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PAPER

Monitoring of bovine serum albumin using ultrasensitive electrochemiluminescence biosensors based on multilayer CdTe quantum dots modified indium tin oxide electrodes†‡

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A novel method for the determination of bovine serum albumin (BSA) is proposed based on the electrochemiluminescence (ECL) of BSA with CdTe quantum dots (QDs) films. Thioglycolic acid (TGA) is used to cap CdTe colloidal solutions to obtain stable water-soluble QDs and intensive anodic ECL emission with a peak value starting from +1.2 V (*vs.* Ag/AgCl) in a carbonate bicarbonate buffer solution (pH 9.32) at an indium tin oxide (ITO) electrode. The ITO glass is modified with CdTe QDs by the layer-by-layer (LbL) self-assembly technique, which is immobilized into a homemade ECL flow chip and used as a receptor of the ECL sensor. The ECL intensity is correlated linearly with the concentration of BSA over the range of 1.0×10^{-9}–1.0×10^{-6} g mL^{-1} and 1.0×10^{-6}–1.0×10^{-5} g mL^{-1}, and the detection limit is 5.48×10^{-10} g mL^{-1}. The relative standard deviation is 1.62% for 4.0×10^{-7} g mL^{-1} BSA ($n = 11$). This simple and sensitive method reveals good reproducibility for ECL analysis. It puts forward a new efficient ECL method for the determination of BSA and opens new avenues for the application of QDs in ECL biosensors.

1 Introduction

Semiconductor nanoparticle-based biotechnology has attracted special interest in the current development of biosensing applications due to the amplification or catalytic effect of the nanostructures and the outstanding size-, shape-optical and electronic performances.[1] Among the miscellaneous functional nanomaterials, luminescent semiconductor quantum dots (QDs) are currently one kind of the most attractive biological fields because of their unique properties compared with organic dyes, such as narrow photoluminescence (PL) spectra, single excitation wavelength for tunable PL, and high stability against photobleaching.[2] These QDs are of technological importance as an active material in optoelectronic devices, such as light emitting diodes and lasers.[3–5] The chemiluminescence (CL) resonance energy transfer[6,7] and electrochemical analytical techniques[8,9] coupled with QDs have been rapidly developed.[10] In order to decorate the materials to have enough structural flexibility and practicality, novel assembly techniques which can be implemented at relatively low cost[11] are required. The self-assembly of nanomaterials has attracted a great deal of attention by bridging different fields of science and engineering for the design and development of outstanding materials, methodologies, and theories.

As is well-known, electrochemiluminescence (ECL) can not only retain the advantages of CL, such as the excellent sensitivity and a wide dynamic concentration response range, but also owns some additional advantages over CL.[12] Owing to the superior features of the ECL technique, such as low cost, wide range of analytes, and high sensitivity,[13] it has been widely used in many fields.[14] A series of co-reactant ECL analytical methods have been developed for clinical diagnostics, environmental assays such as food and water testing, and biowarfare agent detection.[15,16] In recent years, due to the controllable merits of the electrochemical methods, the ECL behaviors of QDs in both organic[17–19] and aqueous[19–23] solutions have attracted considerable interest. Recently, the ECL phenomena of CdS,[24] CdSe,[21–23] and CdTe QDs[25,26] in the presence of different co-reactants such as peroxydisulfate, oxygen, hydrogen peroxide, and amines have been used for the development of the ECL analytical technique. Recent research works have indicated QDs solutions in flow phase which have been used for the determination of several analytes, including oxidase substrates,[27] nitrite,[28] amines[25] and proteins.[29] The method has the properties of rapid determination and excellent sensitivity, but a mass of QDs were wasted as well as being harmful to the environment. In this article, we describe a novel method for preparing luminescent multilayer films using

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aqueous CdTe colloidal solution by the layer-by-layer (LbL) self–assembly technique. There has only been one relevant report at present by our colleagues.[30] Herein, an indium tin oxide (ITO) slide glass was decorated with CdTe QDs by self-assembly which was used as a recognizer of the flow injection electrochemiluminescence (FI-ECL) sensor. The LbL self-assembly methods particularly open new avenues in the applications of QDs to construct ECL analytical systems and ECL biosensors.

