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Electrochemiluminescence of CdTe Quantum Dots and Sensitive Detection of Hemoglobin

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CdTe quantum dots were modified to the surface of glassy carbon electrode (GCE) using Nafion, and the electrochemiluminescence (ECL) behavior of CdTe quantum dots immobilized in Nafion was investigated. Compared with ECL of CdTe quantum dots on the bare GCE, the ECL intensity of CdTe quantum dots modified into the Nafion film was obviously enhanced. Moreover, the ECL behavior of CdTe quantum dots in Nafion films had significant size dependence. CdTe quantum dots–Nafion could generate strong ECL signals in the presence of tripropylamine as the co-reactant in the phosphate buffer solution (PBS) at pH 7.0. A new method for the detection of hemoglobin was established based on hemoglobin could efficiently quench the ECL intensity of CdTe quantum dots. The results showed that the detection signal lg $[(I_0–I)/I]$ and the logarithm of hemoglobin concentration lgC had a good linear relationship between 1.0×10^{-9} mol/L and 5.0×10^{-6} mol/L. The detection limit was 2.6×10^{-10} mol/L.

Keywords: CdTe quantum dots; ECL quenching; tripropylamine; hemoglobin

1. INTRODUCTION

Hemoglobin was one of the most widely distributed proteins in biological field, the main component of the hemoglobin molecular structure was shown in Fig. 1. Hemoglobin plays a key role in the metabolism of people and various organisms. Hemoglobin (Hb) was a vital respiratory protein in red blood cells of vertebrates, which played an important role in oxygen transport, decomposition of H_2O_2, generation of energy and transmission of electrons [1, 2]. Determination of hemoglobin in blood was an important part of poisoning test in criminal cases, which was of great significance to forensic science research. Analytical methods have always been an important part of hemoglobin research. The detection methods reported in the literature mainly included high performance liquid chromatography [3-5], ultraviolet absorption spectroscopy [6-8], and fluorescence analysis [9-11]. Although these methods had high accuracy, they were expensive to test or require complex mathematical processing. Therefore, these methods were subject to a certain degree of restrictions in practical application.

In the past twenty years, electrochemical research has attracted wide attention from researchers [12-16]. The research on ECL is an important part in the electrochemistry study area. ECL was a new detection technology developed on the basis of ECL. The basic principle was that through the electrode to the system containing the ECL substances to impose a certain voltage or current, so that the light-emitting substances were excited. Meaning while, the excited substances were jumped back to the ground state and emitting the photon [17]. ECL not only had excellent electrochemical selectivity [18], but also had the advantages of chemiluminescence with high sensitivity [19]. In recent three decades, the preparation technology of nanomaterials, especially on semiconductor quantum dots, has been developed rapidly and matured. It was possible to develop various signal-sensitive sensors. The combination of ECL and semiconductor quantum dots presented new opportunities for the ultimate resolution of hemoglobin determination challenge. Compared with the traditional organic fluorescent dyes, quantum dots had the advantages of good photostability, high luminous intensity, wide excitation spectrum, larger Stokes shift, narrow emission spectrum and symmetry, long fluorescence lifetime and good biocompatibility, and its light color could be changed by adjusting its size [20-22]. Based on the excellent optical properties of quantum dots above, quantum dots related to the application of research aroused widespread concern. At present, quantum dots have been widely used in ultra-sensitivity, high stability and long-life fluorescent probe [23-28]. According to the literature reported, the information of ECL coupled with quantum dots is still limited [16]. Moreover, the research on the detection of hemoglobin by quantum dots ECL has not been reported.

