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Electrochemiluminescence Immunosensor Based on CdSe Nanocomposites

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A novel strategy for the enhancement of electrochemiluminescence (ECL) was developed by combining CdSe nanocrystals (NCs), carbon nanotube−chitosan (CNT−CHIT), and 3-aminopropyl-triethoxysilane (APS). A label-free ECL immunosensor for the sensitive detection of human IgG (HIgG) was fabricated. The colloidal solution containing CdSe NCs/CNT−CHIT composite was first covered on the Au electrode surface to form a robust film, which showed high ECL intensity and good biocompatibility. After APS as a cross-linker was covalently conjugated to the CdSe NCs/CNT−CHIT film, the ECL intensity was greatly enhanced. And, an intensity about 20-fold higher than that of the CdSe NCs/CNT−CHIT film was observed. After antibody was bound to the functionalized film via glutaric dialdehyde (GLD), the modified electrode could be used as an ECL immunosensor for the detection of HIgG. The specific immunoreaction between HIgG and antibody resulted in the decrease in ECL intensity. The ECL intensity decreased linearly with HIgG concentration in the range of 0.02−200 ng mL^{-1}, and the detection limit was 0.001 ng mL^{-1}. The immunosensor has the advantages of high sensitivity, speed, specificity, and stability and could become a promising technique for protein detection.

Semiconductor nanocrystals (NCs) or quantum dots (QDs) have been extensively studied because of their unique size-dependent electronic, magnetic, optical, and electrochemical properties.[1–3] Highly luminescent semiconductor NCs have gained increasing attention for the use in light-emitting devices and tagging applications.[4–6] Recent work has demonstrated that the layer-by-layer assembly of CdSe NCs in sandwiched polyelectrolyte architecture can improve their photoluminescence property and be used for the detection of paraoxon.[7,8] Some bioinorganic conjugates prepared with CdSe/ZnS core−shell NCs and antibodies have shown potential applications in fluoroimmunoassays.[9–12] Luminescent properties of semiconductor NCs are usually investigated by photoluminescence (PL),[13] electrogenerated chemiluminescence (ECL),[14–16] and cathodoluminescence. ECL is a useful technique for both fundamental study and analytical applications of semiconductor NCs.[17–22] Investigation of NCs ECL has attracted increasing interest because NCs have a great potential for the development of novel ECL sensors and biological labels for ECL detection. In recent years, the ECL phenomena of QDs in organic and aqueous media have been observed.[19–28] At the same time, great effort has been focused on the ECL behavior and mechanism of QDs. However, the reports concerning the detection of biomolecules using ECL of QDs are relatively scarce, though ECL analysis has many advantages over PL due to the absence of background from unselective photoexcitation. The reasons are partially that the ECL intensity of semiconductor NCs is lower than that of conventional luminescent reagents such as luminal or $Ru(bpy)_3^{2+}$ and that NCs in ECL processes with a high excited electrochemical potential are unstable. Thus, it is important to select some new semiconductor NCs and effective methods to enhance

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ECL that are urgently needed for the development of ECL biosensors. The thin-film technique of semiconductor NCs may provide an effective way for the construction of QD-based ECL sensors in aqueous solutions. The efficiency of chemiluminescence can be dramatically enhanced by applying a cathodic potential to the layers made from CdSe or CdSe/CdS core−shell NCs.[29]

Recently, owing to their remarkable optical properties,[30–33] CdSe NCs have been the subject of intense study and proven to be attractive candidates for constructing ECL biosensors. In order to improve the ECL intensity and biocompatibility of NCs, carbon nanotubes (CNTs) have received particular interest due to their remarkable nanostructure, high electrical conductivity, and good chemical stability.[34,35] It is reported that CNTs can be used to enhance the ECL of CdS QDs film by reducing the injection barrier of electrons to the QDs.[36] Furthermore, the QDs−CNTs composites have promising applications in photoelectric devices, biological sensors, and catalytic materials.[37–39] In addition, owing to the excellent film-forming ability and susceptibility to chemical modifications of chitosan (CHIT), a biocompatible solubilization of CNTs in CHIT has been extensively used in biosensing.[40,41] Taking into account the above advantages, the CdSe NCs/CNT−CHIT composite will probably display high ECL intensity, good biocompatibility, and high stability, which is promising for constructing novel ECL biosensors.

Nowadays, sensitive measurement of proteins is a critical need in many aspects of biochemical and biomedical research. The ECL immunosensor which combines the high sensitivity of ECL detection with the specificity of immunoreaction has been paid much attention and proven to be a remarkable detector.[42,43] To the best of our knowledge, no immunosensor using ECL of NCs/CNT−CHIT composite film has been reported.

