Determination of Furosemide Based on CdS QDs-Luc/MoSe2 Electroluminescent Detection Platform

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Keywords: Furosemide, electroluminescence, CdS quantum dots, MoSe2

Abstract: In this paper, a novel composite modified glassy carbon electrode (GCE) based on CdS QDs-Luc / MoSe2 was constructed for the quantitative determination of furosemide by electroluminescence. The CdS QDs-Luc / MoSe2 modified GCE was prepared by dropping 2 μL MoSe2, fluorescein (Luc) and CdS QDs solution to the glassy carbon electrode in turn. The electrochemical response characteristics of the sensor were characterized by electrochemiluminescence (ECL) and cyclic voltammetry (CV), and the preparation and detection conditions were optimized. The results show that the linear range of this method under the optimal conditions is 0.0 – 100 μM, and the linear equations are y= − 5822. 43x + 14475.94 (0.0 - 1.0 μM) and y = − 33. 60x + 8491.46 (1.0 - 100 μM), respectively. The correlation coefficients are R² = 0.99465 and R² = 0.99247, respectively. The new detection platform has the advantages of rapid response, high sensitivity, low detection limit, good stability and low cost, and has good application prospects.

1. Introduction

Furosemide is a kind of drug that promotes the discharge of electrolyte and water from the body, increases urine volume, and eliminates edema. It is mainly used for the treatment of edema caused by various reasons in clinic, and can also be used for the treatment of some non-edema diseases (such as hypertension, renal calculi, and hypercalcemia)[1]. At the 1988 Seoul Olympics, furosemide as a kind of stimulant was banned by the International Olympic Committee[2]. In addition, in recent years, furosemide has also been added to weight loss health products by illegal merchants to increase the weight loss effect. Since the product does not indicate the type and dosage of the added drugs, the users will eat without knowing, which will bring great harm to the body. For example, the long-term or excessive use of furosemide will lead to a decrease in blood volume due to excessive diuresis, resulting in hypotension, shock, renal failure and sudden death. Therefore, the determination of furosemide is of great significance in drug research, doping detection and health products detection. The current quality standard of furosemide tablets is Part II of Chinese Pharmacopoeia 2020, with a specification of 20 mg / tablet. The content is determined by UV spectrophotometry, and there is no content uniformity test item[3-6]. At present, it has been reported that the determination method of furosemide content is mainly HPLC method[7-8], 'Chinese Pharmacopoeia' 2010 edition contains the tablet and injection content determination, respectively,
using UV spectrophotometry and HPLC method\cite{9}, Wu Liping et al.\cite{10} using HPLC-MS method for determination of diuretics in functional foods (including furosemide), but the existing methods generally have the disadvantages of long analysis time, low sensitivity and poor anti-interference effect.

Electrochemiluminescence (ECL) is an analytical technique that uses electrochemical methods as an excitation method to induce chemiluminescence. Due to the advantages of low background, high sensitivity, simple equipment and controllable potential, ECL has become one of the research fields that analytical chemists focus on. Electrochemiluminescence (ECL) is a combination of electrochemistry and chemiluminescence, which endows ECL with many advantages: (1) Compared with photoluminescence, ECL does not require additional excitation light source, so there is no interference such as stray light in signal determination, which makes the analytical sensitivity and selectivity of ECL significantly improved; (2) It has the characteristics of simple experimental device of electrochemical technology and easy automation, intelligence and miniaturization; (3) The time and position of electrochemiluminescence can be regulated by experimental conditions; (4) In many cases, electrochemiluminescence agent can be regenerated after luminescence, and electrochemiluminescence is a non-expendable method.

