Electrochemiluminescence Detection of *o*-aminophenol Based on PAMAM/CdSe QD/*β*-CD Modified Electrode

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Summary: A stable and sensitive electrochemiluminescence (ECL) detection protocol of *o*-aminophenol was developed, which was based on CdSe quantum dots (QDs) and the cavity structure of *β*-cyclodextrin. In this experiment, the working electrode was prepared by PAMAM and CdSe QD and *β*-cyclodextrin using layer self-assembly method under the infrared lamp. Under the optimum conditions, a good linear relationship with the concentration of *o*-aminophenol from 1.0×10^{-7} mol·L^{-1} to 8.0×10^{-6} mol·L^{-1} and the detection limits as low as 2.3×10^{-8} mol·L^{-1} (3σ) were achieved. Good selectivity and repeatability are advantages of the sensor which is feasible.

Keywords: PAMAM; CdSe QD; *β*-cyclodextrin; Electrochemiluminescence (ECL); *o*-aminophenol

Introduction

Nowadays, the increasing pollution of the environment with organic contaminant has created an urgent public health concern in living systems. *O*-aminophenol, as a kind of organic and dye intermediates, is widely used in the voltammetry enzyme-linked immune analysis system [1] and production of acid, sulphur yellow brown, fluorescent whitening agent EBF etc. It is considered to be one of the world's environmental priority controls of toxic organic pollutants owing to its high toxicity in the environment and the ecosystem. Thus, the development of a highly sensitive, facile, and practical sensor for *o*-aminophenol remains a challenge. Currently, some methods have been already reported in the literature for the determination of *o*-aminophenol such as the high performance liquid chromatography [2], electrochemical analysis [3], amperometric sensor [4], etc.

Electrochemiluminescence (ECL), or electrogenerated chemiluminescence, is the process in which reactants are generated during the high-energy electron-transfer reactions occurring at the surface of the working electrodes without the need of an external light source [5]. It has been one of the most attractive luminescent methods, because of its low background and controllable procedure [6] and wide range of analytes, low cost and ease of reaction control by applying electrode potential. In addition to advantages of low cost and easy preparation, ECL detection with QDs has received considerable attention in bioanalytical and analytical chemistry [7, 8].

PAMAM is a new kind of three-dimensional tree structure of macromolecules, which attracted widespread attention due to its own characteristics. Because it has many advantages, such as properties stability, symmetrical structure, molecular size and shape control, the internal functional cavity and the external functional groups with a higher density and so on, it can be used to prepared metal nanocomposites by confined space.

Cyclodextrin is composed of cyclic oligosaccharides having a cavity-like structure, with the most commonly available having six, seven, or eight glucopyranose units (*α*-, *β*-, *γ*-cyclodextrin, respectively) [9]. Organics (or their moiety) may be including by the cyclodextrin because it has an axial open cavity of hydrophobic character [10, 11]. Cyclodextrin can be used for detecting organic compounds as a electron transfer vector on account of the unique cavity that commonly increases the solubility of organics and enhance bioavailability of organic pollutants [12, 13]. Additionally, cyclodextrin, as a kind of natural substance, is considered nontoxic and biodegradable without secondary environmental concerns [14, 15].

Herein, we present a novel highly sensitive and selective enhanced ECL method to detect *o*-aminophenol on the basis of doughnut-shaped structure of the cyclodextrin, using CdSe quantum dots as a luminophores. The detection sensitivity can be significantly improved to 2.3×10^{-8} mol·L^{-1} (3σ) by using this method. This method which has good

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selectivity and reproducibility can be used to detect aminophenol actual samples.

