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## Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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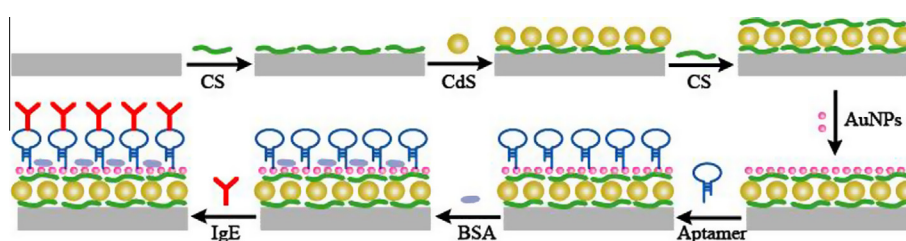
## Petal-like CdS nanospheres-based electrochemiluminescence aptasensor for detection of IgE with gold nanoparticles amplification

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## HIGHLIGHTS

- A label-free ECL aptasensor for sensitive detection of IgE was developed.
- Petal-like CdS nanospheres were prepared and utilized as ECL indicator.
- AuNPs co-immobilized with CdS enhanced the ECL intensity.
- The method was applied for the determination of IgE in human serum sample.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

## Article history:

Received 1 March 2015

Received in revised form 2 June 2015

Accepted 25 June 2015

Available online 29 June 2015

## Keywords:

Electrochemiluminescence

Label-free aptasensor

Petal-like CdS nanospheres

IgE

## ABSTRACT

A facile label-free electrochemiluminescence (ECL) aptasensor was designed for sensitive detection of human immunoglobulin E (IgE) using petal-like CdS nanospheres and Au nanoparticles (AuNPs) as sensing platform. To construct the aptasensor, petal-like CdS nanospheres as ECL emitter were firstly synthesized and immobilized on the chitosan-coated glassy carbon electrode (GCE) surface. Chitosan was coated on the CdS/CS/GCE again with two coating numbers to produce a stable ECL signal and facilitate subsequent AuNPs immobilization. The construction of aptasensor was achieved after IgE aptamer was adsorbed onto the AuNPs. The detection of IgE was performed upon the incubation of the interface with target protein IgE. Under the optimum conditions, the ECL signal decreased depending linearly on the logarithmic value of IgE concentration ranging from  $5.0 \times 10^{-13}$  to  $1.0 \times 10^{-9}$  M with a regression equation of  $I = -15254.8 - 2129.3 \log c$  ( $R^2 = 0.996$ ). The detection limit was experimentally found to be  $8.0 \times 10^{-14}$  M. The applicability of the constructed aptasensor was demonstrated in the determination of IgE in human serum samples.

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## 1. Introduction

Highly sensitive and selective measurement of protein biomarkers holds great promise for disease diagnosis, biomedical research, and biodefense applications. In the past years, many efforts, involving fluorescent [1], colorimetric [2], electrochemical [3], chemiluminescent [4], and electrochemiluminescent (ECL) [5]

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detection techniques, have been endeavored to develop sensitive biosensors for the assay of low-abundance biomarkers. Of these, ECL has attracted great attentions due to its inherent features such as high sensitivity, simplified optical setup, excellent temporal and spatial controllability [6–8]. Besides the conventional ECL reagents such as Ru complexes, luminol and its derivatives, several semiconductor nanocrystals (NCs) including CdS, CdSe, and CdTe NCs, have become promising ECL indicators for ECL aptasensors [9]. Although the NCs-based ECL aptasensors have exhibited various advantages in protein detection, the ECL efficiency of these indicators still could not come to those of conventional luminescent

reagents. Thus, finding a way to obtain high ECL intensity of NCs for biochemical analysis is the constant driving force of this area.

Recently, several materials such as TiO<sub>2</sub> nanotubes [10], carbon nanotubes [11], graphene [12], Au nanoparticles (AuNPs) [13] promoting the electrode transfer between NCs and the electrode or enhancing the loading capacity of the NCs have been explored for improving the ECL intensity of NCs. For instance, Song et al. proposed a sensitive ECL immunosensor based on CdS NCs decorated TiO<sub>2</sub> nanotube array which largely amplified ECL signals to detect IgG with a detection limit of 1.0 fg mL<sup>-1</sup> [10]. Jie et al. developed a CdS NCs-based ECL biosensor for sensitive detection of low-density lipoprotein by increasing sensitivity with AuNPs amplification [13]. Therefore, the combination of NCs with nano-materials provides an ingenious and effective approach to amplify ECL signal.