Serum albumins are the major soluble protein constituents of the circulatory system and have many physiological functions.[31] The most important property of this group of proteins is that they serve as transporters for a variety of compounds. Various methods based on chromatographic analysis,[32] electrochemical techniques,[33] UV–vis absorption and fluorescence spectroscopy[34] have been developed for the determination of bovine serum albumin (BSA). Some of them are complex and time consuming, and the processes limit the application of these methods. Thus, developing new analytical methods for sensitive and rapid detection of BSA combined with multifarious techniques is still an attractive objective.

Our work studied the sensitizing effect of BSA on the anodic ECL property of thioglycolic acid (TGA) -capped CdTe QDs dissolved in aqueous solution. At an ITO electrode, the QDs showed a stable anodic ECL signal. A new type of ECL flow chip with an ITO working electrode was designed and constructed in this new FI-ECL system (See Fig. 1A). The system was established by overcoming several drawbacks of conventional ECL systems.[35] The ECL flow chip had very little dead volume, IR drop, and flow resistance. The working electrode (WE) was parallelly placed with a sheet-type counter electrode (CE), which contacted adequately with the samples. The samples passed through the channel horizontally, which eliminated the dead volume. The WE, reference electrode (RE) and CE were placed so close that the IR drop was decreased remarkably. The inlet and outlet were placed on the top of the chip, which could not produce the gas bubbles and could greatly reduce the flow resistance. After being established, the new FI-ECL system has become a very attractive method to detect analytes for its high sensitivity, good stability and small analyte consumption. To our best knowledge, this is the first FI-ECL method for BSA detection and further detection of proteins in biological samples.

2 Experiments

2.1 Apparatus

The ECL measurements were carried out on a flow injection luminescence analyzer (IFFM-E, Xi'an Remex Electronic Instrument High-Tech Ltd., Xi'an, China) equipped with a CHI760D electrochemical workstation. The detection system was a configuration consisting of a CdTe QDs-decorated ITO WE, a platinum CE, and an Ag/AgCl (saturated KCl) RE. The observation window for the homemade ECL flow chip was placed in front of the photomultiplier tube (PMT). Integrated PL measurements were performed at room temperature using a LS-55 spectrofluorometer (P.E. USA). UV–vis absorption spectra were recorded with a UV-3101 spectrophotometer (SHIMADZU, Japan).

2.2 Chemicals

All chemicals used were analytical grade or the highest purity available. Thioglycolic acid (TGA) and 3-aminopropyltrimethoxysilane (APS) were obtained from Alfa Aesar China Ltd. Tellurium (Te) power, sodium borohydride ($NaBH_4$), cadmium chloride ($CdCl_2 \cdot 2.5H_2O$), $Cd(ClO_4)_2$ and rhodamine 6G were purchased from Shanghai Chemical Reagent Company

