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Detection of biothiols in human serum by QDs based flow injection "turn off–on" chemiluminescence analysis system ☆

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ABSTRACT

In the present work, a flow injection (FI) "turn off–on" chemiluminescent method was developed for the determination of glutathione (GSH). Strong chemiluminescence (CL) signals were observed from the hydrogen peroxide and CdTe quantum dots (QDs) system under basic condition, addition of trace amount of Cu(II) could caused significant CL quenching of the CdTe QDs–H_2O_2 system. In the presence of biothiols, Cu(II) can be removed from CdTe QDs surface via forming Cu(II)–S bond with thiols, and the CL signal of CdTe QDs–H_2O_2 system was recovered. Thus, the CL signals of CdTe QDs–H_2O_2 system were turned off and on by the addition of Cu(II) and biothiols respectively, and a flow injection CL analysis system for the determination of biothiols was established. Under the optimum conditions, the CL intensity and the concentration of GSH have a good linear relationship in the range of 2.0×10^{-9}–6.5×10^{-7} mol L^{-1} ($R^2=0.9993$). The limit of detection for GSH is 1.5×10^{-9} mol L^{-1} (S/N=3). This method has been applied to detect GSH in human serum with satisfactory results.

1. Introduction

In recent years, biothiols sensors have been paid great attention owing to their significant role in the physiological systems. Biothiols, the thiols-containing amino acid and peptides, participate in the process of reversible biological redox homeostasis and vital cellular functions including detoxification and metabolism [1–3]. For example, GSH is the most predominant sulfhydryl tripeptide in the biological fluids, which serves as an antioxidant and a crucial indicator of cellular oxidative stress [4]. Therefore, GSH analysis in biomedical systems has a crucial practical significance for the early diagnosis of a variety of diseases, such as cancer, AIDS, Alzheimer's and cardiovascular disease [5,6]. During the past decade, lots of methods have been developed for the determination of biothiols in human serum, including electrochemical methods [7–9], optical spectroscopy [10], enzymatic methods [11], high-performance liquid chromatography (HPLC)-based methods [12]. However, many approaches suffer from limitations in terms of performance, equipment costs, complicated, expensive and time-consuming. Therefore, novel available methods for biothiols detection are required with high selectivity and sensitivity in biological fluids. Compared with other analysis methods, CL analysis has a number of excellent advantages, such as high sensitivity, wide linear range, simple instrumentation and no interference from background scattering light [13,14]. The classical oxidants, luminol [15], luci-genin, peroxalate and potassium permanganate have been widely developed in the past decades. Zhao et al. determined GSH and other intracellular thiols by microchip electrophoresis with luminol CL system [16]. However, the broad applications of CL assay have been greatly limited due to the weak CL intensity of many traditional CL systems. Recently, a number of researchers have focused their interests on exploring the nanomaterials CL systems for the purpose of enhancing the selectivity and stability [17]. The nanomaterials can be used as catalyst, reductant, luminophor, and energy accepter in the CL systems [18–21].

As a new kind of nanomaterial, QDs have attracted great attention in the past decade, due to their unique properties [22–24], such as narrow, symmetrical and size-tunable emission spectrum, broad excitation spectrum, large stokes shift, high photo-bleaching threshold and good chemical stability. As an excellent optical material, QDs have been widely used to detect various substances in the analytical field, such as small molecules [25] and proteins [26]. It has been reported that CdTe QDs could be directly oxidized by some oxidants, such as H_2O_2 and $KMnO_4$ [27]. The oxidized CL of CdTe QDs/H_2O_2 system and its size-dependent, surfactant-sensitizing effects in aqueous solution have been investigated by Wang et al. [28]. However, most of CdTe QDs based CL systems are based on simple oxidation mechanism and thus lead to low selectivity.