Experimental

Material

NaBH$_4$ and Se and CdCl$_2$ H$_2$O were purchased from Sanhe Chemical Reagent Co. (Yantai, China). Cysteamine (AET) was purchased from Aladdin Reagent Company and used as received. Dendrimer (PAMAM) was synthesized by Chenyuan Molecular New Materials Company in Weihai. The *β*-cyclodextrin was abtained from Aladdin Reagent Company. The *o*-aminophenol was bought from Sanhe Chemical Reagent Co. in Yantai. The *o*-aminophenol was prepared by doubly distilled water and stored in dark as soon as it was used. Phosphate buffer solutions (0.1 mol·L^{-1}) with various pH values were prepared by mixing stock standard solutions of K$_2$HPO$_4$ and KH$_2$PO$_4$. The base solution contained 0.1 mol·L^{-1} phosphate buffer solution and 0.05 mol·L^{-1} K$_2$S$_2$O$_8$ solution. All of the other reagents were of analytical grade and doubly distilled water was used throughout all of the experiments.

Apparatus

ECL singals were achieved by a MPI-A ECL analyzer (Xi'An Remax Electronic Science & Technology, Xi'An, China) using a three-electrode system and photomultiplier tube voltage was 600 V. An Au electrode (diameter 4 mm) acted as a working electrode. A Pt wire acted as a counter electrode. An Ag/AgCl electrode acted as a reference electrode. Transmission electron microscopy (TEM) images were recorded on a JEM 1200EX transmission electron microscope (JEOL, Japan). Adjustable pipettes and automatic dual water distiller were purchased from Brocade Equipment Co., Ltd. in Zhengzhou.

Synthesis of CdSe Quantum Dots

Synthesis of NaHSe

All glasswares were cleaned with HCl–HNO$_3$ (3:1, v/v) and soaked overnight before they were used. A 7 mL centrifuge tube containing 37 mg NaBH$_4$ and 32 mg Se was ventilated with nitrogen for 30 minutes. 1 mL doubly distilled water was slowly added in 7 mL centrifuge tube. Until the bubble disappeared, 3 mL doubly distilled water was added. Subsequently, the reaction was heated to 80 degrees for 30 minutes and then colorless solution was obtained. The condition of the whole process is no oxygen.

Synthesis of CdSe Quantum Dots

All glasswares were cleaned with HCl–HNO$_3$ (3:1, v/v) and soaked overnight before they were used. The Synthesis of NaHSe was rapidly added to a 60 mL solution containing 0.8 mmol CdCl$_2$. Then the pH of the solution was adjusted to 5.5~6.0 and 0.2182 g cysteamine (AET) was added to the solution. Whereafter, the solution was heated to boiling point with stirring vigorously for 3 h. The condition of the whole process was no oxygen and the color of the reaction turned to yellow. Then the solution was precipitated with excess acetone and the precipitate was isolated by centrifugation and then dried under argon at room temperature. Finally, the powder was redissolved and stored in the dark at 4 °C for used.

Preparation of Au Electrode (the Work Electrode)

In a typical experiment, an Au electrode was polished with 1.0, 0.3, 0.05 μm *α*-Al$_2$O$_3$ powder. After washed ultrasonically with doubly distilled water, the electrode was dried with nitrogen gas to remove any remaining impurities. The prepared Au electrode was used as work electrode for electrochemiluminescence detection.

1.0 mg/mL PAMAM and 40 mg/L CdSe QDs and 8 mg/mL *β*-cyclodextrin were prepared by doubly distilled water before used. Subsequently, 0.02 mg/cm^2 PAMAM solution was dropped onto the surface of Au electrode under the lamp for 1 h. After PAMAM/Au electrode dried, 0.8 mg/cm^2 CdSe QDs solution was dropped onto the surface of PAMAM/Au electrode. After PAMAM/CdSe QDs/Au electrode dried again, 0.16 mg/cm^2 *β*-cyclodextrin solution was dropped PAMAM/CdSe QDs/Au electrode. Then the preparation of Au electrode was accomplished.

