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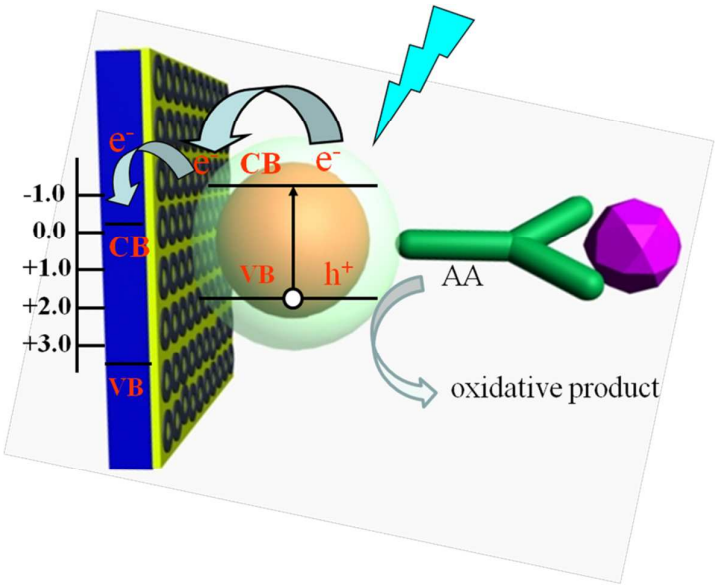

A label free PEC biosensor based on the amplification of mesoporous conductive material and core-shell QDs as light-harvesting architecture.

A Sensitive Photoelectrochemical Immunoassay Based on Mesoporous Carbon/Core-shell Quantum Dots as Donor-acceptor Light-harvesting Architectures

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Abstract

Herein we demonstrate the protocol of a label-free photoelectrochemical (PEC) immunoassay on the basis of ordered mesoporous carbon (CMK-3) and water-soluble CdSe@ZnS core-shell quantum dots (QDs) coupled with a bio-specific interaction for the ultrasensitive detection of human immunoglobulin (antigen, H-IgG) as a model protein. The CMK-3 was dispersed with chitosan（CS-CMK-3）which containing a large amount of amino-group (-NH_2), and the CdSe@ZnS QDs was treated by three thioglycolic acid which containing carboxylic-group (-COOH). The layer by layer assembling of CdSe@ZnS QDs and CS-CMK-3 achieved through the covalent binding of -COOH and -NH_2 was employed as the photoactive antibody (Ab) immobilization matrix. Improved sensitivity was achieved through the synergy effect of the excellent electrical conductivity and large specific surface area of CMK-3, as well as the high photon-to-electron conversion efficiency of CdSe@ZnS QDs. The photoexcitation of CMK-3/CdSe@ZnS QDs modified ITO electrode potentiostated at 0 V (vs. Ag/AgCl) under white light led to a stable anodic photocurrent. To perform the immunoassay, anti-human immunoglobulin (antibody, anti-H-IgG) was conjugated onto CdSe@ZnS QDs modified electrode by using EDC/NHS coupling reactions between -COOH of CdSe@ZnS QDs and -NH_2 of the antibody. The concentrations of H-IgG were measured through the decrease of photocurrent intensity resulted from the increase of steric hindrances due to the formation of the immunocomplex. Under the optimal conditions, a linear relationship between photocurrent decrease and H-IgG concentration was obtained in the range of 10

pg/mL~100 ng/mL with a detection limit of 5 pg/mL. This strategy opens a simple perspective for the application of mesoporous conductive material and core-shell QDs as light-harvesting architecture, which might be of great significance in PEC bioanalysis in the future.

Keywords: ordered mesoporous carbon; CdSe@ZnS; light-harvesting architecture; label-free; photoelectrochemical

Introduction

Among the various biosensing techniques, the newly developed yet dynamically developing photoelectrochemical (PEC) detection is of special interest for its potential application in bioanalysis[1]. The PEC method is evolved from the electrochemistry but is different from the traditional electrochemical methods. PEC sensor has the advantages of both optical methods and electrochemical sensors for its coupling photoirradiation with electrochemical detection. Due to reduced background signals obtained from the separation of the excitation source and detection signal, the PEC technique possesses potentially higher sensitivity than the conventional electrochemical methods.[2] The detection principle of PEC biosensors is based on the photocurrent change caused by the biological interactions between biosensing elements and their corresponding target analytes[3]. Due to the different energy form of the excitation source and detection signal, the PEC method is not only simple but also quite sensitive[3b,3d,4]. Given its desirable advantages, much efforts have been exerted to develop this method for different analytes in the past decade, such as DNA oligonucleotides[1b, 5], enzyme inhibitors[4a], cells and some small molecules[6], anticholera toxin antibody[6a,7], α-fetoproteinmouse[4c], and IgG[8] have been successfully determined accordingly. In these investigations, the label-free method, has been chosen preferred for the purpose of simplificationand costless[1b,3b,3d,6a-6c]. Despite the good simplicity, the deficiencies of these PEC label-free protocols, including low capacity of analyte loading and inability for amplification, need to be conquered instantly. Hence, employ the materials with large specific surface area and high photon-to-electron conversion

efficiency might be a useful route for signal enhancement.