In order to improve the selectivity of CdTe QDs based CL systems, we present a "turn off–on" CL system in this work. "Turn off–on" mode has been used widely in the fluorescence analysis

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field with high selectivity and sensitivity. But it is rarely employed in the CL system. A significant enhancement in CL signal was observed when CdTe QDs were added to H_2O_2–NaOH mixed solution. H_2O_2 reacted with NaOH to generate superoxide radical (O_2^-). And, H_2O_2 reacted with the reactive intermediate and leading to the formation of hydroxyl radical (OH·). Superoxide radical and hydroxyl radical got in touch with CdTe QDs to formed electron-injected CdTe QDs and hole-injected CdTe QDs respectively. Then, the CL emission occurred. Cu(II) can inhibit this CL process and behave as a CL quencher. As a result, the CL intensity is dependent on Cu(II) concentration. When GSH was added into above system, Cu(II) has a very high affinity for thiol of GSH, which result in the CL recovery of the CdTe QDs-based CL system. Thus, a new, simple, sensitive and selective CdTe QDs based "turn off–on" CL system was established for the detection of GSH.

2. Experimental section

2.1. Chemicals

Mercaptosuccinic acid (MSA) (99%) was bought from J&K Chemical Co. and tellurium powder (~200 mesh, 99.8%), $CdCl_2$ (99%) and $NaBH_4$ (99%) were purchased from Aldrich Chemical Co. Hydrogen peroxide (30%), Sodium hydroxide were purchased from Beijing Chemical Plant, China. Cupric chloride was purchased from Tianjin Fuchen Chemical Reagent Factory, China. Glutathione was purchased from Beijing Dingguo Biotechnology Ltd., China. The water used in all experiments had a resistivity higher than 18 MΩ cm^{-1}. All chemicals were used of analytical reagent grade without further purification.

2.2. Apparatus

CL analyses were conducted on a flow-injection analysis processor, FIA-3110 (Beijing Titan Instruments Co., Ltd.). Flow-injection analysis (FIA) system consisted of two peristaltic pumps, sixteen-hole eight-way injection valve and a digital-system to maintain the time of flow and pressure of each pump. PTFE tubing (0.5 mm i.d. Shenyang Zhaofa Institute of Automatic Analysis, China) was used as connection material in the FI-CL system. The flow cell was homemade coil, made by coiling 30 cm of colorless glass tube (1 mm i.d. and 2 mm o.d.) into a spiral disk shape with a diameter of 2 cm and located directly facing the window of the photomultiplier tube. Emitted CL light is measured with the PMT operating at 1000 V and 25 °C with no wavelength discrimination. Resulting peaks are recorded with a FIA monitor/data processing apparatus. CL detection system was a computerized ultra-weak luminescence analyzer (Type BPCL manufactured at the Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). Ultra-weak luminescence analyzer consists of a weak luminescence measure room, a photoelectricity switch-box, an electricity-pulse counterchange electrocircuit, a high-pressure power supply of high stability and an intellectualized measure system. Fluorescence measurements were performed on a Shimadzu RF-5301 PC spectro fluorophotometer. In this experiment, a 1 cm path-length quartz cuvette was used. In order to obtain CL emission signals, the excitation light source was turned off.

2.3. Preparation of CdTe QDs

Water-compatible CdTe QDs used in our study were synthesized as described in previous papers. CdTe QDs were synthesized by refluxing routes as described in detail in Ref. [29]. In brief, sodium hydrogen telluride (NaHTe) was produced in an aqueous solution by the reaction of sodium borohydride ($NaBH_4$) with tellurium powder at a molar ratio of 2:1 at first. Later, freshly synthesized oxygen-free NaHTe solution was mixed with nitrogen-saturated 10 mM $CdCl_2$ aqueous solution at pH 11.2, with MSA as a stabilizing agent. The molar ratio of Cd^{2+}/MSA/HTe^- was charged at 1:1.5:0.2. Then the CdTe precursor solution was subjected to reflux at 100 °C under open-air conditions with condenser attached, and different sizes of CdTe QDs were obtained at different refluxing times. Stable water-compatible (MSA) capped CdTe QDs with emission maximum at about 609 nm were used in the present experiment.

2.4. Human serum samples

The human blood samples were supplied from a local hospital and segregated by centrifugation at 10,000 rpm for 10 min after adding acetonitrile in samples (CH_3CN: serum=1:1). Finally, all supernatant serum samples were subjected to a 1000-fold dilution with 0.01 mol L^{-1} phosphate-buffered saline solutions (PBS, pH 7.4) before analysis, and different concentrations of GSH were added to prepare the spiked samples.