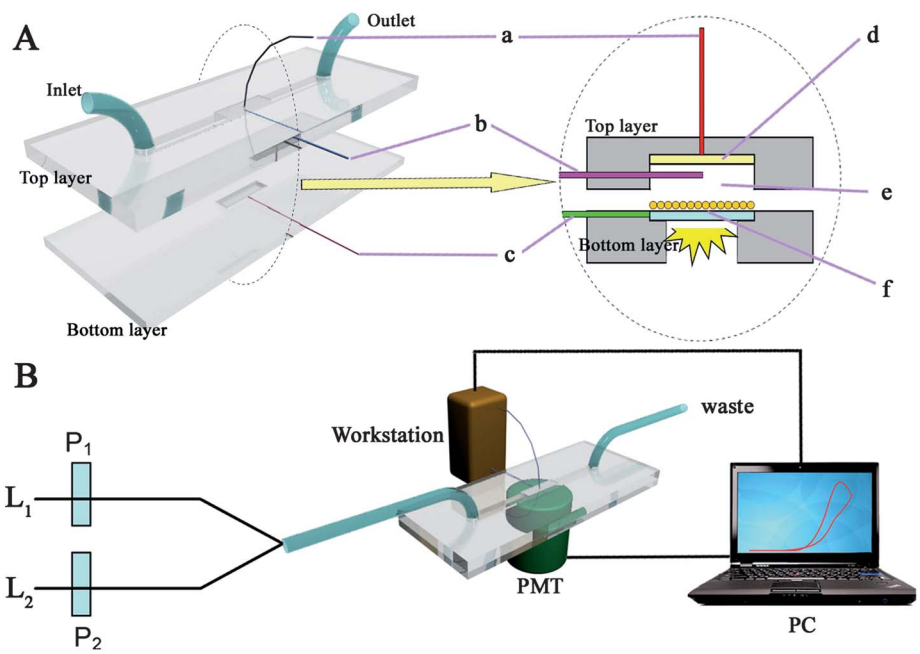

Fig. 1 Schematic diagram of the ECL (A) flow chip and ECL system (B). (A): a, c: wire; b: reference electrode (RE); d: slice counter electrode (CE); e: channel; f: ITO working electrode (WE). (B): L_1: carbonate bicarbonate buffer solution; L_2: BSA solution; P: peristaltic pump; PMT: photomultiplier tube.

(Shanghai, China). Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), alcohol, toluene were purchased from Tian Jin Da Mao Chemical Reagent Factory (Tian Jin, China). BSA was supplied from Sigma. All solutions were prepared using Milli-Q water (Millipore) as a solvent. 0.1 M carbonate bicarbonate buffer solution was used throughout the work, and the pH was adjusted by changing the ratio of Na_2CO_3 and $NaHCO_3$.

2.3 Preparation of water-soluble CdTe QDs

A TGA-capped CdTe (green to red-emitting) QDs aqueous colloid was prepared using a procedure described in detail in a preceding paper.[36] In a typical standard synthesis, 0.0262 g Te powder and 0.0730 g $NaBH_4$ were dissolved in 10 mL of water, and the solution was reacted in 60 °C water bath under oxygen-free until the solution turned into magenta. 0.1060 g of $CdCl_2 \cdot 2.5H_2O$ was dissolved in 35 mL of water, and 80 μL of TGA was added in the water, followed by adjusting the pH to 11.3 by 1 M solution of NaOH. The solution was placed in a three-necked flask fitted with a septum and valves and was deaerated by N_2 bubbling for 20 min, and the upper NaHTe precursors were rapidly injected into the vigorously stirred and oxygen-free solution, and the CdTe QDs are formed upon refluxing at 100 °C. The diverse colors (particle sizes) of the nanocrystals were obtained through different refluxing times. The obtained QDs solution could be rather stable for more than 3 months in a refrigerator at 4 °C.

2.4 Preparation of CdTe QDs films on the ITO surface

The ITO slide glass was cut into 3.0 × 1.0 cm slides with a surface resistance of 30–60 Ω cm^{-2}. The surface treatment of the ITO slide glass was performed using the procedure described in detail in a preceding paper.[37] After N_2 drying, the substrates were cleaned with water, acetone, and ethanol, respectively. Then they were immersed into 1 M solution of NaOH for 20 min, and then were rinsed with water and dried at 120 °C for 2 h.

CdTe QDs films on the ITO surface were prepared according to the literature.[38] The schematic diagram of self-assembling processes is shown in Fig. 2. The substrates were first dipped into the toluene solution of APS for 20 min to obtain an APS layer. In this stage, the thickness of the APS layer was controlled by adjusting the dipping time. Then the modified substrate was immersed in an aqueous solution of TGA (0.15 M, pH ~ 10, containing Cd^{2+} as a form of $Cd(ClO_4)_2$) for 3 min. In this stage, the amino group in APS was linked with the carboxyl group in TGA together with the hydrolysis of APS on the surface. Accordingly, TGA molecules play an important role for the hydrolysis of the APS layer and the linkage between the amino and carboxyl groups. The sample was then immersed in the aqueous QDs colloidal solution for 5 min to form a QD layer. After that, the sample was dipped into the the toluene solution of APS again for 5 min. Each step was interrupted with water rinsing and N_2 drying. *n* dipping cycles result in *n* double layers of absorbed material.