Optimization of Experimental Conditions

The ECL efficiencies were strongly influenced by several experimental conditions such as the pH value of the bottom solution, the amount of PAMAM, the amount of quantum dots, and the amount of the *β*-cyclodextrin. All of these experimental conditions were optimized to obtain a more sensitive, accurate *o*-aminophenol sensor.
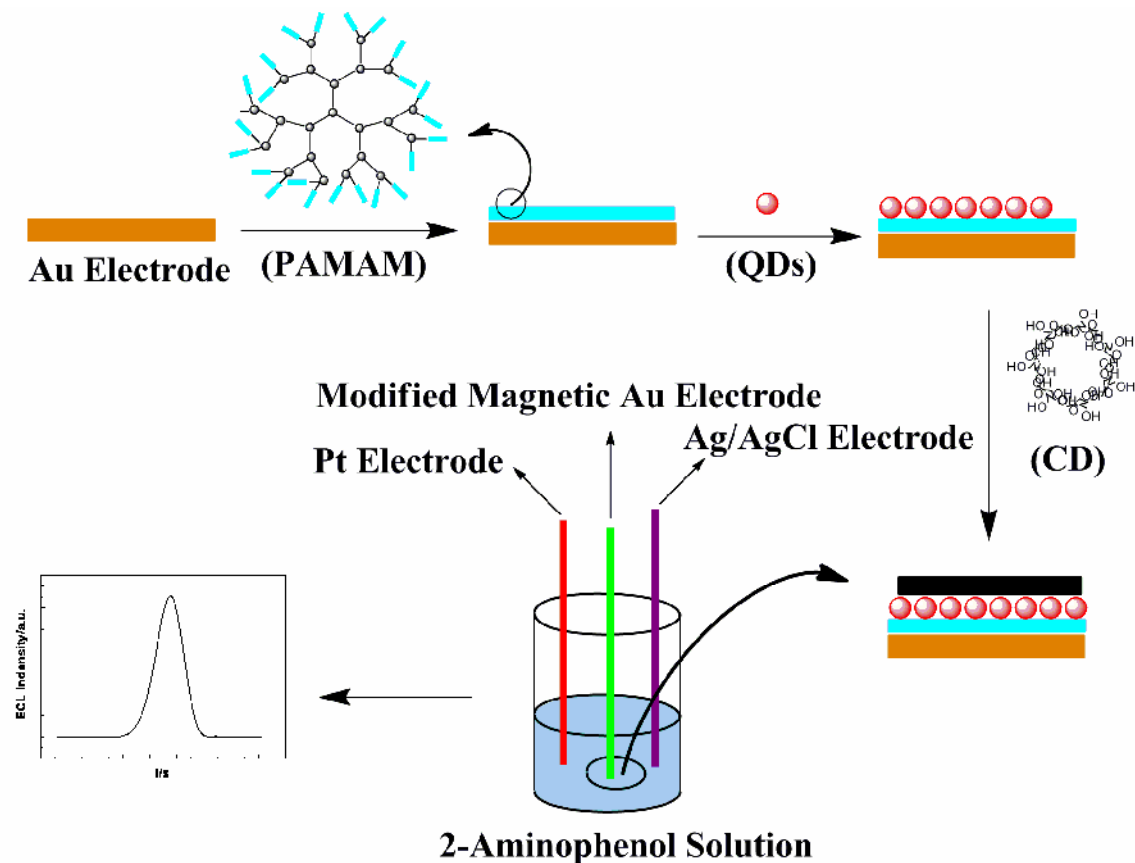
Scheme-1:   The experimental schematic.

ECL Measurements

Finally, the ECL measurement was in contact with pH=7.0 0.1 M base solution and scanned from -1.5 V to 0 V. ECL signals related to the *o*-aminophenol concentrations could be measured.

Results and Discussion

The Principle of the Reaction

The principle of the machine is shown in Scheme 1. As seen in Scheme-1, PAMAM was uniformly distributed on the surface of the Au electrode. When CdSe QDs was dropped onto the surface of PAMAM/Au electrode, CdSe QDs were accommodated by PAMAM due to the internal functional cavity to form PAMAM-CdSe quantum dot composites, which was more stable than CdSe QDs. Subsequently, *o*-aminophenol would be accommodated by *β*-cyclodextrin with its axially open hydrophobic cavity when *β*-cyclodextrin was uniformly distributed on the surface of the

PAMAM-CdSe quantum dot composites. Consequently, ECL signal was triumphantly linked to *o*-aminophenol concentrations. There is electrostatic interaction between positively charged PAMAM and negatively charged quantum dots. Therefore, the ECL signal can be enhanced obvious.

