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Electrochemiluminescence Detection of Melamine with Electropolymerized Poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ Modified Electrode

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In this paper, a PSA/Ru(bpy)$_3^{2+}$ modified electrode was prepared by electrochemical polymerization and used as the working electrode in ECL detection of melamine. Under the optimal conditions, the sensor shows a wide linear range of $1.0\times10^{-7}\sim1.0\times10^{-5}$ mol/L, detection limit of 2.5×10^{-8} mol/L with good reproducibility and stability. The method is expected to be a new method for the detection of melamine, which is simple and convenient.

Keywords: ECL; Poly-sulfosalicylic acid; Ru(bpy)$_3^{2+}$; melamine

1. INTRODUCTION

Since 2008, an increased incidence of kidney stones and renal failure in infants has been reported in China, believed to be associated with the ingestion of infant formula contaminated with melamine. It appears that nitrogen rich melamine was added to raw milk to boost its apparent protein content, which is assessed through determination of the nitrogen content by the Kjeldahl method [1].

There are three main methods for the detection of melamine: ①High performance liquid chromatography (HPLC) [2-3]. The detection limit is 2mg·kg^{-1}, and the time is about 10min; ②High performance liquid phase (HPLC-MC) [4-5]. The detection limit of HPLC-MC is 0.01 mg·kg^{-1}. This method can be the samples at the same time in the qualitative to quantitative, so the accuracy is high, the possibility of miscalculation, HPLC-MC high sensitivity, strong anti-interference ability, in the part of the laboratory used as confirmatory method. ③Gas chromatography - mass spectrometry (GC-MS)[6]. GC-MS method has a detection limit of 0. 05 mg · kg^{-1}, and it need to deal with the derivatization. By the way, there is also an enzyme-linked immunosorbent assay [7-8]. These methods are highly sensitive, stable, but the process is more cumbersome.

Electrochemiluminescence, also known as ECL[9], is the electrochemical means and chemiluminescence method of combining a new research technology. ECL has the advantages of high sensitivity, wide linear range and simple instrument. The electrochemical luminescent system of $Ru(bpy)_3^{2+}$ is widely used because it has the advantages of simple reaction mechanism, high luminous efficiency and good solubility in aqueous solution and organic solution. The traditional $Ru(bpy)_3^{2+}$ electrochemiluminescence system is based on the reaction in liquid phase (especially aqueous solution). Solid-phase electrochemiluminescence based on immobilized $Ru(bpy)_3^{2+}$ [10-12] Reusable, and high luminescence intensity. The immobilization of various $Ru(bpy)_3^{2+}$ modified electrodes is of great significance. Using the method of electropolymerization, the glassy carbon electrode (GCE) was scanned by cyclic voltammetry in 0.01 mol/L sulfosalicylic acid[13] and 0.1 mmol/L $Ru(bpy)_3^{2+}$ to obtain poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode and detect melamine. The linear range was $1.0 \times 10^{-7} \sim 1.0 \times 10^{-5}$ mol/L, and the detection limit was 2.5×10^{-8} mol/L. The poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode also has good reproducibility and stability.

2. EXPERIMENTAL

2.1. Chemicals and materials

Sulfosalicylic acid, melamine, ethanol and $Ru(bpy)_3^{2+}$ were obtained from Aladdin Industrial Corporation(China). They and all other chemical reagents(analytical-reagent grade, Aladdin Industrial Corporation, China) were used without further purification. All aqueous solutions were prepared in doubly distilled water.

2.2. Apparatus

CHI 660D Electrochemical Workstation (Shanghai Chen hua instrument co., LTD, China) was used for electrochemical measurements. MPI-B multi-parameter chemiluminescence analysis test system (Xi’an Remex Analysis Instrument co., LTD, China) was used for ECL test. JSM-6360LA scanning electron microscope (JEOL, Japan) was used for taking SEM figure. Nicolet iN10 micro FTIR spectrometer (Thermo Fisher Scientific Inc., U.S.A) was used for Infrared spectrum measurement. A conventional three-electrode system was employed with a GCE (3.0 mm in diameter) as the working electrode, a platinum wire as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode. All potentials reported in this paper were referenced to the SCE.

2.3. Preparation of modified glassy carbon electrode

Prior to modification, the bare glassy carbon electrode was polished successively with 0.3 and 0.05μm Al_2O_3 slurry on the tracing paper. Then it was rinsed with doubly distilled water, and sonicated in acetone and doubly distilled water for 10 min, respectively. After being cleaned, the electrode was immersed in 0.01 mol / L sulfosalicylic acid and 0.1 mmol/L $Ru(bpy)_3^{2+}$ and was conditioned by cyclic sweeping from −1.5 to 2.0 V at 100 mV/s for 30 scans. After doing above, we got poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode.

