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Determination of melamine based on electrochemiluminescence of $Ru(bpy)_3^{2+}$ at chemically converted graphene modified glassy carbon electrode

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Electrochemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ at chemically converted graphene (CCG) modified glassy carbon (GC) electrode has been employed for the determination of melamine. It gave a linear response (R^2=0.99) for melamine concentration from 1.0×10^{-15} mol/L to 1.0×10^{-11} mol/L with a remarkable detection limit 1.0×10^{-15} mol/L in 0.1 mol/L phosphate buffer (pH = 10). This is much lower compared to other detection methods.To check its applicability, the proposed method was employed to the determination of melamine spiked into the milk samples, and a remarkable detection limit 5.0×10^{-15} mol/L was achieved with good reproducibility and stability. All these provide a possibility to develop an ECL method for the determination of melamine at the chemically converted graphene (CCG) modified GC electrode.

Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) is a nitrogen-containing hetrocyclic triazine with a nitrogen content of 66.7%, used commonly for production of melamine resins. Although melamine is not a type of food additive, it is often found to be added into various dairy products (such as milk, cookies cheese and animal feeds) as an illegal non-protein N source, due to its high nitrogen content. Which leads to an incorrectly high reading in the measurement of protein content based on total nitrogen, causing some serious melamine residue related problems.[1] Violative residues of melamine can cause a variety of pathological diseases including renal failure,[2] carcinoma of urinary bladder,[3] kidney stone[4] and liver necrosis[5] etc. Therefore, it has achieved highest attention from medical scientist, biologist, biochemist, and environmental chemist for developing a rapid and sensitive determination of melamine. Many detection methods such as high-performance liquid chromatography (HPLC),[6-9] gas chromatography[10,11], enzyme-linked immunoassays[12,13] and mass spectrometry[14-16] etc have been developed to measure melamine. However, most of them are very time-consuming and need some special training for their operation.

Previous reports have shown that carbon-based nanocomposites have good sensing ability of improving electron transfer between the analyte and electrode.[17,18] In our previous work,[19] electrochemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ has been employed for the determination of melamine at bare GC electrode and single-wall carbon nanotube (SWNTs) modified GC electrode. It is found that the detection limit is 1.0×10^{-10} mol/L at bare GC electrode, and the detection limit can be reduced further to 1.0×10^{-13} mol/L after modification of the GC electrode by SWNTs. All of these results provide the possibility of developing a novel ECL detection method for melamine.

Compared with SWNTs, chemically converted graphene (CCG) has more excellent properties due to its larger superficial area and unique structure of two-dimensional sheets composed of sp^2-bonded carbon atoms. CCG has fascinated many researchers in number fields since first discovery by Novoselov et al,[20] owing to its extraordinary physical and chemical properties, such as high carrier mobility, excellent thermal conductivity and specific surface area.[21-23] In order to continue our research on carbon-based nanomaterials modified GC electrodes, CCG was employed to modify GC electrode for determination of melamine based on ECL of $Ru(bpy)_3^{2+}$. It was found that the determination limit for melamine can be further down to 1.0×10^{-15} mol/L, which is 2 orders of magnitude lower than that of SWNTs modified GC electrode. And the reproducibility and stability are quite satisfactory.

Experimental section

Materials and Reagent

The Cl^- salt of $Ru(bpy)_3^{2+}$ was synthesized in our lab. Graphene oxide was purchased from XianFeng Nanomaterials Corporation in Nanjing, China. PVA (polyvinyl alcohol, average M_w 17000, 99% hydrolyzed) were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, China. Other chemicals and solvents were obtained from commercial sources without further purification unless specifically mentioned. Milks were commercial samples obtained from a local supermarket.

