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Electrogenerated Chemiluminescence from a CdSe Nanocrystal Film and Its Sensing Application in Aqueous Solution

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Electrogenerated chemiluminescence (ECL) of semiconductor quantum dots in aqueous solutions and its first sensing application were studied by depositing CdSe nanocrystals (NCs) on a paraffin-impregnated graphite electrode (PIGE). The CdSe nanocrystal thin film exhibited two ECL peaks at -1.20 (ECL-1) and -1.50 V (ECL-2) in pH 9.3, 0.1 M PBS during the cyclic sweep between 0 and -1.8 V at 20 mV s^{-1}. The electron-transfer reaction between individual electrochemically reduced nanocrystal species and oxidant coreactants such as H_2O_2 and reduced dissolved oxygen led to ECL-1. When mass NCs packed densely in the film were reduced electrochemically, assembly of reduced nanocrystal species could react with coreactants to produce another ECL signal, ECL-2. ECL-1 showed higher sensitivity to the concentration of oxidant coreactants than ECL-2 and thus was used for ECL detection of coreactant, H_2O_2. A linear response of ECL-1 to H_2O_2 was observed in the concentration range of 2.5×10^{-7}–6×10^{-5} M with a detection limit of 1.0×10^{-7} M. The fabrication of 10 CdSe nanocrystal thin-film modified PIGEs displayed an acceptable reproducibility with a RSD of 1.18% obtained at H_2O_2 level of 10 μM.

Highly luminescent semiconductor nanocrystals (NCs), often referred to as quantum dots (QDs), have received tremendous attention for their possible luminescent application in aqueous solution.[1,2] Some bioinorganic conjugates made with CdSe/ZnS core−shell NCs and antibodies have shown potential application in fluoroimmunoassays.[3−6] 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] Semiconductor NCs are complementary and in some cases may be superior to the existing fluorophores,[1] further explorations on their superior emitting properties by different methods were of great importance.

Due to the controllable merits of electrochemical method, the electrochemical study of the optical behaviors of semiconductor NCs has been extensively carried out.[9−13] Electrogenerated chemiluminescence (ECL) is a useful technique for both fundamental study and analytical application of semiconductor NCs.[14−19] Recent works have indicated the semiconductor NCs are electrically excitable in nonaqueous media[16−19] and aqueous systems[20] containing supporting electrolyte. The reduced and oxidized Si NCs[16] or CdSe NCs,[17] generated at some electrochemical potentials, can react with some coreactants to produce ECL. ECL behavior of semiconductor NCs is more sensitive to and dependent on their surface states than their photoluminescence property.[16−18] The electron-transfer reaction between electrochemically formed nanocrystal species and coreactants implies that QDs have great potential for development of novel ECL sensors. However, so far no ECL sensor based on semiconductor NCs has been reported, though ECL analysis has many advantages over photoluminescence due to the absence of background from unselective photoexcitation.

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Although ECL has been observed in many systems, reports concerning ECL from semiconductor NCs are relatively scarce mainly due to their low solubility and the instability of electrochemically formed nanocrystal species.[16,17] Thin-film technique is probably the most useful method to resolve this problem for electrooptic applications of semiconductor NCs.[20] An electron injection by applying cathodic potential to the nanoparticulate layers made from CdSe or CdSe/CdS core−shell NCs can dramatically enhance the efficiency of nanocrystal chemiluminescence and allow the achievement of efficient and stable ECL from CdSe nanoparticulate layers in aqueous solution.[20] As electron injection into semiconductor nanocrystal film can be reversibly controlled by an electrochemical method,[21,22] the thin film of semiconductor NCs provides an effective way for the construction of QD-based ECL sensors in aqueous solutions. This work reports the first QD-based ECL sensor in aqueous system, which is based on the electron-transfer reaction between reduced nanocrystal species formed in CdSe nanoparticulate thin film and hydrogen peroxide.