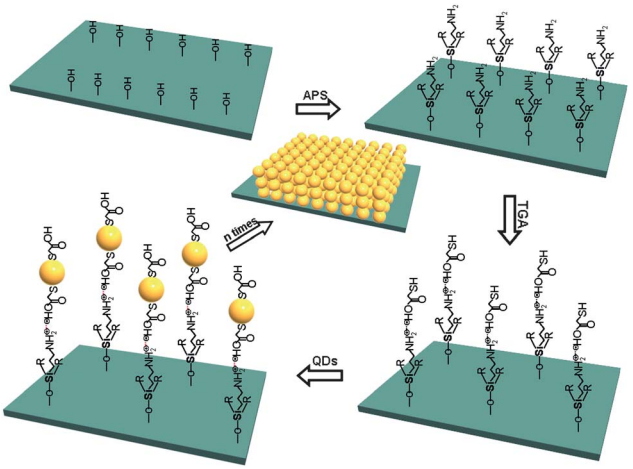

Fig. 2 Schematic diagram of LbL self-assembled processes of multilayer QD films.

2.5 Analytical procedures

Investigations of ECL behaviors were performed using the system shown schematically in Fig. 1B. Flow tubes (L_1, L_2) were connected with carbonate bicarbonate buffer solution and BSA standard or sample solution, respectively. The BSA or sample solution merged with buffer solution with a Y-piece. The mixture passed the ECL chip to produce an ECL spectrum at +1.2 V during the scanning process of cyclic voltammetry. The CHI 760D electrochemical workstation was used as the power supply and provided a cyclic voltammetry mode (Init E: 0.8 V, Low E: 0.8 V, High E: 2.0 V, scan rate: 0.1 V s^{-1}, scan number: 2 (for one peak), sensitivity: 1.e – 0.005 A V^{-1}). The ECL flow chip was placed in front of the detection window of the PMT (−800V).

3 Results and discussion

3.1 Characterization of TGA-capped CdTe QDs

UV–vis spectra and PL spectra were performed according to the procedure described in detail in preceding papers[39,40] (See ESI, Figure S.1.1†). The PL bands are sufficiently narrow (full width at half maximum are in the range of 38–50 nm) indicating again the relatively narrow size distribution of the QDs. The photoluminescence quantum yield (PL QY) was described in detail in a preceding paper.[41] The PL QY was 38.9–74.6% for the prepared CdTe QDs colloid upon refluxing from 1 min to 120 min. Compared with another aqueous synthesis method,[42] the CdTe QDs synthesized had a remarkable QY. In this experiment, the CdTe QDs that refluxed for 1 h were chosen due to their high PL QY (74.6%) and better dispersion characteristic. The X-ray diffraction (XRD) patterns (Figure S.1.3†) demonstrate that a single-phase CdTe compound with a zinc blende structure has been formed after ball milling elemental Cd and Te mixture powders for 1 h.

3.2 Electrochemical and ECL behaviors of CdTe QDs films

The feasibility of the method could be forecasted through the electrochemical and ECL behaviors of CdTe QDs films, and the relative ECL intensity is directly related to the sensitivity of the method. The results were shown in Fig. 3. In air-saturated pH 9.32 carbonate bicarbonate buffer solution, the cyclic voltammogram of CdTe QD films at an ITO electrode began to show

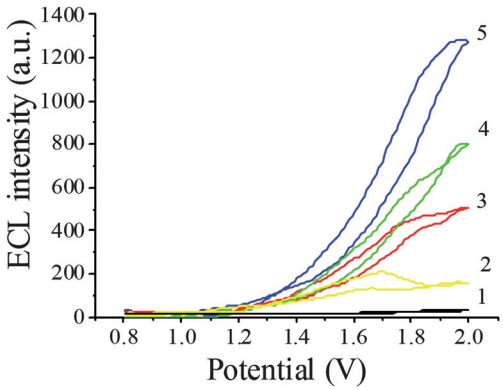

Fig. 3 The ECL curves of CdTe QD films. 1: pH 9.32 carbonate bicarbonate buffer solution + water; 2: pH 9.32 carbonate bicarbonate buffer solution + 1.0×10^{-7} g mL^{-1}BSA; 3: pH 9.32 carbonate bicarbonate buffer solution + 5.0×10^{-7} g mL^{-1} BSA; 4: pH 9.32 carbonate bicarbonate buffer solution + 8.0×10^{-7} g mL^{-1} BSA; 5: pH 9.32 carbonate bicarbonate buffer solution + 5.0×10^{-6} g mL^{-1} BSA.

a weak ECL emission at +1.2 V (curve 1). When 1.0×10^{-7} g mL^{-1} BSA was added into the solution, an intensive ECL signal occurred (curve 2). When the concentration of BSA increased to 5.0×10^{-7} g mL^{-1}, the ECL signal was enhanced (curve 3). With the increasing of the concentration of BSA, the ECL signal was enhanced further (curve 4, curve 5). At that point, the anodic ECL signal increases along with the augmentation of BSA in concentration. Consequently, a novel method of the determination of BSA was set up on anodic ECL of CdTe QD films.