Herein, we present a novel strategy for the enhancement of NCs ECL in aqueous solution by combining CdSe NCs with CNT−CHIT and 3-aminopropyl-triethoxysilane (APS) and successfully developed a label-free ECL immunosensor for the sensitive detection of human IgG (HIgG). The interfusion of CNT−CHIT in CdSe NCs film not only improved the ECL intensity, biocompatibility, and stability but also facilitated the modification of APS for ECL immunosensor. After APS was conjugated to the CdSe NCs/CNT−CHIT composite film, the ECL intensity was further greatly enhanced. It is for the first time that the unique function of APS was explored and used to develop the promising ECL immunosensor. After antibody (Ab) was linked to the CdSe NCs/CNT−CHIT/APS composite film via glutaric dialdehyde (GLD), the electrode could be used as an ECL immunosensor for HIgG (Ag). The immunosensor provided a convenient, low-cost, sensitive, and specific method for protein detection, which could be an alternative technique in the clinical laboratory.

EXPERIMENTAL SECTION

Chemicals and Materials. Multiwalled CNTs (CVD method, purity >95%, diameter 30−60 nm, length 0.5−15 μm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). Chitosan was obtained from Aldrich (Milwaukee, WI). Human IgG (Ag) and goat anti-human IgG (Ab) were purchased from Ningbo Xinzhi Biochemical Reagents (Ningbo, China). Bovine serum albumin (BSA, 96−99%) and GLD were obtained from Sigma (St. Louis, MO). APS ($RC_3H_7-NH_2$) was obtained from Shuguang Chemical Co. (Nanjing, China). All other reagents were of analytical reagent grade and used without further purification. Phosphate-buffered saline (PBS), 0.1 M, with various pH values was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4 and then adjusting the pH with 0.1 M NaOH and H_3PO_4. PBS (0.1 M, pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl was used as the electrolyte. Doubly distilled water was used throughout the experiments.

Apparatus. The electrochemical measurements was carried out on a CHI 812 electrochemical working station (Shanghai CH Instruments Co., China) using a three-electrode system. The electrodes were a 4 mm diameter Au disk working electrode modified with CdSe NCs composite film, a saturated calomel reference electrode (SCE), and a Pt counter electrode. The ECL emission was detected with a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature. The spectral width of the photomultiplier tube (PMT) was 200−800 nm, and the voltage of the PMT was 500−800 V in the detection process. Electrochemical impedance spectroscopy (EIS) was carried out with a CHI 660A electrochemical workstation (Shanghai CH Instruments Co., China), using the same three-electrode system as that in the ECL detection. UV absorption spectra were acquired with a Ruili 1200 photospectrometer (Peking Analytical Instrument Co., Peking, China). Photoluminescence spectra were obtained on an RF-540 spectrophotometer (Shimadzu). Fluorescence microscopy images were taken from a Nikon TE2000-U inverted optical microscope (Japan). Field emission scanning electron microscopy (FESEM, JEOL JSM-6340 F) was used to study the morphology of CNT and CNT−CHIT.

Preparation of Soluble CNT−CHIT. A 0.50 wt % chitosan stock solution was prepared by dissolving chitosan flakes in hot (80−90 °C) aqueous solution with 0.05 M HCl. After the solution was cooled to room temperature, the pH was adjusted to 3.5−5.0 with NaOH solution. The chitosan solutions were filtered using a 0.45 μm Millex-HA syringe filter unit (Millipore) and stored in a refrigerator (4 °C) when not in use. All chitosan solutions were colorless.

CNTs were chemically shortened by ultrasonic agitation in a mixture of sulfuric acid and nitric acid (3:1) for 4 h. The resulting CNTs were separated and washed repeatedly with distilled water

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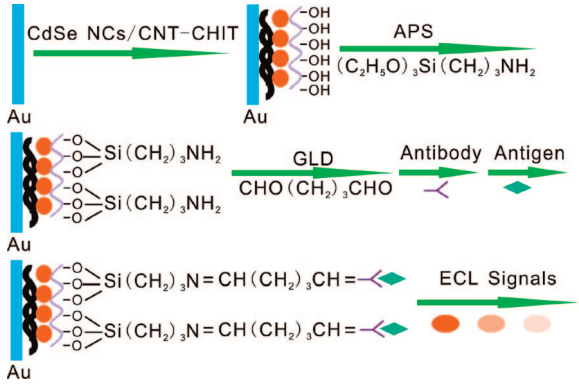
Scheme 1. Fabricating Steps of the ECL Immunosensor



by centrifugation until the pH was ~7. The purified CNTs were solubilized in chitosan solutions (0.50 mg mL^{-1}) using sonication for 15 min to give a homogeneous black solution.

