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Carbon nanospheres enhanced electrochemiluminescence of CdS quantum dots for biosensing of hypoxanthine

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ABSTRACT

This work developed a novel method to greatly enhance the electrochemiluminescence (ECL) of CdS quantum dots (QDs). The ECL amplification was achieved by the assembly of QDs on poly (diallyldimethylammonium chloride)-functionalized carbon nanospheres (PFCNSs), and successfully employed for sensitive ECL biosensing of oxidase substrates. The carbon nanospheres were prepared by a "green" method, and the high loading of QDs on carbon nanospheres led to a 4-times increased ECL intensity with dissolved O_2 as the coreactant. Using xanthine oxidase (XOD) as a model, an ECL biosensor was fabricated by immobilizing the enzyme on the mixing membrane of PFCNSs and QDs. The ECL biosensor showed a fast response to hypoxanthine with a linear concentration range from 2.5×10^{-8} to 1.4×10^{-5} M. The limit of detection was 5 nM at a signal-to-noise ratio of 3. The assay results of hypoxanthine in fish samples were in a good agreement with the reference values by amperometric technique. This facile approach to prepare the PFCNSs/QDs system for ECL biosensing could be of promising application in bioanalysis and electronic device.

1. Introduction

Since the electrogenerated chemiluminescence (ECL) of Si quantum dots (QDs) was reported in 2002 [1], the QDs-based ECL emission has attracted considerable attention. The ECL emission has become a very powerful analytical technique owing to the advantages, such as good stability against photobleaching, simplified optical setup, low cost, and low background noise [2,3]. Multifunctional QDs as ECL signal transduction emitters have been used for construction of versatile biosensing platforms [4]. For example, a QDs-based ECL biosensor for detection of lysozyme has been developed by associating aptamer-lysozyme bioaffinity complexes at an Au electrode [5]. A competitive QDs-based ECL immunoassay coupled with enzymatic amplification has also been proposed for the detection of human IgG [6]. To efficiently enhance the ECL emission, several carbon nanomaterials such as carbon nanotubes (CNTs) [7–11] and graphene [12–15] have been used to load the QDs for improving the sensitivity and the analytical performance of ECL biosensors, especially in the detection of low-abundant proteins. This work used poly (diallyldimethylammonium chloride)-functionalized carbon nanospheres (PFCNSs) to load QDs and develop a high sensitive ECL biosensing method for detection of oxidase substrates.

Carbon-based nanomaterials have extensively been used in improving the ECL emission of QDs due to their unique chemophysical properties and remarkable conductivity. By facilitating CdTe QDs oxidation and trigger $O_2^{\bullet-}$ generation, graphene oxide can produce enhanced ECL emission for selective sensing of glutathione from thiol-containing compounds [13]. Based on the ECL amplification of N-doped carbon nanotubes the CdSe QDs modified electrode shows a five-times stronger cathodic ECL emission, which is more efficient than the three-times stronger ECL emission of CdSe QDs enhanced by CNTs [8]. Compared with CNTs and graphene, carbon nanospheres possess better tunability of particle size and porous nanostructure. The electrical conductivity and chemical stability of CNSs are also acceptable. Thus such nanomaterials have been applied in the electrochemical assays [16,17]. Here CNSs were used for the first time to amplify the ECL emission of QDs, leading to a sensitive ECL biosensor for the detection of oxidase substrates by using hypoxanthine as a model analyte.

Hypoxanthine is an essential metabolite to degrade adenine nucleotide, which is an indicator for the quality control of meat or fish products in food industries. Therefore, it is significant to develop a quick and effective detection method for the determination of hypoxanthine. Various methods have been proposed for the detection of hypoxanthine concentration, such as chromatography [18,19], capillary electrophoresis [20] and electrochemistry [21,22]. In this work, a simple, sensitive and portable ECL method based on CNSs enhanced ECL emission of CdS QDs with dissolved O_2 as a coreactant and consumption of O_2 via xanthine oxidase (XOD)

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enzymatic reaction was developed for the detection of hypoxanthine by layer-by-layer assembly of PFCNSs and QDs on a glassy carbon electrode. The PFCNSs/CdS QDs system provided an efficient platform for practical application of ECL biosensing.