3.3 Optimization of the reaction conditions

A series of experiments were performed to select the optimum analytical conditions using a 1.0×10^{-6} g mL^{-1} BSA solution. The optimal conditions including the voltage range of cyclic voltammetry, the layers of CdTe QD films on ITO glass, pH, and flow rate are investigated below.

The low potential was fixed at +0.8 V and the effect of high potential was measured from +1.2 V to +2.8 V. The ECL intensity was increased with raising the high potential up to +2.0 V, while higher than +2.0 V, the ECL intensity had a tailing peak or a double peak. When the sweep range was tiny, the ECL intensity was too small to be detected. Therefore, the +0.8–+2.0 V voltage range was used for the further work. It is very important to control the thickness of the sol–gel glass layer used to prepare films with a high concentration of QDs because the thick layer results in a decrease in the concentration of QDs. In this LbL self-assembling processes, amino and carboxy play important roles. The layers of CdTe QDs films on ITO glass were examined over 1–7 ranges, and the ECL intensity reached up to a maximum value when the ITO glass was modified by 4 layers (Figure S.2†). Therefore, these multilayer QDs films with 4 layers were adopted in the experiment.

The ECL reaction was performed from acidic to alkaline conditions, while the ECL intensity was very weak in acidic or neutral conditions. The effect of pH on the ECL reaction was examined in the range from 8.11 to 10.23 (Figure S.3†). The results showed that the optimum pH of carbonate bicarbonate buffer solution was 9.32 since a maximal ECL signal could be obtained under this alkalinity. The flow rate was shown schematically in Fig. 1(B), which influences the analytical sensitivity. The flow rate of pump 1 represents the volume of carbonate bicarbonate buffer solution, and the flow rate of pump 2 represents the volume of BSA solution. The effect of the flow rate of pump 1 and pump 2 on the ECL intensity was examined in the range of 0.6–2.4 mL min^{-1}. The results showed that, when the flow rate of pump 1 was less than 1.8 mL min^{-1}, the ECL signal increased with the increasing of the flow rate, and when the flow rate of pump 1 was greater than 1.8 mL min^{-1}, the ECL signal decreased with the increasing of the flow rate (Figure S.4†). So, a flow rate of 1.8 mL min^{-1} which was the suitable flow rate of pump 1 was selected as optimum. In the same way, the optimal flow rate of pump 2 was obtained for 1.5 mL min^{-1} (Figure S.5†).

3.4 Analytical performance

Under the optimal conditions, the analytical performance was investigated. The relative ECL intensity was linear to BSA concentration over the range of 1.0×10^{-9}–1.0×10^{-6} g mL^{-1} and 1.0×10^{-6}–1.0×10^{-5} g mL^{-1}. The relationship between ECL intensity and BSA concentration was shown in Fig. 4. The regression equation was $\Delta I_{ECL\ 1} = 123.1 + 8.866 \times 10^{8}\ c$ (c/g mL^{-1}) ($r = 0.9972$), $\Delta I_{ECL\ 2} = 977.7 + 5.625 \times 10^{7}\ c$ (c/g mL^{-1}) ($r = 0.9944$). The detection limit was 5.48×10^{-10} g mL^{-1}. The relative standard deviation was 1.62% by 11 replicate determinations of 4.0×10^{-7} g mL^{-1} BSA.

3.5 Interference studies

The influence of foreign species was studied by analyzing a standard solution of 1.0×10^{-6} g mL^{-1} BSA to which increasing amounts of foreign species were added. A substance was considered not to be an interferent if the variation of the ECL intensity was within ±5%. The influence of some common amino acids, metal ions and vitamins was studied. The tolerable concentration ratios of foreign species to 1.0×10^{-6} g mL^{-1} of BSA was over 1000-fold for L-lysine, L-leucine, L-valine, L-threonine, Ca^{2+}, Ag^{+}, Zn^{2+}, 500-fold for L-arginine, L-phenylalanine, L-proline, V_{B1}, V_{B12}, Pb^{2+}, Se^{2+}, Cu^{2+}, 100-fold for Fe^{3+}, Hg^{2+}, triglyceride, 50-fold for lactose, 10-fold for L-tryptophan, L-cysteine and V_{B6}, 1-fold for casein.