Preparation of the CdSe NCs/CNT–CHIT Composite Solution. Mercaptoacetic acid-capped CdSe NCs (CdSe/TGA) were synthesized using a slightly modified procedure reported previously.[44] After 20 mL of 5 mM $CdCl_2$ was mixed with 20 μL of TGA, 1 M NaOH was added to adjust its pH to 10. The clear solution was diluted to 50 mL and bubbled with highly pure N_2 for 30 min. Then, 0.5 mL of 0.1 M Na_2SeSO_3 was injected into this mixture to obtain a clear light yellow solution of CdSe/TGA NCs. The final molar ratio of Cd^{2+}/TGA/Se^{2-} was 1:2.5:0.5. The sizes of the obtained NCs could be tuned by simply varying the reflux time.

The 0.05 mM CdSe NCs solution was first adjusted to pH 5.5 with 1 M HCl. Then the above CNT–CHIT and the CdSe NCs solutions were mixed in the volume ratio of 1:3 under sonication for 30 min, and the CdSe NCs/CNT–CHIT composite solution was obtained.

Preparation of the ECL Immunosensor. A gold disk electrode with 4 mm diameter was polished carefully with 1.0, 0.3, and 0.05 μm α-Al_2O_3 powder on fine abrasive paper and washed ultrasonically with water. Before modification, the bare electrode was scanned in 0.5 M H_2SO_4 between −0.2 and 1.5 V until a reproducible cyclic voltammogram (CV) was obtained. After the electrode was rinsed thoroughly with doubly distilled water and allowed to dry at room temperature, 10 μL of CdSe NCs/CNT–CHIT composite solution was dropped on the electrode and dried in the air, followed by immersing the electrode in 2% APS solution for 30 min to introduce the amine functional groups. After rinsing with redistilled water, the electrode was dipped in 2.5% GLD solution at room temperature for 15 min and then in 0.5 mg/mL antibody solution (50 mM PBS, pH 7.4) at 4 °C for at least 12 h. Finally, it was rinsed with pH 7.4 PBS and incubated in 20 μL of 2 wt % BSA at 37 °C for 1 h to block nonspecific binding sites. After being rinsed with pH 7.4 PBS, the electrode was used as an ECL immunosensor and incubated in 40 μL of HIgG (Ag) samples at 37 °C for 50 min. Scheme 1 outlines the fabrication of the ECL immunosensor. This includes the formation of the CdSe NCs/CNT–CHIT composite film on the Au electrode, the linkage of APS with the film, the covalent conjugation of GLD with APS, the immobilization of antibody on the electrode by GLD, and the specific immunoreaction.

ECL Detection. The modified electrodes above were in contact with 0.1 M PBS (pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl and scanned from 0 to −1.5 V. ECL signals related to the HIgG concentrations could be measured.

RESULTS AND DISCUSSION

Characterization of the CNT–CHIT Architecture. *FESEM Images.* As a biomacromolecule, chitosan is insoluble at alkaline and neutral pH, but it can well dissolve in acidic solution when the pH is lower than 6. In acidic medium, it behaves as a polycation. As we know, CNTs functionalized with carboxylic acid groups are negatively charged. When CNTs and chitosan were mixed by ultrasonication, chitosan molecules were adsorbed on the surface of CNTs by electrostatic interaction, which resisted CNTs aggregating. As a result, a black solution could be observed. The SEM image of the pure CNTs film in Figure 1A displays an enlaced structure of small bundles or single tubes. The surface in the side walls was smooth, and the diameter of the CNTs was about 40 nm. As shown in Figure 1B, the more porous and homogeneous structure could be observed for the CNT–CHIT

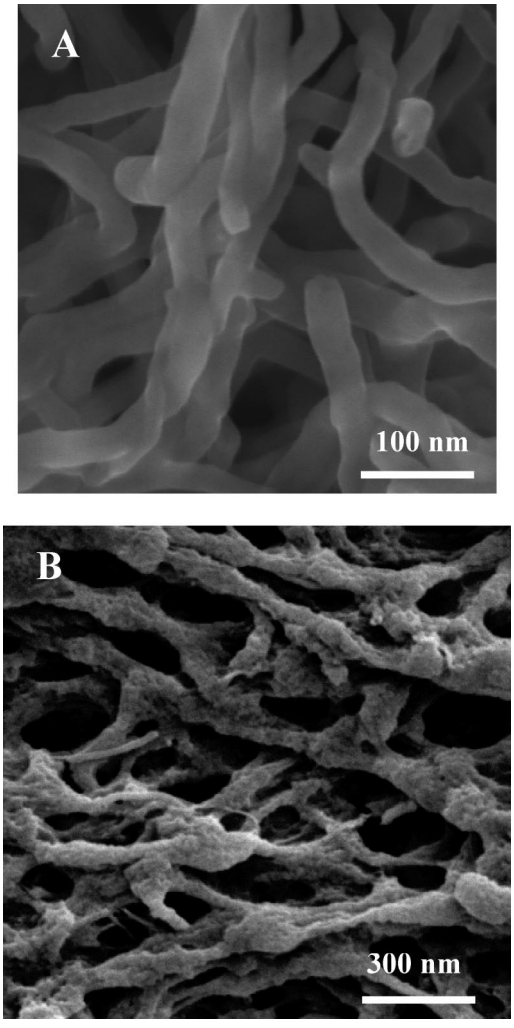


Figure 1. Representative FESEM images of CNTs (A) and CNT–CHIT (B).