Quantum dots (QDs), as one kind of semiconductor nanocrystals with unique size-dependent properties[9], have emerged as a significant new class of materials over the past decade. QDs exhibited a wide range of electrical and optical properties, and QDs-based bioassay has become one of the most exciting forefront fields in analytical chemistry. Especially II-VI semiconductors (e.g., CdSe, CdS, HgS, ZnS, ZnSe) have been the focus in the research field of bioelectrochemistry and bioimaging[10]. The QDs are often passivated by a second semiconductor material (e.g., ZnS)[11] to protect the core from oxidation and bleaching[12]. The band gap energy of the shell is higher in order to confine the exciton generation and relaxation to the core and thus increase the quantum yield[11a-11b,13].

Recently it has been shown that CdSe@ZnS QDs can not only be used as fluorescence labels for biomolecules[14], but can also be attached to metal electrodes for PEC studies[15]. Among them, the photocurrent can be switched by the introduction of cytochrome c[15a,15d]. and different enzymes were combined with QDs-modified electrodes [15b-15c] . However, not only expensive and sophisticated instrumentation (lock-in amplifier) was necessary to detect the photocurrent in these detection systems, but also the photocurrent intensity of CdSe/ZnS QDs was small (about 10^{-9}-10^{-8}A). Thus, developing indirect and sensitive PEC method with QDs based on immunoassay for the detection of general protein is of great significance.

In recent years, carbon materials, due to their extraordinary electronic and mechanical properties, have been applied in broad range of applications with different desirable

functionalities such as energy conversion/fuel storage, catalysis, biotechnology and optoelectronic nanodevices[16]. With the discovery of photoinduced charge transfer between semiconductor quantum dots (SQDs) as the donor and carbon materials as the acceptor[16a,17], carbon-based donor-acceptor architectures have been successfully fabricated as photochemical energy conversion systems with the aim of increasing photoresponses of SQDs[18]. Carbon materials can effectively increase charge generation at the interface and transport pathways for the photoinduced electrons to the electrode[16a]. Ordered mesoporous carbon, CMK-3, posses the properties of excellent mechanical stability, high specific surface area, large pore volume and adjustable pore size contribution. Due to the numerous edge-plane-like defect sites which make the electron transfer easier, CMK-3 shows a fast electron transfer rate and larger current response[19]. Besides, it can act as a scaffold in splitting and transferring flow of photoinduced charge carriers[20], which could reduce the recombination probability of electron-hole pairs of QDs effectively.

In this work, a facile assembly of CdSe@ZnS QDs onto the pores and surface of CMK-3 as an efficient photo-current conversion architecture was demonstrated. In order to obtain uniformly modified ITO electrode as well as to facilitate further modification, CMK-3 was dispersed with chitosan (CS-CMK-3) which having good film-forming property and containing a large amount of amino-group ($-NH_2$). The thioglycolic acid (TGA)-capped water-soluble CdSe@ZnS QDs were assembled on the electrode simply by the covalent bonding between the -COOH of CdSe@ZnS QDs and the $-NH_2$ of CS-CMK-3. Then, Human immunoglobulin (H-IgG) was

conjugated onto CdSe@ZnS QDs modified electrode by using EDC/NHS coupling reactions. The H-IgG concentrations were directly (label-free) measured through the decrease in photocurrent intensity resulting from the specific immunoreaction. Results indicated that the proposed label-free PEC biosensor showed good performance in the monitoring of H-IgG with a rapid response and wide concentration range, which could successfully be applied to the detection of other proteins. The established method provides an approach for the assembly of QDs with other nano-materials owning special structure for rapid electronic transportation, and for the further designation of simple QDs-based PEC biosensors.

2. Experimental Section

2.1 Reagents and Chemicals

Cadmium oxide (CdO) (99.99%), selenium (99.9%, powder), zinc dimethyldithiocarbamate ($Zn(DMSC)_2$) (97%, powder), trioctylphosphine (TOP) (90%), oleic acid (OA) (90%) and 1-octadecene (ODE) (90%) were purchased from Sigma-Aldrich and used as received. 3-thioglycolic acid (TGA), $NaBH_4$, mesoporous carbon (CMK-3) and thiourea were from Sinopharm Chemical Reagent Beijing Co., Ltd., China. Human immunoglobulin antigen (H-IgG) and anti-human immunoglobulin (anti-H-IgG) were purchased from Shanghai Guyan technology Co., LTD. 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Bovine serum albumin (BSA, 96-99%) and ascorbic acid (AA) were from Sigma-Aldrich. Phosphate buffered saline (PBS, 0.1 M, pH 7.4) was used

as electrolyte for all electrochemical measurements and for the preparation of the Ag and Ab solutions. Ultrapure water was used throughout the experiments. All other chemicals were of analytical reagents grade.

