Ultrasensitive Electrochemiluminescence Sensor for Specific Detection of Rutin Based on CdS Modified Pencil Graphite Electrode

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In this paper, A CdS modified pencil graphite electrode(CdS/PGE) was fabricated using solvothermal route through simple procedure. The CdS/PGE showed unexpectedly electrochemiluminescent behaviour with potassium persulfate ($K_2S_2O_8$) as a coreactant. Based on this principle that ECL was quenched when rutin was present in this system, an ECL sensor for detecting rutin had been developed. The effects of buffer solution and pH were studied in detail. Under the optimum conditions, a possible mechanism of ECL quenching was also clearly discussed. The linear correlation between the quenched ECL intensity and the logarithm of rutin concentration was observed over the range of $4 \times 10^{-9} \sim 8 \times 10^{-7}$ mol/L with the limit of detection (LOD) of 2×10^{-9} mol/L. This proposed method was applied in the analysis of rutin in compound rutin tablets, with recoveries of 98%~105%. The experimental results demonstrated that the ECL sensor showed good stability and reproducibility.

1. INTRODUCTION

Rutin (vitamin P) is a kind of commonly used clinical drug with the physiological functions, including anti-oxidant, anti-viral, anti-tumor, anti-inflammatory, and anti-allergic, in the meantime, it is a stimulant of the immune system[1]. Many methods such as capillary electrophoresis[2], chemiluminescence[3], spectrophotometry[4] and electrochemistry[5] have been devised for the sensitive rutin detection. Although these methods have advantages of sensitivity and accuracy, some of these methods involved complicated operation, high cost with large amounts of toxic organic solvents

used or the separation processes. Therefore, it is essential to develop effective methods for highly sensitive and quick determination of rutin.

In recent years, ECL has become an important and useful determination method in analytical chemistry due to its particular advantages, such as high selectivity, low background signal, wide linear and easy control due to its inherent features, which original from electrochemistry (EC) and chemiluminescence (CL). Since Bard and co-workers first reported ECL properties of Si NCs in organic solvent and proposed the mechanism and the nature of ECL from NCs[6]. Many efforts have been made to some research on the preparation and application of various semiconductor NCs such as CdS[7], CdSe[8], CdTe[9]. Semiconductor nanocrystals (NCs) have been extensively studied because of their unique size-dependent electronic, optical and electrochemical properties[10]. Among these nanocrystals, cadmium sulfide (CdS) semiconductor nanocrystals have attracted the most intensely attention owing to their intrinsic properties, such as good chemical stability, easy preparation and high luminescence efficiency and it can ben used in ECL for determination of H_2O_2[11], dissolved oxygen[12], glucose [13], H_2S[14], et al.

Compared with the traditional electrodes such as gold electrode[15], glass carbon electrode[16], screen-printed electrodes[17], pencil graphite electrodes (PGEs) possess the advantages of high electrochemical reactivity, good mechanical rigidity, disposability, low cost and easy to be prepared [18]. Additionly, it was reported that PGEs offer a renewal surface easily [19]. Pencil graphite electrode not only provided an easy renewable modified electrode surface, but also easy batch production. Therefore, recently many scientists have expanded the application of PGEs on the determination of bio-analytical analytes, such as determination of glucose[20], Vitamin B_{12}[21], NADH[22], adenine[23], anticancer drug[24], et al.

In the present study, a simple and facile approach for the synthesized nanorod CdS by the reaction of thioacetamide and cadmium nitrate in ethylenediamine was proposed. A CdS/PGE is obtained by immobilizing CdS nanorods on PGE. The optimal prepared CdS/PGE shows unexpected eletrochemiluminescence behavior as electrochemical sensor for rutin. A novel method for determination of rutin in compound rutin tablets is proposed. This proposed method is used to detect rutin in compound rutin tablets with satisfying recovery.

2. EXPERIMENTAL

2.1. Reagent and apparatus

Thioacetamide, cadmium nitrate tetrahydrate and $K_2S_2O_8$ were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Rutin was purchased from Ruji (Shanghai, China), The 0.5 wt% nafion solution was prepared by diluting 5 wt% nafion stock solution with absolute ethanol. All reagents and solvents are analytical grade and used without further purification.