2.5. Analytical procedure

The FIA-CL system used in this work was shown in Fig. 1. In order to achieve good mechanical and thermal stability, the instruments were allowed to run for at least 30 min before the first measurement was made. As shown in Fig. 1, flow lines a–d were connected with standard or sample solution, CdTe QDs solution, Cu(II) solution, NaOH solution, H_2O_2 solution correspondingly. The mixture passed through the flow cell, while the CL signal was recorded by CL analyzer and displayed on the computer screen simultaneously. The CL intensity $\Delta I = I - I_0$ (where I and I_0 represents the CL intensity of CdTe QDs–H_2O_2–Cu(II) system in the presence and absence of GSH, respectively.) was proportional to the concentration of GSH.

3. Results and discussion

3.1. Optimization of experimental parameters

A series of experimental parameters were investigated and optimized for obtain the maximum sensitivity of the CL system. The concentration of CdTe QDs plays an important role in the CL system. The effect of the concentration of CdTe QDs on the CL intensity was studied in the range of 0.0–1.0 × 10^{-3} mol L^{-1}. It can be seen that the CL intensity increased with the increasing of CdTe QDs concentration (Fig. 2). Considering that the low concentration of CdTe QDs would result in instability of the CL signals and narrow detection range, and the high concentration of CdTe QDs would lead to the lower sensitivity. In order to meet the analysis requirements

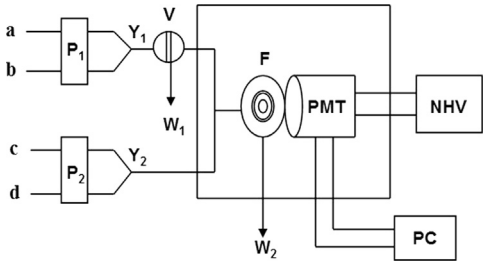

Fig. 1. Schematic diagram of the FIA-CL system. (a) QDs; (b) Cu(II); (c) NaOH; (d) H_2O_2; P_1 and P_2, peristaltic pump; V, eight-way injection valve; Y_1, Y_2, confluence point; F, flow cell; W, waste water; PMT, photomultiplier tube; PC, personal computer; NHV, negative high voltage.

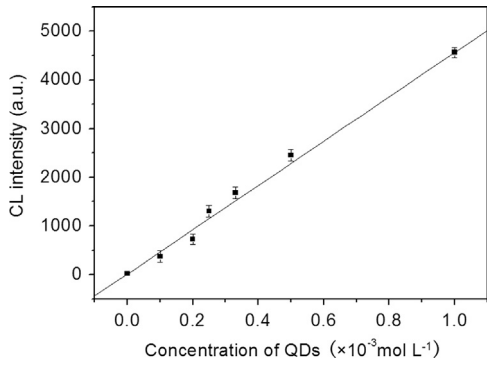

Fig. 2. Effect of concentration of QDs on the CL intensity of the QDs–H_2O_2 CL system. Conditions: H_2O_2, 0.89 mol L^{-1}; flow rate, 3.8 mL/min.

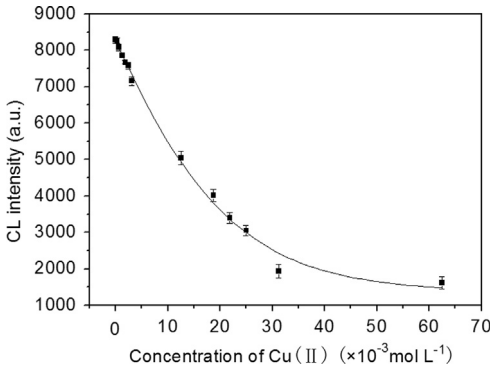

Fig. 3. Effect of concentration of Cu(II) on the CL intensity of the CL system. Conditions: QDs, 2.5×10^{-4} mol L^{-1}; H_2O_2, 0.89 mol L^{-1}; flow rate, 3.8 mL/min; NaOH, 0.4 mol L^{-1}.

and control reagents consumption, the concentration of CdTe QDs solution was selected to be 2.5×10^{-4} mol L^{-1} in this study.