2.2 Apparatus

PEC measurements were performed with 30 W LED light (Tengwei light, China). Photocurrent was measured on a CHI760D electrochemical workstation (Shanghai CHI Instruments Co., China) with a three-electrode system: a modified ITO electrode with a geometrical area of 0.25 ± 0.01 cm^2 used as the working electrode, a Pt wire used as the counter electrode and a saturated Ag/AgCl electrode used as the reference electrode. All the photocurrent measurements were performed at a constant potential of 0 V (versus Ag/AgCl). A 0.1 M PBS containing 0.1 M AA was used as the blank solution for photocurrent measurements. Transmission electron microscope (TEM) images were obtained from a Hitachi H-800 microscope (Japan). The UV-vis absorption spectra were obtained on a TU-1901 UV-vis photo-spectrometer (Beijing's general instrument co., LTD.). Photoluminescence (PL) spectra were collected on an LS-45/55 PL spectrometer (Perkin Elmer, America).

2.3 Synthesis of Water-Soluble CdSe@ZnS Core-Shell Quantum Dots

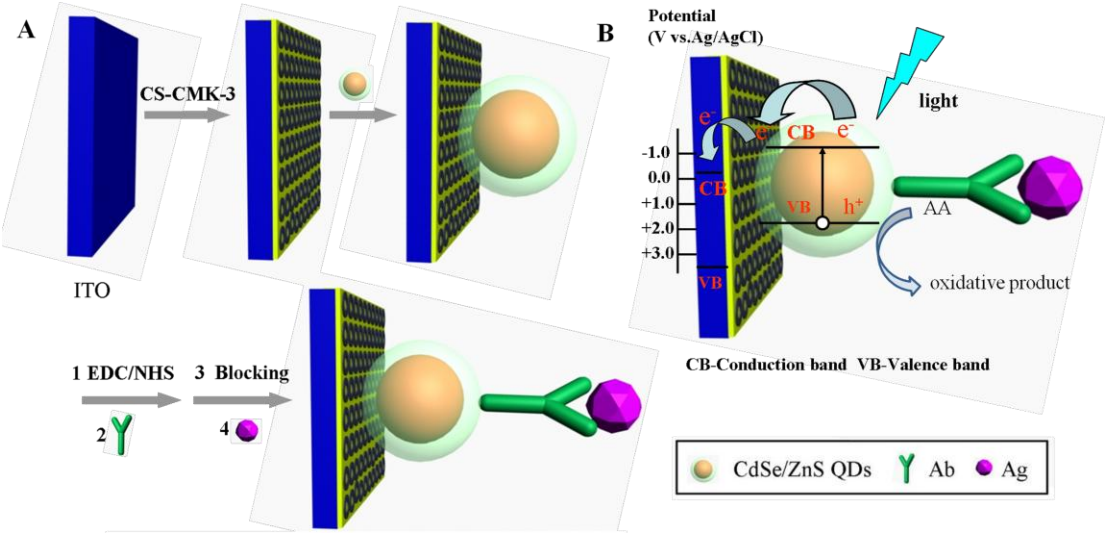
CdSe@ZnS Core-Shell Quantum Dots were synthesized by using a slightly modified procedure reported by Bong-Hyun Jun et.al[21]. Typically, CdO (1×10^{-3} M, 0.1284 g) and $Zn(DMSC)_2$ (5×10^{-3} M, 1.520 g) were dissolved in OA (1.695 g) and ODE (20 mL), treated with N_2 gas, and heated to 100 ℃ under vacuum for 1 h. The solution was further heated to 320 ℃ to form a transparent solution and injected into a

precursor solution, which was prepared by dissolving Se (1×10^{-3} M, 0.078 g) in TOP (1 mL). The growth temperature was set to 300 ℃ for 5 min and then the solution was cooled to room temperature. To prepare Water-Soluble Single QDs, the purified QDs were dispersed in $CHCl_3$ to obtain a 0.1×10^{-6} M QDs solution. Tetramethylammonium hydroxide (TMAH) (100 mg) was mixed well with TGA in $CHCl_3$. After 15 min, a clear and colorless aqueous layer (about 10 % of total volume) was formed above the $CHCl_3$ layer. The biphasic solution was mixed by vigorous shaking and was allowed to stand for 1 h in order to equilibrate. The lower organic phase, which contained deprotonated TGA, was transferred into a vial for the ligand exchange reaction with the QDs. 100 μL of TOP-capped QDs (0.1 μM in $CHCl_3$) was added to the TGA-$CHCl_3$ solution and mixed well. The solution was allowed to stand at room temperature for 1-5 h. After the reaction, the TGA-capped QDs that had separated from the $CHCl_3$ solutions were collected, washed with $CHCl_3$ (three times), and dispersed in 1.0 mL of water.

