temperature resulted in its denaturation. 38 °C is an optimal temperature for the immunoreaction between AFP and Anti-AFP. Thus, 38 °C was chosen for the incubation temperature in the further study.

The incubation time would affect the degree of immunoreaction. GCE/Cu2O-PDA-Au/anti-AFP/BSA was incubated with 10 ng mL^{-1} AFP standard solution at 38 °C for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25 and 30 min. From Figure 5C, we can see that the oxidation peak currents before 16 min have a rapid decline. But after 16 min, the current respond leveled off slowly. It was due to that the immunoreaction has reached equilibration state. Therefore, we select 16 min as the incubation time in the further study.

Figure 5. Oxidation peak currents of CVs of the proposed electrode at different detection solution pH (A), incubation temperature (B) and incubation time (C).

3.4. Performance of the immunosensor

Under the above optimal conditions, the proposed modified electrode was incubated with various concentrations of AFP solutions. The CVs were shown in Figure 6. It was found that the oxidation peak current decreased with the increasing of AFP concentration and a linear relationship between the oxidation peak current change and the logarithm of AFP concentration was obtained from 0.001 to 100 ng mL^{-1}, meanwhile the regression equation is $\Delta I(\mu A)=3.60\times logc_{AFP}(ng\ mL^{-1})+12.63$ ($R^2=0.9973$) and a detection limit of 0.6 pg mL^{-1} at the signal-to-noise ratio of 3. Compared with other AFP electrochemical immunosensors (Table 1),

the fabricated Cu_2O-PDA-Au nanocomposite modified electrode immunosensor exhibited wide dynamic measurement range and low detection limit, which mainly due to that the new kind of Cu_2O nanocrystals have excellent electrocatalytic activity, and the stability and biocompatibility have been enhanced by the wrapping of PDA. By taking advantages of the excellent biocompatibility of PDA and the large surface area and high biocompatibility of Au NPs the immunosensor fabricated with Cu_2O-PDA-Au composites exhibited excellent performance.

3.5. Reproducibility, stability and selectivity of the immunosensor

The reproducibility and stability are important standards in estimating the immunosensor. In this study, five successive measurements at AFP concentration of 10 ng mL^{-1} have been repeated under the optimal experimental conditions, and the relative standard deviation (RSD) was 2.7%. At the same time, five electrodes of different batches have been tested in the same conditions, and the RSD was 4.0%. In addition, 90 continuous cycles' CV scan was carried out in the same conditions. As compared with the initial oxidation peak current, the value decreased about 3.1%. Then the immunosensor was measured every 3 days, and the data was collected about 30 days. It also showed that the oxidation peak current decreased only 6.8%. These results indicate that the proposed immunosensor has good reproducibility and stability.

The selectivity of the immunosensor was also estimated by using 3 non-specific absorption of other biological substances: DA (20 ng mL^{-1}), carcino-embryonic antigen (CEA) (20 ng mL^{-1}) and BSA (20 ng mL^{-1}). Cu_2O-PDA-Au nanocomposite modified electrodes were separately incubated 16 min with 10 ng mL^{-1} AFP solutions with interference and without interference. The oxidation peak current of CVs in the two solutions showed a very low relative deviation of 4.0%. The result indicate that the developed immunosensor represented good selectivity because the antigen-antibody immunoreaction has high specificity.

4. Conclusions

This study introduces a novel electrochemical immunosensor based on Cu_2O rhombic dodecahedra–polydopamine–gold nanoparticles nanocomposite. Cu_2O rhombic dodecahedra nanoparticles with more efficient active sites and excellent catalytic activity were firstly used for immunosensor. It was found that the Cu_2O rhombic dodecahedra demonstrated excellent electroactivity and the as-prepared immunosensor fabricated with Cu_2O-PDA-Au composites

exhibited low detection limit, wide dynamic measurement range and high sensitivity.

Acknowledgements

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Figure Captions

Scheme 1. Schematic representation of the preparation process of Cu_2O-PDA-Au nanocomposite and AFP immunosensor.

Figure 1. SEM image of Cu_2O rhombic dodecahedra (A), TEM images of Cu_2O rhombic dodecahedra (B) and Cu_2O-PDA-Au NPs (C)

Figure 2. CVs of different electrodes in 1/15 mol L^{-1} PBS (pH 6.5): a) bare GCE; b) GCE/Cu_2O-PDA-Au; c) GCE/Cu_2O-PDA-Au/anti-AFP; d) GCE/Cu_2O-PDA-Au/anti-AFP/BSA