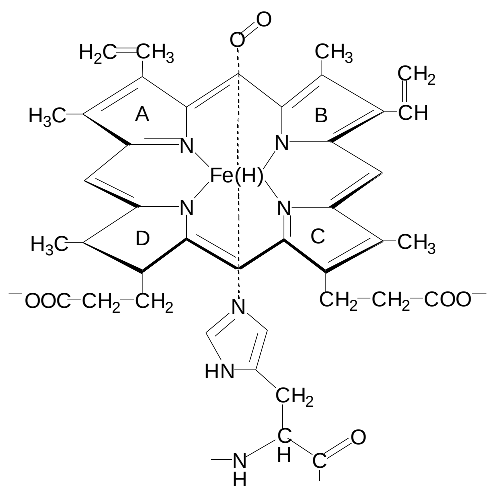

Figure 1. Structure of heme group in hemoglobin

In the present study, CdTe quantum dots were modified in the Nafion membrane by the good film-forming ability of Nafion [29], and the Nafion-CdTe quantum dot modified GCE was prepared. The ECL behavior of CdTe quantum dots on Nafion film modified electrode was studied by using tripropylamine as co-reactant. At the same time, the size dependence of the ECL behavior of CdTe quantum dots in Nafion films was also investigated. The results showed that the hemoglobin could inhibit the ECL intensity of Nafion–CdTe quantum dots, and the linear relationship between the inhibition degree and the hemoglobin concentration was established, and a new method for the determination of hemoglobin by ECL of CdTe quantum dots was established.

2. EXPERIMENTAL SECTION

2.1 Instruments and reagents

The fluorescence spectra were measured with a LS-55 fluorescence spectrophotometer (Perkin Elmer, USA). The absorbance spectra were performed on an UV-2100 ultraviolet-visible spectrophotometer (Beijing Beifen-Ruili Analytical Instrument Co., Ltd.). The electrochemical detection was carried out on a CHI620b electrochemical analyzer (Shanghai Chenhua Instrument Co., Ltd.). Three-electrode system: the platinum wire electrode was the counter electrode, the glassy carbon electrode was the working electrode, and the Ag/AgCl electrode was the reference electrode. TEM was obtained on an EM420 transmission electron microscopy (Netherlands Philips). XRD was performed by a PW3040/60 X-ray diffraction (Netherlands Philips). Hemoglobin (Sigma, USA). $CdCl_2$, thioglycolic acid, $NaBH_4$ and NaOH were purchased from Sinopharm Shanghai Chemical Reagent Co., Ltd. All the chemical reagents were of analytical grade. The water used in the experiment was two-distilled water.

2.2 Synthesis and modification of quantum dots

The synthesis and modification of CdTe quantum dots was consistent with the reported in our previous literature [18]. First, the reaction of sodium borohydride with tellurium powder in an ice bath was continued for more than 4 h until the solution color was white. After standing for 10 min, the obtained colorless transparent upper solution was NaHTe. Subsequently, the thioglycolic acid stabilizer was injected into a nitrogen-saturated 2×10^{-2} mol/L $CdCl_2$ aqueous solution at pH 11.2. The molar ratio of Cd^{2+}: Te^{2-}: TGA was 1: 0.5: 2.5. The whole process N_2 was continuously inlet and reacted in an ice bath. Finally, the obtained cadmium telluride precursors are transferred to a polytetrafluoroethylene pot. The CdTe quantum dots with different sizes and stable properties were obtained by heating at 140 ℃ for 80 min, 135 min, 175 min and 220 min, respectively. The concentration of CdTe quantum dot was estimated to be 5.0×10^{-3} mol/L using Te^{2-} as reference.

2.3 Preparation of CdTe quantum dots modified electrode

The GCE was first ground with 0.05μm Al_2O_3 suspension and polished. Then, The GCE was ultrasound 5 min with two distilled water and ethanol, respectively. After dried by nitrogen, a certain amount of the prepared CdTe quantum dots solution was deposited onto the surface of the treated GCE and dried at room temperature. Subsequently, Nafion-CdTe quantum dots-GCE modified electrode was prepared by adding 10 μL 5% Nafion to the modified electrode.