2.4 Fabrication of PEC sensor

The ITO slices (type N-STN-S1-10, Zhuhai Kaivo Electronic Components Co., Ltd, Guangdong, China, sheet resistance ≤ 10 Ω/cm^2) were sonicated in acetone, NaOH (1 M) in 1:1 (v/v) ethanol/water, and water for about 30 min consecutively, and dried with nitrogen flow. To fabricate the CS-CMK-3 composite film modified ITO, the CMK-3 powder was ultrasonically dispersed in chitosan solution (0.25%, wt) to obtain homogeneous solution of CS-CMK-3 (1 mg/mL). 4 μL of the CS-CMK-3 was coated onto the ITO electrode and dried at room temperature, then 4 μL of the

CdSe@ZnS QDs was coated onto CS-CMK-3 modified ITO electrode. Conjugation of antibodies onto a CdSe@ZnS QDs modified electrode was achieved by using the EDC/NHS as the coupling agent. Briefly, the CS-CMK-3/CdSe@ZnS QDs modified electrode was activated by immersion in a solution containing 10 mg/mL of EDC and 20 mg/mL of NHS for 50 min. Then the activated electrode was thoroughly rinsed with distilled water and dried. Following this step, 4 μL of anti-H-IgG (10 μg/mL) was dropped on the electrode surface and the electrode was incubated at 4 ℃ for 1 h. After incubation, the electrode was rinsed with the distilled water. 4 μL of BSA (1%, wt) was incubated on the modified electrode for 1 h at room temperature to block non-specific binding sites, and then washed with distilled water thoroughly. Finally, 4 μL of analyte (H-IgG) solution with different concentrations was droped on the electrode and incubated at 37 ℃ for 1 h followed by washed with distilled water. A detailed description of the immunosensor process is illustrated in Scheme 1A.



Scheme 1 (A) Schematic diagram of the stepwise immunosensor fabrication process. (B) Photocurrent generation mechanism of CS-CMK-3/CdSe@ZnS QDs.

3. Results and discussion

3.1 Characterization of Water-Soluble CdSe@ZnS Core-Shell Quantum Dots

After the synthesis of carboxylated CdSe@ZnS QDs according to the process mentioned above, a series of characterization technology were adopted to further analyze its relevant properties. As shown from the TEM image in Fig. 1A, the size of the CdSe@ZnS QDs was found to be about 5 nm with good monodispersity. The fluorescence microscopy image was performed to further confirm the excellent fluorescence properties and further the high quantum yield of CdSe@ZnS QDs (Fig. 1B). The optical property of QDs (the inset of B) exhibited a broad absorption spectrum (covering the range of wavelength <700 nm) with a characteristic peak at around 650 nm and a narrow PL emission band centered at 680 nm, which potentially indicated the effective absorption and photo-electron transfer under visible wavelength. The narrow emission spectrum indicated that high degree of monodispersity of QDs present.

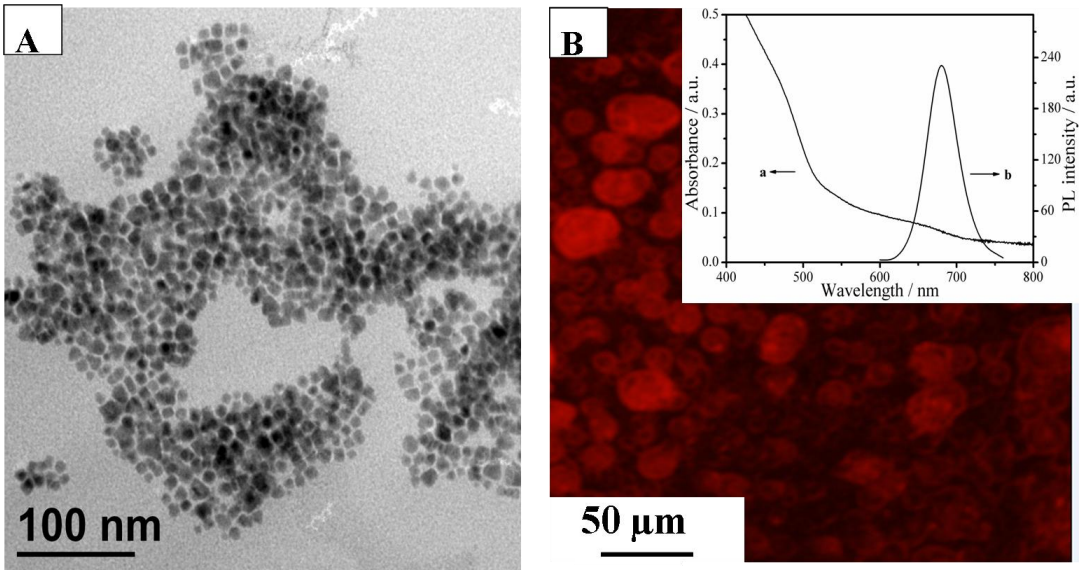


Fig.1 The TEM (A) and fluorescence microscopy image (B) of the prepared CdSe@ZnS QDs. The

inset is the room-temperature PL spectra (b) and UV-vis spectrum (a) of CdSe@ZnS QDs.