Human immunoglobulin E (IgE), an antibody subclass, which is found only in mammals, plays a very important role in allergic reactions and other related diseases. However, the determination of IgE in human serum is still challenging due to the protein is the least abundant of the immunoglobulins in serums [14]. The development of sensitive and selective method for analyzing IgE is critical in biochemical research and clinical diagnosis. Although the antibody have been used extensively as affinity reagents for IgE analysis [15,16], the drawbacks of antibody associated with its production, stability, and manipulation have prompted researchers to seek alternative. Aptamer, a new class of affinity elements, is becoming an alternative to antibody. Up to now, aptamer-based methods have been widely reported, such as colorimetry [17], electrochemistry [18,19], fluorescence [20], and capillary electrophoresis [21]. Among these methods developed, the label-free aptamer-based ECL biosensor has received an increasing attention due to its inherent advantages such as simplicity, high sensitivity, high specificity, and low cost. The development of novel, label-free ECL aptasensors for sensitive detection of IgE is promising.

Herein, we developed a novel strategy to fabricate a label-free ECL aptasensor for IgE detection based on petal-like structure CdS nanospheres and AuNPs. The petal-like structure CdS nanospheres and AuNPs provide signal amplification for ECL detection due to the high ECL efficiency of CdS and good conductivity and large electroactive surface area of AuNPs. The proposed aptasensor was used in determination of IgE in human serum samples.

## 2. Materials and methods

### 2.1. Reagents and materials

IgE was purchased from Athens Research and Technology (Athens, GA, USA). The aptamer (5'-SH-(CH<sub>2</sub>)<sub>6</sub>-TTTTTTTTTGGGG CACGTTTATCCGTCCCTCCTAGTGGCGTGGCCC-3') for IgE was prepared by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai,

China). Human platelet-derived growth factor-BB (PDGF-BB) was obtained from Peprotech (USA). Human thrombin (TB) was from Sigma-Aldrich (St. Louis, MO, USA). Carcinoembryonic antigen (CEA) was donated by Zhengzhou Immuno Biotech Co., Ltd. (Zhengzhou, China). Bovine serum albumin (BSA, Mr = 67,000), Cd(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, thiourea, polyvinyl pyrrolidone (PVP), hydrogen tetra chloraurate (III) trihydrate (HAuCl<sub>4</sub>·3H<sub>2</sub>O), and chitosan (CS) were from Shanghai Reagent Company (Shanghai, China). AuNPs were prepared by citrate reduction of HAuCl<sub>4</sub> in aqueous solution [22]. All aqueous solutions were prepared using ultrapure water (Kangning water treatment solution provider, China).

### 2.2. Apparatus

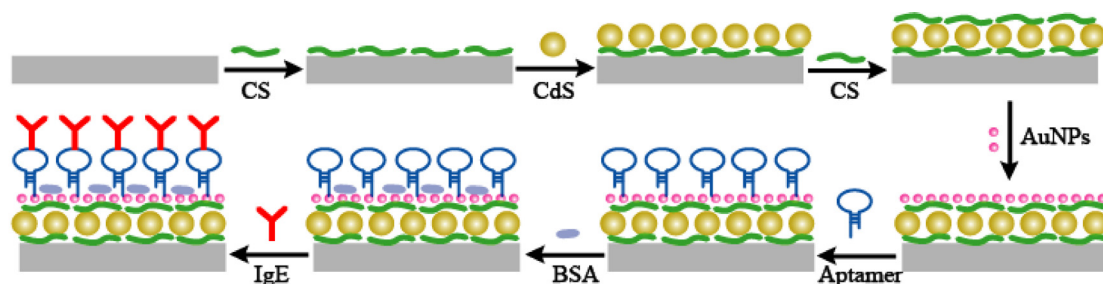
Scanning electron microscopy (SEM) images were obtained with S-4800 (Hitachi, Tokyo, Japan). Transmission electron microscopy (TEM) images were taken using a Tecnai G<sup>2</sup> F20 TEM system (FEI Co., America). X-ray powder diffraction (XRD) patterns were carried out on a RigakuD/Maxr-A X-ray diffractometer (Tokyo, Japan). Raman spectra were measured on a Renishaw-in-Vcia Raman microscope (UK), excited with an excitation laser wavelength of 532 nm at room temperature. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed on a RST5200 electrochemical workstation (Zhengzhou Shiruishi Technology Co., Ltd., China). ECL experiments were carried out in a self-made system consisting of a RST5200 electrochemical workstation, a photomultiplier tube (PMT, CR 120, Binsong Photonics, China) and BPCL ultra weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China). All electrochemical experiments were conducted on conventional three-electrode system.