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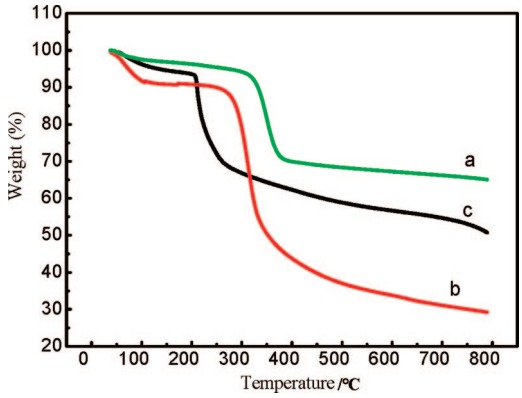

Figure 2. TG curves of (a) CNT, (b) CHIT, and (c) CNT−CHIT samples.

composite film, and the surface was much rougher, which was favorable for the construction of the ECL immunosensor.

Thermogravimetric Curves. Figure 2 presents the comparative thermogravimetric (TG) studies of the solid-state samples of CNT, CHIT, and CNT−CHIT. The TG graph of CNT sample (Figure 2, curve a) shows that there is mass loss in the temperature range from 320 to 400 °C, which could be ascribed to the destruction of functional groups attached to CNTs.[45] This result indicated the good stability and high purity of the CNT. The remaining material at higher temperatures corresponded to transition metals that were introduced during the synthesis of CNTs.

The TG graph of the CHIT sample (Figure 2, curve b) shows that the thermal decomposition of chitosan took place in two steps after the initial evaporation of residual water. These steps could involve depolymerization and decomposition of glucosamine units of chitosan at 100−250 °C, which was followed by the oxidative decomposition of the residues in the temperature range from 250 to 350 °C.[46]

The TG of the CNT−CHIT composite sample in Figure 2, curve c, shows that it decomposed within the temperature range from 220 to 800 °C in two steps, which generally resembled the thermal decomposition of the CHIT sample.

Characterization of the CdSe NCs. *PL and Absorption Spectra.* Figure 3 shows the PL and absorption spectra (inset) of the CdSe NCs in aqueous solution. The PL emission peak at 616 nm (λ_{ex} = 450 nm) and absorption maximum at 496 nm indicate the consequence of quantum confinement.[32] The size of the CdSe NCs, estimated from the first adsorption peak in UV−vis spectra and the empirical equations,[47] is about 3.0 nm.

Fluorescence Image. Figure 4 shows the fluorescence image of the CdSe NCs in aqueous solution. The red-orange light emitted by the CdSe NCs was consistent with the above PL spectra.

Electrochemical and ECL Behaviors of the CdSe NCs Composite Film. The curve in Figure 5 (inset) shows the CVs of CdSe NCs/CNT−CHIT composite film on the Au electrode. One cathodic peak appeared at −1.02 V, corresponding to the reduction of $S_2O_8^{2-}$.[28]

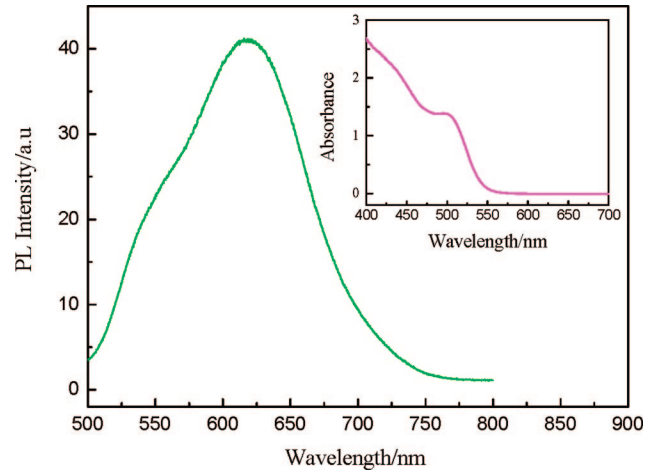

Figure 3. Photoluminescence and absorption (inset) spectra of the CdSe NCs in aqueous solution. Excitation wavelength: 480 nm.