2. Experimental

2.1. Materials and reagents

Xanthine oxidase (EC 1.1.3.22, from microbial source, 8.1 U/mg), hypoxanthine (≥99%), mercaptopropionic acid (MPA), chitosan (≥85% deacetylation) and poly (diallyldimethylammonium chloride) (PDDA, MW 200,000–350,000, 20% (w/w) aqueous solution) were purchased from Sigma Chemical Co. (MO, U.S.A.). Cadmium chloride ($CdCl_2{\cdot}2.5H_2O$) was purchased from Alfa Aesar Co., Ltd. (China). Thioacetamide was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. D-(+)-Glucose, ascorbic acid and uric acid was purchased from Sinopharm Chemical Reagent Co., Ltd. The N_2-saturated solution was obtained by bubbling highly pure N_2 into air-saturated solution for 30 min. Other reagents were of analytical grade and used as received. The ultrapure water (≥18 MΩ, Milli-Q, Millipore) was used throughout the work.

2.2. Apparatus

The electrochemical and ECL measurements were carried out on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Co., Ltd.) at room temperature with a conventional three-electrode system. The three-electrode system was consisted of a GCE (5 mm in diameter) as working, a platinum wire as counter and an Ag/AgCl (saturated KCl solution) as reference electrodes. Electrochemical impedance spectroscopic (EIS) measurements were carried out on a PGSTAT30/FRA2 system of Autolab Electrochemical Analyzer (Ecochemie, BV, Netherlands) in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 0.1 M KCl as the supporting electrolyte. After coated with Au film to improve the conductivity, the sample films were examined under a Hitachi S-4800 scanning electron microscope (SEM, Hitachi, Japan). Photoluminescence (PL) spectra were performed on a RF-5301 PC fluorometer (Shimadzu Co., Japan). UV–vis spectra were obtained on a Shimadzu UV-3600 UV–vis-NIR photospectrometer (Shimadzu Co., Japan).

2.3. Preparation of PFCNSs

The CNSs were prepared by a "green" method under hydrothermal conditions according to the method reported by Sun and Li [23]. In brief, 4 g glucose was dissolved in 40 mL water and placed in a 50 mL Teflon-sealed autoclave, which was maintained at 180 °C for 6 h. The resulting black product was isolated and purified by centrifugation, redispersed in alcohol, and oven-dried at 80 °C overnight. The as-prepared CNSs were further treated with 3:1 H_2SO_4/HNO_3 (v/v) to induce carboxylic groups on the surface by stirring for 4 h, followed by filtering and washing repeatedly with water until the pH reached 7. Then, 1 mg/mL of carboxylated CNSs was dispersed in a 0.5% PDDA aqueous solution containing 0.5 M NaCl by stirring for 30 min to give a homogenous brown suspension. Residual PDDA was removed by high-speed centrifugation, and the complex was thrice washed with ultrapure water to obtain PFCNSs.

2.4. Preparation of MPA capped CdS QDs

The synthesis of MPA capped CdS QDs was performed according to a method reported previously [9] with slight modification. Briefly, 86 μL MPA was added in 20 mL of 0.02 M $CdCl_2$ solution followed by adjusting the pH to 10 with 1 M NaOH solution. Then, 20 mL of 0.02 M thioacetamide solution was injected into the solution with extensive sonication in air for 30 min. The resulting solution was refluxed at 80 °C for 10 h to obtain MPA capped CdS QDs. The as-prepared QDs colloid was dialyzed exhaustively against deionized water over one week at room temperature for purification. Finally, the product was condensed by ultrafiltration at 10,000 rpm for 10 min, obtained by decanting the upper phase and kept at 4 °C.

2.5. Fabrication of ECL biosensor

The GCE was polished carefully with 1.0 and 0.05 μm alumina slurry (Beuhler), followed by rinsing thoroughly with deionized water. After successive sonication in acetone and deionized water, the electrode was rinsed with deionized water and allowed to dry under N_2 stream. A 15 μL of 1.0 mg/mL PFCNSs dispersion was spread on the pretreated GCE, which was allowed to dry at room temperature. Then 25 μL of 5 μM CdS QDs solution was covered on the modified layer and dried in air. 10 μL of 3 mg/mL XOD and 10 μL of 0.05% chitosan solutions (both prepared in 0.1 M pH 7.4 PBS) were subsequently dropped onto the CdS QDs/PFCNSs modified electrode to obtain the ECL biosensor. The function of chitosan is to protect the enzyme molecules from leakage. The biosensor was stored in 4 °C refrigerator when not in use.