Characterization of the CdSe Quantum Dots

In order to demonstrate that the CdSe QDs was successfully synthesized, TEM method was performed to characterize immanent state of the nanoparticles. Fig. 1 is the TEM imagine of CdSe QDs, it revealed that average diameter of CdSe QDs was about 5 nm.

Characterization of the Modified Au Electrode

Modification processes on Au electrode surface were characterized in 0.1 M PBS (pH 7.0) containing 0.05 M $K_2S_2O_8$. As shown in Fig. 2(a), there were almost no ECL signals when pure

PAMAM was uniformly distributed on the surface of the Au electrode. Compared to pure CdSe quantum dots (b), ECL signals were greatly enhanced when PAMAM-CdSe quantum dot composites(c) were modified Au electrode. There were a large number of amino on the surface of the PAMAM which promoted and accelerated the electron transfer of the ECL reaction. Reduction of $S_2O_8^{2-}$ by NH_2 group of PAMAM on the electrode [1] produced a strong oxidant $SO_4^{-\bullet}$[18], which can then react with the negatively charged $CdSe^{-\bullet}$ by injecting a hole into the highest occupied molecular orbital, producing an excited state (CdSe*) to emit light. ECL signal was significantly reduced when β-cyclodextrin was uniformly distributed on the surface of the PAMAM-CdSe quantum dot composites (shown in Fig. 2(d)).
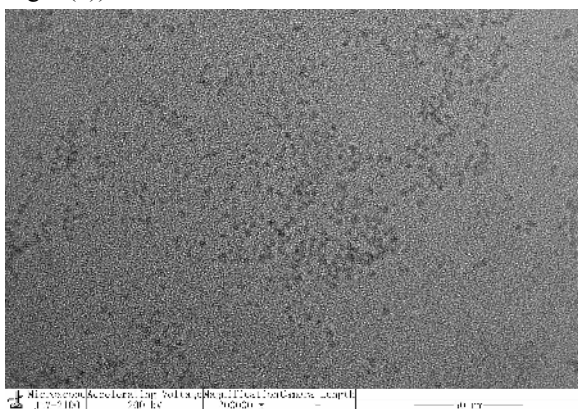

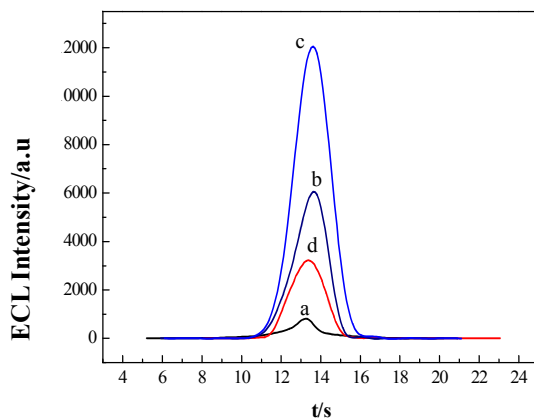Fig. 1:   The TEM imagine of CdSe quantum dots.

Fig. 2:   ECL-time curves for (a) PAMAM (b) Pure CdSe QDs (c) PAMAM/CdSe QDs complexes modified Au electrode (d) modified electrode after dropping β-cyclodextrin in 0.1 M PBS (pH 7.0) containing 0.05 M $K_2S_2O_8$.

Optimum of Experimental Conditions

It was found that the concentration of PAMAM has great effect on the ECL efficiencies during the experimental process. Consequently, influence of the PAMAM's concentration range from 0.1 mg/mL to 2.0 mg/mL was researched. As shown in Fig. 3(A), the ECL intensity increased with the increase of the PAMAM's concentration when it was under 1.0 mg/mL because more and more CdSe QDs can be accommodated by the cavity and the intensity began to reduce when it was above 1.0 mg/mL due to the thick PAMAM layer. At last, 1.0 mg/mL were regarded as the optimum PAMAM's concentration in view of the ECL efficiencies.