3. RESULTS AND DISCUSSION

3.1 Spectral characterization of CdTe quantum dots

CdTe quantum dots with different sizes were synthesized by changing the reaction time. The CdTe quantum dots emit green, yellow, orange, and red fluorescence with the heating time increases (Fig. 2), respectively. The absorption and fluorescence emission spectra were shown in Fig. 2. It can be seen from Fig. 2 that the fluorescence emission spectra of CdTe quantum dots were symmetrical and the half-width was narrow. Absorption spectra with significant absorption peaks showed that the synthesized CdTe quantum dots performed a uniform distribution. The fluorescence emission peaks of prepared CdTe quantum dots were 521, 562, 593, and 613 nm for heating 80, 135, 175 and 220 min, respectively. The maximum absorption peaks of these CdTe quantum dots were 485, 536, 563, and 583 nm, respectively. According to the calculated method reported in the literature [13], the particle size of CdTe quantum dots was about 1.87, 3.07, 3.37, and 3.53 nm, respectively. It can be seen that with the increase of quantum dots size, the maximum absorption peak and fluorescence emission peak shifted to longer wavelengths, respectively, which was consistent with the quantum size effect.

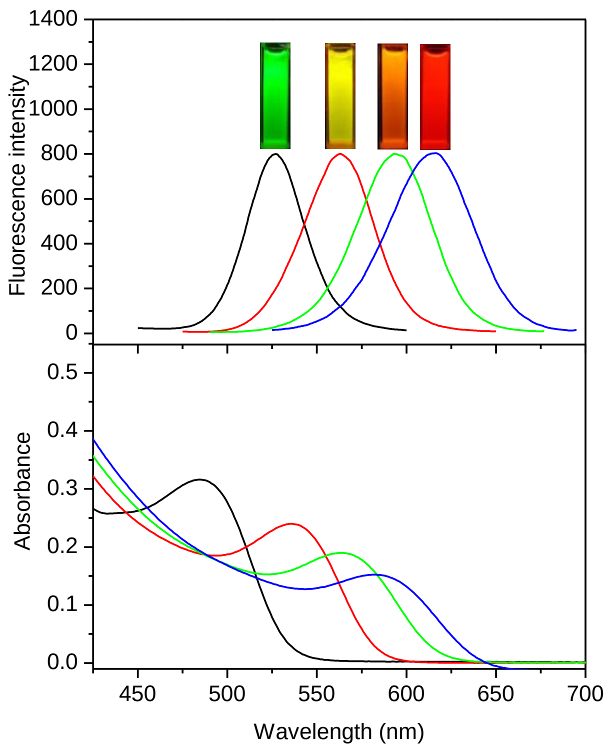

Figure 2. Absorption and fluorescence emission spectra of four thioglycollic acid capped CdTe quantum dots with heating (a) 80 min, (b) 135 min, (c) 175 min, and (d) 220 min, respectively

3.2 Structure characterization of CdTe quantum dots

Fig. 3 showed TEM of the CdTe quantum dots with the emission peak at 613 nm. It can be seen that the synthesized CdTe quantum dots were spherical, better dispersion, and narrow particle size distribution. The average particle size was about 3-4 nm, which was consistent with the empirical formula. The corresponding SAED electron diffraction pattern (inset) showed a dotted polycrystal diffraction pattern, indicating that the quantum dots were a cubic sphalerite crystal structure.

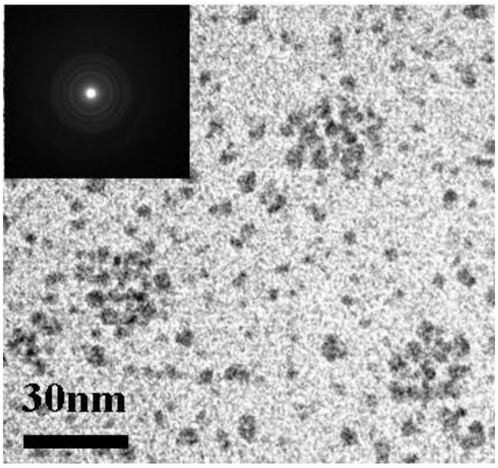

Figure 3. TEM image of CdTe quantum dots for heating 220 min

Fig. 4 showed the X-ray diffraction pattern of CdTe quantum dots synthesized by heating at 140^{o}C for 220 min. It can be seen from the Fig. 3 that the nanocrystals belonged to the cubic structure. The 2θ values of CdTe quantum dots were 23.854, 40.632, and 45.946, respectively, which corresponded well to the three crystal planes of (111), (220) and (311) of cubic CdTe with the standard card (03-065-1046). The X-ray diffraction peak broadens indicated that the synthesized CdTe quantum dots were a very small nanocrystal.