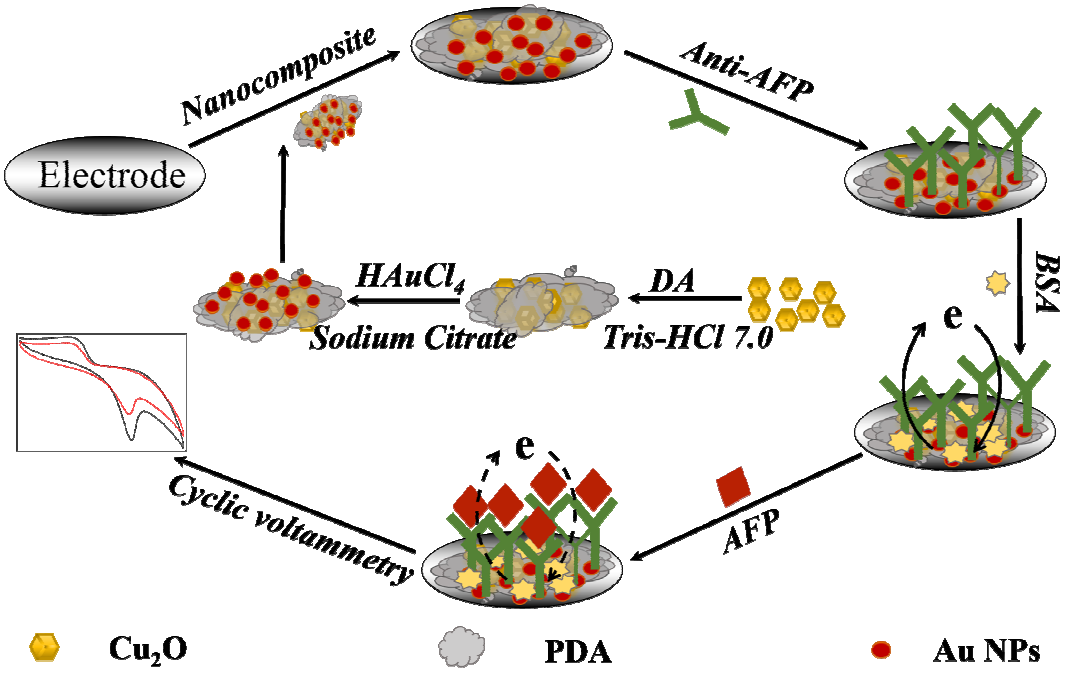
Figure 3. EIS of different modified electrodes in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ solution: a) bare GCE; b) GCE/Cu_2O-PDA-Au; c) GCE/Cu_2O-PDA-Au/anti-AFP; d) GCE/Cu_2O-PDA-Au/anti-AFP/BSA

Figure 4. CV of the proposed electrode at different scan rates (from inner to outer): 20, 30, 50, 80, 100, 120, 150, 200, 250, and 300 mV s^{-1} in 1/15 mol L^{-1} PBS (pH 6.5) Inset: the dependence of oxidation peak currents on the square root of scan rate

Figure 5. Oxidation peak currents of CVs of the proposed electrode at different detection solution pH (A), incubation temperature (B) and incubation time (C).

Figure 6. CVs of different concentration of AFP. Inset: peak current vs. logarithm of AFP concentration in 1/15 mol L^{-1} PBS (pH 6.5).

Table 1. Comparison of the performance of electrochemical immunosensor for the determination of AFP.



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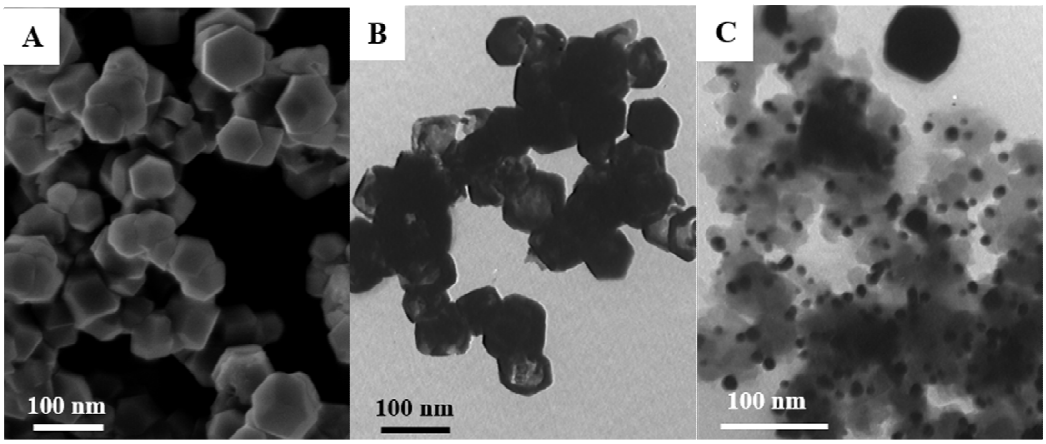