3.2 Photoelectrochemical behavior of CS-CMK-3/CdSe@ZnS QDs modified electrode

In order to confirm the effect of CS-CMK-3 on the PEC behavior of CdSe@ZnS QDs, the photocurrent response and the corresponding fluorescence emission spectrum of both the two substances respectively and their assembling were studied. As shown in Fig.2A, compared to the CdSe@ZnS QDs modified electrode (b), there was an obvious enhancement in the photocurrent of the CS-CMK-3/CdSe@ZnS QDs modified electrode (c). On the basis of electron transfer in CdSe QDs-C_{60} nanocomposites[22], we suspect that the enhancement may be ascribed to the donor-acceptor assemblies of CMK-3 and CdSe@ZnS QDs. To further investigate the effect of CMK-3 on the PEC properties of the CdSe@ZnS QDs, emission quenching of CdSe@ZnS QDs is a good measure to probe the photoinduced electron transfer process in donor-acceptor assemblies[23]. For pristine CMK-3 (a), no luminescence peak is perceived, while the CdSe@ZnS QDs (b) exhibits a high luminescence with a maximum emission at 680 nm. It is interesting to note that upon the adding of CMK-3 into the CdSe@ZnS QDs solution (c), an obviously reduced emission intensity at 680 nm was observed, which is assigned to electron-hole recombination in CdSe@ZnS QDs. Significant emission quenching seen in this experiment indicated that the dramatically increased photocurrent stems from effective charge carrier separation via electron transfer of the CMK-3.

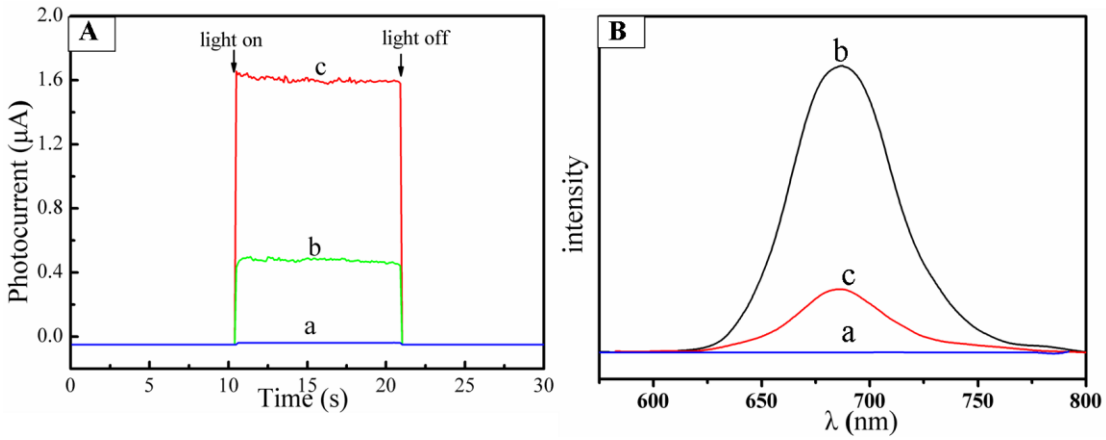


Fig.2 (A) Photocurrent response of (a) CS-CMK-3, (b) CdSe@ZnS QDs and (c) CS-CMK-3/CdSe@ZnS QDs. (B) The corresponding fluorescence emission spectrum.

The photocurrent generation mechanism of CS-CMK-3/CdSe@ZnS QDs was shown in Scheme 1B. When CdSe@ZnS QDs absorbed photons with energy higher than that of its band gap, electrons are excited from the (occupied) valence band to the (empty) conduction band and formed the electron-hole pairs. Once the process happened, the electron-hole pairs would recombine or the charges would be transferred. In this study, the electron transferred to the CMK-3 fast and further to the ITO electrode.

3.3 Photoelectrochemical Immunosensor

In this study, CS-CMK-3, CdSe@ZnS QDs, Ab and BSA were modified onto the electrode successively. The interfacial behavior of each sensor fabrication step was probed by recording the electrochemical impedance spectra and photocurrent. EIS was used to characterize the fabrication process of biosensor. As shown in Fig.3A, compared to bare ITO (a), after the deposition of CS-CMK-3 (b), the semicircular diameter of the Nyquist plot that represents the electron transfer resistance (R_{et})[24] became much smaller due to the better conductivity of CS-CMK-3. While, upon the

assembling of CdSe@ZnS QDs (c), the semicircular diameter dramatically increased, suggesting the CdSe@ZnS QDs successfully deposited on the outerlayer of CS-CMK-3 and in turn hindered the electron transfer. After the immobilization of Ab and the subsequent BSA blocking, the R_{et} increases gradually again (curves d-e), turning out the successful assembling of sensing elements on the electrode surface. The reason for the resistance increase is that the nonconductive properties of the proteins obstruct the mass transport and electron transfer of the electrochemical probe to the electrode surface by elevating the hindrance effect.