The ECL efficiencies were strongly impacted by the concentration of CdSe quantum dots. The concentrations of CdSe quantum dots between 10 mg/mL to 60 mg/mL were selected in order to research the influence on the ECL intensity. As shown in Fig. 3(B), the ECL intensity increased with increasing the concentration of CdSe quantum dots, however when it was above 40 mg/mL the intensity of ECL tend towards stability. Finally 40 mg/mL were regarded as the optimum concentration of CdSe quantum dots.

The β-cyclodextrin's concentration between 4 mg/mL to 12 mg/mL was investigated in order to discover β-cyclodextrin's concentration influencing on ECL efficiencies. As shown in Fig. 3(C), the higher the concentration of the β-cyclodextrin, the higher the ECL signals when it was under 8.0 mg/mL and the intensity tend to decline when it was above 8.0 mg/mL. For this reason, 8.0 mg/mL was admitted as the optimum β-cyclodextrin's concentration in view of the ECL efficiencies.

Signal intensity of the ECL was strongly influenced by the pH value of base solution. So effect of the pH value was studied. The effect of the base solution in the pH range from 6.0 to 8.0 was researched. As shown in Fig. 3(D), the ECL intensity increased with increasing pH when it was fewer than 7.0 and the intensity tends to decline when it was above 7.0. In the end, pH 7.0 was chosen for the optimum pH value in consideration of the ECL efficiencies.
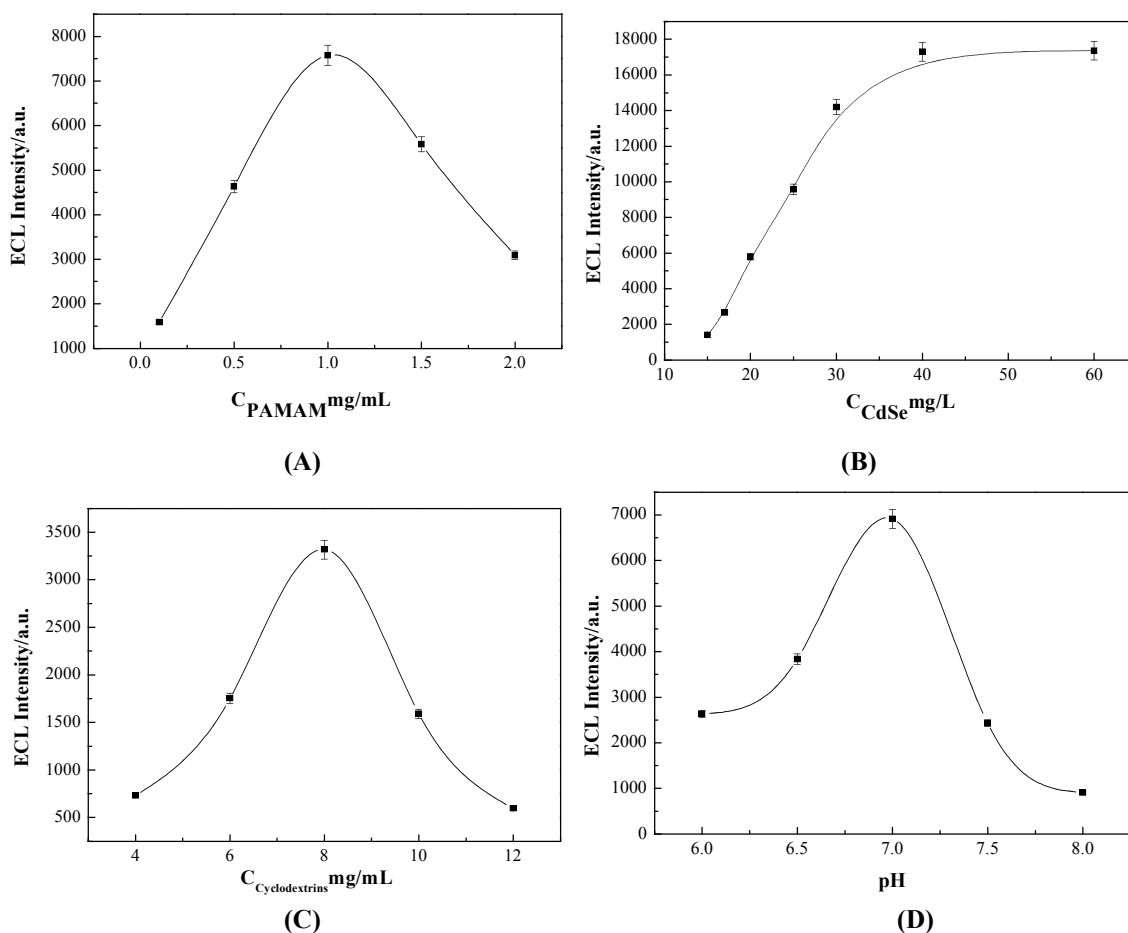
(A)

