Calcium Carbonate–Gold Nanocluster Hybrid Spheres: Synthesis and Versatile Application in Immunoassays

Juan Peng,[a] Li-Na Feng,[a] Kui Zhang,[b] Xing-Hua Li,[a] Li-Ping Jiang,*[a] and Jun-Jie Zhu[a]

Abstract: Fluorescent gold nanoclusters (AuNCs) were incorporated into porous calcium carbonate spheres through electrostatic interaction. The resulting CaCO₃/AuNCs hybrid material exhibited interesting properties, such as porous structure, excellent biocompatibility, good water solubility, and degradability. These properties make the CaCO₃/AuNCs hybrid material a promising template to assemble horseradish peroxidase/antibody conjugates (HRP-Ab₂). By using CaCO₃/AuNCs/HRP-Ab₂ bioconjugates as probes, a versatile immunosensor was developed for fluorescent and electrochemical detection of the cancer biomarker neuron-specific enolase (NSE). The detection limits of the sensor were 2.0 and 0.1 pg mL⁻¹ for fluorescent and electrochemical detection, respectively. The immunosensor shows high sensitivity and offers an alternative strategy for the detection of other proteins and DNA.

Keywords: calcium carbonate · electrochemistry · fluorescent probes · gold · immunoassays

Introduction

Gold nanoclusters (AuNCs) have attracted great attention in the past decade. Research interest ranges from fundamental properties such as photoluminescence,[1–4] optical chirality,[5–7] ferromagnetism,[8] and quantized double-layer charging behavior[9,10] to potential applications in optoelectronics,[11] sensing,[4,12–15] and bioassays.[16–18] Compared to semiconductor quantum dots, which have larger size (3–100 nm) and contain toxic metal species (e.g., cadmium, lead), AuNCs are highly attractive because of their smaller size and nontoxicity.[19] Moreover, AuNCs exhibit other fascinating features, including ease of synthesis, good water solubility, and surface functionalities, which also give them great promise in bioassays. However, direct bioconjugation of these AuNCs with biomolecules may induce aggregation during the cross-linking reaction, which influences the stability, precision, and reproducibility of the assay. Thus, a promising strategy is to use a porous template to encapsulate AuNCs.

Calcium carbonate (CaCO₃) is one of the main biomineral components of seashells. Polymorph control of CaCO₃ biomineral in seashells is achieved naturally by complex cellular cues. CaCO₃ has three different crystalline phases: vaterite, calcite, and aragonite. Porous CaCO₃ microspheres can be used as effective hosts for the fabrication of biocompatible hybrid materials due to their occurrence in nature as biominerals and their porous structure, which makes them suitable for the loading of other materials.[20,21] Porous CaCO₃ microspheres have wide applications in drug delivery,[22] biosensing,[21,23–25] and protein encapsulation.[20] The difficulty in using this attractive material for the design of multifunctional composites lies in its polymorphic character. The most attractive phase of CaCO₃ for the fabrication of multifunctional materials is the metastable vaterite form featuring spherical morphology and porous surface. Vaterite easily undergoes phase transition to the more thermodynamically stable phase of CaCO₃, calcite, which does not show the spherical, porous morphology of vaterite.[20] This phase transformation usually occurs overnight for vaterite samples in water,[20] which greatly limits their further application. CaCO₃ also shows good degradability, because it can be easily dissolved in ethylenediaminetetraacetic acid (EDTA) solution under mild conditions. Much research has been carried out on the fabrication and application of CaCO₃ microspheres with large size (larger than 3 μm).[20,26] However, the large size limits their application in bioassay. Thus, a facile way of preparing CaCO₃ spheres of smaller size is highly required and of great challenge.

Hybrid materials with specific catalytic, magnetic, or optical properties have been fabricated.[27–30] To the best of our knowledge, no report has been made on the fabrication of CaCO₃/AuNCs hybrid spheres. Due to the good biocompatibility of both CaCO₃ spheres and AuNCs, excellent fluores-
cent property of AuNCs, and porous structure of CaCO₃/AuNCs hybrid material could find potential applications in drug delivery, bioassay, and biological systems.

Immunoassays based on specific molecular recognition of an antigen by its antibody have been widely used for quantitative analysis of protein biomarkers for clinical purposes. Numerous immunoassays have been developed for enhancement of detection sensitivity by signal amplification or employment of different detection technologies. Nanoparticle-based labeling systems have attracted special interest in immunoassays due to the outstanding optical, electronic, and biocompatible performance of nanoparticles.[31,32] Here, CaCO₃/AuNCs hybrid spheres were employed to construct a labeling system for the fabrication of dual fluorescent and electrochemical detection platform.