### 2.3. Synthesis of CdS nanoparticles

The petal-like structure CdS nanospheres acting as the ECL emitter was synthesized through a simple one-step hydrothermal method according to the previous report with some modifications [23]. Briefly, 1.30 g of Cd(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O was dissolved in 42 mL of ethylene glycol and stirred for 10 min. 0.32 g of thiourea and 0.47 g of PVP were then added to the solution under stirring. Subsequently, the mixture was transferred to a 50 mL Teflon-lined autoclave and allowed to react at 100 °C for 24 h. Finally, CdS nanoparticles was collected by centrifugation and further washed in turn by water and ethanol.

### 2.4. Fabrication of ECL aptasensor

Glass carbon working electrode (GCE, Φ = 3 mm) was used to fabricate the ECL aptasensor. Prior to surface modification, the electrode was polished to mirror with 0.05 μm alumina slurry (Beuhler) and followed successive sonication in 1:1 nitric acid/water, ethanol and ultrapure water. Afterward, the GCE



**Scheme 1.** Fabrication procedure of the ECL aptasensor.

surface was modified by casting 5  $\mu\text{L}$  of 0.1 mg/mL CS in 1.0% (v/v) acetic acid solution and dried under infrared lamp. 5  $\mu\text{L}$  of 3.0 mg/mL CdS was pipetted onto surface of the electrode. After dried at room temperature, the CS coating procedure same as the above mentioned was repeated several times as needed. 8  $\mu\text{L}$  prepared AuNPs was then dropped onto the electrode and kept at room temperature for 1 h. After rinsing the electrode thoroughly with ultrapure water, 8  $\mu\text{L}$  SH-Apt1 was introduced onto the electrode through Au–S bond. Subsequently, it was rinsed with 10 mmol/L pH 7.4 phosphate buffer solution (PBS) and then incubated with 8  $\mu\text{L}$  of 1% BSA blocking buffer solution at 37  $^{\circ}\text{C}$  for 1 h to block unbound sites. The finished aptasensor was stored at 4  $^{\circ}\text{C}$  when not in use. For IgE detection, the fabricated aptasensor was incubated with 8  $\mu\text{L}$  of the different concentrations of IgE at 37  $^{\circ}\text{C}$  for 2 h. The fabrication procedure of the aptasensor was shown in Scheme 1.

### 2.5. ECL measurement

ECL measurement was performed in 0.1 mol/L pH 7.4 PBS containing 0.08 mol/L  $\text{KNO}_3$  and 30 mmol/L  $\text{H}_2\text{O}_2$ . ECL signals were acquired by a CV mode with potential varying from  $-1.7$  to 0 V and scan rate at  $100 \text{ mV s}^{-1}$ . The curves of CV and ECL were recorded simultaneously.

## 3. Results and discussion

### 3.1. Characterization of CdS nanoparticles

Fig. 1 shows SEM and TEM images of the synthesized CdS nanoparticles. It can be observed from Fig. 1A that the spherical

CdS particles were obtained with the average diameter around 200 nm. The images in Fig. 1B reveal that the CdS particles possess petal-like structure.

XRD was used to investigate the phase and structure of the CdS nanoparticles. As shown in Fig. 2A, all the diffraction peaks of the CdS nanoparticles match well with those of the hexagonal CdS phase. Two Raman active peaks at about 295 and 589  $\text{cm}^{-1}$  from the Raman spectrum in Fig. 2B are the characteristic LO phonon mode and two-phonon overtone of nanosize CdS materials. These results indicate the formation of CdS nanoparticles.