2.6. Determination of hypoxanthine in real sample

A piece of fish meat was homogenized in 15 mL of ultrapure water for 30 min. The solution was filtered through a filter membrane (0.2 μm pore size). Then ultrapure water was added into the filtrate producing a total volume of 50 mL homogenized sample solution. A mixture containing equal volumes of fish extract and 0.1 M PBS was applied for the freshness analysis. After spiking the standard solutions of hypoxanthine into the samples, the concentrations of hypoxanthine and the recovery of the assay were measured from the decrease of ECL emission.

3. Results and discussion

3.1. Morphology and spectroscopic characterization of CNSs and QDs

The SEM image of carboxylated CNSs showed a relatively narrow size distribution with the average diameter of 200–250 nm (Fig. 1A). The surface of CNSs exhibited a homogeneous spiky and porous structure, which should benefit to the loading of QDs on the CNSs and the diffusion of analytes through interconnected millipores, leading to a sensitive biosensing.

The formation of MPA capped CdS QDs was characterized by UV–vis and PL spectra (Fig. 1B). The UV–vis absorption peak occurred at 391 nm (curve a). According to Peng's empirical equations [24], the size and the concentration of QDs were estimated to be 3.1 nm and 5.8 μM, respectively. The PL spectrum of MPA capped CdS QDs showed a relatively narrow emission with a maximal intensity at 560 nm (curve b). The short PL excited wavelength at 400 nm indicated that the PL emission came from the excited state of QD core [25].

3.2. EIS characterization of modified electrodes

The EIS characterization could demonstrate the modification procedure of the electrode (Fig. 2). The diameter of semicircle in the Nyquist plot at high frequency corresponds to the electron-transfer resistance R_{et}, which can be calculated according to the equivalent circuit (inset of Fig. 2). The bare GCE showed a relatively small R_{et} of 230 Ω (curve a). When PFCNSs were assembled onto GCE,

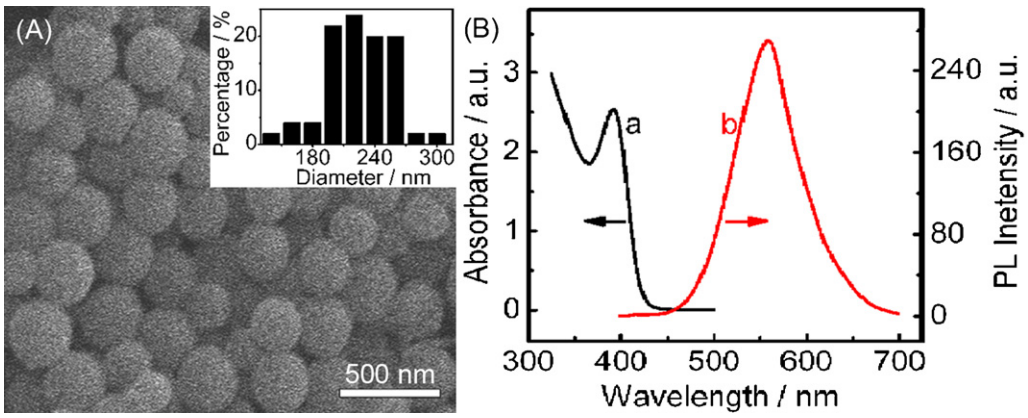

Fig. 1. (A) SEM image of CNSs. Inset: size distribution of 50 CNSs. (B) UV–vis absorption (a) and PL (b, λ_{ex}: 400 nm) spectra of as-prepared MPA capped CdS QDs with dilution by 30 times.