The ECL signals were acquired on an MPI-E electrochemiluminescence analyser system (Xi'an Remax Electronic High-Tech Co., Ltd., Xi'an, China). The working voltage of the photo-multiplier tube was set at 600 V for detection. A conventional three-electrode system was used in the ECL experiments with a platinum foil as counter electrode, an Ag/AgCl (3 mol L^{-1} KCl) as reference electrode. The working electrode was CdS modified pencil graphite electrode (CdS/PGE). ECL

measurements were obtained in 0.1 mol L^{-1} phosphate buffer solution (PBS pH 9.0) containing 1×10^{-3} mol L^{-1} $K_2S_2O_8$ in a voltage range from 0 to -1.6 V using cyclic voltammetry at a scanning rate of 0.1 V s^{-1}. The structure and morphology of CdS were charactered by SEM (JSM-7500F) and XRD (DX-2700).

2.2. Synthesis of CdS nanorods

CdS was prepared according to the procedure described in previous literature[13]. In a typical procedure, 0.3080 g $Cd(NO_3)_2 \cdot 4H_2O$ and 0.2250 g NH_2CSNH_2 (molar ratio 1: 3) were added into a teflon-lined stainless steel autoclave. Then ethylenediamine was added to the autoclave up to 80% volume of autoclave. The obtained solution was kept in a furnace at 180 °C for 1.5 h. After cooling to room temperature, the precipitates were centrifuged at 10000 rpm for 3 min, washed with ultra-pure water for 3 times to remove the excessive ions and byproducts. Finally, the precipitates were dried in a vacuum drier at 60 °C for 5 h to obtain the CdS nanorods. The resulting powder was used for the following experiments.

2.3. Fabrication of PGE and CdS/PGE

A 2B pencil graphite with a diameter of 2 mm purchased from a local bookstore was inserted into silent stock tube; electrical contact with the pencil graphite is achieved by soldering a copper wire to the metallic holder of the working electrode and then sealed with epoxy resin. The electrical contact was obtained by the disc at the tip of the PGE. The PGE is polished by 0.3 μm and 0.05 μm Al_2O_3 powder, and finally sonicated in ethanol and double-distilled water in an ultrasonic bath for 3min each. For immobilization of CdS nanorods onto the electrode surface, an optimum amount of CdS nanorods was dispersed in a nafion solution (0.5 wt% in ethanol) and the resulting solution is sonicated for 30 min. 5 μL above CdS nonacrystals suspension was dropped on the surface of bare PGE, and then dried in the air to get the CdS/PGE.

2.4. preparation and analysis of rutin samples

The rutin sample solutions were prepared by grinding two pieces of compound rutin tablets (20 mg/tablets) in a mortar, and then 10 mL ethanol was added into the sample solution. The prepared solution was diluted with distilled water to 100 mL to get the rutin stock solution. The rutin stock solution was stored in refrigerator for further detection.

3. RESULT AND DISCUSSION

3.1. Characterization of the CdS nanorods

SEM was used to characterize the size and shape of CdS nanorods, as can be seen in Fig. 1(A), the obtained samples possessed the rod-like structure with length of 50—150 nm. All the diffraction

peaks could be well distinguished and agreed with the JSPDS Card (80-0006). The majority of the products collected from the CdS nanorods preparation are well crystallized and XRD patterns are in agreement with CdS
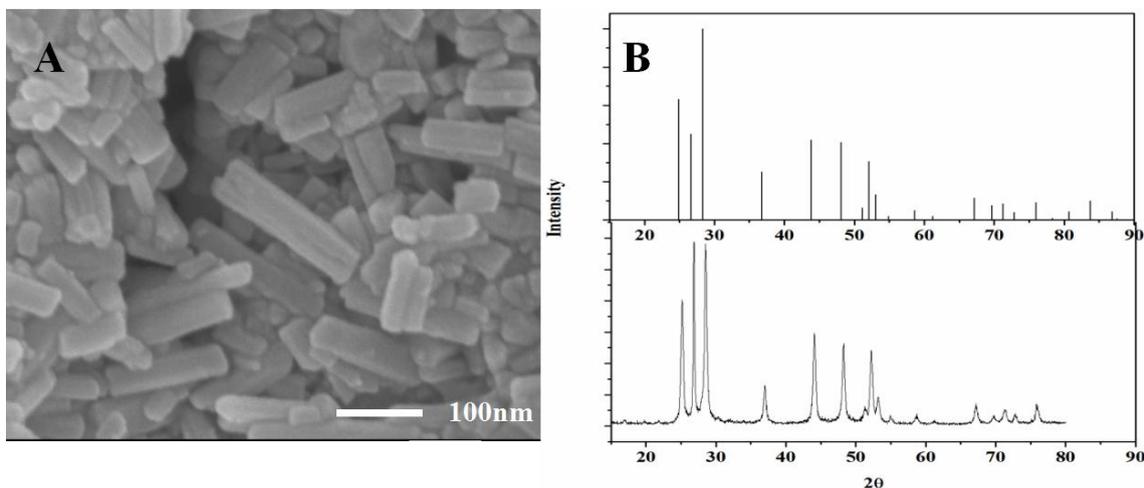
Figure 1. SEM photographs (A) and XRD photographs (B) of CdS nanorods