### 3.2. EIS and ECL measurements

EIS was used to monitor the change of electron-transfer resistance in the stepwise assembly process of the electrode. As shown in Fig. 3A, the electron-transfer resistance of the bare GCE was very low (curve a). After CS, CdS, CS were modified onto the surface of the bare GCE step by step, gradually increased electron-transfer resistances were observed owing to insulating effect of CS or the low conductivity of semiconductors (curve b–d). The addition of AuNPs on the electrode gave a decreased resistance due to the excellent conductivity of AuNPs (curve e). In the cases of aptamer and BSA were introduced onto the electrode separately, the electron-transfer resistance increased because of conductivity-decreased surface of the self-assembled layers (curve f and g), suggesting success of the layer-by-layer assembly. When the as-prepared aptasensor was incubated with IgE, the electron-transfer resistance increased further (curve h), demonstrating the bioconjugate of aptamer with IgE.

The procedure of the aptasensor construction could also be monitored by ECL responses. As shown in Fig. 3B, a weak ECL signal

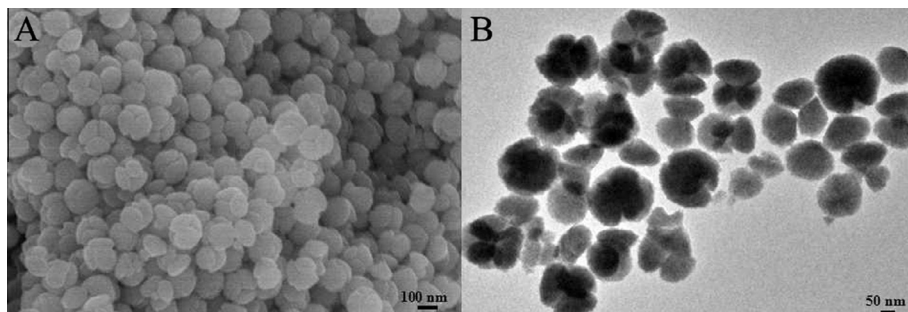


Fig. 1. SEM (A) and TEM (B) images of the prepared CdS nanoparticles.

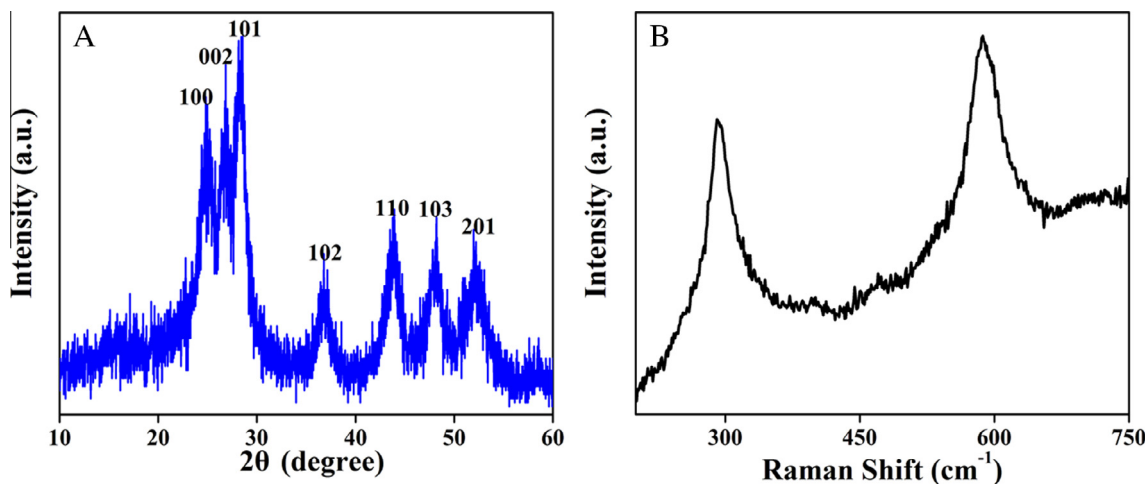


Fig. 2. XRD (A) and Raman (B) spectra of the CdS nanoparticles.