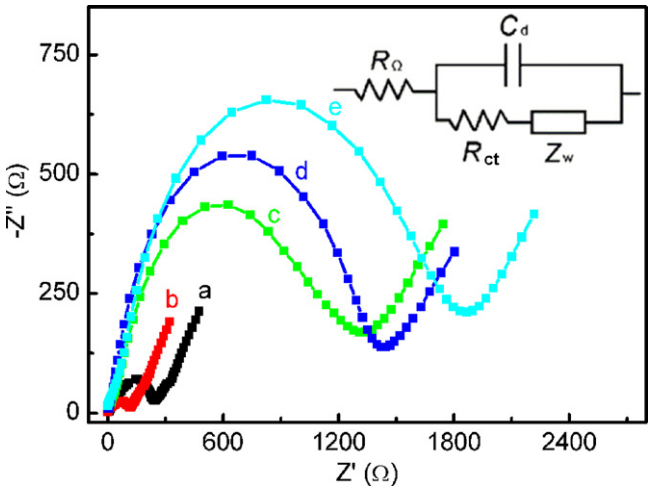

Fig. 2. EIS of bare GCE (a), GCE/PFCNSs (b), GCE/PFCNSs/QDs (c), GCE/PFCNSs/QDs/XOD (d) and GCE/PFCNSs/QDs/XOD/chitosan (e) in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$. The impedance spectra were recorded in the frequency range of 10^{-1}–10^5 Hz with the amplitude of 5 mV. Inset is the equivalent circuit applied to fit impedance measurements. R_Ω, the resistance of the electrolyte solution; C_d, the double-layer capacitance; Z_w, Warburg impedance; R_{et}, the electron transfer resistance.

R_{et} reduced to 115 Ω (curve b), implying that CNSs were excellent conductor for accelerating the electron transfer. The QDs film with its intrinsic semiconducting property decreased the charge transfer rate, thus exhibited a relatively large R_{et} value of 1300 Ω (curve c). Further, both the immobilization of XOD as the non-conductive protein and chitosan as a kind of insulating polymer hindered the electron-transfer kinetics of the redox probe at the electrode surface, resulting in the increased R_{et} with values of 1443 and 1865 Ω as the stepwise modification of XOD and chitosan, respectively (curves d and e), which also verified the immobilization of XOD and chitosan on CdS QDs/PFCNSs modified GCE.

3.3. ECL behavior of GCE/PFCNSs/QDs

As shown in inset of Fig. 3A, the reduction current of GCE/PFCNSs/QDs at −1.0 V was about 3 times higher than that of the intrinsic QDs modified GCE, indicating that the condensed state and low electron-transfer resistance of CNSs could efficiently facilitate the generation of electron-injected QDs, which led to much larger ECL emission of QDs in air-saturated 0.1 M pH 8.0 PBS (Fig. 3A). Obviously, PFCNSs greatly enhanced the ECL emission of QDs at −1.15 V from 5610 a.u. at GCE/QDs to 21,120 a.u. at GCE/PFCNSs/QDs due to the higher loading of QDs on larger surface area and the better conductivity of the modified film. The strong and stable ECL of GCE/PFCNSs/QDs with dissolved O_2 as endogenous coreactant could be desirable to construct the enzyme-based ECL biosensing platform.

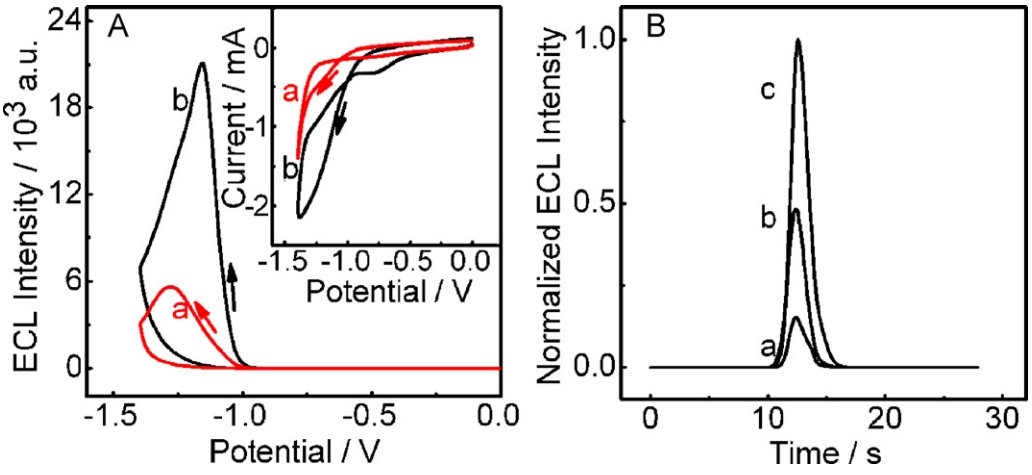

Fig. 3. (A) ECL–potential curves of GCE/QDs (a) and GCE/PFCNSs/QDs (b) in air-saturated 0.1 M pH 8.0 PBS. (B) ECL–time curves of GCE/QDs in air-saturated (a), 320 μM H_2O_2 in N_2-saturated (b) and O_2-saturated (c) 0.1 M pH 8.0 PBS. Inset in A: corresponding cyclic voltammograms. Scan rate: 0.1 V/s. PMT bias: 600 V.