2.4. Methods

In this paper, a three-electrode system was used, the poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode as the working electrode, the platinum wire electrode as the auxiliary electrode and the saturated calomel electrode as the reference electrode. The modified electrode was placed at 0.1 mol / L PBS buffer (pH = 10.0), using a cyclic voltammogram from 0.1 V to 1.5 V, sweeping 0.1 V/s, and recording the ECL intensity. As seen in fig.1, when different concentration of melamine was added, the ECL intensity changed, too.

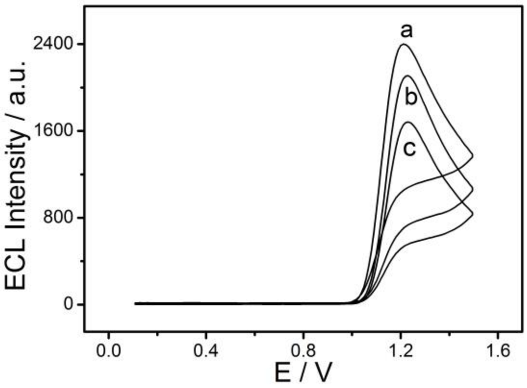

Figure 1. ECL of poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode in 0.1 mol/L PBS buffer (pH = 10.0) with (a) 0 mmol/L, (b) 0.1 μmol/L and (c) 10 μmol/L melamine, cyclic sweeping 0.1 V / s from 0.1 V to 1.5 V

3. RESULTS AND DISCUSSION

3.1. Characterization of poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode

In order to confirm poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode was successful produced, we took the SEM figure of bare glassy carbon electrode in Fig.2(left) and the SEM figure of modified electrode in Fig.2(right) We can easily find electrosynthesized film on modified electrode but nothing on bare glassy carbon electrode, which means that poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ modified electrode produced successfully.

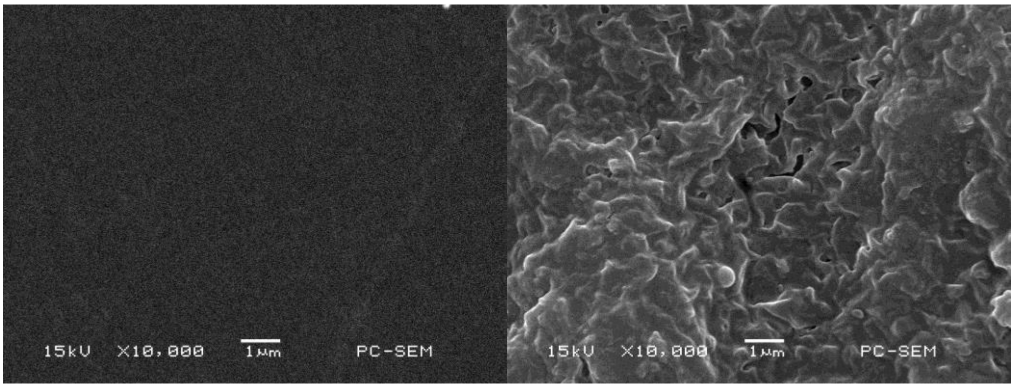

Figure 2. SEM image of bare glassy carbon electrode(left) and poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ modified electrode(right)

The peaks at 700-900 cm^{-1} are C-H stretching vibration peaks. They are obvious different between the two films, which means Ru(bpy)$_3^{2+}$ had been modified on the electrode.

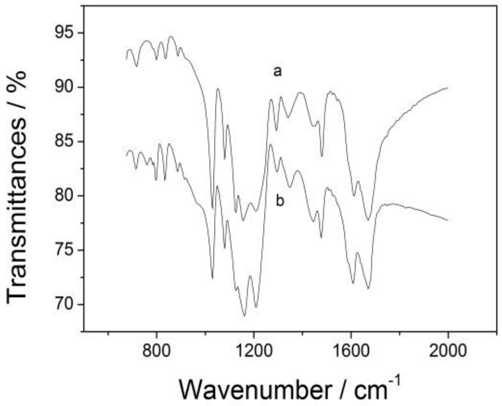

Figure 3. Fourier transforms infrared spectra of sulfosalicylic acid film(a) and poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ film(b)

3.2. Influence of electrochemical polymerization scanning segment on △ECL

The amount of Ru(bpy)$_3^{2+}$ fixed on the electrode determines ECL intensity of the electrode[14-15]. In this paper adsorption of Ru(bpy)$_3^{2+}$ influenced the experimental results, and it affected by electrochemical polymerization scanning segment. In Fig.4, we can find △ECL intensity increasing with circle number increasing from 10 to 30 because of the increasing adsorption of Ru(bpy)$_3^{2+}$. When the scanning circle number is greater than 30, △ECL intensity did not change a lot because the adsorption of Ru(bpy)$_3^{2+}$ was saturated. In addition, with the increase of polymerization scanning segment, the poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ film became thicker and the conductivity became worse[16] So 30 was chosen to be the suitable electrochemical polymerization scanning segment.