In this work, a novel CaCO₃/AuNCs multifunctional material was synthesized and further used for the encapsulation of horseradish peroxidase/antibody conjugates (HRP-Ab₂) to fabricate CaCO₃/AuNCs/HRP-Ab₂ bioconjugates (Scheme 1). Fluorescent AuNCs were incorporated into CaCO₃ porous spheres. The resulting CaCO₃/AuNCs hybrid material retained the porous spherical structure, which provided an efficient template for the assembly of HRP-Ab₂ to construct a labeling system. The ability of CaCO₃/AuNCs/HRP-Ab₂ to act as probes was further examined by performing a sandwich bioaffinity immunoassay, as shown in Scheme 1b. Neuron specific enolase (NSE) is highly specific for neurons. Elevated NSE levels in serum can be attributed to cerebral injury due to physical damage or ischemia caused by infarction or cerebral hemorrhage, coupled with increased permeability of the blood/brain barrier. The serum concentration of NSE has also been reported to correlate with the extent of damage and neurological outcome.[33] Therefore, NSE was chosen as a protein model to evaluate the immunoassay. The coating antibody (Ab₁) was immobilized on polyethylenimine (PEI)-functionalized, nitrogen-doped multiwalled carbon nanotubes (PNCNs). Then, NSE was bound with Ab₁ through immunoreaction. Finally, CaCO₃/AuNCs/HRP-Ab₂ bioconjugates were captured during the specific binding event. The sensing signals could be detected by fluorescence and electrochemical differential pulse voltammetry (DPV).

Results and Discussion

Synthesis and characterization of the porous CaCO₃/AuNCs hybrid spheres: The porous CaCO₃ spheres were synthesized by a one-pot approach.[34] Figure 1A shows TEM image of the CaCO₃ spheres, which have diameters in the range of 500–700 nm. A high-resolution TEM (HRTEM) image (inset in Figure 1A) showed that the CaCO₃ sphere has a unique structure and porous surface with pore size around 30 nm. The nanosized pores and channels in the CaCO₃ spheres provide efficient templates for encapsulation of biomacromolecules such as enzymes and proteins through physical adsorption and pore diffusion.[35] As shown in Figure S1 (Supporting Information), fluorescent AuNCs around 2 nm in size were synthesized and well dispersed in deionized water. For the adsorption of AuNCs, poly(allylamine hydrochloride), PAH, was used to make the surfaces of the CaCO₃ spheres positively charged. Loading with AuNCs was carried out by dispersing the CaCO₃ spheres in an aqueous solution of AuNCs under sonication. Since the CaCO₃ spheres have positive surface charge (+16.5 mV) and AuNCs negative surface charge (−13.5 mV), the driving force for adsorption of AuNCs into the pores was mainly attributed to electrostatic interaction. Figure 1B shows TEM and HRTEM images of the CaCO₃/AuNCs hybrid spheres. The AuNCs incorporated into the CaCO₃ particles can be
clearly observed. The spherical and channel-like interior structure can also be observed in the HRTEM image (inset of Figure 1B). This result confirms that the AuNCs were successfully loaded into the CaCO₃ spheres.

The fabrication process was monitored by fluorescence spectroscopy. Figure 2A shows fluorescence spectra of the as-prepared AuNCs solution (curve a) and the supernatant of the AuNCs solution after adsorption onto CaCO₃ particles (curve b). A great loss in fluorescence intensity was observed after loading of AuNCs, which indicates incorporation of the AuNCs into the CaCO₃ spheres. No fluorescence was observed for the CaCO₃ spheres (curve a in Figure 2B). In contrast, the CaCO₃/AuNCs hybrid spheres showed strong fluorescence (curve b in Figure 2B), indicating effective loading of AuNCs, which are responsible for the strongly fluorescent property in the hybrid spheres. The loading amount of AuNCs in the CaCO₃ particles could be calculated by the difference in peak intensity of AuNCs solution before and after loading (Figure 2A). According to the concentration difference before adsorption (344 μg mL⁻¹) and after adsorption (56 μg mL⁻¹), a rough estimate indicated that 1.0 mg of CaCO₃ particles could accommodate 288 μg AuNCs.[36,37] Confocal laser-scanning fluorescence microscopy was also employed to demonstrate the distribution of the AuNCs in the CaCO₃ spheres (Figure 3). This showed that the AuNCs could penetrate into the inner pores of CaCO₃. The porous structure and open channels in the CaCO₃ spheres offer suitable microenvironments for AuNCs adsorption, and resulted in a large loading amount of AuNCs.