Preparation of standard solutions and milk samples

The 1.0×10^{-3} mol/L standard stock solution of melamine were prepared by accurately weighting 3.16 mg of melamine, added into 25 ml volumetric flask and dissolved in 10 mL acetonitrile, then the 25 mL volumetric flask was diluted with acetonitrile to the scale. Then, the stock solutions of melamine was diluted with acetonitrile to obtain five standard solutions, 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9} and 1.0×10^{-8} mol/L respectively. All the solutions were kept at room temperature.

The milk samples were prepared according to a modified

method of the literature.[24,25] Firstly, 20 mL milk was mixed with 4 mL 20% (4.2 × 10^{-3} mol/L) trichloroacetic acid and shaked for 10 min to make protein coagulation. Then 10 mL sample-extracting solvent (the volume ratio of ethanol and acetonitrile is 1:1) was added and the mixture was vortexed for 10 min. Followed by vortexing 10 min, the mixture was centrifuged at 16, 000 rpm for 10 min and heat-treated 3 min at 70 ºC to inactivate endogenous substances. After that, the supernatant was transferred to a 50 mL volumetric flask and the residue was further extracted with 16 mL sample-extracting solvent (8 mL × 2). The extract was collected and combined with the supernatant. Then the corresponding melamine sample was added to the volumetric flask. The volumetric flask was filled to the final volume with sample-extracting solvent.

Preparation of CCG

CCG was synthesized according to the literature.[26] In short, 12.5 mg purified GO was added to 50 mL deionized water and ultrasonicated for 1 h to make GO disperse evenly into water. Then 150 μL of ammonia solution (28 wt% in water) and 14 μL of hydrazine monohydrate were added into the GO solution. The mixture was stirred at 95 ºC for 1 h. After reaction, a homogeneous black dispersion with some black precipitate was obtained. The black dispersion was filtered through glass cotton to remove the precipitate and yield a stable black aqueous dispersion of CCG. The resultant CCG solution (0.25 mg/L based on the initial GO weight) was employed for the preparation of modified electrode.

Preparation of CCG modified GC electrode

GC working electrodes (3.0 mm in diameter) were first polished with a slurry of 0.05 mm alumina, then ultrasonicated, and rinsed with deionized water. Then the electrode was successively ultrasonicated in 1:1 nitric acid and doubly distilled water, and allowed to dry at room temperature. 1 mL of the CCG solution (0.25 mg/L) was taken to mix with 2 mL 5% PVA aqueous solution, to obtain a homogeneous, well-distributed suspension, then 10 μL of this suspension was dropped onto the surface of the pretreated GC electrode, and the solvent was allowed to evaporate at room temperature in the air.

ECL measurement

ECL measurements were performed on a MPI-B multifunctional ECL detector (Xi'an Remex Analyse Instrument Corporation, Xi'an, China). All experiments were carried out at room temperature. A commercial cylindroid quuartz cell was used as an ECL cell, which contained a conventional three-electrode system consisting of CCG modified GC electrode as the working electrode, a KCl-saturated Ag/AgCl electrode and a platinum wire electrode were used as the reference and the auxiliary electrode, respectively.

The ECL measurement was performed according to the literature.[27-29] The corresponding standard solution (5.0 μL) of melamine or milk sample containing melamine was added into 5.0 mL 1.0 × 10^{-3} mol/L $Ru(bpy)_3^{2+}$ in 0.1 mol/L phosphate buffer (pH = 10). The mixture was transferred to an ECL detection cell for ECL determination. Cyclic potential sweep experiments were carried out in the potential region from 0 to 1.5 V and then back to 0 at a scan rate 100 mV/s, the ECL signals and CV vs time were measured repeatedly for at least 7 times, and the average reading were used for the creation of plots.

Results and discussion

Optimization of detection conditions

According to the literature,[30-31] pH and scan rate can affect directly the ECL over a wide range. In order to achieve the best ECL performance, the pH and scan rate were optimized at first. It is noted that both the highest ECL signal and the highest signal to background noise ratio were reached at pH 10 (0.1 mol/L phosphate buffer and scan rate 100 mV/s, this is in good agreement with our previous work.[19] Therefore, pH 10 (0.1 mol/L phosphate buffer) and scan rate 100 mV/s was employed for all the detections below.