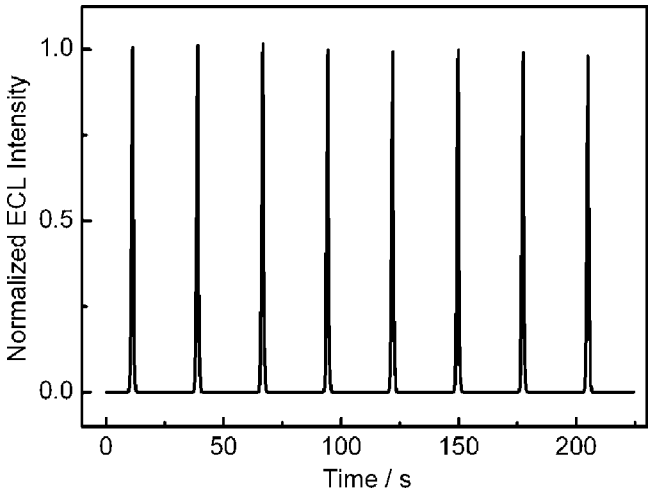

Fig. 4. Continuous cyclic ECL curves of GCE/PFCNSs/QDs/XOD/chitosan in air-saturated 0.1 M pH 8.0 PBS. Scan rate: 0.1 V/s. PMT bias: 600 V.

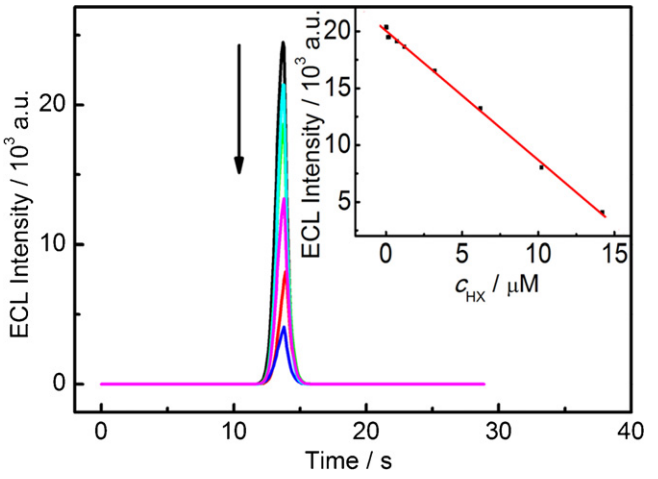

Fig. 5. ECL responses of GCE/PFCNSs/QDs/XOD/chitosan to different concentrations of hypoxathine in air-saturated 0.1 M pH 8.0 PBS. Inset: linear calibration curve for hypoxathine detection. Scan rate: 0.1 V/s. PMT bias: 600 V.

Although O_2 and H_2O_2 can act as the ECL coreactants, the efficiencies of the two coreactants are different since O_2 can capture more electrons from the electrochemically reduced QDs than H_2O_2 and their reaction rates with the reduced QDs also differ [26]. Fig. 3B shows the discrepancy of ECL signals enhanced by O_2 and H_2O_2 by immersing the QDs film in the detection solution (curve a) or deaerated detection solution containing 320 μM H_2O_2 (curve b), which approximates the saturated concentration of dissolved O_2 at room temperature under standard atmospheric pressure. The ECL peak intensity of the GCE/QDs in O_2-saturated PBS (curve c) was twice that in N_2-saturated PBS containing 320 μM H_2O_2 solution. Thus the dissolved O_2 is a more efficient coreactant to enhance the ECL emission of QDs. According to the previous report [26], the possible ECL route of CdS QDs (R) using O_2 as the coreactant was proposed as follows:

$$\text{CdS QDs} + \text{ne}^- \rightarrow \text{nR}^{\bullet -} \quad (1)$$

$$O_2 + 2R^{\bullet -} + 2H^+ \rightarrow 2R^* + H_2O_2 \quad (2)$$

$$H_2O_2 + 2R^{\bullet -} \rightarrow 2R^* + 2OH^- \quad (3)$$

$$R^* \rightarrow R + h\nu \quad (4)$$

3.4. ECL-based enzyme biosensor

The ECL emission from GCE/PFCNSs/QDs/XOD/chitosan in air-saturated 0.1 M pH 8.0 PBS over consecutive cyclic potential scans from 0 to −1.4 V was shown in Fig. 4. The strong and stable ECL signal indicated that the enzyme biosensor was suitable for ECL detection. Upon addition of hypoxanthine, the enzymatic reaction consumed dissolved O_2 (Eq. (5)), which resulted in a quenching effect on the ECL emission and a method for ECL biosensing of hypoxanthine.