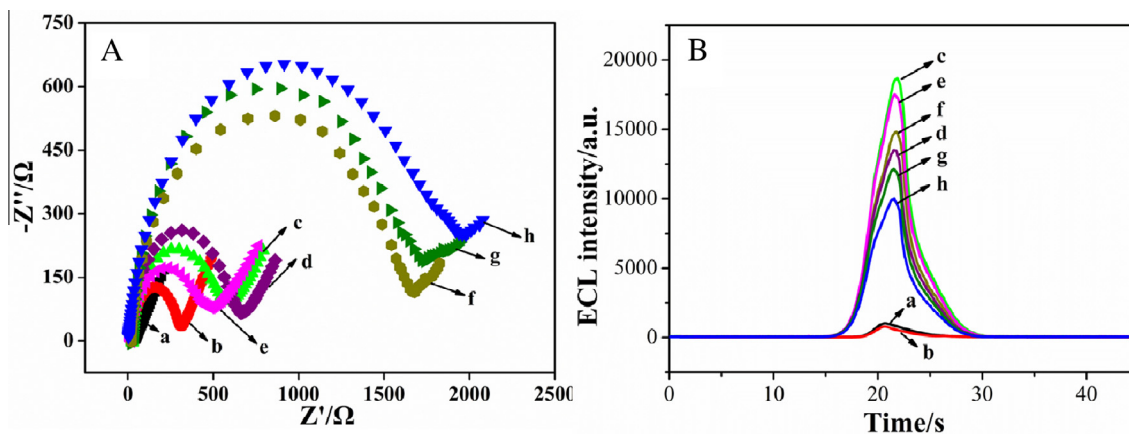


**Fig. 3.** EIS (A) and corresponding ECL response (B) of the electrodes: (a) bare GCE, (b) CS/GCE, (c) CdS/GCE, (d) CS/CdS/GCE, (e) AuNPs/CS/CdS/GCE, (f) SH-Apt/AuNPs/CS/CdS/GCE, (g) BSA/SH-Apt/AuNPs/CS/CdS/GCE, and BSA/SH-Apt/AuNPs/CS/CdS/GCE after incubation with 6  $\mu\text{L}$  of 1.0 pM IgE (h). The EIS experiments were performed in 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing 1 M KCl. The frequency range in EIS experiment is 1 Hz–125 kHz.