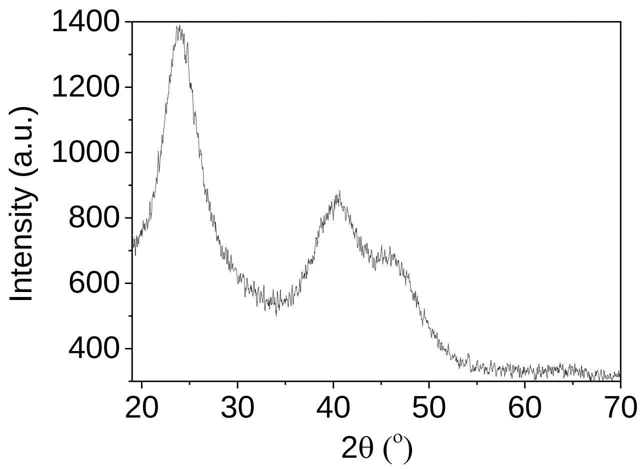

Figure 4. The XRD diagram of CdTe quantum dots powder

3.3 ECL of size-dependent CdTe quantum dots immobilized in Nafion-GCE

The ECL of four CdTe quantum dots with different sizes in air saturated solution was investigated on Nafion-GCE. As shown in Fig. 5, the ECL intensities of four CdTe quantum dots increased with the size of CdTe quantum dots, indicating that ECL of CdTe quantum dots had a size-dependency. It can be seen from Fig. 6 that the ECL intensity of CdTe quantum dots increased not only with the size of quantum dots, but also the on-set potential and the peak potential increased with the increase of the CdTe quantum dots size.