3.6 Stability and reproducibility exploration of FI-ECL sensor

The stability and reproducibility of the ECL intensity of the FI-ECL sensor was shown in Fig. 5. Under the optimal

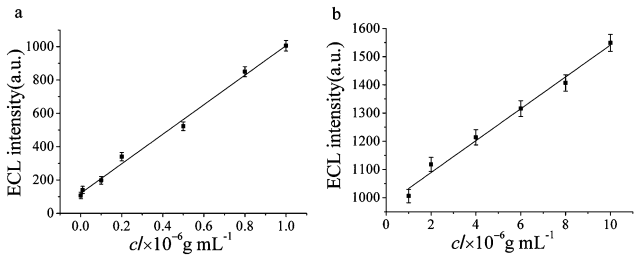

Fig. 4 Relationship between ECL intensity and BSA concentration.

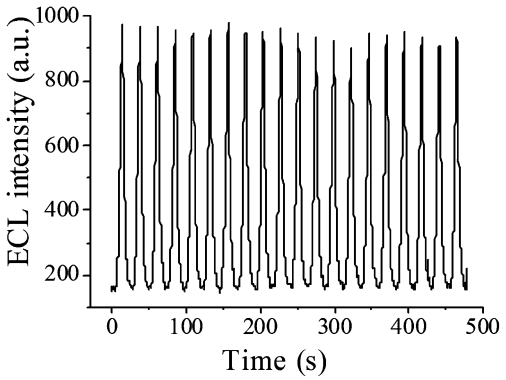

Fig. 5 ECL emissions from the CdTe QD films in 0.1 M pH 9.32 carbonate bicarbonate buffer solution containing 1.0×10^{-6} g mL^{-1} BSA under continuous cyclic scans between 0.8 and 2.0 V for 20 cycles at 100 mV S^{-1}.

conditions, twenty measurements of ECL emission upon continuous cyclic scan in 0.1 M pH 9.32 carbonate bicarbonate buffer solution containing 1.0×10^{-6} g mL^{-1} BSA showed coincident signals with a relative standard deviation (RSD) of 3.12%, indicating the reliability and stability of the signal. During the experiment, the QDs film sensor was used for hundreds of cycles, only a slight decrease in ECL intensity was observed with a decrease to about 94% of its initial value, indicating a good precision and stability. These observations suggest that the sensor exhibits excellent ECL behavior.

3.7 Application

In order to evaluate the applicability and accuracy of the proposed method, the proteins in two milk samples were determined. In the experiment, BSA was used to present the amount of various proteins in milk, which have similar structure and property. The two samples were diluted 1.0×10^5 times with water, and were determined with the proposed FI-ECL method. The results of the determinations and the recovery tests were shown in Table 1. As can be seen in Table 1, the recoveries of BSA can be quantitative and the *t*-test assumed that there is no significant difference between recovery efficiency and 100% at a confidence level of 95%.

3.8 Elucidation of the ECL mechanism

The ECL behaviors of CdTe QDs films containing carbonate bicarbonate buffer solution and different concentrations of BSA solution were measured (Fig. 4). In order to confirm whether the BSA was denatured or not in this condition, the BSA was heated to 85 °C for 5 min to make it thermally denatured, and the ECL emission was very weak and not often detected even in ECL transients (Fig. 6, curve a). However, the untreated BSA showed an intensive ECL emission at the surface of the QDs modified ITO electrode (Fig. 6, curve b) with a concentration of 1.0×10^{-6} g mL^{-1}. It can be seen that the BSA was not denatured in the rapid determination in such conditions. Based on the aforementioned experiments, the possible mechanism of the QDs ECL reaction was proposed.