The fabrication of the immunosensor could also be monitored by PEC experiments (Fig. 3B). After immobilization of the Ab and BSA on the CS-CMK-3/CdSe@ZnS QDs modified electrode, the photocurrent intensity decreased (curve b and c). This could be explained by the fact that the immobilization of the proteins on the CS-CMK-3/CdSe@ZnS QDs modified electrode hindered the diffusion of electron and resulted in the decrease in the photocurrent intensity. After the as-obtained biosensor was incubated with the corresponding antigen (curve d), the photocurrent further decreased. On the basis of the photocurrent decrease due to the formation of the immunocomplex, a label-free PEC immunosensor was achieved.

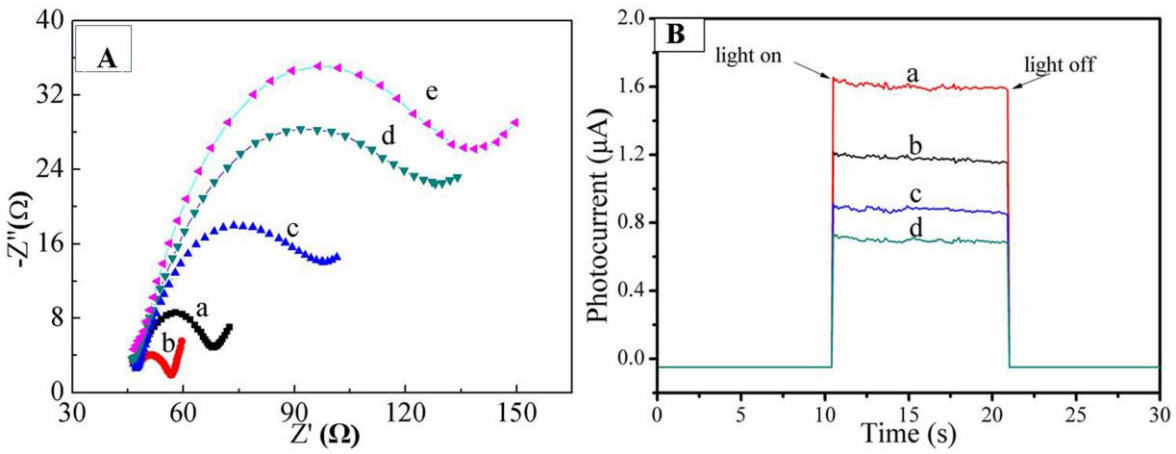


Fig.3 (A) EIS of (a) CS-CMK-3 modified electrode electrodes, (b) bare ITO electrodes, (c) CS-CMK-3/CdSe@ZnS QDs modified electrode electrodes, (d) after Ab immobilization, (e) after anchoring the Ag corresponding to 10 ng/mL. The EIS measurements are carried out in 0.1 M KCl containing 5.0 mM $K_3Fe(CN)_6/K_4$ $Fe(CN)_6$ (1:1). The frequency range was between 0.1 and 100 000 Hz with an applied voltage of 5 mV. (B) Photocurrent response of CS-CMK-3/CdSe@ZnS QDs modified electrodes (a) before and (b) after Ab immobilization, (c) after further blocking with BSA, (d) after anchoring the Ag corresponding to 10 ng/mL.

3.4 Optimization of Experimental Conditions

In order to obtain high sensitivity for this PEC detection system, the experimental parameter in the fabricating and detecting process must be investigated and optimized. The effect of pH on the assay performance was studied over a pH range from 5.4 to 10 with a universal PBS buffer. To obtain high signal-to-background ratios (Fig. 4A), pH 8 was chosen to be the optimum PEC measurement conditions in this test. Besides, the concentration of CMK-3, which acted as the matrix of the ITO electrode for the subsequent combination of QDs, has a great influence on the charge generation at the interface and transport pathways for the photoinduced electrons to the electrode. The photocurrent sharply increased as the concentration of CMK-3 increased from 0.5

mg/mL to 2 mg/mL and then trended toward a platform at 2 mg/mL (Fig. 4B). Giving an overall consideration, in this study, 2 mg/mL of the CMK-3 was chosen all through the PEC measurement.