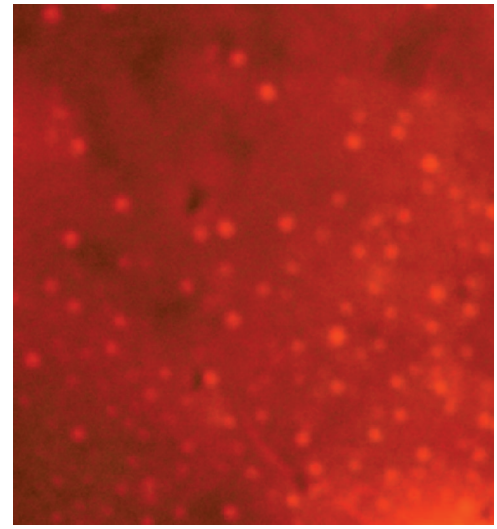

Figure 4. Fluorescence image of CdSe NCs.

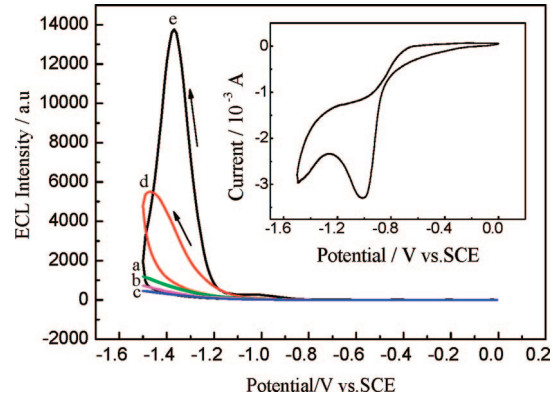

Figure 5. ECL−potential curves of (a) the bare Au electrode, (b) the CNT−CHIT/Au electrode, (c) the CNT−CHIT/APS/Au electrode, (d) the CdSe NCs/Au electrode, and (e) the CdSe NCs/CNT−CHIT/Au electrode in 0.1 M PBS (pH 7.4) containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. Inset: cyclic voltammogram of the CdSe NCs/CNT−CHIT/Au electrode. Scan rate: 100 mV s^{-1}. The voltage of the PMT was 700 V.

Figure 5, curves d and e, shows the ECL−potential curves of the pure CdSe NCs and CdSe NCs/CNT−CHIT composite film, respectively. The quantities of CdSe NCs are equal in both films.

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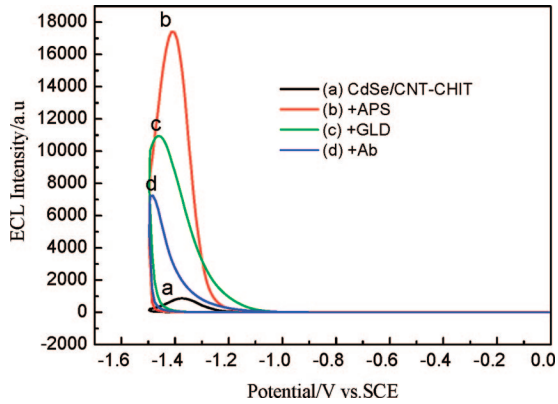
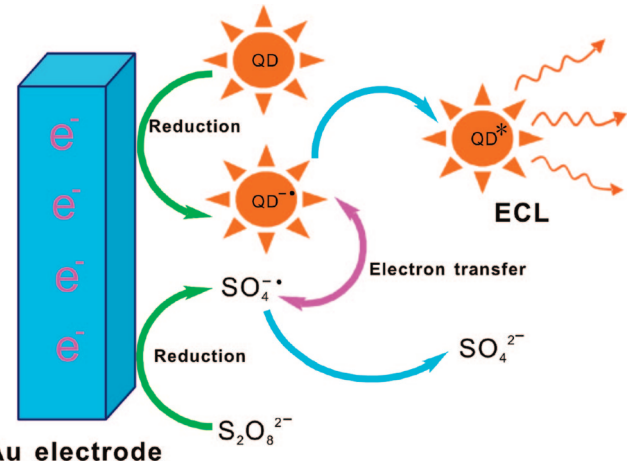


Figure 6. ECL−potential curves of (a) CdSe NCs/CNT−CHIT, (b) CdSe NCs/CNT−CHIT/APS, (c) CdSe NCs/CNT−CHIT/APS/GLD, and (d) CdSe NCs/CNT−CHIT/APS/GLD/Ab modified Au electrodes in 0.1 M PBS (pH 7.4) containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. (The voltage of the PMT was 500 V.) Scan rate: 100 mV s^{-1}. (The voltage of the PMT was specially set at 500 V because of the 17 700 counts of maximum scale of the ECL analyzer.)