H_2O_2 is an important oxidant in the CL system. The effect of H_2O_2 concentration on the CL intensity was studied in the range of 0.0–1.6 mol L^{-1}. It can be seen in Fig. S1 that the CL intensity increased with the increasing of H_2O_2 concentration until the concentration of H_2O_2 reached 0.89 mol L^{-1}. Then, the CL intensity achieved stability with further increase of the H_2O_2 concentration. Thus, 0.89 mol $L^{-1}H_2O_2$ was selected as the optimum concentration in the further experiments.

In order to obtain high CL intensity, the flow rate was investigated in the range of 0.6–6.0 mL/min. As shown in Fig. S2, the CL intensity increased with the increasing of the flow rate at first. Then, it was closed to reach a platform in the range of 3.8–6.0 mL/min. Under lower flow rate, the CL reaction has already partially occurred before the reagent solution entered into flow cell. Hence, 3.8 mL/min was chosen for the flow rate.

The CL intensity of CdTe QDs–H_2O_2 system could be greatly decreased by addition of Cu(II) as inhibitor. The effect of Cu(II) concentration was studied in the range of 3.1×10^{-8}-3.1×10^{-5} mol L^{-1}. The results are shown in Fig. 3. It can be seen that the CL intensity decreased with the increase of Cu(II) concentration and reached a stable value at the concentation of 3.1×10^{-5} mol L^{-1}. The results showed that the electron almost completely transfer from $1S_e$ quantum-confined orbital of CdTe QDs to Cu(II) when the concentration of Cu(II) reached 3.1×10^{-5} mol L^{-1}. Consequently, 3.1×10^{-5} mol L^{-1} Cu(II) was used in the following experiments.

The effect of the NaOH concentration on the determination of GSH was studied in the range of 0.0–0.8 mol L^{-1}. As shown in Fig. S3, the CL intensity increased with the increase of NaOH concentration and reached a maximum at 0.2 mol L^{-1}. The CL system contained more OH^- with the increasing concentration of NaOH, when CdTe QDs surface was more negatively charged, the electron transfer from the conduction band of CdTe QDs was easier [22]. Furthermore, superoxide radical is more stable in high concentration of NaOH solution, which benefit to the CL emission and leading to the increasing CL intensity. When the concentration of NaOH was higher than 0.2 mol L^{-1}, too many anions loaded on the surface of CdTe QDs, which would suppress the approach of negatively charged superoxide radical to the CdTe QDs surface. In addition, superoxide radical had difficulty in donating one electron to the $1S_e$ quantum-confined orbital of CdTe QDs to form electron-injected CdTe QDs, and then the CL emission was totally quenched [30]. Therefore, the maximum CL intensity was observed at 0.2 mol L^{-1} NaOH, which was chosen in the further work.

3.2. GSH detection

Under the optimum conditions, we studied the CL recovery capability of GSH on CdTe QDs–H_2O_2–Cu(II) system. From Fig. 4, it can be seen that the CL intensity of CdTe QDs–H_2O_2–Cu(II) system increased with the increasing concentration of GSH, The CL intensity was linearly proportional to the GSH concentration in the range of 2.0×10^{-9} mol L^{-1}–6.5×10^{-7} mol L^{-1}. The regression equation was $I=2781.28+126.41C(\times 10^{-6}$ mol $L^{-1})$ (I being the CL intensity and C being the GSH concentration), with the regression coefficient of 0.9993. The detection limit (S/N=3) for GSH was 1.5×10^{-9} mol L^{-1}. As shown in Fig. 4, the chemiluminiscence time trace for the GSH calibration curve shows the good repeatability and stability of the measured signal.