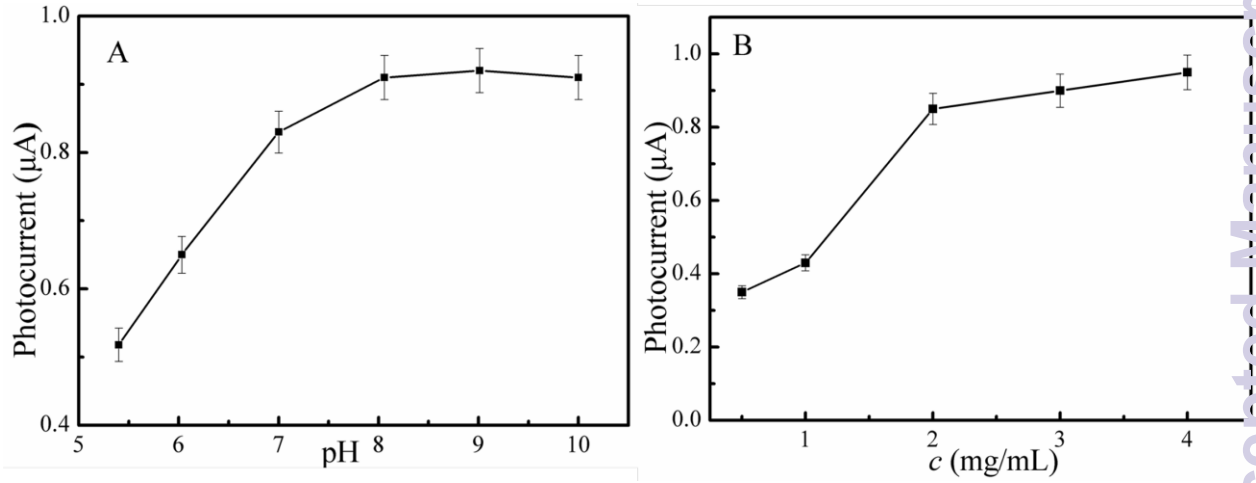


Fig.4 (A) Effects of the pH of 5.4, 6.1, 7.0, 8.06, 9.0, 10.0 on the photocurrent response of the modified electrodes after incubation in 10 ng/mL H-IgG. (B) Effects of concentration of CMK-3 of 0.5, 1, 2, 3, 4 mg/mL on the photocurrent response of the modified electrodes after incubation in 10 ng/mL H-IgG. The error bars showed the standard deviation of five replicate determinations.

The degree of photocurrent variation in this PEC immunoassay is directly related to the concentration of target Ag. Fig. 5A presents the photocurrents after incubation with Ag of variable concentrations. The photocurrent decreases with the increase of the concentration of Ag. As shown in Fig. 5B, the photocurrent decrement was proportional to the concentrations of Ag in a linear range from 10 pg/mL to 100 ng/mL. Upon the treatment of the sensing interface with increasing Ag concentration, more antigen-antibody immunocomplexes could be induced onto the electrode interface. The reduction of photocurrent was prone to saturation over 100 ng/mL. The detection limit was experimentally found to be 8 pg/mL. In addition, the sensitivity of

the presented PEC immunosensor here was comparable and even better than those of many reported immunoassay methods for human IgG. The analytical performances of various H-IgG immunoassays were listed in Table 1. The comparison results showed that this PEC assay was promising for the determination of human IgG in clinical application.

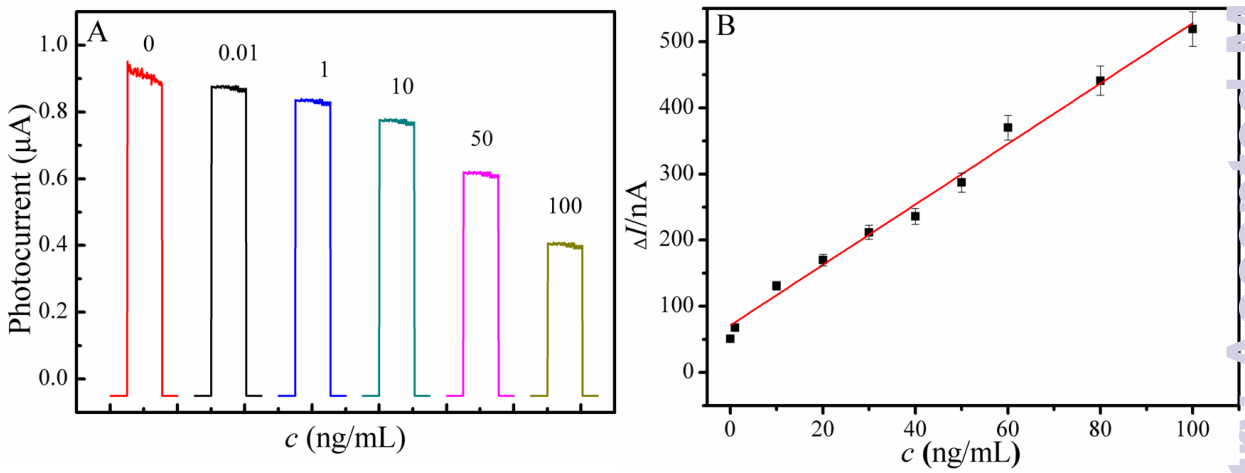


Fig.5 (A) Effect of different concentrations of Ag on the photocurrent responses. (B) The corresponding calibration curve, ΔI was the change of photocurrent before and after Ag immobilization. All photocurrent responses were measured in 0.1 M PBS (pH=8.06) containing 0.1 M AA with an applied potential of 0 V under white light.

