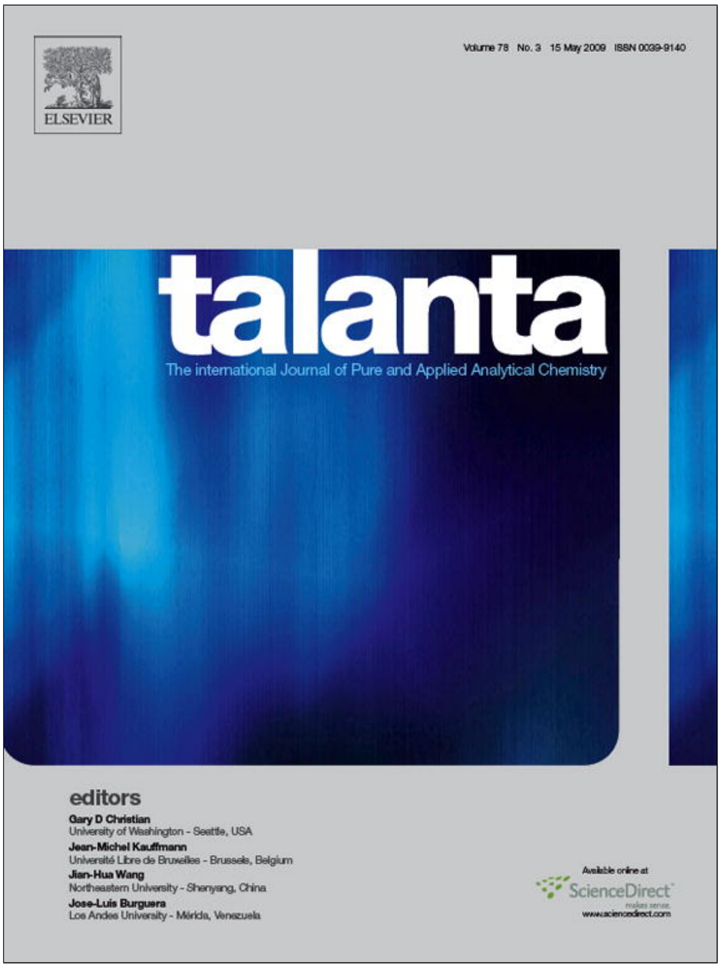

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Determination of nitrite based on its quenching effect on anodic electrochemiluminescence of CdSe quantum dots

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ABSTRACT

A novel method for electrochemiluminescent (ECL) detection of nitrite was proposed based on its quenching effect on anodic ECL emission of CdSe quantum dots (QDs). The ECL emission could be greatly enhanced by sulfite and dissolved oxygen in a neutral system and occurred at a relatively low potential in comparison with traditional anodic ECL emitter, leading to high sensitivity and good selectivity. The quenching mechanism followed an "electrochemical oxidation inhibition" process, which was completely different from those of some analytes on the ECL emission of QDs. The coincidence of photoluminescence and ECL spectra of the QDs indicated that the ECL emission resulted from the redox process of QDs core and the sulfite acted as a coreactant. The nitrite quenched ECL emission could be analyzed according to the treatment of Stern–Volmer equation with a linear range from 1 μM to 0.5 mM for detection of nitrite. This work presented a new efficient ECL methodology for quencher-related detection.

1. Introduction

Electrochemiluminescence (ECL) technique has been widely used in many fields [1]. The ECL processes of quantum dots (QDs) have attracted considerable interest due to their reproducible [2] and size-dependent properties [3]. These QDs provide potential alternatives for developing new ECL emitters [2,4–11] and preparing new ECL sensors. Recently, some biosensors based on the cathodic ECL emission of II–VI QDs film immobilized on electrode surfaces have been developed using O_2 [4], H_2O_2 [5–7] and $S_2O_8{}^{2-}$ [8,9] as coreactants. The anodic ECL emission produced from water soluble CdTe QDs in presence of dissolved oxygen [10] and amines [11] has also been reported. These ECL biosensors and anodic ECL emission have been used for detection of several analytes, including oxidase substrates [4], hydrogen peroxide [5], thiol compounds [7], proteins [8,9], dopamine [10] and amines [11]. Compared with cathodic ECL emission of QDs, the anodic ECL emission is relatively weak, and only the anodic ECL emission of CdTe QDs was studied [10,11]. Thus the overwhelming majority of anodic ECL luminophores are still focused on $Ru(bpy)_3{}^{2+}$ derivatives in presence of reductive coreactant [12], and most of the reported anodic ECL systems undergo high-excited potentials, which was unfavorable for analytical application.