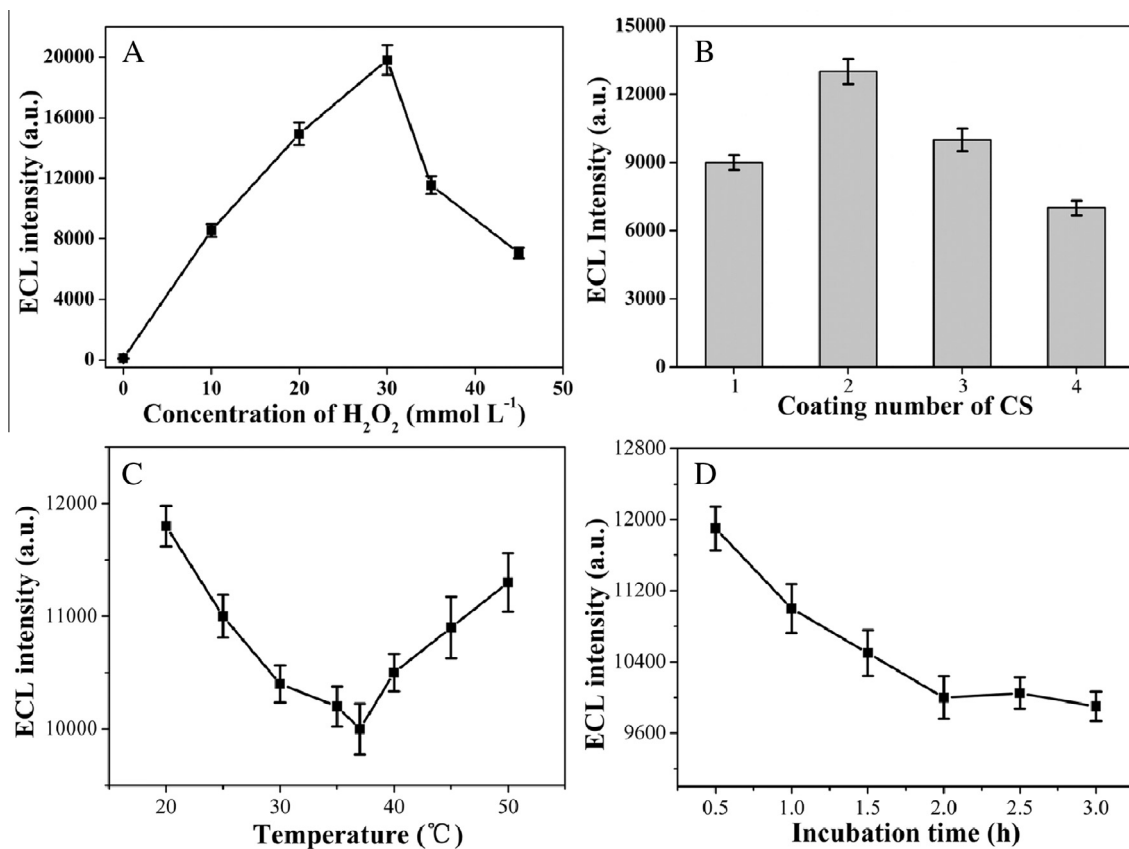


**Fig. 4.** Effects of  $\text{H}_2\text{O}_2$  concentration (A), coating number of CS (B), incubation temperature (C) and time (D) on ECL responses.

was observed on the bare GCE (curve a). After CS was modified on the electrode, the ECL signal decreased (curve b). The ECL signal was enhanced dramatically when CdS was modified onto the electrode (curve c), thanks to the excellent ECL property of the CdS nanospheres. While CS was coated on, the ECL signal decreased (curve d). After AuNPs was subsequently modified on the electrode, a clearly increased ECL signal was obtained (curve e), because AuNPs play an important role similar to a conducting wire which made it easier for the electrode transfer. However, when aptamer, BSA, and IgE were modified on the electrode in order, a successive decline of the ECL signal was detected (curve f–h).

This could be attributed to the fact that the immobilization of those organics partly obstructs diffusion of the ECL coreactants towards the electrode surface.

### 3.3. Optimization of experimental conditions

$\text{H}_2\text{O}_2$ , coreactant of the CdS-based ECL system in this work, has important influence on ECL intensity. The concentration of  $\text{H}_2\text{O}_2$  from 0 to 45 mmol L<sup>-1</sup> was tested. As shown in Fig. 4A, the more  $\text{H}_2\text{O}_2$  contained in the detection solution, the greater was the ECL increase we obtained, demonstrating that more S vacancies were

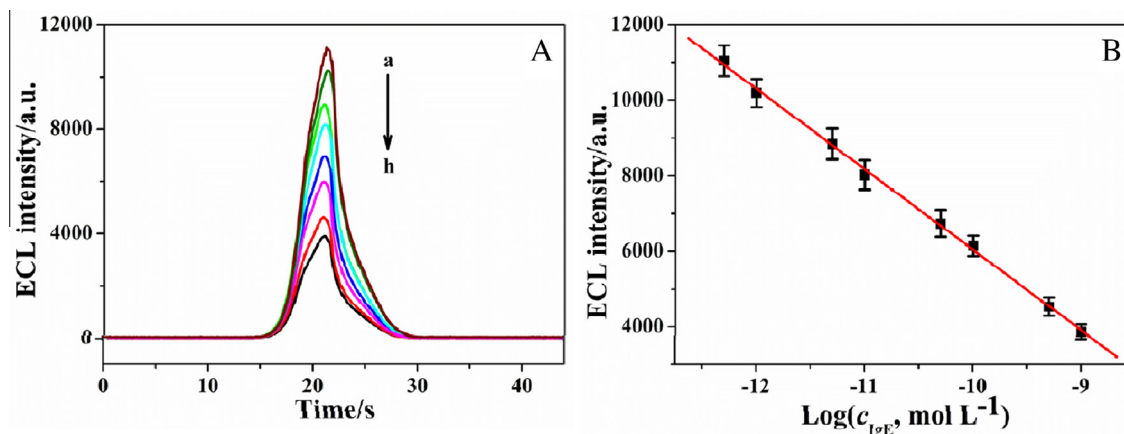


**Fig. 5.** ECL profiles (A) and calibration curve (B) of the proposed aptasensor. The concentration of IgE: (a) 0.0005 nM, (b) 0.001 nM, (c) 0.005 nM, (d) 0.01 nM, (e) 0.05 nM, (f) 0.1 nM, (g) 0.5 nM and (h) 1 nM. All ECL signals were measured in 5 mL PBS (pH 7.4) with 0.08 M KNO<sub>3</sub> and 30 mM H<sub>2</sub>O<sub>2</sub>.