Because of the porous structure, the CaCO₃ spheres have a high surface area of 27.21 m² g⁻¹ and pore volume of 0.1227 cm³ g⁻¹ (curve a in Figure S2, Supporting Information). After incorporation of AuNCs, the CaCO₃/AuNCs hybrid spheres had a surface area of 20.60 m² g⁻¹ and a pore volume of 0.0926 cm³ g⁻¹ (curve b in Figure S2, Supporting Information), which are smaller than those of the CaCO₃ spheres. In addition, a narrower pore-size distribution (inset of Figure S2, Supporting Information) and a sharp decrease in the number of large pores were observed, which might be attributed to infiltration of the AuNCs into the CaCO₃ particles. SEM images of both the CaCO₃ spheres and the CaCO₃/AuNCs hybrid spheres are shown in Figure 4. As can be seen in Figure 4A, the surface of the CaCO₃ particles is porous and consists of numerous carbonate nanoparticles that form the specific morphology. This unique structure played an important role in the assembly of AuNCs. After incorporation of AuNCs, the surface became smooth (Figure 4B). It was found that loading with AuNCs could enhance the stability of CaCO₃ spheres consisting of the vaterite polymorph. When the CaCO₃ particles were stored in water for five days (Figure 4C), calcite began to form. In contrast, the CaCO₃/AuNCs hybrid particles were stable as the vaterite polymorph in water for more than two months (Figure 4D). This result is confirmed by the XRD data in Figure S3 (Supporting Information). It is speculated that for-

Figure 2. A) Fluorescence spectra of a) as-prepared AuNCs and b) the supernatant after AuNCs adsorption. B) Fluorescence spectra of a) CaCO₃ particles and b) CaCO₃/AuNCs suspension. Inset of B) is a photograph of CaCO₃/AuNCs aqueous solution under UV light.

Figure 3. Confocal laser-scanning fluorescence image of CaCO₃/AuNCs spheres. Inset: high-magnification confocal image of a CaCO₃/AuNCs sphere.

Figure 4. SEM images of A) vaterite CaCO₃ spheres, B) CaCO₃/AuNCs hybrid spheres, C) calcite CaCO₃ acquired after five days, and D) CaCO₃/AuNCs spheres after two months.
mation of thermodynamically stable compounds between the stabilizer and CaCO₃ is the main reason for the good stability of the hybrid. It is reported that a solid solution is formed between calcite carbonate and inorganic materials on the vaterite surface.[38] These solid solutions show a lower heat of formation than calcite, and thus prevent dissolution of the vaterite polymorph. For comparison, gold nanoparticles (AuNPs) with a size of 20 nm were used to fabricate CaCO₃/AuNPs hybrid spheres. The SEM image of the CaCO₃/AuNPs hybrid spheres (Figure S4, Supporting Information) indicated that the calcite polymorph of CaCO₃ could easily form after one week. AuNPs of larger size less readily penetrate into the inner pores of CaCO₃ than AuNCs of smaller size, and hence the loading of AuNPs in CaCO₃ is greatly reduced. Thus, incorporation of AuNCs can efficiently stabilize the vaterite polymorph of CaCO₃.

Assembly of protein on CaCO₃/AuNCs hybrid spheres for biolabeling system: The CaCO₃/AuNCs hybrid spheres inherited advantages from their parent materials, such as satisfactory biocompatibility, good solubility in water, and porous structure. Thus, the CaCO₃/AuNCs hybrid spheres could be expected to be promising templates for protein loading. Then HRP-Ab₂ was encapsulated into the hybrid spheres and the obtained bioconjugates were used as versatile probes for immunoassay. Since the pI of HRP is 8.8,[39] HRP-Ab₂ is positively charged at pH 7.0, and thus could be easily adsorbed on the hybrid spheres through electrostatic adsorption and interactions between AuNCs and the amino groups of the protein. Compared to CaCO₃/AuNCs hybrid spheres, a smaller BET surface area (13.62 m² g⁻¹) and lower pore volume (0.0682 cm³ g⁻¹) for CaCO₃/AuNCs/HRP-Ab₂ indicated effective loading of HRP-Ab₂. Because of their small size, HRP-Ab₂ could penetrate into the pores, resulting in a decrease in the pore volume. Calculating the difference in enzyme concentration before and after adsorption revealed that about 180 μg of HRP-Ab₂ was captured by 1.0 mg of CaCO₃/AuNCs hybrid spheres from a protein solution with a concentration of 0.4 mg mL⁻¹ HRP-Ab₂. When the hybrid material was conjugated with HRP-Ab₁ and kept in water for one month, vaterite was still the dominant polymorph. To demonstrate the potential application of CaCO₃/AuNCs/HRP-Ab₂ bioconjugates in bioassay, a sandwich immunoassay was developed.