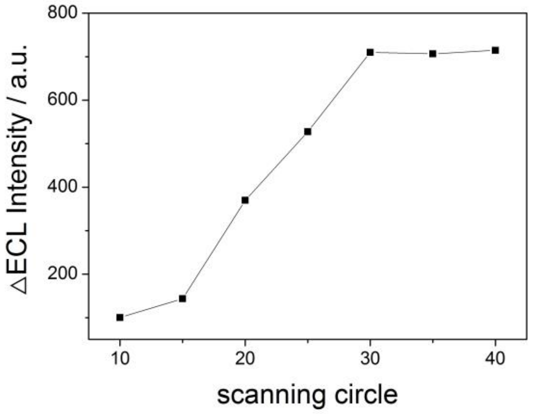

Figure 4. △ECL of poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ modified electrode with different electrochemical polymerization scanning segment in 0.1 mol/L PBS buffer (pH = 10.0), cyclic sweeping 0.1 V/s from 0.1 V to 1.5 V, 10 μmol/L melamine

3.3. Influenceof pH on △ECL

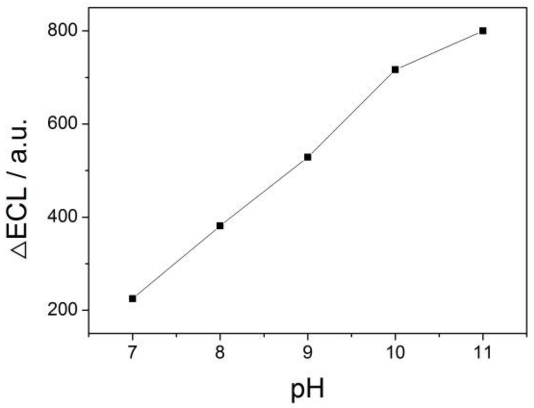

Figure 5. △ECL of poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ modified electrode in 0.1 mol/L PBS buffer with different pH, cyclic sweeping 0.1 V/s from 0.1 V to 1.5 V, 10 μmol/L melamine

The protonation of melamine also influences the strength of △ECL because the protonation of amine is closely related to pH of the solution. As shown in Fig. 5, in 0.1 mol/L phosphate buffer solution, the △ECL intensity increased gradually with the increase of pH because Ru(bpy)$_3^{2+}$ have good luminescence properties under the alkaline condition[17]. At pH=10.0, the △ECL luminescence intensity was large and the value was stable. After that, although △ECL intensity is still increasing, it is no longer stable because melamine can be hydrolyzed in alkali conditions[18]. So in this paper we selected pH=10.0 phosphate buffer solution.

3.4. Influence of scanning rate on ECL

Cyclic voltammetry was used to find suitable scanning rate for stable result of ECL, the result was shown in Fig.6. In 0.1 mol/L PBS buffer solution with pH 10.0, ECL intensity increased with the increase of scanning rate. But with the increase of scanning speed, ECL intensity fluctuated greatly, And the reproducibility and stability are very good at 0.1 V/s. With the increase of scanning rate, the oxidation peak current increased. But when the scanning rate was too high, the background current was too large and shape variation, the luminous effect also affected[19]. Therefore, we choose 0.1 V/s as the experimental test conditions.

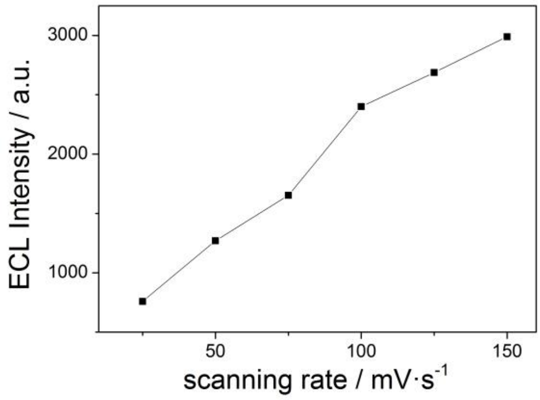

Figure 6. ECL of poly(sulfosalicylic acid)/Ru(bpy)$_3^{2+}$ modified electrode in 0.1 mol/L PBS buffer (pH = 10.0), cyclic sweeping different scanning rate from 0.1 V to 1.5 V