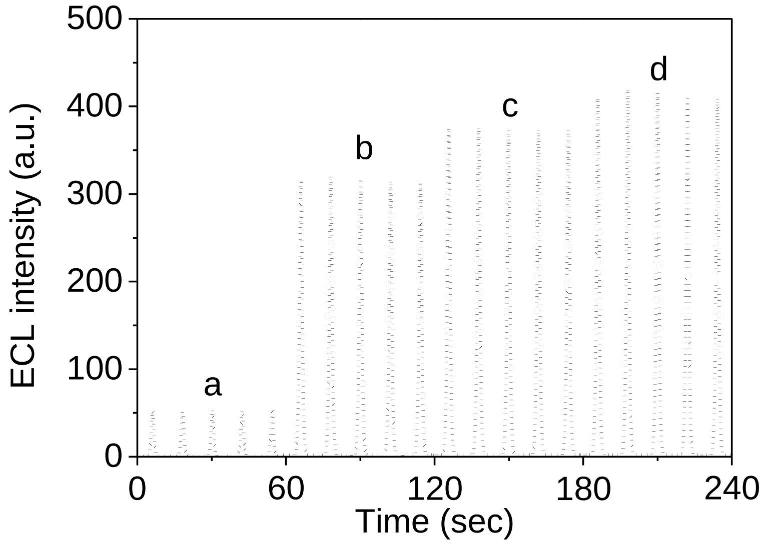

Figure 5. ECL curves of CdTe quantum dots with four sized on Nafion-GCE

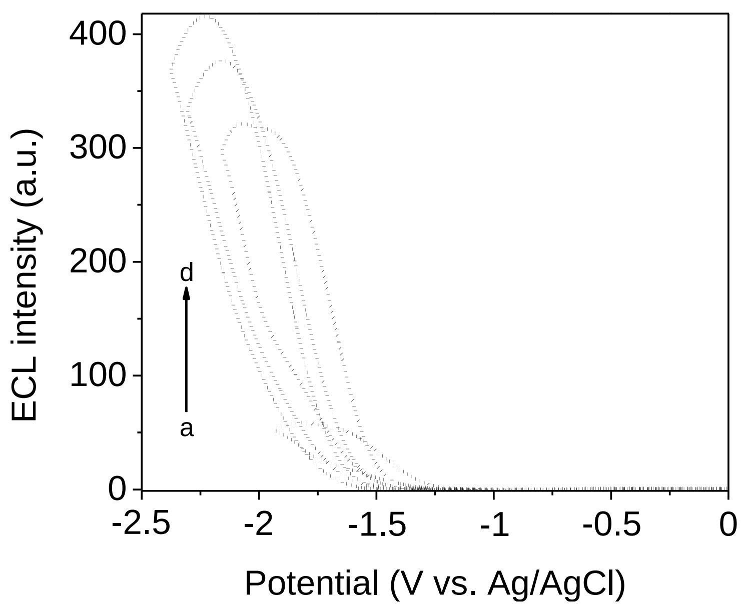

Figure 6. Effects of potential and ECL intensity on Nafion-GCE with four sized CdTe quantum dots

3.4 Optimization of detection conditions

The optimal experimental conditions for the determination of hemoglobin by ECL were investigated, and the maximum ECL signal of the CdTe quantum dots was taken as the index. According to the experimental results, CdTe quantum dots for heating 220 min was used as the optimal ECL sensor, the concentration of CdTe quantum dots was 1.0×10^{-5} mol/L, the pH of 0.1 mol/L PBS solution was 7.0, and the concentration of co-reactant tripropylamine was 0.02 mol/L, .

3.5 Enhanced ECL of CdTe quantum dots by Nafion modified electrode

The direct ECL behavior of CdTe QDs was investigated using bared GCE and Nafion- GCE, respectively (Fig. 7a, b, c, and d). Using the Nafion modified electrode, the cathodic current was significantly enhanced, which was shown in curves c and d. The cyclic voltammetric curves (a, b) showed that the on-set potential of the CdTe quantum dots also shifted positive, although the information reflected by the cyclic voltammograms was limited. ECL curves showed that the Nafion layer on the surface of the GCE can promote the ECL of CdTe quantum dots well. It can be seen that the ECL intensity of CdTe quantum dots on the Nafion modified electrode was much stronger than that on the bare electrode. Moreover, the Nafion modified electrode was used to make the ECL of the on-set potential and peak potential were positive shifted. Therefore, the ECL sensor can generate ECL of CdTe quantum dots at a low negative potential, so as to effectively avoid the high potential interference.

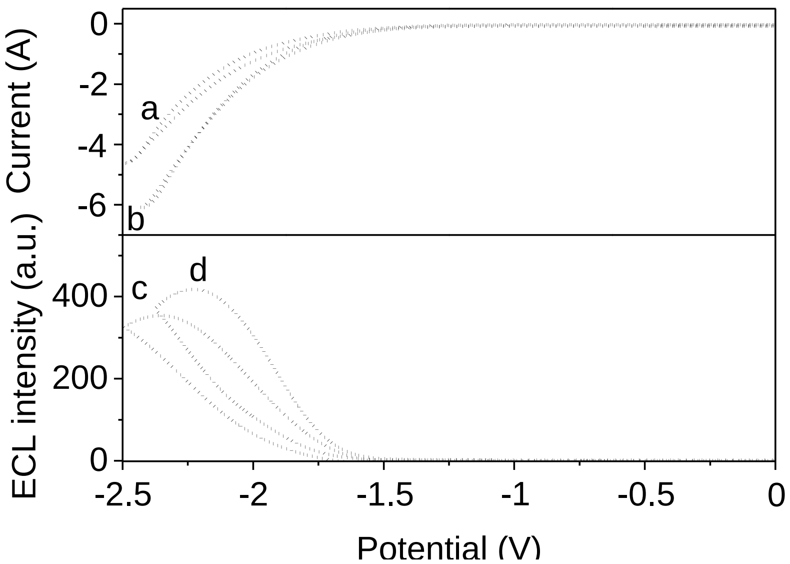

Figure 7. The direct cyclic voltammetric and ECL behavior of CdTe quantum dots was investigated in bared GCE and Nafion-GCE. (a, c was performed in bared GCE, and b, d was obtained in Nafion- GCE).