In order to solve the shortcomings of traditional methods for the determination of furosemide content, such as long determination time, low sensitivity and poor anti-interference effect, a method for the determination of furosemide based on CdS QDs-Luc / MoSe\textsubscript{2} electroluminescence detection platform was constructed in this paper. A novel composite CdS QDs-Luc / MoSe\textsubscript{2} modified glassy carbon electrode was prepared by using the unique photophysical properties of CdS QDs, Luc catalytic oxidation of fluorescein to oxidized fluorescein and the characteristics of bioluminescence and the strong adsorption ability of MoSe\textsubscript{2}. The electrochemical response characteristics of the sensor were studied by electrochemiluminescence (ECL) and cyclic voltammetry (CV), and the preparation and detection conditions were optimized. Through the research on the performance of the new electroluminescence detection platform, it is proved that it has the advantages of rapid response, high sensitivity, low detection limit, good stability and low cost. It can be widely used in the determination of furosemide content in food and medicine, and has important application prospects.

2. Experimental part

2.1. Experimental drugs and instruments

Experimental materials and reagents : sodium molybdate (Na\textsubscript{2}MoO\textsubscript{4}·2H\textsubscript{2}O, 99 %, Sinopharm Chemical Reagent Co., Ltd.); selenium powder (Se, 99%, Chemical reagent Co., Ltd.); ethylenediamine (C\textsubscript{2}H\textsubscript{8}N\textsubscript{2}, 99 %, chemical reagent Co., Ltd.); hydrazine Hydrate (N\textsubscript{2}H\textsubscript{4} · H\textsubscript{2}O, 98 atom %, Chemical reagent Co., Ltd., China Pharmaceutical Group); cadmium chloride (CdCl\textsubscript{2}, 99.99 %, chemical reagent Co., Ltd.); sodium hydroxide (NaOH, ≥ 96 %, Chemical reagent Co., Ltd.); sodium sulfide (Na\textsubscript{2}S, 99 %, chemical reagent Co., Ltd.); sodium hydroxide (NaOH, ≥ 96 %, Chemical reagent Co., Ltd.); sodium sulfide (Na\textsubscript{2}S, 99 %, chemical reagent Co., Ltd.); isopropanol (C\textsubscript{3}H\textsubscript{8}O, 99.8 %, chemical reagent Co., Ltd.); nitric acid (HNO\textsubscript{3}, 69 %, Chemical reagent Co., Ltd.); ethanol (C\textsubscript{2}H\textsubscript{6}O, ≥ 99.5 %, Chemical Reagent Co., Ltd.); fluorescein (Luc, 50 mL, Chemical reagent Co., Ltd.); deionized water (50 mL, China Pharmaceutical Group Chemical Reagent Co., Ltd.); PBS buffer (500 mL, China Pharmaceutical Group Chemicals Co., Ltd.).

electric heating constant temperature blower dryer (intelligent, Gongyi Yuhua Instrument Co., Ltd.); programmable intelligent temperature controller (KBC-I, J-KEM); x-ray diffractometer (D/max-2400 model, excitation light source: CuKα, λ = 0.15406 nm, China Institute of Metrology); transmission electron microscope (TECNAIG2TF20, China Institute of Metrology); dual-beam UV-visible spectrophotometer (TV-1901, Pusan General Instrument Co., Ltd.); fluorescence spectrophotometer (RF-540, General Instrument Co., Ltd.); chemiluminescence immunoassay analyzer (BHP 9507, General Instrument Co., Ltd.); uV-visible spectrophotometer (UV-2450, Metler-Toledo Group).

2.2. Synthesis of MoSe₂

As shown in figure 1, Firstly, 0.4765 g Na₂MoO₄·2H₂O was dissolved in a mixture of 40 mL deionized water and 5 mL ethylenediamine. Next, 0.311 g selenium powder was dispersed into 10 mL hydrazine hydrate and stirred overnight until the solution color became dark red and unchanged. Thirdly, under severe stirring, the hydrazine-selenium hydrate solution was added to 45 mL Na₂MoO₄·2H₂O mixture drop by drop at 90 °C. Next, the mixture was transferred to a 100 mL autoclave with Teflon lining, and hydrothermally treated at 200 °C for 10 h. The solvent was removed by vacuum filtration and washed with deionized water and ethanol. The product was dried overnight at 80 °C. Finally, the prepared samples were treated at 600 °C for 1 h, and then treated at 800 °C for 1 h to obtain MoSe₂ composites with good crystallization. Dispersed into 1 mg / mL solution for future use.