Our previous work observed the sensitizing effect of sulfite on the anodic ECL emission of CdTe QDs, which led to a relatively low anodic potential for sensitive detection of tyrosine by combining an enzymatic cycle with a quenching effect based on an energy transfer process [13]. Another work also observed the sensitizing effect of sulfite on the anodic ECL emission of CdSe QDs, producing a novel method for dopamine detection with the same quenching mechanism [14]. This work further studied the sensitizing mechanism and observed a quenching effect of nitrite on the sensitized ECL emission. The quenching process followed a completely different mechanism from dopamine, producing a method for ECL detection of nitrite.

Nitrite detection is of great importance in environmental and public health fields, and various methods based on spectrophotometric [15], chromatographic [16], chemiluminescent [17] and electrochemical techniques [18,19] have been developed for determination of nitrite ions. Some of them are complex, time consuming and require tedious sample pretreatment [18]. Although enzyme electrode can be utilized for nitrite detection [20], this technique has not been widely accepted yet due to its expensive cost and instability under operation conditions [21]. Thus, development of new analytical methods for selective and sensitive detection of nitrite by combining with multifarious techniques is still an attracted object. Herein, the strong anodic ECL emission of CdSe QDs in the presence of sulfite could be produced at a relatively low potential. The quenching effect of nitrite on the ECL emission via an "electrochemical oxidation inhibition" mechanism made it be able to be detected at relatively low potential, which inhibited greatly the interference from the oxidation of some electroactive compounds. To our best knowledge, this is the first ECL method for nitrite detection, thus extending the application field of QDs.

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2. Experiments

2.1. Chemicals

Thioglycolic acid (TGA), Se powder, $CdCl_2 \cdot 2.5H_2O$, sodium sulfite and $NaBH_4$ were of analytical grade. Tris(hydroxymethyl)-aminomethane hydrochloride and Tris(hydroxymethyl) aminomethane (Tris, reagent grade) were purchased from Sigma. Doubly distilled water and 0.1 M HCl–Tris buffer containing 0.1 M KNO_3 was used throughout the work. 1 M HCl and 0.1 M Tris were used to adjust solution pH.

2.2. Preparation of water-soluble CdSe QDs

The water-soluble CdSe QDs were prepared using TGA as stablizing agent according to the previous report [7]. The Se source was obtained from the reaction between Se powder and $NaBH_4$ in air-free doubly distilled water. After refluxed at 100 °C for 4 h the product was purified by ultrafiltration at 6000 rpm for 5 min, and the upper phase was decanted and kept at 4 °C for ECL detection. The obtained QDs solution could be stable for 3 months, and its concentration and the size of CdSe QDs were detected with UV–vis absorption spectroscopy.

2.3. Apparatus and analytical procedures

The electrochemical and ECL measurements were carried out on a MPI-A multifunctional analytical system (Xi'an Remex Analytical Instrument Ltd. Co.) at room temperature with a configuration consisting of an indium tin oxide (ITO) working electrode, a platinum counter electrode, and an Ag/AgCl (saturated KCl solution) reference electrode. The observation window for ECL was placed in front of the photomultiplier tube biased at −1000 V.

ECL spectrum was obtained by collecting the ECL data at +0.927 V during cyclic potential sweep with six pieces of filters at 490, 535, 550, 580, 600 and 630 nm. Their transparent efficiency was around 88%. UV–vis absorption spectrum was recorded with UV-3600 UV–vis-NIR photospectrometer (Shimadzu Co., Japan). Photoluminescence (PL) spectrum was obtained on a Jasco FP 820 fluorometer (Jasco Co.).

3. Results and discussion

3.1. Characterization of TGA-modified CdSe QDs

The PL spectrum of 20-times diluted QDs solution showed a maximum intensity at 560 nm, and the UV–vis absorption peak occurred at 424 nm (Fig. 1). From the absorption peak the size and concentration of CdSe QDs product were estimated to be 1.75 nm and 5.76 μM [22].