(B)

(C)

(D)

Fig. 3:   Optimization of experimental conditions: (A) PAMAM concentration; (B) CdSe quantum dots concentration; (C) *β*-cyclodextrin concentration; (D) pH value.

Sensitivity and Feasibility of o-Aminophenol Analysis

As shown in Fig. 4, the higher the concentration of the *o*-aminophenol, the higher the ECL signals. Under the optimum conditions, a good linear relationship between ECL signal and the concentration of *o*-aminophenol from 1.0×10^{-7} M to 8.0×10^{-6} M was achieved (as shown in Fig. 4). The linear regression equation was calculated as Y = 114 C + 5861 with a correlation coefficient as 0.992 (n = 10, Y is the relative ECL signal in a.u. and C is the concentration of *o*-aminophenol 10^{-7} M). A detection limit was 2.3×10^{-8} M for *o*-aminophenol according to 3σ rules.

The Selectivity of o-Aminophenol Analysis

The selectivity of the method has also been investigated by testing interfering substances, including phenol, *m*-aminophenol, *p*-aminophenol, *o*-nitrophenol, Na^+, Cl^- under ten times conditions of *o*-aminophenol. The results demonstrated there was almost no change of the ECL signal intensity in the case of the presence of

phenolic compounds as shown in Fig. 5. It was an excellent selectivity and strong anti-interference ability of the proposed method over phenolic compounds.

The Comparison of Several o-aminophenol Methods

To demonstrate the performance of the developed method, a comparison of the linear range, and detection limit obtained by several methods for *o*-aminophenol detection was made in Table-1. From the table we know that the sensor based on CdSe quantum dots exhibit excellent performance.

The Recovery of o-Aminophenol Methods

Recovery testing was carried out to further demonstrate the validity of the proposed method. The recoveries for the four water samples were in the range of 97.4-104.2% as shown in Table-2, indicating that the developed approach has high accuracy in measuring *o*-aminophenol concentrations in samples.
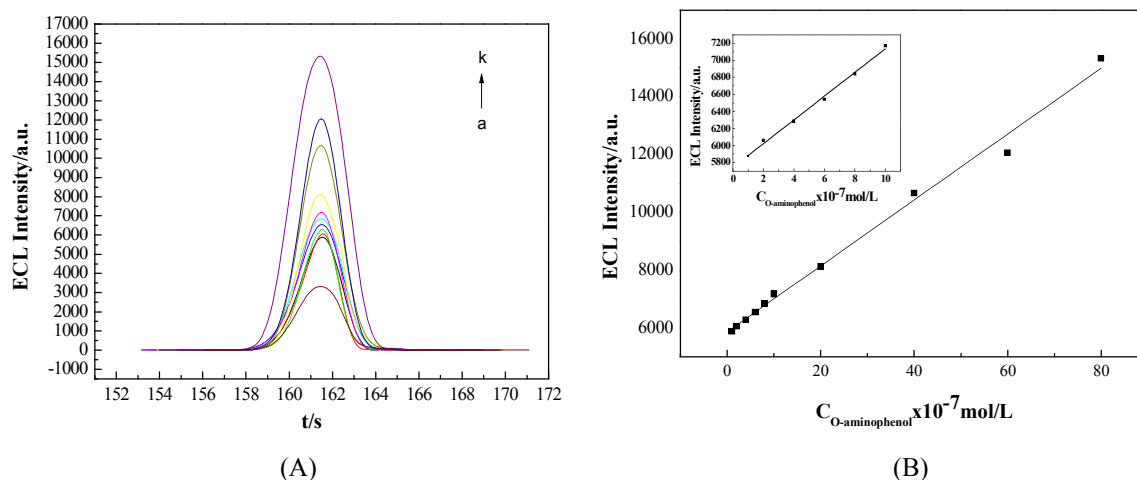
(A)   (B)