The accurate and rapid determination of hydrogen peroxide is of practical importance in bioanalytical and environmental fields.[23] Although H_2O_2 can be detected by many enzyme-based biosensors,[24−26] even a nanomolar detection limit could be achieved by using a simply designed amperometric biosensor,[27] the enzymes cannot obviously provide a complete long-term stability of the sensors due to their inherent instability. Here, we studied the ECL behaviors of CdSe nanocrystal thin films in aqueous solution. Upon addition of hydrogen peroxide to the solution, a great enhancement of the intensity of ECL emission of CdSe nanocrystal thin films at −1.20 V was observed, which leads to a novel application of CdSe NCs or semiconductor quantum dots in chemosensing in aqueous solutions.

EXPERIMENTAL SECTION

Materials. All reagents were of analytical grade, and doubly distilled water was used throughout. CdSe NCs were synthesized by an ultrasonic procedure similar to that developed by Zhu et al.[28] at higher ultrasonic intensity. Briefly, 50 mL of 10 mg mL^{-1} $CdCl_2$ was mixed with 5 mL of 30% ammonia, 10 mL of 0.2 M Na_2SeSO_3, and 35 mL of water. When yellow turbidity occurred, the solution was irradiated with a JY92-IID ultrasonic cell disruptor (Ningbo Scientz Biotechnology Ltd. Co., Ningbo, China) under ambient air for 30 min and dark red precipitates were obtained. The precipitates were centrifuged, washed with water and acetone in sequence, and then dried in air. For the nanocrystal thin-film preparation, the resulting powders were ultrasonically dispersed in ethanol and then filtered to obtain a colloidal solution of CdSe NCs, which was kept in a refrigerator at 4 °C.

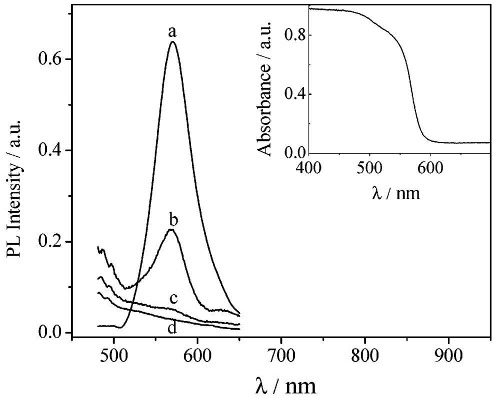

Figure 1. PL spectra of CdSe nanocrystal powder (a), CdSe nanocrystal thin films prepared with 10 (b) and 1 μL (c) of CdSe nanocrystal colloidal solution on PIGE, and bare PIGE (d) at λ_{ex} of 355 nm. Inset: UV spectrum of CdSe nanocrystal powder.

Modified Electrode Preparations. The paraffin-impregnated graphite electrode (PIGE, 3.0-mm diameter) was made according to the literature.[29] After the working electrode was smoothed on a weighing paper and rinsed with water, a drop of the colloidal solution of CdSe NCs was placed on the surface of PIGE and then let dry slowly at 4 °C under ambient pressure to form an optically clear thin film.[30]

The CdSe nanocrystal modified carbon paste electrode (CPE) was prepared by mixing 50 mg of graphite powder with 14 mg of CdSe nanocrystal powder and 36 mg of solid paraffin and then packing the obtained paste firmly into an electrode cavity (2.5-mm diameter, 5-mm depth).

Apparatus and Optical Measurements. ECL was conducted with a homemade ECL system containing a three-electrode system, which consisted of a CdSe nanocrystal thin-film modified electrode, a platinum counter electrode, and a Ag/AgCl reference electrode. During measurement, the potential was applied to the working electrode via a CHI 812 electrochemical working station (Shanghai, China), and the ECL emission was detected with a luminescence analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi′an, China) at room temperature. UV absorption spectra were acquired with a Ruili 1200 photospectrometer (Peking Analytical Instrument Co., Peking, China). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu). Scanning electron micrographs (SEMs) of CdSe nanocrystal thin films were obtained with a Hitachi X-650 scanning electron microscope (Hitachi Lid., Tokyo, Japan). Nitrogen was bubbled through the solution for different times and kept flowing over the solution during the ECL experiments.