2.3. Synthesis of CdS QDs

As shown in figure 2, N₂ was injected into 50 mL 0.01 M CdCl₂ solution for 30 min, and then the pH value of the solution was adjusted to 11 with 1.0 M NaOH. The solution changed from turbidity to clarification. Subsequently, 5.0 mL 0.1 M Na₂S was injected into the mixture, heated to 110°C and refluxed for 4 h to obtain water-soluble CdS QDs. Finally, CdS QDs solution was precipitated with isopropanol and collected by centrifugation. The precipitate was freeze-dried and prepared into 1 mg / mL solution for subsequent use.
3. Preparation and properties of CdS QDs-Luc / MoSe₂

3.1. Preparation of CdS QDs-Luc / MoSe₂

As shown in figure 3, Before the electrode was modified, the surface of glassy carbon electrode (GCE) was polished on the velvet with 0.05 μm alumina slurry until the mirror was glossy. Then the electrode was ultrasonically treated with nitric acid (v/v = 1 : 1), ethanol and ultrapure water for 5 min. The prepared MoSe₂ solution (1 mg / mL) was absorbed by 2 μL drop and added to the prepared GCE. drying at 60 °C. Luc was prepared into aqueous solution (1 mg / mL), absorbed 2 μL, added to the electrode drop by drop, dried at 60 °C. The prepared CdS QDs solution was also absorbed by 2 μL and added into the electrode to dry under the same conditions. CdS QDs/Luc/MoSe₂ modified GCE was obtained.

3.2. Performance analysis of CdS QDs-Luc/MoSe₂

In order to test the performance of prepared CdS QDs-Luc / MoSe₂, a total of 10 groups of control experiments were carried out. In each group of experiments, with or without addition of thiosulfate (S₂O₈²⁻) as the variable, the corresponding volt-ampere characteristic curve and...
electroluminescence intensity curve were measured and plotted, respectively. The performance of CdS QDs-Luc / MoSe₂ was analyzed by comparison between groups. The results are as follows:

Figure 4: (A) ECL response and CV response of bare GCE + PBS and bare GCE + S₂O₈²⁻ + PBS. (B) ECL response and CV response of MoSe₂ / BC + PBS and MoSe₂ / BC + S₂O₈²⁻ + PBS.

Figure 5: (C) ECL response and CV response of Luc + PBS and Luc + S₂O₈²⁻ + PBS. (D) ECL response and CV response of CdS QDs + PBS and CdS QDs + S₂O₈²⁻ + PBS.

Figure 6: (E) ECL response and CV response of Luc + MoSe₂ / BC + PBS, Luc + MoSe₂ / BC + S₂O₈²⁻ + PBS. (F) ECL and CV responses of CdS QDs + MoSe₂ / BC + PBS, CdS QDs + MoSe₂ / BC + S₂O₈²⁻ + PBS.
Figure 7: (G) ECL and CV responses of CdS QDs + Luc + PBS, CdS QDs + Luc + S$_2$O$_8^{2-}$ + PBS. (H) ECL and CV responses of CdS QDs + Luc + MoSe$_2$/BC + PBS, CdS QDs + Luc + MoSe$_2$/BC + S$_2$O$_8^{2-}$ + PBS. The concentrations of S$_2$O$_8^{2-}$ and PBS (pH 7.0) were 0.1 M and 0.01 M, respectively.

Figure 8: (I) CV response of different modified electrodes in 100 mM K$_2$S$_2$O$_8$ solution (PBS, 0.01 M, pH = 7.0). (J) ECL response of different modified electrodes in 100 mM K$_2$S$_2$O$_8$ solution (PBS, 0.01 M, pH = 7.0).

S$_2$O$_8^{2-}$ as an initiator in the experiment, its specific role is to react with fluorescein, resulting in electron transfer, to achieve the purpose of causing luminescence. PBS buffer acts as an electrolyte to conduct electricity and regulate the PH value of the reaction environment, so that the reaction can proceed smoothly.