3.2. ECL behavior of CdS/PGE
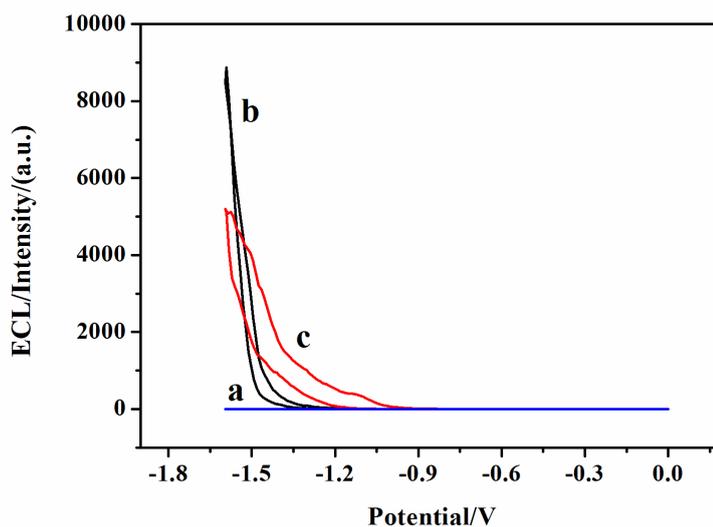
Figure 2. ECL curves of (a) bare PGE, (b) CdS/PGE , (c) 2×10^{-8} mol L^{-1} rutin and CdS/ PGE Solution: 0.1 mol L^{-1} pH 9.0 PBS containing 0.01 mol L^{-1} $K_2S_2O_8$. scanning rate: 0.1 V s^{-1}.

The ECL properties of bare PGE, CdS/PGE and influence of rutin were investigated (Fig. 2). Fig 2a, showed that no background emission was observed from bare PGE, but a stronger ECL peak was observed on CdS/PGE at −1.5 V (Fig. 2b). It had been reported that the emission was original from the excited state of CdS (CdS*) [13]. When 2×10^{-8} mol L^{-1} rutin was added into the solution, the

ECL emission intensity decreased dramatically (Fig. 2c). Based on this principle, a novel method for detection of rutin can be developed here.

3.3. The possible reaction mechanism

And the possible mechanism involved was discussed according to the previous report[25]. Nanocrystals can be reduced and oxidized by charge injection during the potential cycling at the electrodes[26]. When the potential is scanned in the negative direction, CdS immobilized on the electrode is reduced to nanocrystalline species $CdS^{\bullet-}$, while the coreactant $S_2O_8^{2-}$ is reduced and generated a strong oxidant ($SO_4^{\bullet-}$). Then $SO_4^{\bullet-}$ could react with the electrogenerated species ($CdS^{\bullet-}$) through electron transfer to produce the excited state CdS (CdS^*), The CdS^* returns to its ground state accompanying with releasing of a photon[27]. The schematic representation of the luminescent process is presented in Scheme 1. When rutin was added to the solution, oxidation of rutin to the corresponding o-quinone and consume of the strong oxidant radical SO_4^{*-} result in the decrease of ECL. Which is consistent with reported literature that o-benzoquinone residues can efficiently quench the fluorescence emission of nanocrystals by energy transfer [28].
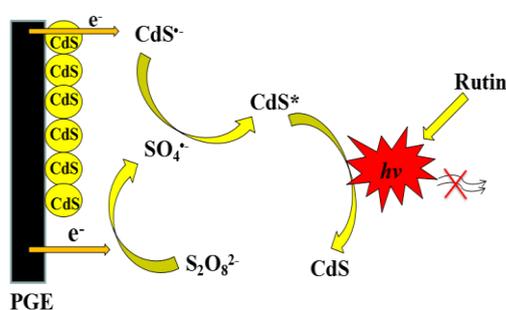
Scheme 1. The possible reaction mechanism