Fig. 4:   (A) The ECL signal of different o-aminophenol concentrations (a) 0 M, (b) 1.0×10^{-7} M, (c) 2.0×10^{-7} M, (d) 4.0×10^{-7} M, (e) 6.0×10^{-7} M, (f) 8.0×10^{-7} M, (g) 1.0×10^{-6} M, (h) 2.0×10^{-6} M, (i) 4.0×10^{-6} M, (j) 6.0×10^{-6} M, (k) 8.0×10^{-6} M. (B) The standard curve of *o*-aminophenol detection, which concentration corresponding to the A.
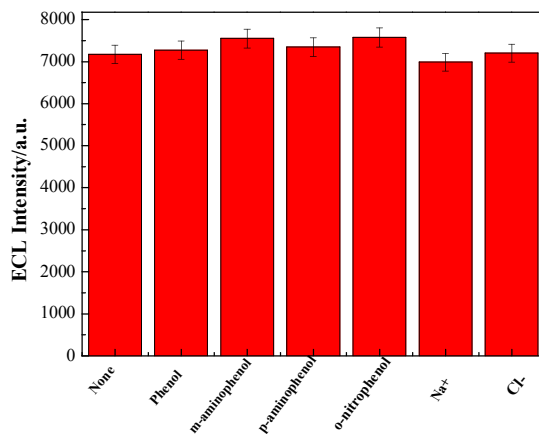

Fig. 5:   The interference detection of *o*-aminophenol.

Table-1:   Performance comparison of the developed sensor for *o*-aminophenol detection with the reported method

Method	Linear range (mol·L^{-1})	Detection limit (mol·L^{-1})
This method	1.0×10^{-7}-8.0×10^{-6}	2.3×10^{-8}
Montmorillonite-modified expanded graphite electrode [4]	1.0×10^{-4}-1.0×10^{-3}	5.0×10^{-7}
DNA–CdSe/TiO₂ electrode[16]	4.0×10^{-7}-2.7×10^{-5}	8.0×10^{-8}
Nano-Zr-ZSM-5/CPE[17]	1.2×10^{-7} -5.0×10^{-4}	3.0×10^{-8}

Table-2:   Determination of *o*-aminophenol in water samples.

Sample	*o*-aminophenol (μM)	Added *o*-aminophenol (μM)	Measured *o*-aminophenol (μM)	Recovery (%)	RSD (%)
1	0.00	5.00	5.21	104.2	3.2
2	0.00	5.00	4.87	97.4	2.2
3	0.00	5.00	5.15	103.0	3.6
4	0.00	5.00	5.09	101.8	2.5

Conclusion

The part was of an electrochemiluminescence (ECL) sensor based on CdSe quantum dots to detect *o*-aminophenol. Using CdSe quantum dots as luminescent materials, *β*-cyclodextrin as identification unit for its cavity structure electrochemiluminescence (ECL) sensor was achieved to detect *o*-aminophenol. Under the optimum conditions, the linear regression equation was calculated as $Y = 114\,C + 5861$ with a correlation coefficient as 0.992 (n = 10; Y is the relative ECL signal in a.u. and C is the concentration of *o*-aminophenol 10^{-7} M). The detection sensitivity can be significantly improved to 2.3×10^{-8} mol·L^{-1} (3σ) by using this method. Good selectivity and repeatability are advantages of the sensor which is feasible.

Acknowledgments

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