Table 1 Analytical performance of various human IgG immunoassays

analyte	measurement protocol	linear range	detection limit	reference
human IgG	Photoelectrochemical immunoassay	10 pg/mL~100 ng/mL	8 pg/mL	This work
Goat antihuman	LRET-based immunoassay	3~67 μg/mL	0.88 μg/mL	25

IgG				
human IgG	flow immunoassay	5.0×10^{-6}~9.6×10^{-4} M	8.0×10^{-6} M	26
human IgG	electrochemical immunoassay	0.01~15 M	5.0 pM	27
goat antihuman IgG	chemiluminescence resonance energy transfer immunoassay	0.2~4.0 nM	2.9×10^{-11} M	28
human IgG	fluoroimmunoassay	0.2~12 μg/mL	10 ng/mL	29
human IgG	Electrochemical impedance	10~1 μg/mL	5 ng/mL	30

Control experiments revealed that the developed PEC sensor does not exhibit any obvious changes in photocurrent when incubating the as-fabricated immunosensor in sample solutions containing a 100-fold excess of different interfering agents such as glucose, cholesterol, human hemoglobin, and alpha-fetoprotein (Fig. 6A,). The results indicated the good selectivity of the developed PEC sensor. The reproducibility of this PEC H-IgG immunoassay was also assessed by an interassay relative standard deviation (RSD). By assaying the same concentration of H-IgG with five electrodes at identical experimental conditions, RSD of 5.5 % were obtained, indicating a good reproducibility of the fabrication protocol. Meanwhile, it is also worth mentioning that the photocurrent response of the PEC sensor was fairly reversible and stable under several on/off irradiation cycles for 300 seconds. As shown in Fig. 6B, the current could reproducibly increase violently under each irradiation and recover

rapidly in the dark, indicating the structural stability of the developed PEC sensors and their potential for biosensing experiments.

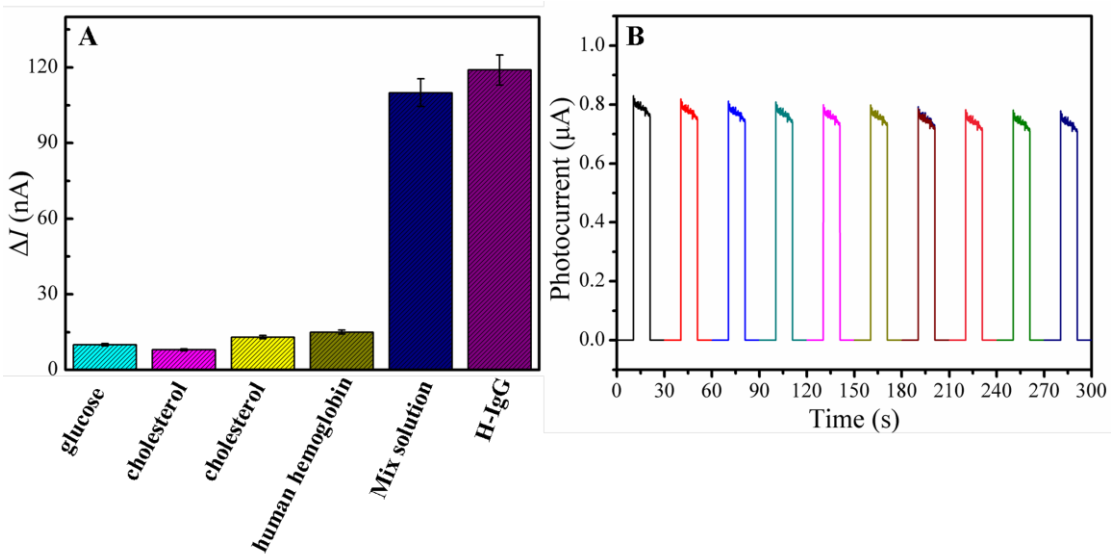


Fig.6 (A) Selectivity of the proposed immunoassay to human IgG (H-IgG) by comparing it to the interference at the 10 ng/mL level: glucose, cholesterol, human hemoglobin, and the mixed-sample, where *n*=5 for each point. (B) Time-based photocurrent response of PEC immunosensor.

4 Conclusions

In this work, a simple and low-potential label-free PEC immunosensor using H-IgG as a model analyte was successfully achieved based on CMK-3 and water-soluble core-shell CdSe@ZnS QDs. As compared to the conventional enzyme labeled PEC immunoassays, this simple biosensing strategy possesses high sensitivity via CMK-3 with good ability of transmitting and accepting electron, and core-shell CdSe@ZnS QDs with excellent absorption efficiency in visible light. In addition, this

immunoassay also had the advantages of low cost (4 μL of each step and no enzyme is used), and simple (the style of label free is easy to operate) and so on. The fast photoelectronic communication among CdSe@ZnS QDs, CMK-3 and ITO electrode led to a novel method for the PEC detection of H-IgG with good analytical performance. This strategy manifests its excellence by being simple, cost-effective and specific in immunoassays, and showed the promise to open a new perspective for the combination of mesoporous material with superior electrochemical properties and core-shell QDs for biological analysis.

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