3.4. The effect of concentration of CdS
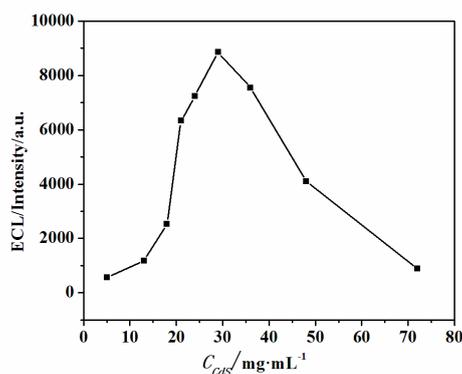
Figure 3. Effect of CdS concentration
Solution: 0.1 mol L^{-1} PBS (pH 9.0) containing 1×10^{-3} mol L^{-1} $K_2S_2O_8$. Scanning rate: 0.1 V s^{-1}.

In order to achieve the optimal ECL response for the detection of analytes, the amounts of CdS deposited on the PGE were investigated. Fig. 3 showed that ECL intensity increased with the increase of CdS concentration on the surface of PGE when concentration of CdS below 30 mg/mL, However, the ECL intensity decreased with the further increase of CdS concentration when the concentration of CdS beyond 30 mg/mL. This suggested that the overloaded CdS may enchance the blocking of the electron transfer and inhibition of the diffusion of co-reactant into the electrode which could hinder the ECL. When concentration of CdS is too high, the efficiency of electron transfer decrease, leading to the reduction of ECL[29]. As a result, 30 mg/mL CdS was used for fabrication of the sensor.

3.5. The effect of scan rate on ECL

The effect of scan rates on ECL were also discussed in our study, as can been seen in Fig. 4. The ECL intensity increases abruptly with the elevated scan rate when the scan rate below 0.1 V s^{-1}, this may be attributed to the rapid formation of CdS*. The ECL intensity increases showly and unstable after 0.1 V s^{-1}. Thus, 0.1 V s^{-1} is used for further experiments.
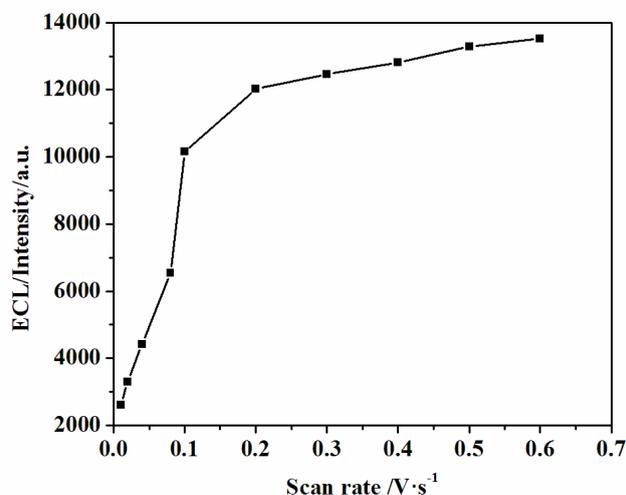
Figure 4. Effect of scan rates on ECL
Solution: 0.1 mol L^{-1} PBS(pH 9.0) containing 1×10^{-3} mol L^{-1} K$_2$S$_2$O$_8$

3.6. Optimization of the ECL response for rutin

In order to achieve the optimal ECL response for rutin determination, the effects of solution pH and various buffer solutions were studied. As shown in Fig. 5A, the ECL intensity increased with an increase of pH from 4.0 to 6.0 when 2×10^{-8} mol L^{-1} rutin was added, and then the increasement became slow when the pH over 6.0. However, the ECL intensity decreased when the pH over 6.0, The highest response was obtained at pH of 6.0. Therefore, this pH was selected and used for subsequent experiments. Furthermore, the effect of different buffer solutions were studied (Fig 5B). The ECL