In order to study the analytical application possibility of the present CL method, the possible interferences were investigated when the concentration of GSH was 1.0×10^{-7} mol L^{-1}. The results were listed in Table 1. It can be seen that no interference has been found when the concentration ratios of the foreign substances to GSH were more than 1000 fold for K^+, Na^+ and glucose; 100 fold for Ca^{2+}, Mg^{2+}, Zn^{2+}, Ba^{2+}, Fe^{2+} and ascorbic acid; 500 fold for glycine; 10 fold for arginine, cysteine, Ag^+, Cd^{2+} and Hg^+ respectively. Due to the concentrations of Ag^+, Cd^{2+} and Hg^+ in human serum are usually very low that we could ignore the interference effects of them on the determination of GSH. The above results demonstrated that the present CL method has high selectivity for the determination of GSH.

3.3. Real samples detection

In order to evaluate the feasibility of the proposed method in real samples detection, it was applied to the detection of GSH

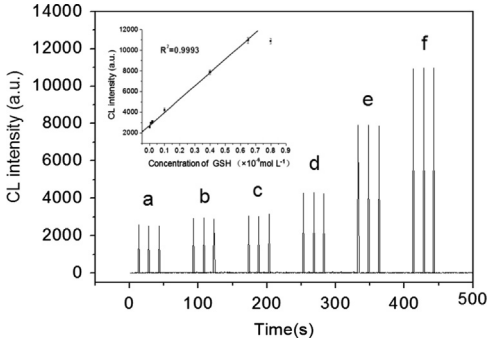

Fig. 4. The chemiluminiscence time trace for the GSH calibration curve. (a) 2×10^{-9} mol L^{-1}GSH; (b) 1×10^{-8} mol L^{-1}GSH; (c) 2×10^{-8} mol L^{-1}GSH; (d) 1×10^{-7} mol L^{-1}GSH; (e) 4×10^{-7} mol L^{-1}GSH; (f) 6.5×10^{-7} mol L^{-1}GSH. Conditions: QDs, 2.5×10^{-4} mol L^{-1}; H_2O_2, 0.89 mol L^{-1}; flow rate, 3.8 mL/min; Cu(II), 3.1×10^{-5} mol L^{-1}; NaOH, 0.2 mol L^{-1}. Inset shows the linear relationship between the CL intensity and the concentration of GSH.

in human serums. The results obtained by standard addition method were shown in Table 2, and the accuracy of the proposed method was evaluated by determining the average recoveries of GSH in real samples. It can be seen that the recoveries were between 100 and 105%. Other methods for the determination of GSH are compared with the present CL method, and were shown in Table 3 [31–35]. From Table 3, it could be seen that the sensitivity of this proposed CL method for the determination of GSH was better than most of the reported methods. As shown in Fig. S4, the chemiluminiscence time trace for the spiked samples also shown the good repeatability and stability of the measured signal.

3.4. Mechanism of the CL system

As shown in Fig. 8A, there was no CL emission when CdTe QDs and H_2O_2 were passed through the flow cell separately, while the CL intensity of CdTe QDs–H_2O_2–NaOH system was extremely strong. This can be explained that hydrogen peroxide could directly oxidize CdTe QDs to produce strong CL emission under basic condition. Superoxide radical and hydroxide radical were generated in the reaction of NaOH and H_2O_2 in oxygenic solution. Superoxide radical can easily donate one electron to the $1S_e$ quantum-confined orbital of CdTe QDs to form electron-injected CdTe QDs. At the same time, hydroxide radical ions injected a hole in the $1S_h$ quantum-confined orbital of CdTe QDs to form hole-injected CdTe QDs. An electron-transfer reaction between electron-injected CdTe QDs and hole-injected CdTe QDs for direct electron-hole recombination produced the excited CdTe QDs. The CL emission appeared when (QDs)* returned to the ground-state [36,37]. The CL intensity significantly depended on the rates of generation and extinction of (QDs)*. As shown in Fig. 5A, when Cu (II) was added to the CdTe QDs–H_2O_2 CL system, the CL intensity was inhibited greatly. When GSH was added to the CdTe QDs–H_2O_2–Cu(II) system, the CL intensity was enhanced. In this work, to get an idea about the inhibitory effect of Cu(II) and the recovery effect of GSH on the CdTe QDs–H_2O_2 CL reaction. The emission spectra of CdTe QDs–H_2O_2, CdTe QDs–H_2O_2–Cu(II), CdTe QDs–H_2O_2–Cu(II)–GSH were examined by fluorospectrophotometer. The results showed that the maximum CL emission intensity appeared at 576 nm for the above three systems (Fig. 5B), which indicated that the luminophor in the CL system was CdTe QDs.