One ECL peak was observed in both curves, resulting from the reaction between CdSe NCs and $S_2O_8^{2-}$. It should be highlighted that the ECL intensity from the CdSe NCs/CNT−CHIT composite film is about 2.5-fold higher than that observed from the pure CdSe NCs film, where the ECL peak from the composite film is at −1.37 V, whereas that from the pure CdSe NCs film is at −1.47 V. The results indicated that the presence of CNT/CHIT not only enhanced the ECL intensity of the CdSe NCs but also decreased the potential barriers of the ECL reaction. The reasons may be that the more porous structure and larger surface area in the composite film facilitated the diffusion of $K_2S_2O_8$ into the membrane, which resulted in the occurrence of ECL signal not only at the interface but also in the nanocrystalline film. Therefore, the CdSe NCs/CNT−CHIT composite film was more favorable for the fabrication of the ECL immunosensor.

Because APS has reactive amine groups, it can usually be recognized as an efficient cross-linker for the conjugation of biomolecules. Herein it was used for antibody immobilization to construct an ECL immunosensor. As shown in Figure 6, when APS was conjugated to the CdSe NCs/CNT−CHIT composite film, the ECL intensity was enhanced about 20-fold higher (Figure 6, curve b) than that observed from the CdSe NCs/CNT−CHIT composite film (Figure 6, curve a). To identify the function of APS for ECL enhancement, control experiments were performed. In the absence of $K_2S_2O_8$, the ECL of the composite film in PBS was very weak, which was hardly enhanced after APS conjugation. The result suggests that $K_2S_2O_8$ is necessary for the strong ECL, and APS only catalyzes the ECL reaction of CdSe NCs with $K_2S_2O_8$. If mercaptopropyltriethoxysilane (MPS) instead of APS was conjugated to the CdSe NCs/CNT−CHIT composite film, a slight decrease of ECL was observed because of the increased impedance, which was different from the ECL enhancement of APS. Since APS and MPS have similar groups except that the former has amine functional groups, whereas the latter has mercapto functional groups, it may be inferred that the amine groups of APS play a key role in the ECL enhancement, which facilitates

Scheme 2. ECL Mechanisms of the CdSe NCs



the radical generation and electron-transfer processes during the ECL reaction.[48,49] This deduction could also be demonstrated by the great decrease in ECL signal (Figure 6, curve c) after reaction of GLD with the amine groups of APS.

In addition, control experiments in the absence of CdSe NCs were also performed. As shown in Figure 5, all the ECL signals in the absence of CdSe NCs were very low, which indicated that the CNT, CHIT, and APS could not generate ECL, and thus ECL was from the CdSe NCs.

According to previous reports, electrochemically reduced and oxidized Si NCs[19] or CdSe NCs[20] can react with coreactants to produce ECL. In this case, upon the potential scan with an initial negative direction, the CdSe NCs immobilized on the electrode were reduced to nanocrystalline species ($CdSe^{-\bullet}$).[20] Reduction of $S_2O_8^{2-}$ by APS ($RC_3H_7-NH_2$) on the electrode produced a strong oxidant, $SO_4^{-\bullet}$, which can then react with the negatively charged $CdSe^{-\bullet}$ by injecting a hole into the highest occupied molecular orbital (HOMO), producing an excited state (CdSe*) to emit light. The possible ECL mechanisms are described in Scheme 2 with the following equations:[19,48,49]

$$\text{CdSe NCs} + ne^- \rightarrow n\text{CdSe}^{-\bullet} \quad (1)$$

$$S_2O_8^{2-} + RC_3H_7 - NH_2 \rightarrow SO_4^{2-} + SO_4^{-\bullet} + RC_3H_7 - NH_2^{+\bullet} \quad (2)$$

$$\text{CdSe}^{-\bullet} + SO_4^{-\bullet} \rightarrow \text{CdSe}^* + SO_4^{2-} \quad (3)$$

$$\text{CdSe}^* \rightarrow \text{CdSe} + h\nu \quad (4)$$

Fabrication of the ECL Immunosensor. *Electrochemiluminescence.* In order to characterize the fabrication process of the ECL immunosensor, ECL signals at each immobilization step were recorded. As shown in Figure 6, when APS was conjugated to the CdSe NCs/CNT−CHIT composite film (Figure 6, curve a), the ECL intensity was greatly enhanced (Figure 6, curve b) because of the APS catalysis on the ECL reaction. Then, when GLD was covalently attached on the amine groups of APS, the

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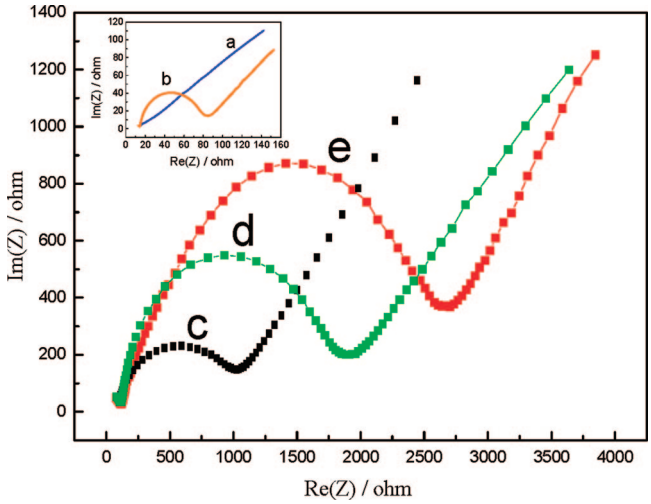