3.6 Stability and reproducibility of ECL of Nafion-CdTe quantum dots-GCE

For the surface ECL, whether the CdTe quantum dots were fixed or not was an important factor affecting the determination. In this study, the fixation degree of CdTe quantum dots was measured by using the stability of the quantum dots ECL on the electrode surface. Fig. 8 showed the results of repeated ECL of CdTe quantum dots in 0.1 mol/L PBS exited 0.02 mol/L tripropylamine, and Nafion-GCE was used as the working electrode. It can be seen that the quantum dots immobilized in the Nafion membrane exhibited good ECL stability and reproducibility, which can meet the requirements of the ECL analysis.

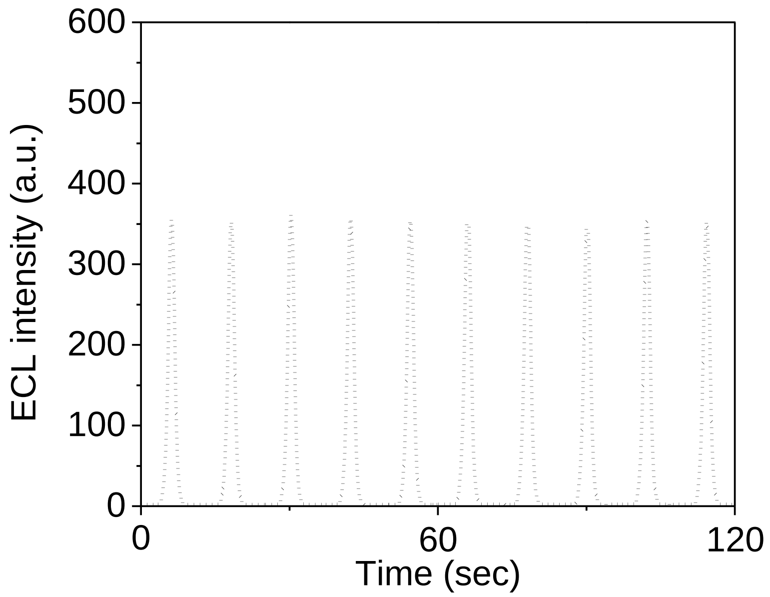

Figure 8. The results of repeated ECL of CdTe quantum dots in 0.1 mol/L PBS with 0.02 mol/L tripropylamine, and Nafion-GCE was used as the working electrode.

3.7 Effect of coexistence substances

In order to study the selectivity of the method for determination of hemoglobin, the effects of metal ions, amino acids and proteins on the measurement of 5.0×10^{-7} mol/L hemoglobin were investigated. The interference level was ± 5%. As shown in Fig. 9, 30-fold concentration of K^+, Na^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Zn^{2+}, Fe^{2+}, NO_3^- and PO_4^{3-} did not interfere with the ECL signal. 25-fold concentration of L-glycine, L-cysteine, L-glutamic acid , L-tryptophan, L-tyrosine, L-lysine, and L-phenylalanine also could not interfere the ECL signal. Similarly, 20-fold concentration of glucose, ascorbic acid, citric acid, BSA, vitamin B and dopamine will not interfere with the ECL signal. The experimental results showed that this method has high selectivity and was suitable for quantitative determination of hemoglobin in real samples.