RESULTS AND DISCUSSION

PL and SEM Characterizations of CdSe Nanocrystal Thin Film. The UV absorption edge of a CdSe NC sample showed an obviously blue shift from bulk band gap of 716 nm to 550 nm (inset in Figure 1), indicating the effect of quantum confinement.[31] The UV absorption at 550 nm suggested the size of the CdSe NCs

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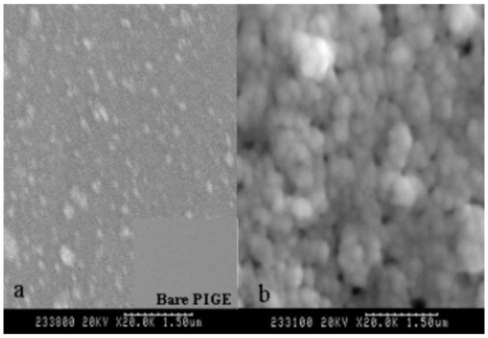

Figure 2. SEMs of CdSe nanocrystal thin films prepared with 1 (a) and 10 μL (b) CdSe nanocrystal colloidal solution on PIGE. Inset: bare PIGE.

to be ~35 Å.[17,31] The PL spectrum of the CdSe nanocrystal film deposited on PIGE (Figure 1b) was similar to that of the nanocrystal powder (Figure 1a) except for the difference in PL intensity. The peak position of immobilized CdSe NCs was almost identical to that of the CdSe nanocrystal powder, suggesting CdSe NCs deposited on the surface of PIGE preserved their light emission feature. With decreasing amount of CdSe NCs in the thin film, the PL intensity decreased (Figure 1c). The SEM of the CdSe nanocrystal thin film formed using 1 μL of nanocrystal colloidal solution showed "isolated" CdSe nanoparticles (Figure 2a), while the CdSe nanocrystal thin film formed using 10 μL of nanocrystal colloidal solution showed a close-packed assembly of CdSe nanoparticles (Figure 2b). From the well-known size-depended PL feature of CdSe NCs,[32] it could be concluded that the CdSe nanocrystal thin film was an assembly of CdSe NCs. The size of the aggregates was between 50 and 300 nm, depending on the amount of CdSe nanocrystals used for preparation of the thin film. Considering the PL intensity and ECL sensitivity of the proposed sensor, the thin film prepared using 10 μL of colloidal solution of CdSe NCs was chosen in the following section.

Electrochemical and ECL Behaviors of CdSe Nanocrystal Thin Film on PIGE. Figure 3 shows the cyclic voltammograms and ECL curves of the CdSe nanocrystal film at 20 mV/s. The bare PIGE electrode showed very low background current, and no light emission was observed in an air-saturated solution (curve a in Figure 3). At the CdSe nanocrystal thin-film modified PIGE, the cathodic current in air-saturated solution was greatly enhanced after −1.10 V and reached a peak value at −1.46 V (curve b in Figure 3). At the same time, two ECL peaks were observed at −1.20 (ECL-1) and −1.50 V (ECL-2), respectively. Thus, CdSe NCs (R) in the thin film could be reduced to produce nanocrystal species ($R^{\bullet-}$)[17] that then reacted with some coreactants to produce excited states (R^*) and to emit light in an aqueous system. Two ECL peaks resulted from the reaction between coreactants and individual reduced nanocrystal species (ECL-1) and the reaction between coreactants and assembly of CdSe reduced nanocrystal species (ECL-2). The latter could be demonstrated from the ECL responses of both CdSe NCs entrapped in carbon paste and the CdSe nanocrystal thin film formed using 1 μL of NC colloidal solution (insets in Figure 3), in which the formation of a close-packed nanocrystal film was impossible and only the ECL-1 was observed. Here the coreactants should be dissolved oxygen or its reduced product OOH^- [29] and some impurities in the film or electrode surface.[17] When dissolved oxygen was removed from the solution by bubbling high-purity nitrogen for 25 min, both peak current and light emission intensity of ECL-1 and ECL-2 decreased dramatically (curve c in Figure 3). When dissolved oxygen was further removed by adding Na_2SO_3 to the solution or bubbling high-purity nitrogen for a prolonged time, the reduction current and ECL intensity further decreased (Figure 4). Thus, dissolved oxygen or its reduced product OOH^- participated in the electrode process and ECL reactions. The addition of H_2O_2 to the deoxygenated solution by bubbling high-purity nitrogen for 25 min could reversibly enhance the reduction current and ECL intensity of CdSe nanocrystal thin-film modified PIGE (curve d in Figure 3). H_2O_2 was also a coreactant to react with individual reduced nanocrystal species and assembly of reduced nanocrystal species to produce ECL emissions (ECL-1 and ECL-2). The increase in the reduction current of CdSe nanocrystal thin-film modified PIGE upon addition of H_2O_2 or the presence of dissolved