Figure 7. EIS of bare (a), CdSe NCs/CNT−CHIT (b), CdSe NCs/CNT−CHIT/APS (c), CdSe NCs/CNT−CHIT/APS/GLD (d), and CdSe NCs/CNT−CHIT/APS/GLD/Ab (e) modified Au electrodes in 10 mM PBS (2.5 mM $Fe(CN)_6^{4-/3-}$ + 0.1 M KCl, pH 7.4). The frequency range is between 0.01 and 100 000 Hz with signal amplitude of 5 mV.

ECL intensity obviously decreased (Figure 6, curve c), which could be attributed to the increased electron-transfer resistance in reaction. Finally, after antibody (Ab) was immobilized onto the electrode by GLD, the ECL intensity decreased again (Figure 6, curve d), suggesting that the protein layer blocked the electron exchange in ECL reaction.

Electrochemical Impedance Spectroscopy. Electrochemical impedance spectroscopy is an effective method for probing the features of surface-modified electrodes. The impedance spectra include a semicircle portion and a linear portion. The semicircle diameter at higher frequencies corresponds to the electron-transfer resistance (R_{et}), and the linear part at lower frequencies corresponds to the diffusion process. Figure 7 shows the EIS of the electrode at different stages. It was observed that the EIS of the bare Au electrode displayed an almost straight line (inset of Figure 7a), which was characteristic of a mass diffusion limiting process. After the electrode was modified with CdSe NCs/CNT−CHIT composite film, the EIS showed a low electron-transfer resistance of about 80 Ω, (inset of Figure 7b), implying that both CNT−CHIT and CdSe NCs possessed good electrical conductivity. Subsequently, APS was covalently conjugated to the composite film, followed by GLD. As a result, the electron-transfer resistance increased (Figure 7, curves c and d). In the final step, the immobilization of antibody generated an insulating protein layer on the assembled surface, which significantly increased the electron-transfer resistance (Figure 7e).

Detection of HIgG (Ag). Figure 8 shows the ECL emission of the immunosensor under consecutive potential scans from 0 to −1.5 V for 13 cycles. The ECL signals are high and stable, suggesting that the sensor is suitable for ECL detection.

Figure 9 shows the ECL responses of the immunosensor before (Figure 9a) and after (Figure 9b−i) reacting with different concentrations of HIgG (Ag). The ECL intensity of the large peak (ECL-1, −1.48 V) in the presence of Ag (Figure 9b) was lower than that in the absence of Ag (Figure 9a) and decreased gradually with the increase of concentrations of Ag (Figure 9b−i). The reason was that the formed immunocomplex after specific immunoreaction greatly inhibited the reaction on the electrode and thus decreased the ECL intensity, which suggested that the Ag concentration could be determined by the ECL-1 measurement. In addition, a small shoulder peak (ECL-2, −1.02 V) was observed after BSA was used to block nonspecific binding sites of the immunosensor. According to the literature,[15] the possible ECL mechanism was that the large peak (ECL-1) resulted from the reaction between individual reduced CdSe NCs and coreactants, whereas the shoulder peak (ECL-2) corresponded to the reaction between assembly of reduced NCs and coreactants.

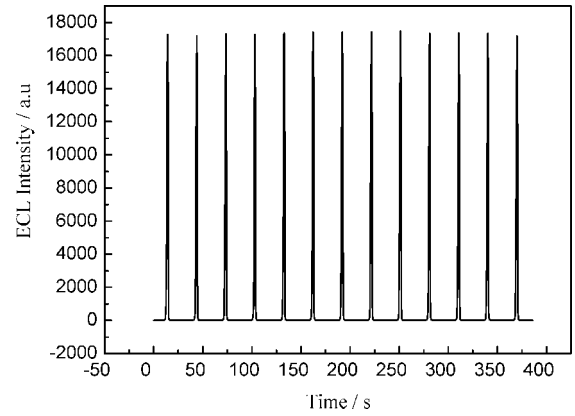

Figure 8. ECL emission from the immunosensor in pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$ under continuous cyclic voltammetry for 13 cycles. Scan rate: 100 mV s^{-1}. The voltage of the PMT was set at 600 V.