It can be seen from Figs. 4 – 7 that the peak values of the experimental results with the addition of S$_2$O$_8^{2-}$ were significantly increased, because the reaction of S$_2$O$_8^{2-}$ in the solution with fluorescein led to the accelerated electron transfer and the luminescence, which significantly increased the conductive effect.

The longitudinal comparison of Figs. 4–7 shows that with the change of experimental materials, from the bare GCE, MoSe$_2$/BC, Luc and CdS QDs at the beginning to the following Luc+MoSe$_2$/BC, CdS QDs+MoSe$_2$/BC and CdS QDs + Luc, and then to the last CdS QDs+Luc+MoSe$_2$/BC, the electrochemiluminescence effect is getting better and better, indicating the unique photoelectric chemical properties of CdS QDs. The novel composite CdS QDs-Luc / MoSe$_2$ modified glassy carbon electrode has the characteristics of high sensitivity and rapid reaction, which is prepared by the oxidation of fluorescein catalyzed by Luc to fluorescein oxide and the emission of biological fluorescence and the super adsorption capacity of MoSe$_2$. In order to compare the performance differences of various materials more intuitively, the experimental results
in Figs. 4 – 7 are plotted on the same coordinate axis, as shown in Fig. 8. It can be seen from the figure that the electrochemiluminescence effect of CdS QDs+Luc is about three times that of MoSe₂ / BC, Luc, CdS QDs, Luc + MoSe₂/BC and CdS QDs+MoSe₂, while the electrochemiluminescence effect of CdS QDs + Luc + MoSe₂ / BC is significantly better than that of CdS QDs+Luc. This further shows that the new material CdS QDs-Luc/MoSe₂/BC has the best electrochemiluminescence effect and high sensitivity and reaction speed.

4. Electrochemiluminescence detection of furosemide by CdS QDs-Luc / MoSe₂

The prepared CdS QDs/Luc/MoSe₂ electrode was placed in PBS buffer containing different concentrations of furosemide for 5 min, and then rinsed with water. ECL detection voltage range is -0.6 - 0 V, scanning speed is 100 mV / s. All measurements were performed at room temperature. The concentration-luminescence intensity diagram drawn from the experimental data is as follows:

![Figure 9: (A) ECL response of CdS QDs-Luc/MoSe₂ under different concentrations of furosemide. (B) The linear relationship between the concentration of furosemide and the ECL intensity of CdS QDs-Luc/MoSe₂.](image)

The experimental results showed that with the increase of furosemide concentration, the ECL intensity of CdS QDs-Luc / MoSe₂ showed two linear relationships with the concentration of 1 μM as the boundary. linear range: 0.0 – 1.0 μM; 1.0 – 100 μM, and the corresponding linear equations are $y = -5822.43x + 14475.94$ ($R^2 = 0.99465$); $y = -33.60x + 8491.46$ ($R^2 = 0.99247$). Therefore, the determined concentration of furosemide can be uniquely determined by the ECL intensity of CdS QDs-Luc/MoSe₂ with high accuracy.

5. Conclusion

In this paper, a novel composite CdS QDs-Luc/ MoSe₂ was proposed and prepared as a functional sensor for electrochemical determination of furosemide. The CdS QDs-Luc / MoSe₂ modified GCE was prepared by dropping 2 μL MoSe₂, Luc and CdS QDs solution to the glassy carbon electrode in turn. The electrochemical response characteristics of the sensor were studied by electrochemiluminescence (ECL) and cyclic voltammetry (CV), and the preparation and detection conditions were optimized. The ECL intensity of CdS QDs-Luc / MoSe₂ showed a good linear relationship ($R^2 = 0.99465$ and $R^2=0.99247$) in the range of 0.0–100μM of furosemide concentration. The ECL intensity decreased sharply in the range of 0.0–1.0μM, and decreased slowly in the range of 1.0–100μM. The sensor has the advantages of rapid response, high sensitivity, low detection limit, good stability and low cost, and has good application prospect.
References