ECL performance of $Ru(bpy)_3^{2+}$ in the presence of melamine at CCG modified GC electrode

Cyclic voltammograms and the corresponding ECL performance of 1.0 × 10^{-3} mol/L $Ru(bpy)_3^{2+}$ for a certain amount of melamine at CCG modified GC electrode is shown in Fig. 1. When the electrode potential was scanned positively close to 1.0 V, upon the oxidation of $Ru(bpy)_3^{2+}$, an ECL signal was observed, which was in accordance with the literature.[19] The anodic current increased along with the increase of the oxidation potential, and significant ECL enhancement was observed when the electrode potential was close to 1.2 V. The ECL intensity of $Ru(bpy)_3^{2+}$ at CCG modified GC electrode increased with increase in the concentration of melamine in phosphate buffer.

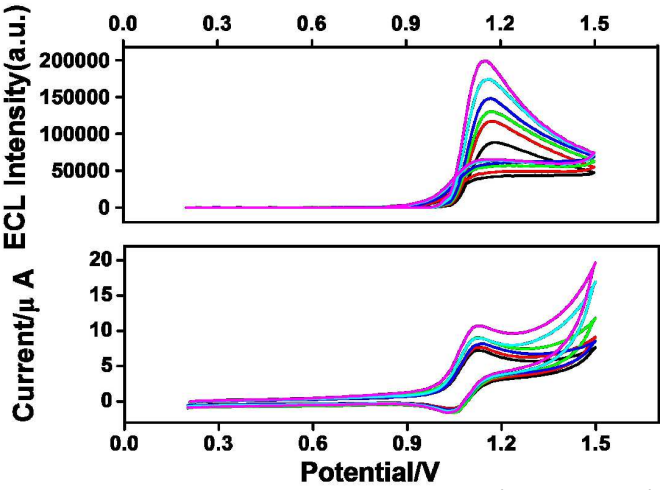

Fig. 1 Cyclic ECL and voltammetric curves of 1.0 × 10^{-3} mol/L $Ru(bpy)_3^{2+}$ and corresponding melamine co-reactant in 0.1 mol/L phosphate buffer (pH 10) at CCG modified GC electrode: $Ru(bpy)_3^{2+}$ alone (black), addition of 1.0 × 10^{-15} mol/L (red), 1.0 × 10^{-14} mol/L (green), 1.0 × 10^{-13} mol/L (blue), 1.0 × 10^{-12} mol/L (blue-green), 1.0 × 10^{-11} mol/L (pink) melamine, respectively; Scan rate = 100 mV/s.

The ECL intensity at CCG modified GC electrode reached up to 3.2 times stronger (black line) than that at the SWNTs modified GC electrode for 1.0 × 10^{-3} mol/L $Ru(bpy)_3^{2+}$ alone, and it can be further improved to 7 times (pink line) after the addition of 1.0 × 10^{-11} mol/L melamine.[19] All these can be ascribed to the remarkable higher specific surface area and also the oxygen functionalities existed on the surface of CCG than that of SWNTs [21-23]. According to the literature[32], the surface of CCG is normally negatively charged due to the presence of oxygen functionalities, so that the positively charged $Ru(bpy)_3^{2+}$ can be absorbed easier on CCG through electrostatic interaction. Meanwhile, more melamine can be simultaneously immobilized on the higher specific surface of the CCG through π-π interaction,[19] providing an enrichment effect on both reactants and alter the intermolecular interaction between $Ru(bpy)_3^{2+}$ and melamine in solution to something like 'both intra-

and intermolecular' interaction on the CCG modified GC electrode surface.[19]