3.5. Linear range and detection limit

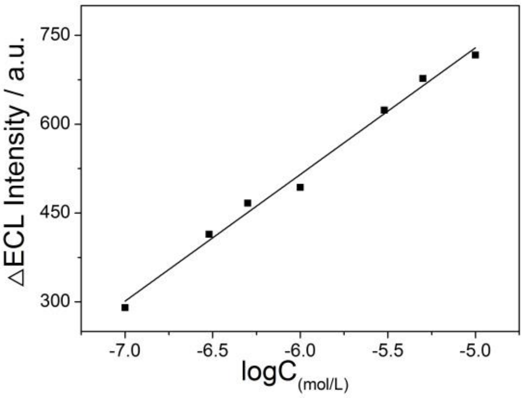

Figure 7. △ECL intensity with different concentrations of melamine in 0.1 mol/L PBS buffer (pH = 10.0), cyclic sweeping 0.1 V/s from 0.1 V to 1.5 V

Under the optimum condition, the effect of melamine on ECL was observed by adding different concentrations of melamine. The high voltage of photomultiplier tube was 800 V and the scanning voltage was 0.1 ~ 1.5 V. Set the difference between ECL intensity in 0.1 mol/L PBS buffer (pH = 10) and ECL intensity in 0.1 mol/L PBS buffer (pH = 10.0) with melamine as △ECL intensity. The linear relationship between the △ECL intensity and the logarithm of melamine concentration was obtained when the concentration of melamine was in the range of 1.0×10^{-7} ~ 1.0×10^{-5} mol/L. The result shows in Fig.7, the linear regression equation was $\Delta ECL=213.84\log C_{(mol/L)}+1798.04$ (R=0.995) and the detection limit was 2.5×10^{-8} mol/L.

3.6. Determination of melamine

Weigh 1.0 ml of commercially available milk uniform sample in a 100 mL volumetric flask and measure the concentration of melamine in the sample with 100 mL of PBS buffer at pH 10.0. The results are shown in Table 1. The relative standard deviation (RSD) of each sample was less than 5%, which indicated that the precision of the method was good. The recoveries of spiked samples ranged from 97% to 103%, which indicated that the method had good accuracy. Based on results above, the method has the advantages of low limit of detection, high sensitivity and good selectivity, and can be used for accurate quantitative analysis of melamine in the sample.

Table 1. Determination results of melamine in injections

Sample	Add（$\times10^{-6}$ mol/L）	Found（$\times10^{-6}$ mol/L）	RSD(%)	Recovery(%)
Milk 1	1.00	0.97	1.9	97
Milk 2	1.50	1.51	3.1	101
Milk 3	2.00	1.97	3.5	98.5

3.7. Analytical Performance of the Proposed Method

Many analytical methods were proposed for melamine detection in milk and other dairy products. The main analytical parameters in linear range and sensitivity of our method were compared with other reported methods. As shown in Table 2, the limit of detection (LOD) for melamine in this work is lower than that of most reported methods. The proposed method also exhibits a wide linear range. In addition, compared with most of reported methods, the detection process of melamine in milk with poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode in this paper is much simpler.

Table 2. Comparison of the proposed method with other methods for determination of melamine.

Methods	Linear range (μmol/L)	r	LOD (μmol/L)	RSD (%)	Ref.
FT-IR	198-4956	0.99	19.8	-	Ref. [20]
HPLC-UV	0.39-19.8	-	0.23	3.6	Ref. [21]
ELISA	0.16-3.9	-	0.16	10.0	Ref. [21]

Au nanoclusters	0.09-100.0	0.999	0.032	2.9	Ref. [22]
Turn-on fluorescence/Au nanoclusters	0.5-10.0	0.997	0.15	3.7	Ref. [23]
Spectrophotometric	3.9-15.9	0.993	0.45	4.6	Ref. [24]
Colorimetric/AuNPs-TMB-H_2O_2	0.001-0.8	0.9991	0.0002	4.4	Ref. [25]
Colorimetry/label-free AgNPs	2.0-250.0	0.996	2.32	2.32	Ref. [26]
3,4-dihydroxyphenylacetic acid recognition element	0.01-5.0	0.997	0.003	2.3	Ref. [27]
Electrospray ionization tandem MS	3.96-79.2	0.9992	0.79	8.9	Ref. [28]
poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$	0.1-10.0	0.995	0.025	2.8	This work

4. CONCLUSIONS

In this study, poly(sulfosalicylic acid)/$Ru(bpy)_3^{2+}$ modified electrode was successfully fabricated with a new way of one step electrochemical polymerization, and the electrochemical luminescence sensor for detection of melamine was constructed. Under the optimal experimental conditions, the sensor has a wide linear range of $1.0 \times 10^{-7} \sim 1.0 \times 10^{-5}$ mol/L and a detection limit of 2.5×10^{-8} mol/L for melamine. The method is simple and easy to operate, and has been successfully applied to the detection of melamine in milk, which is expected to provide a new idea for the detection of melamine.

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