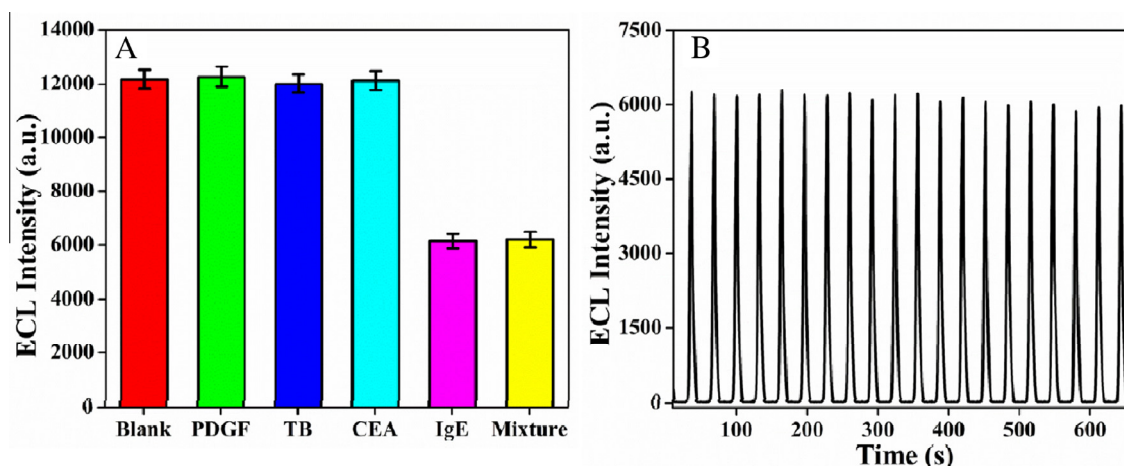


**Fig. 6.** Selectivity evaluation of the aptasensor against the interference molecules (A), PDGF (100 nM), TB (100 nM), CEA (100 nM), and their mixture with IgE (0.1 nM). Stability of the proposed aptasensor under consecutive cyclic potential scans for 20 cycles (B).

formed in the surface of CdS nanosphere [24]. However, when the H<sub>2</sub>O<sub>2</sub> concentration was higher than 30 mM, the CdS modified electrode was unstable and thus produced a decreased ECL signal. The similar phenomenon has also been observed by the previous report [24]. Thus, 30 mmol L<sup>-1</sup> was used.

To produce stable ECL signal and immobilize the AuNPs onto the surface of CdS/CS/GCE, positively charged CS was coated on the CdS/CS/GCE to adsorb the AuNPs with negative charge. To optimize the addition amount of CS, different coating number of CS were investigated. The ECL intensity of CS/CdS/CS/GCE electrode with different coating numbers of CS was shown in Fig. 4B. As can be seen, CdS/CS/GCE electrode with two coating numbers of CS exhibited the highest ECL emission. After the coating number was further increased, the ECL emission gradually decreased owing to the increased diffusion resistance for electron transfer. Therefore, two coating numbers of CS were selected.

Incubation temperature and time for the aptamer-IgE interaction greatly influence the sensitivity of the developed aptasensor. Fig. 4C shows the effect of incubation temperature ranging from 20 to 50 °C on the ECL intensity. The ECL intensity decreased with increasing temperature up to 37 °C, which is attributed to the increasing reaction rate between aptamer and IgE. The decreased ECL intensity could be ascribed to more IgE immobilized onto the electrode. However, the photocurrent response increased since

the temperature was over 37 °C. The reason may be that the high temperature results in an irreversible denaturation of IgE. In the subsequent experiments, 37 °C was employed as the optimum incubation temperature. As shown in Fig. 4D, the ECL intensity decreased with incubation time and reached a plateau at 2 h. So, 2 h was chosen.

### 3.4. Analytical performance

The analytical performance of the aptasensor was examined upon the addition of different concentrations of IgE. As depicted in Fig. 5A, the ECL intensity decreased with the increasing concentration of IgE. It was found that the ECL intensity is proportional to the logarithm of IgE concentration in the range of  $5.0 \times 10^{-13}$ – $1.0 \times 10^{-9}$  M, and the linear equation is  $I = -15254.8 - 2129.3 \log c$  ( $R^2 = 0.996$ ), where  $I$  is the ECL intensity and  $c$  is the concentration of IgE (shown in Fig. 5B). The limit of detection was experimentally found to be  $8.0 \times 10^{-14}$  M, which is lower than 37 pM for a label-free electrochemical aptasensor [19], 211 fM for a fluorescent method [20], 4.4 pM for an electrokinetic concentration-enhanced aptamer affinity probe electrophoresis assay [21], 2.89 nM for an aptamer based dot-blot assay [25].