Fabrication of a sandwich immunosensor by using CaCO₃/AuNCs/HRP-Ab₂ as probes: The utility of CaCO₃/AuNCs/HRP-Ab₂ bioconjugates for immunoassay was examined by using a model sandwich immunoassay for detection of NSE. Nitrogen-doped multiwalled carbon nanotubes (NCNs) have attracted considerable interest for constructing electrochemical biosensors because of their high electrical conductivity and excellent electrocatalytic effects.[40] To fabricate the immunosensing surface, NCNs were used for immobilization of Ab₁. To increase their solubility and biocompatibility, NCNs were initially acid-oxidized to introduce carboxyl groups on their surface (see Supporting Information for experimental details), followed by the functionalization with polyethyleneimine (PNCNs). Then, by employing glutaraldehyde as cross-linking agent, Ab₂ molecules could be firmly absorbed onto the PNCN surface through the interaction between amino-functionalized PNCNs and primary amino groups of the protein. As shown in Figure S5 (Supporting Information), SEM was employed to characterize the NCNs, PNCNs, and the Ab₁ immobilized on PNCNs (PNCNs/Ab₁). The NCNs showed a well-dispersed one-dimensional structure with diameter in the range of 20–40 nm. For PNCNs, no obvious change was observed in the morphology after PEI functionalization. When Ab₂ was immobilized on the surface, the thickness of PNCNs/Ab₁ clearly increased, which indicated that Ab₂ was effectively bound to the PNCN surface. The immunoassay process is outlined in Scheme 1b. First, Ab₁ was immobilized on the PNCN surface, and then NSE was bound with Ab₁ through the first immunoreaction. Subsequently, CaCO₃/AuNCs/HRP-Ab₂ bioconjugates were captured on the surface by the second immunoreaction. Finally, two applications were demonstrated to detect the target NSE concentration using fluorescent and electrochemical DPV analysis. The details of the procedure are described in the Experimental section.

Fluorescence immunoassay: To be useful as a fluorescence biolabel, it is important that the loaded AuNCs can be released from the particles for fluorescence detection. In our work, AuNCs can be released from the captured CaCO₃/AuNCs/HRP-Ab₂ bioconjugates by dissolution of CaCO₃ templates in aqueous EDTA solution. Thus, the fluorescence of the AuNCs released from the biolabels was detected to determine the NSE concentration. The fluorescence intensity was strongly affected by the assay conditions (Figure S6, Supporting Information). After the Ab₂ concentration increased to 50 μg mL⁻¹, the fluorescence intensity increased and tended to a stable signal at 50 μg mL⁻¹. Thus, an Ab₁ concentration of 30 μg mL⁻¹ was selected for the further studies. The fluorescence intensity increased with the incubation time between 10 and 30 min and then leveled off above 30 min. This result indicated that the interaction between antigen and antibody had reached equilibrium after 30 min, and hence an incubation time of 30 min was selected. The fluorescence response increased with increasing concentration and reached a platform at 50 μg mL⁻¹ of the bioconjugates. Under optimal assay conditions, the fluorescence intensity with CaCO₃/AuNCs/HRP-Ab₂ probes (curve b in Figure 5A) was 8.54 times the signal when using AuNCs/HRP-Ab₂ probes (curve a in Figure 5A). This amplification of the fluorescence signal can mainly be attributed to the high loading amount of AuNCs on CaCO₃ spheres. As shown in Figure 5B and C, the fluorescence response of the immunosensor with CaCO₃/AuNCs/HRP-Ab₂ probes increased linearly with increasing logarithm of the target NSE concentration in the range from 0.005 to 1.0 ng mL⁻¹. The linear regression equation is \( F/N = \frac{428.8 + 174.81 \log(C_{\text{NSE}})}{\text{nmL}^{-1}} \) with a linear regression coefficient of 0.995. The detection limit (\( S/N = 3 \)) is estimated to be 2.0 pg mL⁻¹.
**Calcium Carbonate–Gold Nanoclusters Hybrid Spheres**