The CL mechanism of the present reaction was expressed in the following steps. First, the superoxide radical and hydroxyl radical were produced under NaOH and H_2O_2 existed condition [shown in reactions (1) and (2)]. Then, the superoxide radical injected an electron into the $1S_e$ quantum-confined orbital of CdTe QDs as shown in reaction (3). Meanwhile, the hydroxyl radical injected a hole into the $1S_h$ quantum-confined orbital of CdTe QDs as shown in reaction (4). Finally, the CL emission was produced by the exciton reaction (5)

$$O_2 + 4OH^- \rightarrow 2O_2^- + 2H_2O \quad (1)$$

$$O_2^- + H_2O_2 \rightarrow 2OH\bullet + O_2 \quad (2)$$

$$O_2^- + QDs \rightarrow QDs(e^-_{1se}) + O_2 \quad (3)$$

$$OH\bullet + QDs \rightarrow QDs(h^+_{1sh}) + OH^- \quad (4)$$

$$QDs(e^-_{1se}) + QDs(h^+_{1sh}) \rightarrow (QDs)^* \rightarrow QDs + h\nu \quad (5)$$

When Cu(II) were added to the CdTe QDs–H_2O_2 CL system, the electron transferred from $1S_e$ quantum-confined orbital of CdTe QDs to Cu(II) during the CL process [38–40]. Therefore the exciton reaction (5) was inhibited [shown in reactions (6)]. And the CL signals of CdTe QDs and H_2O_2 reaction was decreased. When GSH was added to the CdTe QDs–H_2O_2–Cu(II) CL system. Cu(II) prefer to combine with GSH during the CL process [reactions (7) and (8)]. The CdTe QDs based exciton reaction was recoveried. From reactions (6) to (8), the inhibitory and recovery effect of CL could be observed. Furthermore, the CL intensity is dependent on the concentration of GSH. Based on this mechanism, CdTe QDs–Cu (II)–GSH CL system has been successfully employed to detect GSH in human serum with satisfactory results

$$QDs(e^-_{1se}) + Cu(II) \rightarrow QDs{-}Cu \quad (6)$$

$$GSH + QDs{-}Cu \rightarrow GSH{-}Cu + QDs \quad (7)$$

$$(QDs)^* \rightarrow QDs + h\nu \quad (8)$$

Table 1
Effects of metal ions and biomolecules on the detection of GSH.

Metal ions	Tolerable concentration ratios	I/I_0	Biomolecules	Tolerable concentration ratios	I/I_0
K^+	1000	0.982	Ascorbic acid	100	0.996
Na^+	1000	1.01	Glucose	1000	1.00
Ca^{2+}	100	0.981	Glycine	500	1.00
Mg^{2+}	100	0.995	Cysteine	10	0.989
Ba^{2+}	100	0.995	Arginine	10	1.00
Zn^{2+}	100	0.982			
Fe^{2+}	100	0.991			
Ag^+	10	0.961			
Cd^{2+}	10	1.01			
Hg^+	10	0.981			

I_0 represents the relative CL intensity of QDs–H_2O_2–Cu(II) system in the presence of GSH and I represents the relative CL intensity of QDs–H_2O_2–Cu(II)–GSH system in the presence of metal ions or biomolecules.

Table 2
Determination of GSH in human serum samples.

Samples	Founded (nmol L^{-1})	Added (nmol L^{-1})	Total founded (nmol L^{-1})	Recovery (%)	RSD (%, $n=3$)
1	4.44	2.00	6.46	101	2.90
2	7.43	10.00	17.8	103	1.10
3	2.43	4.00	8.86	100	2.43
4	6.98	2.00	9.09	105	1.65
5	3.58	3.00	6.68	104	1.28
6	4.11	4.00	8.19	102	2.14

Table 3
Comparison of different methods for the determination of GSH.