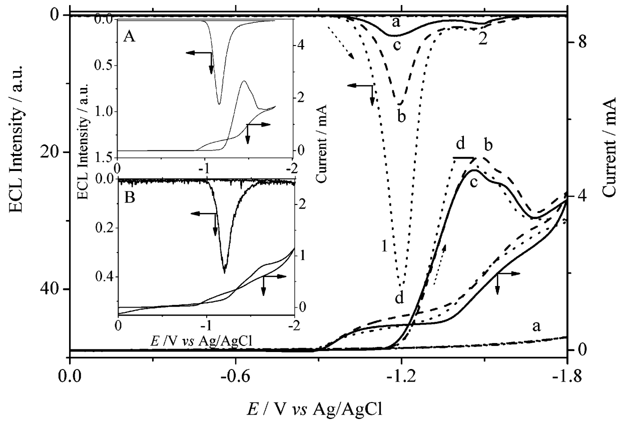

Figure 3. Cyclic voltammograms and ECL curves of bare (a) and CdSe nanocrystal thin-film modified PIGE in air-saturated 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 (b), solution b bubbled with N_2 for 25 min (c), and solution b + 100 μM H_2O_2 (d). Inset: cyclic voltammograms and ECL curves of (A) PIGE modified with 1 μL of CdSe nanocrystal colloidal solution and (B) CdSe nanocrystal modified CPE in air-saturated 0.1 M pH 9.3 PBS containing 0.1 M KNO_3. Scan rate: 20 mV s^{-1}.

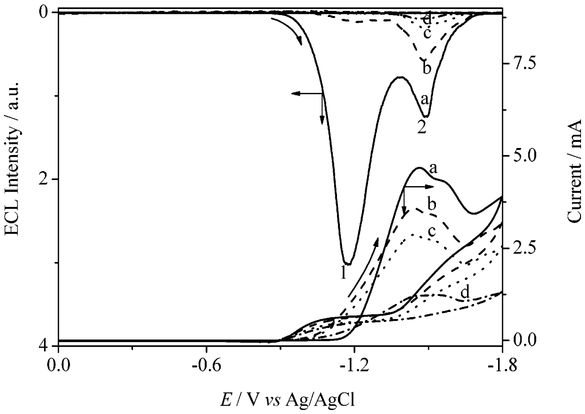

Figure 4. Effect of trace dissolved oxygen on the electrochemical and ECL responses of CdSe nanocrystal thin-film modified PIGE in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 bubbled with N_2 for 25 min (a), solution a + 0.1 M Na_2SO_3 (b), and solution b bubbled further with N_2 for 25 (c) and 50 min (d). Scan rate: 20 mV s^{-1}.