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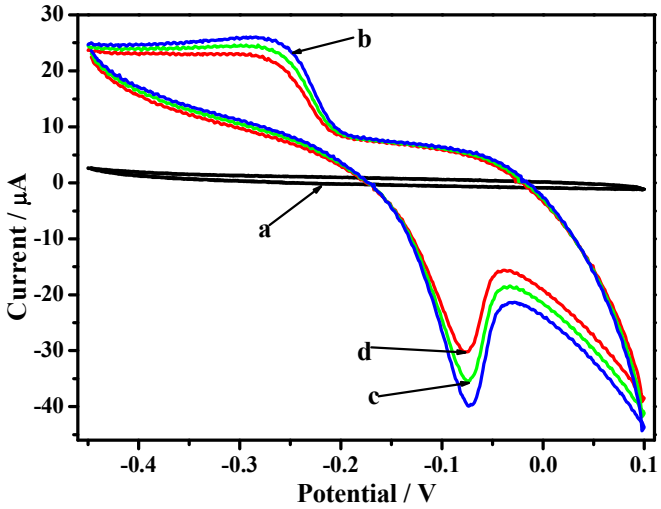

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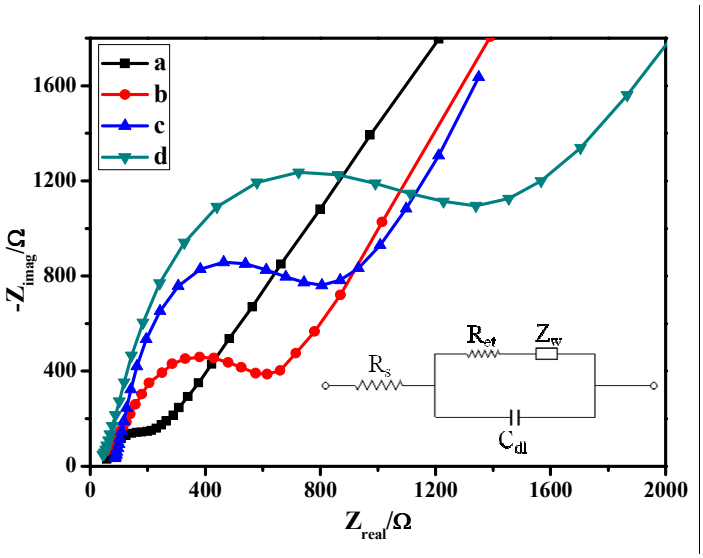
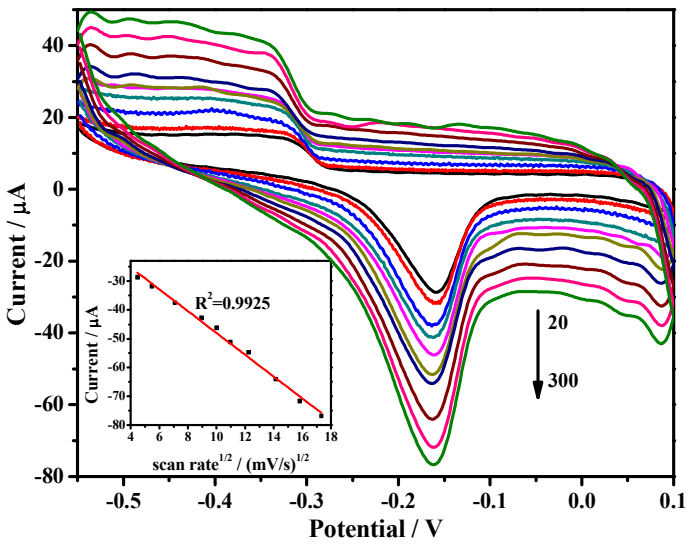


Figure 3. EIS of different modified electrodes in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ solution: a) bare GCE; b) GCE/Cu_2O-PDA-Au; c) GCE/Cu_2O-PDA-Au/anti-AFP; d) GCE/Cu_2O-PDA-Au/anti-AFP/BSA