Methods	Systems	Samples	Linear range	Detection limit	d-Signal/d-concentration	References
Fluorescence	Alizarin Red S/copper ion ensemble	Human serum	5–300 nmol L^{-1}	2.3 nmol L^{-1}	80	[31]
Fluorescence	Fluorescent probe	Human Plasma	0.1–1.2 μmol L^{-1}	–	46.81	[32]
HPLC	N-ethylmaleimide	Human blood	0.1–2 mmol L^{-1}	0.05 mmol L^{-1}	–	[33]
Fluorescence	QDs-Hg(II)	Hela cell	0.6–20 μmol L^{-1}	0.1 μmol L^{-1}	–	[34]
Fluorescent probe	Containing a Se–N bond	Living cells	3–120 nmol L^{-1}	1.4 nmol L^{-1}	77.01	[35]
Flow injection chemiluminesce	QDs-Cu(II)	Human serum	2–650 nmol L^{-1}	1.5 nmol L^{-1}	126.41	This work

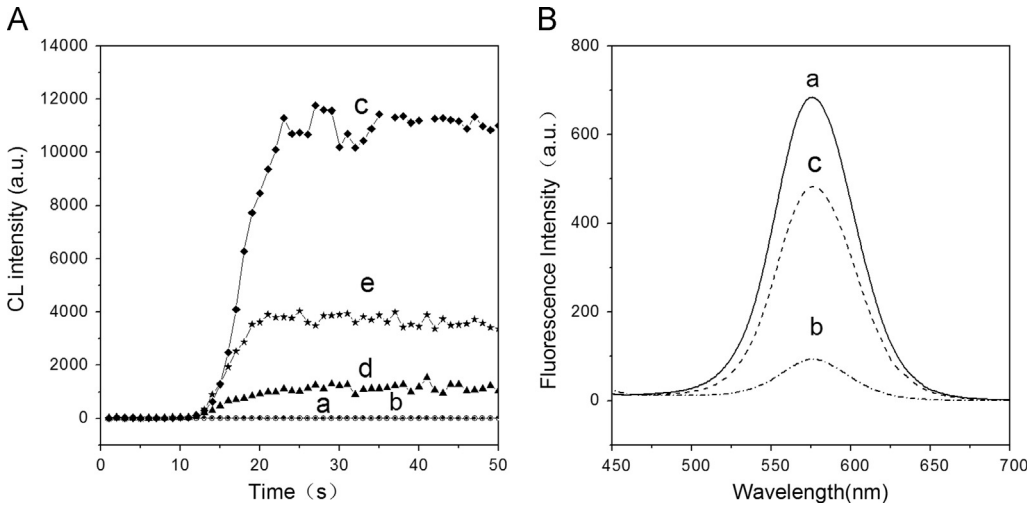

Fig. 5. (A) Kinetic curves of the CL system. (a) QDs; (b) H_2O_2; (c) QDs–H_2O_2; (d) QDs–H_2O_2–Cu(II); (e) QDs–H_2O_2–Cu(II)–GSH. (B) Fluorescence emission spectrum. (a) QDs–H_2O_2; (b) QDs–H_2O_2–Cu(II); (c) QDs–H_2O_2–Cu(II)–GSH. Conditions: QDs, 2.5×10^{-4} mol L^{-1}; H_2O_2, 0.89 mol L^{-1}; flow rate, 3.8 mL/min; NaOH, 0.2 mol L^{-1}; Cu(II), 3.1×10^{-5} mol L^{-1}; GSH, 1.0×10^{-7} mol L^{-1}.

4. Conclusions

Based on the quenching and recovery effect of Cu(II) and biothiols on the CdTe QDs based CL system, a novel "turn off–on" CL system for the detection of biothiols has been established in this paper. The CL reaction conditions including the concentrations of CdTe QDs, H_2O_2 and NaOH and flow rates have been optimized, and the mechanism of CdTe QDs based CL system was discussed. The method has been successfully applied to the determination of biothiols in human serum samples. The proposed CL system is simple, fast and convenient in the real analysis application. And this work is significant for the development of the nanomaterials CL in biothiols analysis.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.05.010.

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