According to the literature,[33-36] a mechanism for the observed ECL performance was proposed (Scheme 1). Melamine first underwent a one-electron oxidation in either step 2 and 3 and formed the melamine cation radical (Melamine$^{\bullet+}$,), which was rapidly deprotonated to form an melamine free radical (Melamine$^{\bullet}$). This highly energetic melamine radical generated the excited state species $Ru(bpy)_3^{2+*}$ through reduction of $Ru(bpy)_3^{3+}$ in step 5. Although melamine is not electrochemically active, it is observed to be oxidized around 1.04 V on CCG modified GC electrode (Fig. S1), provided that CCG modified GC electrode can improve the electrochemical activity of melamine.

$$Ru(bpy)_3^{2+} - e^- \longrightarrow Ru(bpy)_3^{3+} \quad (1)$$

$$Ru(bpy)_3^{3+} + \text{Melamine} \longrightarrow \text{Melamine}^{\bullet+} + Ru(bpy)_3^{2+} \quad (2)$$

$$\text{Melamine} - e^- \longrightarrow \text{Melamine}^{\bullet+} \quad (3)$$

$$\text{Melamine}^{\bullet+} - H^+ \longrightarrow \text{Melamine}^{\bullet} \quad (4)$$

$$Ru(bpy)_3^{3+} + \text{Melamine}^{\bullet} \longrightarrow \text{Melamine}^{+} + Ru(bpy)_3^{2+*} \quad (5)$$

$$Ru(bpy)_3^{2+*} \longrightarrow Ru(bpy)_3^{2+} + h\nu \quad (6)$$

Scheme 1 The proposed mechanism for $Ru(bpy)_3^{2+}$/melamine ECL system

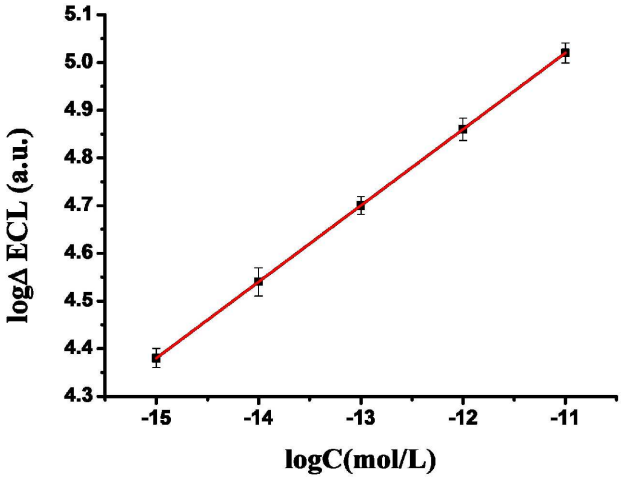

Fig. 2 Dependence of the logarithmic ΔECL increase versus the logarithmic concentration of melamine with 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ in 0.1 mol/L phosphate buffer (pH = 10.0) at CCG modified GC electrode.

A good linear calibration curve (Fig. 2) between the logarithmic of ΔECL intensity (logΔECL, $\Delta ECL = ECL_{\text{after addition melamine}} - ECL_{\text{before addition melamine}}$) and the logarithmic concentration of melamine (log[Melamine]) can be established over the concentration range 1.0×10^{-15}–1.0×10^{-11} mol/L for melamine. The regression equation was logΔECL = 6.78 + 0.16 × log[Melamine] with a linear coefficient $R^2 = 0.99$, the detection limit was down to 1.0×10^{-15} mol/L, which is 2 orders of magnitude lower than that of SWNTs modified GC electrodes[19] and much lower compared to other detection methods.[6-16] This suggests that CCG modified GC electrode is suitable to detect melamine quantitatively in aqueous solution.

To evaluate the reproducibility and stability of the ECL measurement at CCG modified GC electrode, 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ and 1.0×10^{-15} mol/L melamine was scanned by continuously cyclic potential for ten times in 0.1 mol/L phosphate buffer (pH 10) at CCG modified GC electrode. These results were shown in Fig.3. It is noted that there is no significant change for the ECL intensity can be observed in the detection process (RSD 2.15%), illustrating good reproducibility and stability of the ECL measurement on CCG modified GC electrode.