$$\text{Hypoxanthine} + O_2 \rightarrow \text{Uric acid} + H_2O_2 \quad (5)$$

The decrease of ECL signal was proportional to the concentration of hypoxanthine in a range of 2.5×10^{-8} to 1.4×10^{-5} M ($R^2 = 0.992$, $n = 9$) with a detection limit of 5 nM at a signal-to-noise ratio of 3 (Fig. 5). The limit of detection was much lower than that of 3 μM detected at room temperature with a luminol-based ECL biosensor [27].

After store in refrigerator at 4 °C for 7 days, the ECL responses of the biosensor to 20 nM, 200 nM and 5 μM of hypoxanthine at 25 °C did not show obvious decrease, demonstrating good storage stability. The intra-assay precision of the biosensor was evaluated by detecting the analyte with the same concentration (5 μM) for three replicate measurements, and the inter-assay precision was estimated by determining 5 μM analytes with four biosensors. They showed the relative standard deviations of 4.1% and of 8.8%, respectively, indicating acceptable detection and fabrication reproducibility of the ECL biosensor. Common interferents of hypoxanthine detection, such as ascorbic acid and glucose, did not affect the cathodic ECL emission of CdS QDs even at the concentrations of ten-fold hypoxanthine, which suggested that the sensor had good anti-interference ability.

3.5. Analysis of fish samples

To evaluate the analytical reliability and application potential of the proposed method, the biosensor was used to detect hypoxanthine accumulated in fish continuously after death, which directly reflected the freshness of fish. Due to the unavailable experimental

Table 1
Hypoxanthine concentration in crucian samples tested by the proposed method.

Sample	Store condition	Added (μM)	Found (μM)	Recovery (%)
1	Freshly killed	0	3.35	
		5.0	8.07	94.4
2	4 h After killed	0	4.06	
		5.0	8.73	93.4
3	8 h After killed	0	5.90	
		5.0	10.74	96.8
4	12 h After killed	0	9.00	
		5.0	13.62	92.4

Table 2
Hypoxanthine concentration in holds thorn samples tested by the proposed method.

Sample	Store condition	Added (μM)	Found (μM)	Recovery (%)
1	Freshly killed	0	2.14	
		5.0	6.89	95.0
2	4 h After killed	0	3.65	
		5.0	8.22	91.4
3	8 h After killed	0	5.42	
		5.0	9.95	90.6
4	12 h After killed	0	8.47	
		5.0	12.98	90.2

conditions for performing a traditional determination, the recovery experiment was used to identify the reliability of the proposed method. After fish was killed and stored at room temperature for 0, 4 and 12 h, the concentration of hypoxanthine increased from 3.35 to 4.06 and 9.00 μM, respectively (Table 1), which was consistent with 5.55, 5.83, 9.05 μM via amperometric technique [22]. The recoveries for spiking the standard solutions of 5.0 μM were listed in Table 2. All of recoveries were from 96.8% to 90.2%, indicating acceptable accuracy.

4. Conclusions

A carbon nanospheres enhanced QDs platform has been successfully designed to construct an ECL enzyme biosensor for facile, rapid and sensitive assay of hypoxanthine. The biosensor is based on the consumption of dissolved O_2 as coreactant in oxidase catalyzed oxidation reaction. The uniform porous structure of CNSs results in high loading of QDs and quick mass transfer of oxidase substrate through interconnected millpores, which produces a stable ECL emission and sensitive ECL response to the substrate. Thus an ECL method for detection of the oxidase substrate is developed using XOD and hypoxanthine as the target. The proposed method shows a wide linear range with a low detection limit. The biosensor shows good reproducibility and acceptable stability, and has successfully been applied in the detection of hypoxanthine in fish samples.

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