At higher pH the ITO surface was more negatively charged.[43] For the QDs on ITO, the holes are short-lived due to fast hole transfer processes to the trapped electrons in the negatively charged QDs or to ITO, inhibiting the formation of the off state. A direct measurement of QDs under optical illumination shows that in off states QDs are positively charged.[44] It should be noted that suppression of blinking has also been reported for QDs in reducing solution environments, which also provides electron sources to remove any long-lived holes in the QDs.[45,46] The emitter of the ECL emission was the excited state of QDs (QDs*). Here, we suggest that BSA could capture electrons in alkaline conditions, which could transfer to dissolved oxygen molecules to produce O_2^- species. The O_2^- produced by interaction with BSA inject one electron into the quantum-confined orbital of CdTe to form reduced QDs (e^-).[47] The formation of the QDs* was explained for the participation of dissolved oxygen and BSA through the ITO electrode surface. In the anodic process, the ITO acted as a medium for the electron transfer between QDs, dissolved oxygen and BSA.

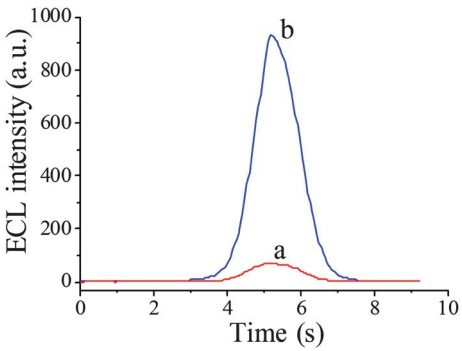

Fig. 6 The ECL curves of the thermal denaturation of BSA (a) and untreated BSA (b).

Table 1 Determination of BSA in milk

Samples	Found[a] (g 100 g^{-1})	RSD (%)	Added ($\times 10^{-6}$ g mL^{-1})	Recovered[a] ($\times 10^{-6}$ g mL^{-1})	Recovery (%)
1	2.963	3.21	0.2	0.1920	96.0
			0.4	0.3932	98.3
			0.6	0.5843	97.4
2	2.527	2.96	0.2	0.1975	98.8
			0.4	0.4071	101.8
			0.6	0.6168	102.8

[a] Average of six measurements.

The CdTe QDs films at an ITO electrode began to show a weak ECL emission at +1.2 V as pH 9.32 buffer solution was injected into the ECL flow chip (Fig. 4, curve 1). However, the ECL signal was enhanced when BSA was added into the mixture, and enhanced further with the increasing concentration of BSA (Fig. 4, curve 2–5). It is confirmed that the anodic ECL emission was related to BSA concentration. The whole process of anodic ECL emission can be simply described with the following equations:

$$\mathrm{In/SnO}_x + \mathrm{CdTe} \xrightarrow{\mathrm{e^-}} \mathrm{CdTe}(\mathrm{h^+}) + \mathrm{In/SnO}_x(\mathrm{e^-}) \quad (1)$$

$$\mathrm{BSA} + \mathrm{OH^-} \rightarrow \mathrm{BSA}\ (\mathrm{e^-}) + \mathrm{H_2O} \quad (2)$$

$$\mathrm{O_2} + \mathrm{BSA}(\mathrm{e^-}) \xrightarrow{\text{electron transfer}} \mathrm{O_2^-} + \mathrm{BSA} \quad (3)$$

$$\mathrm{CdTe} + \mathrm{O_2^-} \rightarrow \mathrm{CdTe}\ (\mathrm{e^-}) + \mathrm{O_2} \quad (4)$$

$$\mathrm{CdTe}\ (\mathrm{h^+}) + \mathrm{CdTe}\ (\mathrm{e^-}) \rightarrow \mathrm{CdTe^*} \quad (5)$$

$$\mathrm{CdTe^*} \rightarrow \mathrm{CdTe} + h\nu \quad (6)$$

4 Conclusions

A sensitive and low-cost method for the determination of BSA was developed using QDs as the ECL probe. ITO glass was decorated by CdTe QDs *via* LbL self-assembly for producing a novel ECL sensor. The modified ITO glass was packing into a homemade ECL flow chip and used as a receptor of the FI-ECL system. With pH 9.32 carbonate bicarbonate buffer solution, the CdTe QDs films showed the largest ECL intensity. The FI-ECL sensor exhibited a low detection limit, wide linear range, high sensitivity, and good reproducibility for the determination of BSA. The analysis procedures were simplified and the analysis time was also shortened. This strategy could be easily realized and opens new avenues for the applications of QDs in establishing ECL analytical methods and ECL biosensors.

Acknowledgements

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