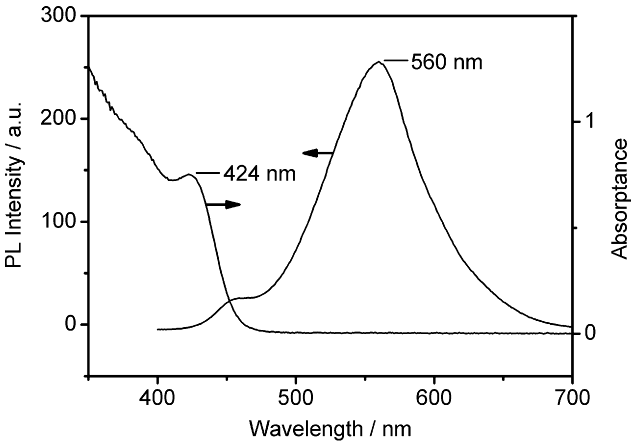

Fig. 1. UV–vis absorption and PL spectra of as-prepared TGA-modified CdSe QDs solution with 20-times dilution.

3.2. Electrochemical and ECL behaviors of CdSe QDs solution

In air-saturated pH 7.0 HCl–Tris buffer, the cyclic voltammogram of CdSe QDs at an ITO electrode showed an irreversible anodic peak at +0.919 V, while no any ECL emission could be observed (curve a, Fig. 2). After 0.2 mM sulfite was added into the solution, an intensive ECL emission peaked at +0.927 V occurred, accompanied with an increase of anodic peak current (curve b, Fig. 2). Sulfite itself could be oxidized at potentials more than +0.461 V, but did not show any ECL signal at the same concentration (curve c, Fig. 2). A control experiment showed that HCl–Tris buffer did not produce any observable response (curve d, Fig. 2). Although the anodic wave of CdSe QDs in presence of sulfite could be approximately considered as the sum of the anodic waves of CdSe QDs and sulfite, the anodic ECL emission of this system was obviously produced from the interaction of CdSe QDs and sulfite. The ECL intensity of this system was weaker than those of the most popular $Ru(bpy)_3{}^{2+/3+}$/TPrA systems, however, it was strong enough for analytical purpose, and the ECL emission peak potential of ~+0.9 V was about 200 mV lower than that of $Ru(bpy)_3{}^{2+/3+}$/TPrA system, indicating a superiority for analysis.

To confirm what was the light emitter of this intensive ECL signal, excited QDs* or $SO_2{}^*$, the ECL and PL spectra were carried out using the same detection solution. The ECL and PL spectra of CdSe QDs showed the peaks at 560.0 and 560.7 nm (Fig. 3), respectively, which were far from the emission range of $SO_2{}^*$ light-emitter from 300 to 450 nm [23]. The good coincidence in peak position of ECL with that of PL emission confirmed that the light-emitter of the enhanced ECL system was the same as that of PL procedure, i.e. excited QDs*. The same peak position and different emission processes of ECL from PL of QDs with core/shell structure indicated the surface of CdSe

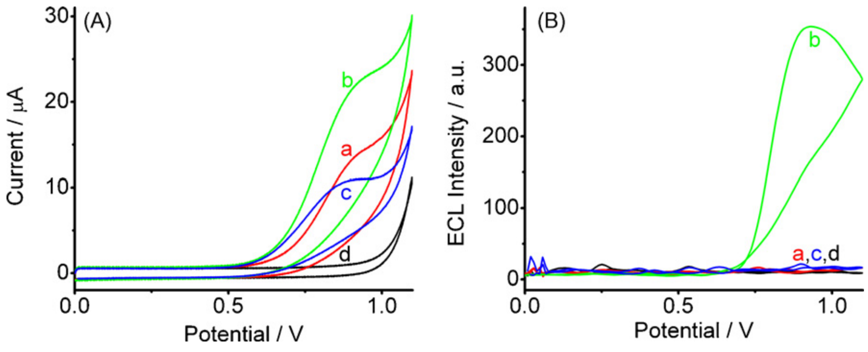

Fig. 2. Cyclic voltammograms (A) and ECL curves (B) of (a) 5.76 μM QDs, (b) 5.76 μM QDs + 0.2 mM sulfite, (c) 0.2 mM sulfite and (d) control in pH 7.0 air-saturated HCl–Tris buffer at ITO electrode. Scan rate: 100 mV s^{-1}.