decreased in britton–robinson (BR) and PBS, while ECL intensity little increase in borate buffer solution (BBS). In general, the ECL intensity decreased significantly in PBS, possibly due to the ability of phosphate anions to diffuse through the PGE in comparison with other ions[30]. Thus PBS was selected for further study.
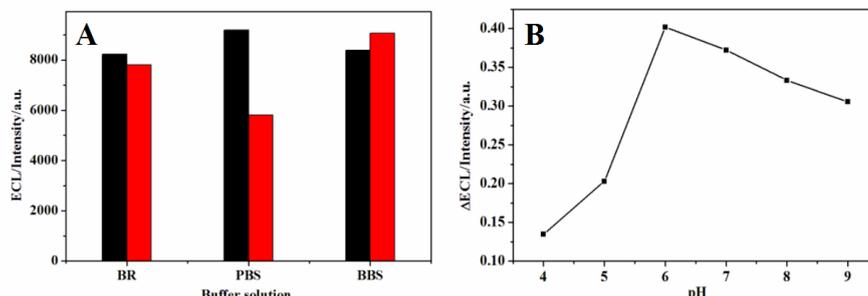
Figure 5. Effects of buffer solution (A) and pH (B) on the ECL intensity Solution: (A) 0.1 mol L^{-1} different buffer solutions (pH 9.0) containing 1 × 10^{-3} mol L^{-1} K$_2$S$_2$O$_8$ (B) 0.1 mol L^{-1} PBS containing 1 × 10^{-3} mol L^{-1} K$_2$S$_2$O$_8$

3.7. ECL detection of rutin

Base on inhibition of rutin on the ECL intensity of CdS/PGE, an effective method for ECL detection of ruitn was proposed. Under the optimal working conditions, it was found that ECL intensity is linear with rutin concentrations in the range from 4 × 10^{-9} to 8 × 10^{-7} mol L^{-1} with a detection limit (*S/N*=3) of 2 × 10^{-9} mol L^{-1} as shown in Fig. 6. The linear regression equation was expressed as Y = −13339.5 log*C*−2362.8 (*C* represents the concentration of rutin) with a correlation coefficient of R = 0.9988.
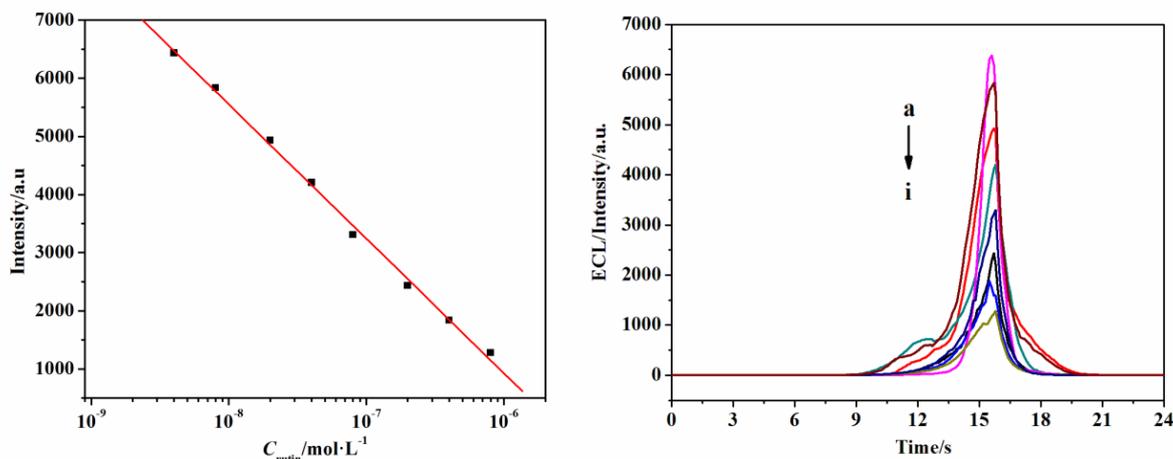
Figure 6. Calibration of rutin. Solution: 0.1 PBS (pH 9.0) containing 2×10^{-3} mol L^{-1} K$_2$S$_2$O$_8$. Rutin concentration (from a to i): 4×10^{-9}, 8×10^{-9}, 2×10^{-8}, 4×10^{-8}, 8×10^{-8}, 2×10^{-7}, 4×10^{-7}, 8×10^{-7} mol L^{-1}

In addition, we compared with other electrochemical methods for determination of rutin, and the results were listed in Table 1. Compared with other method, the proposed ECL sensor owns a better sensitivity, wider linear range and the lowest detection limits over other reported methods.