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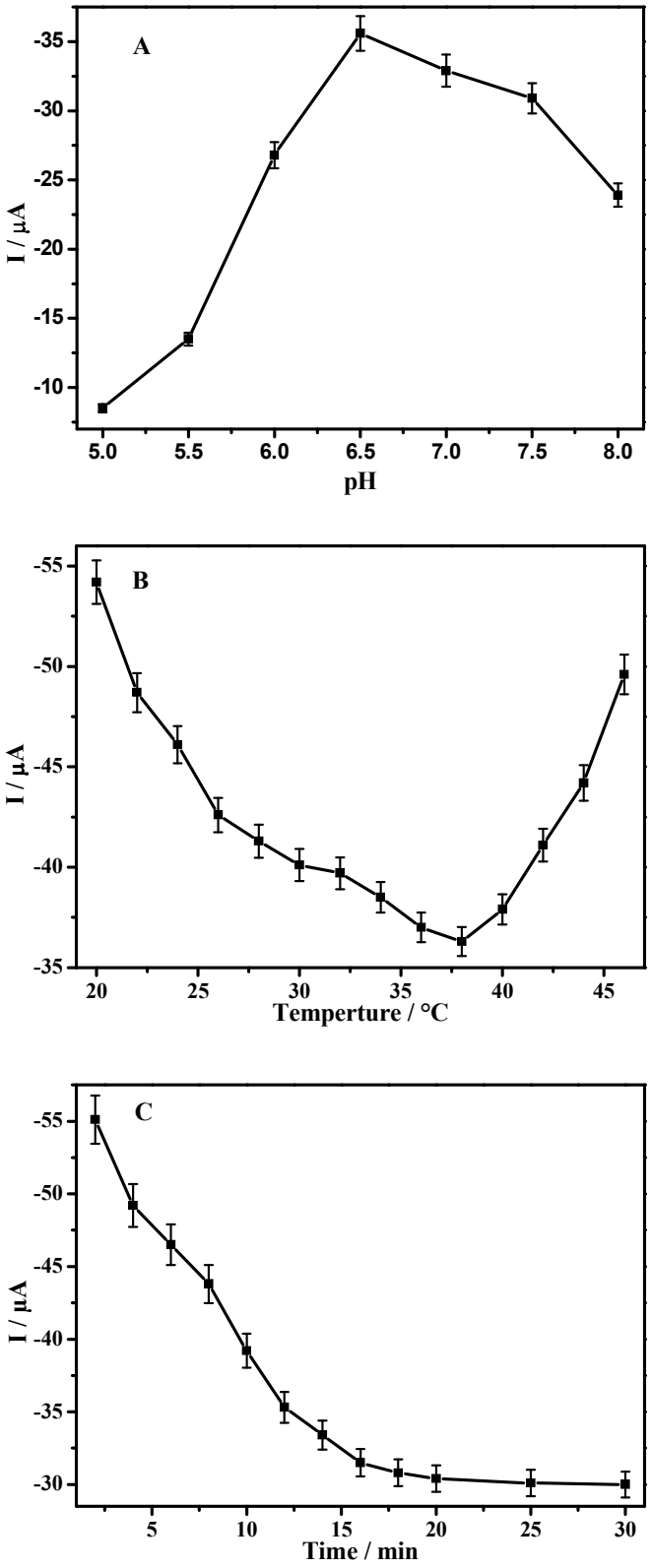

Figure 5. Oxidation peak currents of CVs of the proposed electrode at different detection solution pH (A), incubation temperature (B) and incubation time (C).

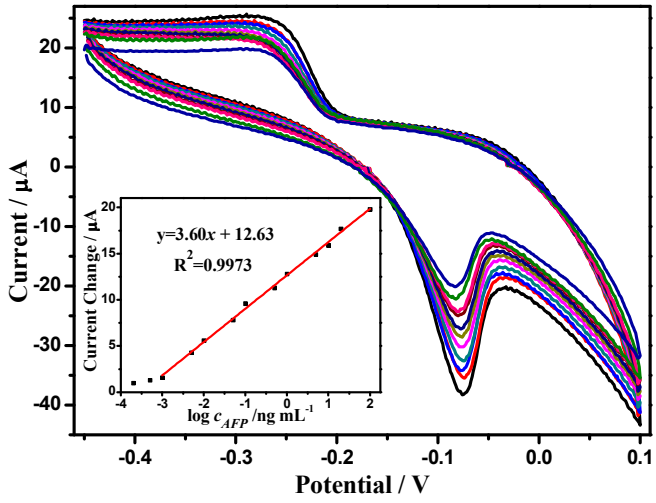

Figure 6. CVs of different concentration of AFP. Inset: peak current vs. logarithm of AFP concentration in 1/15 mol L^{-1} PBS (pH 6.5).

Table 1. Comparison of the performance of electrochemical immunosensor for the determination of AFP. rGO: reduced graphene oxide; Gr: graphene; MPS: mesoporous silica; CNTs: carbon nanotubes; Ab: Antibody

Immunosensor fabrication	Linear range / ng ml^{-1}	Detection limit / pg ml^{-1}	Reference
Pd-rGO/Anti-AFP	0.01-12	5	[33]
Gr/SnO/Au/Anti-AFP	0.02-50	10	[34]
Gr/TMCS-MPS/CNTs/anti-AFP	0.1-100	60	[35]
chitosan/Au/Ab1/AFP/Ab2/Au-Fe_3O_4	0.01-40	2.3	[36]
Cu_2O-PDA-Au/Anti-AFP	0.001-100	0.6	This work

Polydopamine functionalized Cu_2O rhombic dodecahedra as superexcellent electroactive substance for ultrasensitive electrochemical immunosensor