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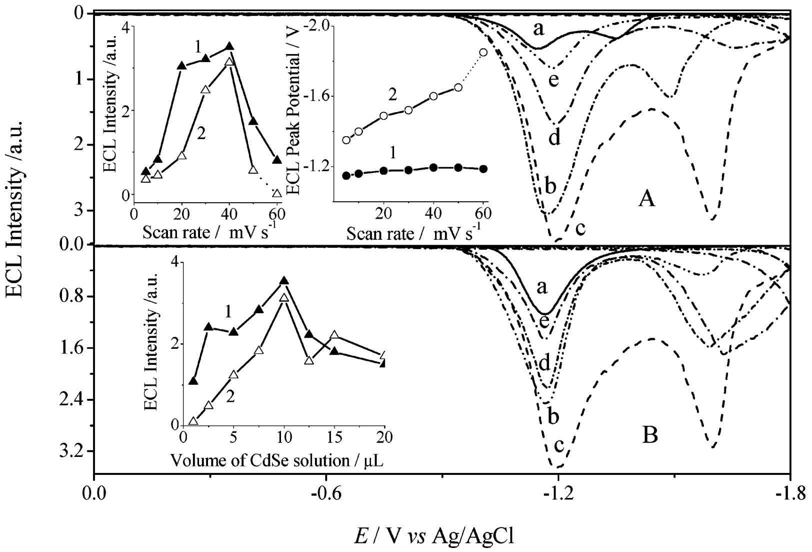

Figure 5. ECL curves of (A) CdSe nanocrystal thin-film modified PIGE in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 bubbled with N_2 for 25 min at (a) 5, (b) 20, (c) 40, (d) 50, and (e) 60 mV s^{-1} and (B) the electrodes prepared with (a) 1.0, (b) 2.5, (c) 10, (d) 12.5, and (e) 20 μL of CdSe nanocrystal colloidal solution at 40 mV s^{-1}. Inset: effects of (A) scan rate on ECL-1 and ECL-2 intensity and peak potentials and (B) amount of the colloidal solution used for preparation of nanocrystal film on ECL-1 and ECL-2 intensity.

oxygen indicated the regeneration of CdSe NCs. Thus, the electrochemical and ECL process can be expressed as follows:[17,29]

$$\text{CdSe NCs} + n\text{e} \rightarrow n\text{R}^{\bullet -} \tag{1}$$

$$O_2 + H_2O + 2e \rightarrow OOH^- + OH^- \tag{2}$$

$$2R^{\bullet -} + OOH^- + H_2O \rightarrow 3OH^- + 2R^* \tag{3}$$

or

$$2R^{\bullet -} + H_2O_2 \rightarrow 2OH^- + 2R^* \tag{4}$$

$$nR^* \rightarrow \text{CdSe NCs} + h\nu \tag{5}$$

From Figure 3, the ECL-1 occurred at −1.20 V, just following the cathodic current increase of CdSe nanocrystal thin-film modified PIGE in air-saturated solution. The cathodic current increase resulted from the formation of individual reduced nanocrystal species; thus, reactions 2−5 produced ECL emission. At the potential more negative than the cathodic peak potential of −1.46 V, most CdSe NCs were reduced to form an assembly of reduced CdSe nanocrystal species; thus, ECL-2 occurred at more negative potential than the cathodic peak potential. Both Figures 3 and 4 showed ECL-1 was more sensitive to the dissolved oxygen and H_2O_2, indicating the reactions between coreactants and individual reduced nanocrystal species was faster than those between coreactants and the assembly of reduced nanocrystal species. Thus, ECL-1 was chosen to detect H_2O_2 for developing ECL sensing application of CdSe nanocrystal thin film.

Effects of Scan Rate on ECL Response. With increasing scan rate, the ECL intensity of both ECL-1 and ECL-2 from CdSe nanocrystal thin-film modified PIGE, observed in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 bubbled with N_2 for 25 min, increased and reached the maximum emission intensity at 40 mV s^{-1} (Figure 5A). The increase in emission intensity was due to the formation of more reduced CdSe nanocrystal species in unit time. In this system, the coreactants were some impurities in the film or electrode surface[17] and the reduced product of rudimentary trace dissolved oxygen. When the scan rate was higher than 40 mV s^{-1}, the emission intensity decreased dramatically. This was because the electrochemical reduction of CdSe NCs was one irreversible electrode process, and high scan rate was unfavorable to the electrochemical reduction of CdSe NCs to produce reduced CdSe nanocrystal species. With increasing scan rate, the difference between maximum emission potentials of ECL-1 and ECL-2 also increased (inset in Figure 5A), indicating the formation of an assembly of reduced CdSe nanocrystal species was more difficult than individual reduced nanocrystal species. In the following experiments, the scan rate of 40 mV s^{-1} was chosen for obtaining the maximum emission intensity.