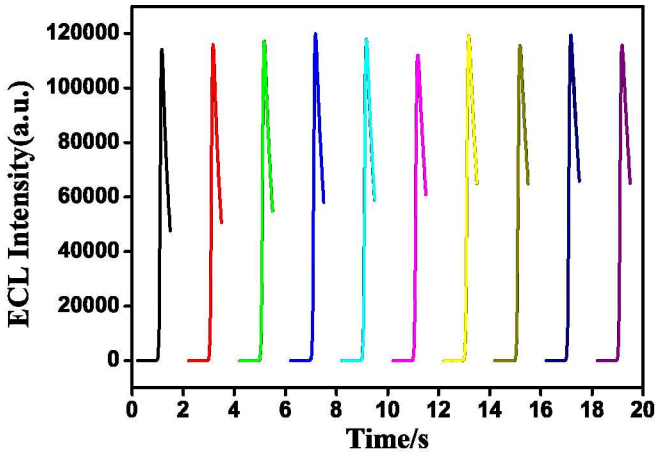

Fig.3 Continuous cyclic scan of CCG modified GC electrode for 10 cycles in 0.1 mol/L phosphate buffer (pH 10) containing 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ and 1.0×10^{-15} mol/L melamine; the scan rate is 100 mV/s.

Interference study

To assess the ability of the proposed method in the analysis of melamine in real samples, the interference effects of coexistence substances, which were expected to be present in real samples, were also examined. The solutions used for this purpose contain 1.0×10^{-13} mol/L melamine, together with 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ and interfering species. A species was not considered to make much interference if it just caused a relative error of no more than ±5% in the measurement of 1.0×10^{-13} mol/L melamine.[37]

The tolerable concentration ratios for detecting 1.0×10^{-13} mol/L melamine were: one hundred million-fold higher concentration by weight for K^+, Na^+, CO_3^{2-}, NO_3^-, Cl^-, vitamin C, lactos and glucose; one million-fold higher concentration by weight for Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}; seven hundred thousand-fold higher concentration by weight for arginine and glycine.

Analytical Applications

To further assess the accuracy of the proposed method, it was applied to the determination of melamine added intentionally into commercial milk samples. Before addition of melamine, the milk sample was measured on LC-MS (Liquid chromatography-mass spectrometry)[38,39] to make sure that no melamine contained. To eliminate interference from high concentrations of calcium and other ingredients, milk samples were prepared as reported,[24,25] but with modifications as described in the experimental section. The analytical results are shown in Fig. 4 and Table 1.

A good linear calibration curve (Fig.4) between the logarithmic ΔECL increasing (logΔECL, $\Delta ECL = ECL_{\text{after addition of the milk samples}} - ECL_{\text{before addition of the milk samples}}$) and the logarithmic concentration of melamine (log[Melamine]) was established over the concentration range from 5.0×10^{-15} to 1.0×10^{-11} mol/L. The regression equation was logΔECL = 6.80 + 0.16 × log[Melamine] with a linear coefficient $R^2 = 0.99$. The melamine detection limit was 5.0×10^{-15} mol/L, which is much lower than others methods.[6-16]

It is noted that the recovery was quite satisfactory for CCG

modified GC electrode (table 1). The relative standard deviations are < 1.70% and the recoveries are 99.80%-100.00% for melamine. All these results underscored the fine accuracy and further demonstrated the potential application of this method for the determination of melamine in milk.