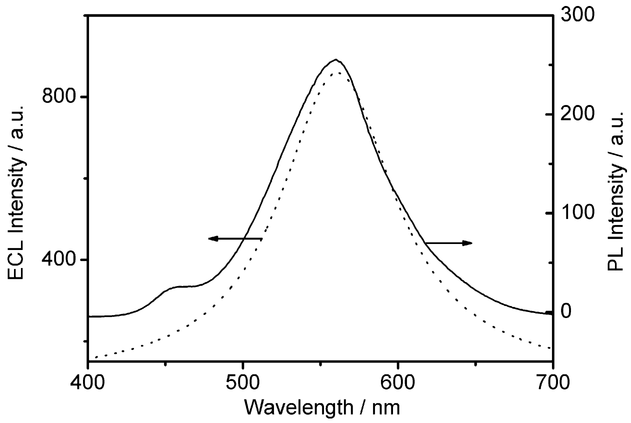

Fig. 3. Coreactant enhanced anodic ECL (dot line) and PL (solid line) spectra of TGA-modified CdSe QDs solution in presence of 0.6 mM sulfite. ECL data were collected at +0.927 V.

QDs was efficiently passivated [24]. During the anodic scanning sulfite as a coreactant could be oxidized to produce $^{\bullet}SO_3{}^-$ species that then reacted with dissolved oxygen to form $^{\bullet}O_2{}^-$ radical [13], which was a key species to produce electron injected QDs (QDs(e$^-$)) [10]. The formed QDs(e$^-$) collided with hole injected QDs (QDs(h$^+$)), electro-oxidization product of QDs, to produce the excited QDs*. When QDs* returned to the ground state the ECL emission occurred.

3.3. Effects of solution components on ECL emission

The anodic ECL intensity of CdSe QDs depended on the concentration of coreactant and pH value of HCl–Tris buffer. The maximum intensity occurred in pH 7.0 solution containing 0.6 mM sulfite, which was used in following work.

The components of buffer also affected the ECL emission of TGA-modified CdSe QDs. In air-saturated pH 7.0 phosphate buffer containing 0.6 mM sulfite and 5.76 μM QDs the ECL emission peak occurred at +1.16 V, 230 mV more positive than that in HCl–Tris buffer (Fig. 4) and close to that of CdTe QDs in PBS [10]. This was possibly due to the absorption of phosphate on ITO surface, which made the electrode surface more negatively charged [25]. So a lower potential for anodic ECL emission was easily achieved by using HCl–Tris buffer, leading to an ECL analytical protocol at a relatively low-anodic potential.

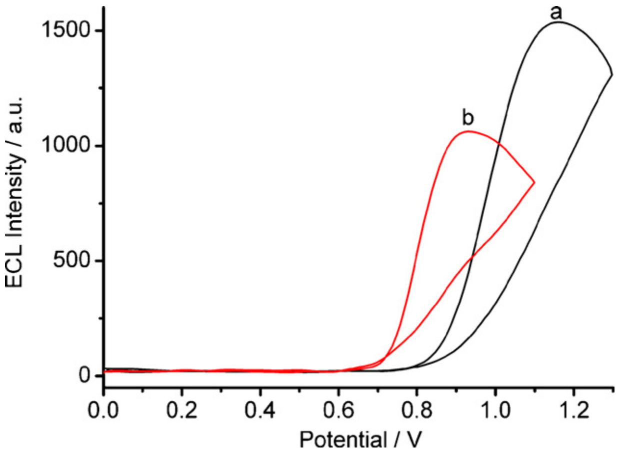

Fig. 4. Cyclic ECL curves of 5.76 μM TGA-modified CdSe QDs in air-saturated pH 7.0 phosphate (a) and HCl–Tris (b) buffer in presence of 0.6 mM sulfite at ITO electrode. Scan rate: 100 mV s^{-1}.

3.4. Nitrite detection based on its quenching effect on the anodic ECL emission

Some compounds such as nitrite have been proved to be electroactive ECL quenchers in Ru(bpy)$_3{}^{2+}$ system via an "electrochemical oxidation inhibition" mechanism [26]. The presence of these compounds produces larger IR drop and makes the practical potential E_w less than the applied potential E [26]:

$$E_w = E - IR = E - (I_q + I_{emitter})R \tag{1}$$

where I_q and $I_{emitter}$ are the oxidation currents of the quencher and light-emitter. The lower E_w decreases the oxidation of light-emitter, leading to weaker ECL emission. Upon addition of nitrite into the detection solution, the ECL intensity at +0.927 V was attenuated (Fig. 5A), indicating an efficient quenching effect.