**FULL PAPER**

**DPV analysis**: The target NSE concentration was also detected electrochemically. It is well known that HRP can catalyze the oxidation of \( \sigma \)-phenylenediamine (OPD) by \( \text{H}_2\text{O}_2 \), and the mechanism of enzymatic catalysis and oxidation was investigated previously.\(^{[41]}\) The DPV response can be tested in \( \text{0.1 m pH 7.0 phosphate-buffered saline (PBS)} \) containing OPD and \( \text{H}_2\text{O}_2 \). The mechanism of the electrochemical oxidation of OPD is illustrated in Scheme 2. Multiple HRP in \( \text{CaCO}_3/\text{AuNCs}/\text{HRP-Ab}_2 \) bioconjugates can catalyze the oxidation of OPD with \( \text{H}_2\text{O}_2 \). Figure 6A shows typical DPV curves for \( \text{AuNCs}/\text{HRP-Ab}_2 \) and \( \text{CaCO}_3/\text{AuNCs}/\text{HRP-Ab}_2 \) bioconjugates. A well-defined peak was observed around \(-0.521 \text{ V (vs. SCE)}\) for the electrochemical oxidation of OPD to \( \text{2,2'-diaminoazobenzene} \), the enzymatic product.\(^{[42]}\) The DPV response was strongly influenced by the assay conditions. Therefore, the concentration of \( \text{Ab}_1 \), incubation time, and concentration of bioconjugates were investigated (Figure S7, Supporting Information). An \( \text{Ab}_1 \) concentration of \( 30 \mu\text{g mL}^{-1} \), an incubation time of 30 min, and a bioconjugate concentration of \( 50 \mu\text{g mL}^{-1} \) were selected as optimal.
assay conditions. The DPV signal with CaCO₃/AuNCs/HRP-Ab₂ probes (curve b in Figure 6A) was 4.24 times that with AuNCs/HRP-Ab₂ probes (curve a in Figure 6A). This also confirms that the signal was amplified by the high loading amount of AuNCs/HRP-Ab₂ on porous CaCO₃. Figure 6B shows typical DPV curves of the immunoassay at different NSE concentrations under optimal conditions. The voltammetric peaks were well defined and the intensity was proportional to the concentration of NSE in the range from 0.0005 to 2.0 ngmL⁻¹. The resulting calibration plots were linear (Figure 6C). The linear regression equation is \( I/\mu A = 7.944 + 2.138 \lg (C_{\text{NSE}}/\text{ng mL}^{-1}) \) with a linear regression coefficient of 0.993. The detection limit (S/N = 3) is estimated to be 0.1 pgmL⁻¹.

Compared to other reported electrochemical immunosensors, the proposed assay showed a lower detection limit. The high sensitivity could be attributed to the following reasons: The porous CaCO₃ sphere provided an efficient host for multiple AuNCs/HRP-Ab₂ loading, which greatly enhanced the detection signal. In addition, the PNCN-modified GCE was an effective matrix to immobilize biomolecules with high stability and bioactivity.

**Specificity and stability of the immunoassay:** The selectivity of the immunoassay was evaluated by using possible interfering substances such as immunoglobulin G (IgG) and α-fetoprotein (AFP) at a concentration of 0.1 ngmL⁻¹. The fluorescence responses for IgG and AFP were 5.8 and 7.1% that of NSE, respectively. The DPV signals for IgG and AFP were 6.2 and 7.4% that of NSE, respectively. This indicates that the proposed sensor has sufficient selectivity for NSE detection, and is capable of distinguishing NSE from its analogues in complex samples. The reproducibility of the immunoassay was estimated by assaying one NSE level for five replicate measurements with relative standard deviations (RSD) of 6.5%. The stability of CaCO₃/AuNCs/HRP-Ab₂ was also investigated by comparing the signals after a sandwiched immunoassay. When the CaCO₃/AuNCs/HRP-Ab₂ bioconjugates were stored in PBS at 4°C, the signals remained at about 93.4% for fluorescence response and 94.6% for DPV signal after one month. This indicates that CaCO₃/AuNCs/HRP-Ab₂ has good storage stability at 4°C.