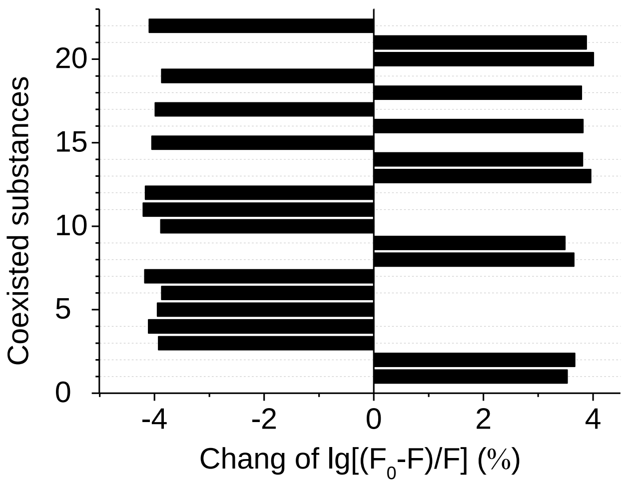

Figure 9. Effects of potential coexistence substances on the measurement of 5.0×10^{-7} mol/L hemoglobin were investigated.

3.8 Analytical applications

Under the optimized experimental conditions, the signal lg [(F_0-F)/F] and logarithm of hemoglobin concentration lg C in the concentration range of 1.0×10^{-9} ~ 5.0×10^{-6} mol·L^{-1} showed a good linear relationship (Fig. 10). The linear regression equation was lg [(F_0-F)/F] = 0.27476lg C + 1.75454 (F_0 was the ECL intensity of blank value, F was the corresponding ECL intensity when adding different concentrations of hemoglobin, C was the concentration of hemoglobin (mol/L)), the linear correlation coefficient was 0.9978, and the detection limit was 2.6×10^{-10} mol/L. The relative standard deviation (RSD) of hemoglobin standard solution with concentration of 5.0×10^{-7} mol/L was 3.92%.

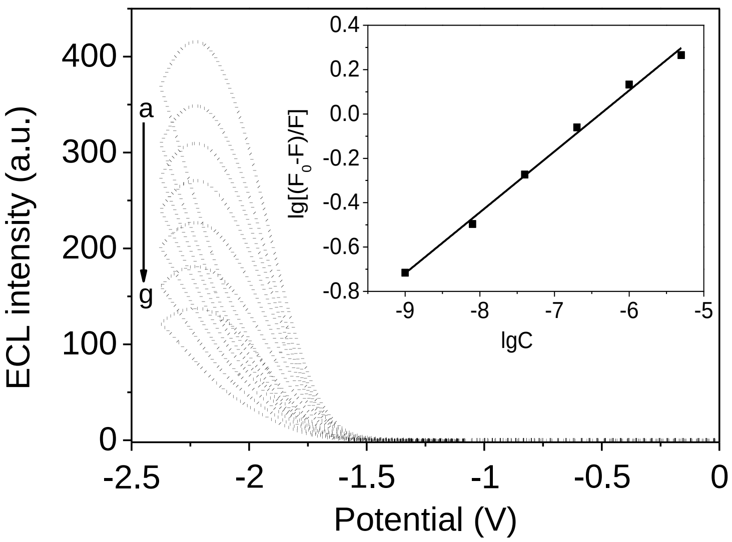

Figure 10. Effect of hemoglobin with different concentrations on ECL spectra of CdTe quantum dots, and the inset was the relationship of difference ECL intensity with the concentration of hemoglobin

4. CONCLUSIONS

In this study, high quality water-soluble quantum dots were synthesized by hydrothermal method. Then, CdTe quantum dots were modified into Nafion membrane to study the ECL behavior of CdTe quantum dots. Under the optimal experimental conditions, the ECL intensity of CdTe quantum dots was high and stable. Based on the quenching effect of hemoglobin on the ECL, a new method for detecting hemoglobin was established. The method had the advantages of high sensitivity, good selectivity and simple operation. The related research opened up a new way for the application of semiconductor quantum dots as ECL probes in the forensic science area.

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