Based on the quenching effect on the anodic ECL emission of CdSe QDs, a rapid analytical method for nitrite detection could be developed. Under the optimal conditions, the plot of the ratio of initial ECL intensity I_0 to the intensity I at a given nitrite concentration *vs.* nitrite concentration ranging from 1 μM to 0.5 mM showed a linear relation (R = 0.997, n = 11, Fig. 5B). The limit of detection (LOD) was 0.1 μM at a quenching degree of 3 times noise. The relative standard deviation for five measurements at the nitrite concentration of 0 and 10 μM was 2.20% and 3.84%, respectively, indicating accept-

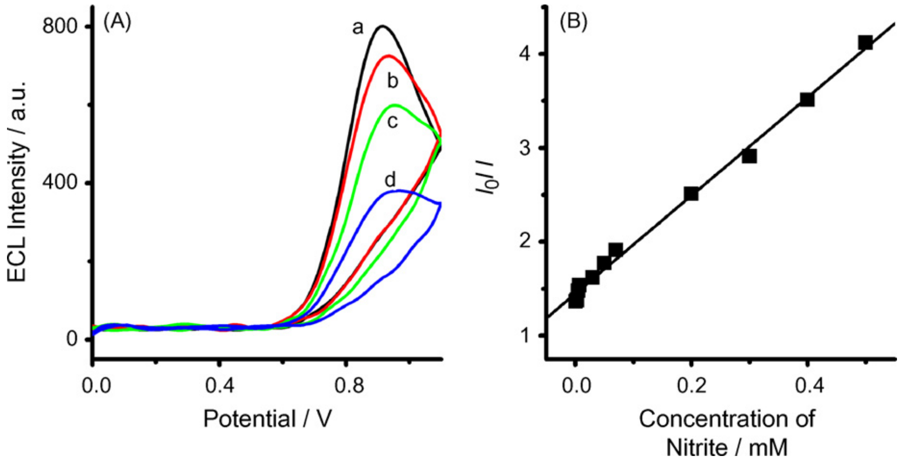

Fig. 5. Cyclic ECL curves of TGA-modified CdSe QDs in air-saturated pH 7.0 solution containing 0.6 mM sulfite and 0 (a), 0.1 (b), 1 (c) and 100 (d) μM nitrite at ITO electrode at 100 mV s^{-1} (A) and calibration plot for nitrite detection (B).

Table 1
Comparison of the proposed method with some electrochemical sensors.

Technique	Linear range (μmol/L)	LOD (μmol/L)	Precision	Ref.
Toluidine blue-based sensor	2.94–2110	1.76	1.8%	[18]
CdS QDs-based sensor	0.30–182	0.08	4.2%	[27]
Oxovanadium(IV)-4-methyl salophen-based sensor	3.90–4050	0.61	–	[19]
Nile blue-based sensor	0.5–100	0.1	3.53%	[28]
ECL technique	1–500	0.1	3.84%	This work

able reproducibility of the ECL system and this analytical method for nitrite detection.

The interference experiments were carried out under the same conditions as the nitrite detection. Common anions of NO_3^-, Cl^- and cations of Na^+, K^+ and NH_4^+ at the concentration of 20 mM did not cause any interference to the determination of 20 μM nitrite, indicating an acceptable selectivity for this method. However, 0.1 mM I^- caused a great interference, where ECL intensity decreased by five times. According to the "electrochemical oxidation inhibition", all coexisted electroactive species with lower oxidation potential than light emitter [26] would act as interferents in nitrite detection when the *IR* drop could not be neglected. Thus, the detection of practical samples should be carried out by combining with effective separation techniques.

As shown in Table 1, the proposed method had lower LOD and wider linear range than several reported electrochemical sensors. Moreover, the detection medium tended to be neutral, which would greatly benefit its application in bioanalytical filed.

4. Conclusions

The mechanism of coreactant sulfite induced anodic ECL of CdSe QDs is studied in neutral system. Not only the concentration of sulfite and solution pH but also the components of buffer play important roles for obtaining the sensitive ECL emission at a relatively low potential. The ECL emitter has been proved to be the excited QDs, which is produced from the collision of hole and electron injected QDs. The electron injected QDs result from the reaction of QDs with O_2^- formed in presence of oxidized sulfite and dissolved oxygen. The anodic ECL emission can be quenched by electroactive quenchers such as nitrite via an "electrochemical oxidation inhibition" mechanism, producing the first ECL method for detection of nitrite, with rapid speed, acceptable sensitivity and selectivity. By combined with effective separation techniques, this method could be further developed for detection of nitrite in complex practical samples. The CdSe QDs provide a potential alternative for developing new ECL emitters. This work significantly extends the application of QDs for constructing new rapid analytical methods of electroactive quenchers.

Acknowledgements

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