**Application of the immunoassay in human serum:** The feasibility of the immunoassay in clinical applications was investigated by analyzing several real samples, in comparison with the ELISA method. Figure 7 shows the correlation between the results obtained by fluorescence immunoassay, electrochemical immunoassay, and the ELISA method. The relative deviations between fluorescence immunoassay and ELISA method were in the range from -4.4 to +4.68%, and the relative deviation between electrochemical assay and ELISA method was 3.2–7.2%. Since there are no significant differences among the results of the three methods, the developed immunoassay may provide an interesting alternative tool for detection of proteins in clinical laboratories.

**Conclusion**

Porous CaCO₃ spheres were used for loading of AuNCs to fabricate CaCO₃/AuNCs hybrid. The vaterite polymorph of CaCO₃ can be well stabilized by AuNCs. The hybrid spheres have the advantages such as porous structure, high porosity, biocompatibility, degradability, and fluorescence property and can be used as templates for the encapsulation of HRP-Ab₂ to fabricate CaCO₃/AuNCs/HRP-Ab₂ bioconjugates. A promising and versatile immunosensor was developed for both fluorescent and electrochemical detection of NSE by using the as-prepared bioconjugates as probes. The CaCO₃/AuNCs hybrid spheres are also expected to find other potential applications such as drug delivery, diagnostics, and optics.

**Experimental Section**

**Chemicals:** H₂O₂ (30% w/v solution), HAuCl₄·H₂O and o-phenylenediamine (OPD) were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Human neuron specific enolase (NSE) antigen, NSE antibody coating (Ab₁, monoclonal), and NSE antibody (Ab₂, polyclone) labeled with HRP (HRP-Ab₂) were purchased from Shanghai Linc-Bio Science Co. Ltd. Poly(allylamine hydrochloride) (PAH, mw = 15000), poly-ethyleneimine (PEI), bovine serum albumin (BSA), glutaraldehyde (GLU), and Tween-20 were obtained from Aldrich Chemical Co. All other chemicals were of analytical grade. Doubly distilled water was used throughout the experiments.

**Synthesis of CaCO₃/AuNCs hybrid spheres:** AuNCs were synthesized according to the literature (see Supporting Information for experimental details).[20] CaCO₃ spheres were synthesized by a one-pot approach.[34] For CaCO₃/AuNCs synthesis, CaCO₃ spheres were dispersed in PAH aqueous solution (2 mgmL⁻¹) and sonicated for 10 min to make the surface positively charged. Then, CaCO₃ (50 mg) was dispersed in 50 mL of AuNCs solution (pH 7.0) and sonicated for 30 min. After centrifugation, the composites were obtained. The composites were further washed with distilled water three times and dried.

**Assembly of HRP-Ab₂ into the CaCO₃/AuNCs spheres:** 10 mg of CaCO₃/AuNCs was dispersed in a solution of HRP-Ab₂ (0.4 ngmL⁻¹) and shaken for 1 h at 4°C for enzyme absorption. The bioconjugates were then centrifuged and washed with distilled water three times. Then, the CaCO₃/AuNCs/HRP-Ab₂ was dispersed in PBS.
After incubation with 60 mCi of 125I, the electrode was immersed in a solution of Ab1 for 1 h for immobilization of Ab1. The electrode was rinsed with doubly distilled water and allowed to dry at room temperature. As shown schematically in Scheme 1, 5.0 mg mL⁻¹ of Ab1 was immobilized to a concentration of 5.0 mg mL⁻¹ on a gold wire as the auxiliary electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum counterelectrode. Electrochemical measurements were performed from 0.3 to 0.8 V with a pulse amplitude of 50 mV and a pulse width of 50 ms.

Immunoassay procedure: GCE/PNCNs/Ab was blocked with 100 mL of 10 mM BSA for 30 min at room temperature and washed with PBST. After incubation with 60 mCi of 125I-labeled antigen, the electrode was rinsed with 2 ml of PBS, 2.0 mM H₂O₂, and then immersed in a 500 mA GLU solution for 30 min. After rinsing with water, the electrode was immersed in a solution of Ab2, for 1 h for immobilization. The immunosensor that was stored at 4°C when not in use.

Acknowledgements

We greatly appreciate the support of the National Natural Science Foundation of China (No. 21075061, 21103088, 21121091) and the Natural Science Foundation of Jiangsu Province (BK2010363). This work is also supported by National Basic Research Program of China (2011CB933502).


Received: September 14, 2011
Revised: December 31, 2011
Published online: March 15, 2012