Table 1. Comparison with other methods for determination of rutin

Methods	Linear range (mol·L^{-1})	LOD	Ref.
CE-ED β-CD-Au@PTCA-	7.5×10^{-6}—1×10^{-3}	4.34×10^{-7}	[31]
SWCNHs/GCE	1×10^{-8} — 1×10^{-5}	4.4×10^{-9}	[28]
MWCNT-IL/CPE	3×10^{-8} — 1.5×10^{-6}	1×10^{-8}	[32]
SWCNT /CILE	1×10^{-7} — 8×10^{-4}	7×10^{-8}	[33]
IL-CCE	3×10^{-7} — 1×10^{-4}	9×10^{-8}	[34]
GR/CILE	7×10^{-8}–1×10^{-4}	2.4×10^{-8}	[35]
PtNPs/RGO/GCE	5.7×10^{-8}–1.2×10^{-4}	2.0×10^{-8}	[36]
Fe$_2$O$_3$/RGO/GCE	1.5×10^{-8}–1.8×10^{-5}	9.8×10^{-9}	[37]
CdS/PGE	$4 \times 10^{-9} \sim 8 \times 10^{-7}$	2×10^{-9}	our work

In addition, the reproducibility and stability of the ECL sensor were examined as shown in Fig.7. A much more stable ECL reaction could be observed. The relative standard deviation for the 9 parallel detection of 2×10^{-8} mol L^{-1} rutin was 2.56% indicating that the CdS/PGE have good stability and repeatability.
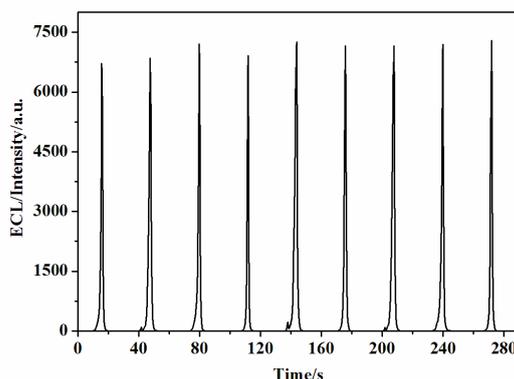
Figure 7. Stability and reproducibility of sensors Solution: 0.1 mol L^{-1} PBS(pH 9.0) containing 1×10^{-3} mol L^{-1} K$_2$S$_2$O$_8$ and 2×10^{-8} mol L^{-1} rutin

3.8. Interference study

The effects of some factors in the determination of rutin were investigated. The absence and presence of various concentrations of concomitants associated with rutin. The results demonstrated that common ions such as a 500−fold concentration common ions such as NO$_3^-$, Cl$^-$, SO$_4^{2-}$, CO$_3^{2-}$, Mn^{2+},

Na$^+$, Ca^{2+}, Zn^{2+}, 50−fold concentration of ethanol, soluble starch, 20−fold ascorbic acid, glucose had no significant interference for determination of rutin.

3.9. Recovery of rutin from Compound rutin tablets

In order to evaluate the practicability of CdS/PGE, recovery experiments were carried out from compound rutin tablets. Compound rutin tablets (20 mg per tablet) were purchased from local drugstore. As shown in Table 2. This study was performed by adding known amounts of standard solutions to the samples, and then followed by analysis using the proposed method. Satisfactory values can reach between 98% and 105%. indicating that the CdS/PGE can be efficiently used for rutin determination in real samples. To test the accuracy, the content of rutin was also detected by HPLC. It was found that the results obtained by HPLC and CdS/PGE were good agreement.

Table 2. Determiantion results of rutin in compound rutin tablets

No.	By HPLC (mg)	By this method (mg)	Samples rutin ($10^{-8}\times$mol L^{-1})	Add ($10^{-8}\times$mol L^{-1})	Found ($10^{-8}\times$mol L^{-1})	Recovery （%）	Average recovery （%）
1	19.85	19.34	2.10	2.00	4.06	98	
2	21.93	21.20	1.89	2.00	3.96	103.5	100.40
3	20.88	20.02	1.92	2.00	4.03	105	

4. CONCLUSIONS

In the present work, rutin was quantitatively determinated by ECL on a disposable fabricated PGE/CdS modified electrode. CdS nanorods with well-defined morphology were synthesized by solvothermal method through the reaction of thioacetamide and cadmium nitrate in ethylenediamine in mild condition. Much sensitive detection for rutin was found on this PGE/CdS ECL sensor with the concentration from 4×10^{-9} to 8×10^{-7} mol/L and the detection limit was 2×10^{-9} mol/L. Moreover, this ECL detection method was much suitable for real samples without large interference and the recovery was 98%～105% which shows fine applicability for the detection of rutin in compound rutin tablets.

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