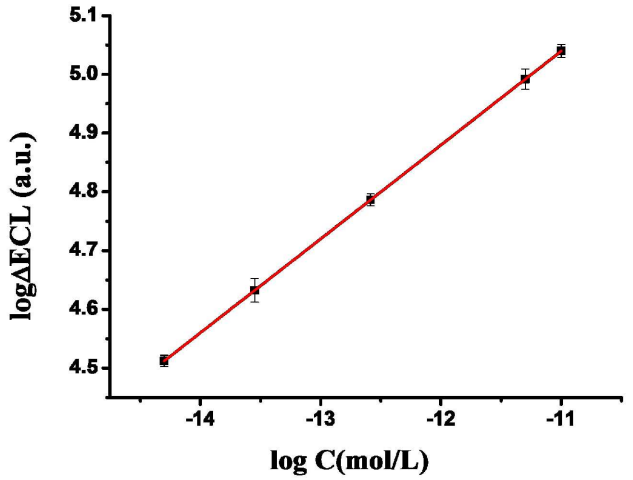

Fig. 4 Dependence of the logarithmic ΔECL increase versus the logarithmic concentration of the melamine in milk samples with 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ in 0.1 mol/L phosphate buffer (pH 10) at CCG modified GC electrode; the scan rate is 100 mV/s.

Table 1 Recovery of the melamine in milk samples detected by ECL of 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ in 0.1 mol/L phosphate buffer (pH 10) at CCG modified GC electrode.[a]

No.	Added (mol/L)	Detected (mol/L)	Average (mol/L)	Recovery %	RSD %
1	5.00×10^{-15}	4.99×10^{-15} 5.01×10^{-15} 4.97×10^{-15}	4.99×10^{-15}	99.80	0.40
2	2.80×10^{-14}	2.79×10^{-14} 2.82×10^{-14} 2.79×10^{-14}	2.80×10^{-14}	100.00	0.60
3	2.60×10^{-13}	2.59×10^{-13} 2.61×10^{-13} 2.59×10^{-13}	2.59×10^{-13}	99.90	0.38
4	5.00×10^{-12}	5.01×10^{-12} 4.98×10^{-12} 5.02×10^{-12}	5.00×10^{-12}	100.00	0.42
5	1.00×10^{-11}	0.99×10^{-11} 1.02×10^{-11} 1.01×10^{-11}	1.00×10^{-11}	100.00	1.70

[a]Average of three samples, each sample was measured repeatedly for at least 7 times, and the averaged readings were used.

Conclusions

In conclusion, ECL of $Ru(bpy)_3^{2+}$ at CCG modified GC electrode has been successfully employed for the determination of melamine in milk samples. The recovery was quite satisfied with good reproducibility and stability. All these provided a possibility of developing CCG modified GC electrode for ECL determination of melamine in milk samples.

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Notes and references

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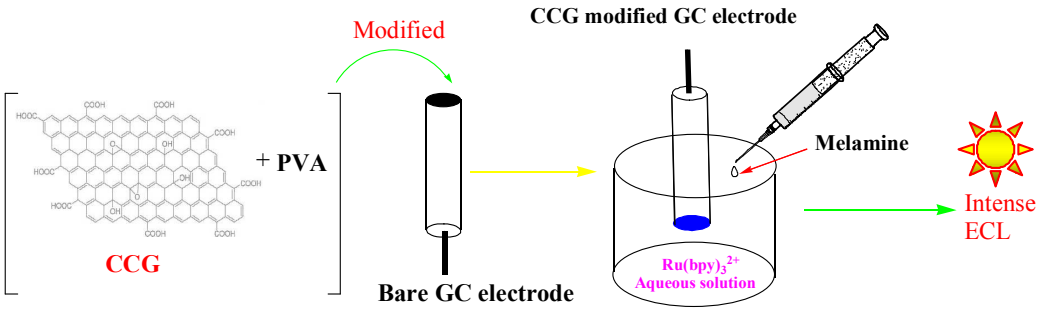
Graphic Abstract



In this paper, Electrochemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ at chemically converted graphene (CCG) modified glassy carbon (GC) electrode has been employed for the determination of melamine in milk with a remarkable detection limit 5.0×10^{-15} mol/L.