**Table 1**

Standard deviation and relative standard deviation for 1 pM IgE.

|                    | Intra-assay (n = 5) | Inter-assay (n = 5) |
|--------------------|---------------------|---------------------|
| Mean value (pM)    | 0.998               | 1.04                |
| Standard deviation | 0.042               | 0.061               |
| RSD                | 4.2%                | 5.9%                |

**Table 2**

Analytical results of 6 human serum samples using the proposed and reference method.

| Sample no.                | 1    | 2     | 3     | 4     | 5     | 6     |
|---------------------------|------|-------|-------|-------|-------|-------|
| The proposed method (nM)  | 1.93 | 0.462 | 0.176 | 0.347 | 0.114 | 0.266 |
| The reference method (nM) | 1.81 | 0.439 | 0.183 | 0.335 | 0.119 | 0.254 |
| Relative errors (%)       | 6.6  | 5.2   | −3.8  | 3.6   | −4.2  | 4.7   |

### 3.5. Specificity, stability and reproducibility

Some interfering proteins including PDGF-BB, TB, and CEA were selected for interference test. As shown in Fig. 6A, no apparent changes were observed in these ECL signals with interfering substances, in comparison with that of the blank test. However, the presence of target IgE caused a substantial decrease in ECL signal. Even when these interfering substances co-existed with IgE, the ECL intensity of the mixture presents neglectable changes compared with that of IgE only. These results demonstrate that the specificity of the aptasensor for IgE detection is satisfactory.

The stability and reproducibility of the aptasensor were also evaluated. The stability was studied by consecutive cyclic potential scans for 20 cycles and the results were presented in Fig. 6B. It can be seen that no obvious change was found in ECL response for the detection of 0.1 nM IgE, demonstrating the good stability of the aptasensor. The reproducibility was tested by determining 1 pM IgE with five aptasensors of same batches (intra-assay) and different batches (inter-assay) under the same experimental conditions. As listed in Table 1, the relative standard deviation (RSD) of intra-assay is 4.2%, the RSD of inter-assay is 5.9%. Hence, the precision and reproducibility of the aptasensor are acceptable.

### 3.6. Application to real-world samples

The applicability of the proposed method was tested by comparing the assay results of 6 human serum samples with the values of reference method obtained by Roche Cobas E601-3 ECL instrument employed in Xinyang Central Hospital. When the level of IgE was over the linear range, the serum samples were appropriately diluted with 0.1 M pH 7.4 PBS. The analytical results were listed in Table 2. It can be seen that there is an acceptable agreement between the results obtained by the two methods. In addition, the recovery experiments were also performed by the standard addition method. Three different concentrations of IgE (0.001, 0.01, and 0.1 nM) were added to the human serum samples with recoveries from 91.5% to 105%, respectively. All these results indicate the satisfactory accuracy and reliability of the method in real biological samples.

## 4. Conclusions

In this work, we provided a new and label-free ECL aptasensor for IgE detection based on CdS nanospheres coupled with AuNPs as sensing platform and aptamer as recognition element. The aptasensor exhibits high sensitivity for IgE detection, which could be ascribed to three main reasons. First, the CS modified on the GCE and that on the CdS films enhance the stability of the CdS and AuNPs immobilized on the electrode. Second, the CdS nanospheres with petal-like structure possess excellent ECL efficiency. Third, the AuNPs immobilized on the electrode accelerate the electron transfer and enhance the loading capacity of the aptamers. The aptasensor features high sensitivity, good selectivity and wide linear range.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (21375114, 21405129), the Project of Science and Technology Development of Henan Province (142300410197), the Foundation of Educational Committee in Henan Province (14A150013), State Key Laboratory of Analytical Chemistry for Life Science (SKLACLS1419), and Program for Innovative Research Team (in Science and Technology) in University of Henan Province (15IRTSTHN001).

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