Effects of Density of Immobilized CdSe NCs on ECL Behaviors. The ECL emission of semiconductor NCs is very sensitive and dependent on the surface properties and the presence of surface states.[16,17] Thus, the architecture of the nanocrystal film has an important effect on its ECL behavior. Figure 5B shows the effect of volume of CdSe nanocrystal colloidal solution used for preparation of the CdSe nanocrystal thin-film modified PIGE on ECL curve and ECL intensity. The strongest ECL intensities of both ECL-1 and ECL-2 were obtained at the modified PIGE prepared with 10 μL of CdSe colloidal solution (inset in Figure 5 B), which was chosen in the modification procedure. With increasing density of CdSe NCs, the formed individual nanocrystal species and assembly of reduced CdSe nanocrystal species increased, leading to increasing ECL intensity. Furthermore, increase in the assembly of reduced CdSe nanocrystal species was more obvious due to the increase of the CdSe nanocrystal density. Thus, the amount chosen of CdSe NCs might improve the ECL behaviors of the obtained CdSe nanocrystal film.[20] When the volume was more than 10 μL, the ECL intensities of both ECL-1 and ECL-2 decreased, which was probably due to

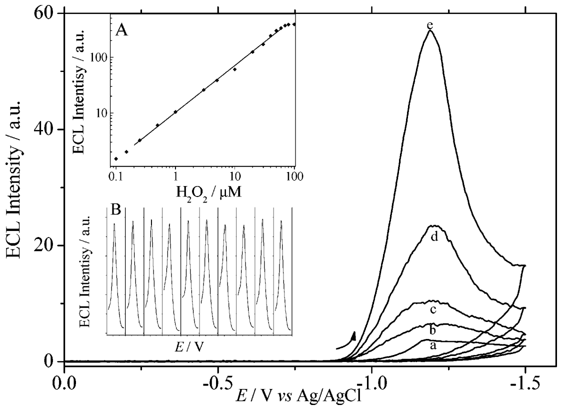

Figure 6. Effect of hydrogen peroxide concentration on ECL-1 intensity of CdSe nanocrystal thin-film modified PIGE in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 and (a) 0.0, (b) 0.5, (c) 1, (d) 3, and (e) 10 μM H_2O_2 bubbled with N_2 for 25 min at 40 mV s^{-1}. Inset: (A) calibration curve for H_2O_2 detection; (B) reproducibility of ECL-1 response of the modified in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 and 10 μM H_2O_2 bubbled with N_2 for 25 min at 40 mV s^{-1}.

the change in surface state of the thin film[18] due to the deposition of a large amount of CdSe NCs on the electrode surface.

Sensing Application of CdSe Nanocrystal Thin Film for ECL Detection of H_2O_2. As shown in Figure 3, H_2O_2 could obviously enhance the intensity of ECL-1 in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 after bubbling with N_2 for 25 min. Thus, the CdSe nanocrystal thin film can be used to develop an ECL sensor for H_2O_2 in aqueous solution. Under optimized fabrication conditions, the pH value of the electrolyte solution showed a great effect on the ECL emission. With an increasing solution pH from pH 5.5 to 10.0, the ECL intensity of the CdSe nanocrystal film in the presence of 10 μM H_2O_2 increased greatly and then trended to a constant value at pH 9.3. The enhancement in ECL emission in the presence of H_2O_2 was mainly due to the adsorption of Lewis bases,[33] which changed the surface states of CdSe nanocrystal film. In the solution bubbled with N_2 for 25 min, the ECL intensity of CdSe nanocrystal film in the absence of coreactants is very low; pH showed little effect on the ECL intensity. Considering the analytical performance of the ECL sensor, pH 9.3 was chosen for the determination procedure.