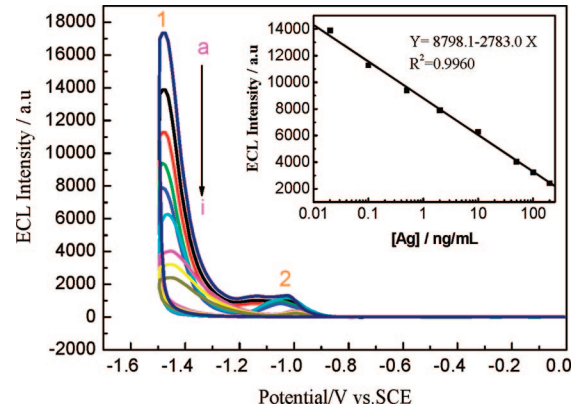

Figure 9. ECL profiles of the immunosensor in the absence (a) and presence (b−i) of different concentrations of Ag in pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. Ag concentration (ng mL^{-1}): (a) 0, (b) 0.02, (c) 0.1, (d) 0.5, (e) 2, (f) 10, (g) 50, (h) 100, (i) 200. The voltage of the PMT was set at 600 V. Inset: calibration curve for Ag determination. Scan rate: 100 mV s^{-1}.

The standard calibration curve for the Ag detection is shown in the inset of Figure 9. The ECL-1 peak intensity was linear with the Ag concentration from 0.02 to 200 ng mL^{-1} with a detection limit of 0.001 ng mL^{-1}. According to the linear equation, Ag concentration could be detected quantitatively. Higher human serum Ag levels were detected with an appropriate dilution with pH 7.4 PBS.

Specificity, Stability, Reproducibility, and Regeneration of the Immunosensor. To further characterize the specificity of the immunosensor, we first mixed 20 ng mL^{-1} goat IgG and 20 ng mL^{-1} HIgG and then detected the ECL response. In comparison with the ECL response obtained from the pure HIgG,

Table 1. Comparison of IgG Determinations on Human Serum Samples by the ECL Immunosensor and ELISA

serum samples	1	2	3	4	5
our method (ng/mL)	0.065	0.95	8.48	49.3	124.5
ELISA (ng/mL)	0.06	0.90	8.05	45.9	116.4
relative deviation (%)	5.6	3.8	3.7	5.1	4.8

no significant difference (RSD = 5.6%) was observed, indicating that the goat IgG could not cause the observable interference, and thus this immunosensor is feasible for the determination of HIgG in serum.

When the immunosensor was stored in pH 7.4 PBS at 4 °C over 30 days, no apparent change in the same HIgG concentration was found, indicating that the immunosensor has good stability.

The reproducibility of the immunosensor was estimated with intra- and interassay precision. The intraassay precision was evaluated by assaying one HIgG level for three replicate measurements. The interassay precision was estimated by determining one HIgG level with three immunosensors made at the same electrode. The intra- and interassay variation coefficients (CVs) obtained from 20 ng mL^{-1} HIgG were 5.8% and 7.4%, respectively. Obviously, the interassay CV showed a good electrode-to-electrode reproducibility of the fabrication protocol, while the low value of intraassay CV indicated that the immunosensor could be regenerated and used repeatedly.

Regeneration of the immunosensor is of interest to immunoanalysis. In the experiment, 0.2 M glycine−hydrochloric acid (Gly−HCl) buffer solution (pH 2.8) was chosen to break the antibody−antigen linkage. After detecting HIgG, the immunosensor was dipped into glycine−hydrochloric acid buffer solution for 5 min to remove HIgG from Ab. It had repeated five times consecutive measurements, and a relative standard deviation (RSD) of 5.8% was acquired.

Application of the Immunosensor in Human IgG Levels. The feasibility of the immunoassay system for clinical applications was investigated by analyzing several real samples. In comparison with the ELISA method, these serum samples were diluted to different concentrations with a PBS of pH 7.4.

Table 1 describes the correlation between the results obtained by the proposed ECL immunosensor and the ELISA method. It obviously indicates that there is no significant difference between the results and ELISA method. Thus, the developed immunosensor could be satisfactorily applied to the clinical determination of IgG levels in human serum.

CONCLUSIONS

The ECL response from the CdSe NCs in aqueous solution could be greatly enhanced by conjugating APS to the CdSe NCs/CNT−CHIT composite film. A novel label-free ECL immunosensor for the rapid, sensitive detection of HIgG was developed. The CdSe NCs/CNT−CHIT composite film displayed high ECL intensity, good biocompatibility, and excellent film-forming ability, which made it a promising candidate for developing the ECL immunosensor. It has been demonstrated here, for the first time, that APS could greatly enhance NCs ECL intensity while it acted as a cross-linker for antibody immobilization. By combining the high sensitivity of NCs ECL detection with the specificity of immunoreaction, the developed immunosensor has been successfully applied to the detection of IgG in a human serum sample. The results were validated by using the conventional ELISA method and showed high consistency. In particular, this highly enhanced ECL from the NCs composite film opens new avenues to apply NCs ECL in analytical systems and ECL biosensors.

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