Figure 6 shows the sensing response of the CdSe nanocrystal thin-film modified PIGE to hydrogen peroxide in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 bubbled with N_2 for 25 min. A linear relation between the ECL-1 intensity and hydrogen peroxide concentration was obtained from 2.5×10^{-7} to 6×10^{-5} M with a correlation coefficient of 0.9992 (inset A). In this figure, the value of ECL-1 intensity was the average of three determinations. The detection limit was 0.1 μM at a signal-to-noise ratio of 3, which was much lower than those of some amperometric biosensors,[24,34–37] a flow injection technique based on electrocatalyzed luminol chemiluminescence,[38] and an ECL sensor based on polycrystalline TiO_2 film.[39] The fabrication of 10 sensors showed an acceptable reproducibility with a relative standard deviation of 1.18% at the 10 μM level (inset B in Figure 6). The CdSe NCs modified electrode showed good stability. After this electrode was stored under dark conditions for three months, the ECL-1 response decreased by only 3.4%. The colloidal solution could be preserved under dark conditions for a long time. After the colloidal solution was stored for six months, the CdSe NCs modified electrode obtained with the same volume of colloidal solution (10 μL) showed 98.4% of the original ECL-1 response. The stable response indicated the efficiency of electron transfer within the coating did not change upon storage.

The effects of some interferences on H_2O_2 determination with this ECL sensor were investigated in 0.1 M pH 9.3 PBS containing 0.1 M KNO_3 and 100 μM H_2O_2 bubbled with N_2 for 25 min. The addition of K^+, Cl^-, NO_3^-, and Br^- increased slightly the ECL intensity at concentrations lower than 0.1 M. Strong oxidants such as $S_2O_8^{2-}$ could increase the intensity of ECL-1 dramatically at the 1 mM level, while 1 mM SO_3^{2-} decreased the ECL intensity obviously. These results indicated the strong oxidants would act as coreactants to interfere with the determination of H_2O_2, while the strong reductant could directly react with H_2O_2 and thus interfere with its determination. The weak redox-active or redox-inactive compounds showed little interference with H_2O_2 determination.

Although the detection performance of the ECL sensor for hydrogen peroxide based on a CdSe nanocrystal thin film at the present stage was not so efficient as that of the luminol–enzyme-based ECL system,[14] improved performance would be achieved by extensively optimizing the size, structure, and surface states dependent ECL features of semiconductor NCs, such as nanocrystal composition, capping ligands, and even the architecture of the nanoparticulate layers. Many luminol-based ECL biosensors for different analytes have been developed based on the involvement of H_2O_2 in ECL process.[14,15] The further optimized ECL performance of the CdSe nanocrystal thin film should possess potential for determination of many analytes, just as those reported QD-based PL sensors or luminol-based ECL sensors.[4,14,15]

CONCLUSIONS

Electrogenerated chemiluminescence of a CdSe nanocrystal thin film deposited on PIGE surface in aqueous solutions exhibits two ECL peaks upon a negative potential scan, which correspond to two different mechanisms. The first ECL peak results from the electron-transfer reaction between coreactants and individual reduced nanocrystal species produced at cathodic potentials more negative than −1.10 V. The second ECL peak is due to the reaction between coreactants and assembly of reduced CdSe nanocrystal species. A close-packed film is required for the formation of the second ECL peak, suggesting ECL from the CdSe nanocrystal film is adjustable by controlling the packing density of nanocrystal thin film. Both the packing density of CdSe CNs and coreactants such as dissolved oxygen and hydrogen peroxide affect greatly the intensity of ECL. Based on the sensitizing of hydrogen peroxide to ECL of CdSe nanocrystal thin film, the first ECL sensing application of semiconductor QDs is developed. The novel

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ECL sensor shows a linear response to H_2O_2 ranging from 2.5×10^{-7} to 6×10^{-5} M with good sensitivity and reproducibility. This work provides an alternative method to avoid the inherent instability of biomolecules for designing of stable biosensors.

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