Sensitive detection of melamine by an electrochemiluminescence sensor based on tris(bipyridine)ruthenium(II)-functionalized metal-organic frameworks

Defen Feng[a], Yeyu Wu[a,*], Xuecai Tan[a,*], Quanyou Chen[a], Jun Yan[a], Min Liu[a], Chenhao Ai[a], Yanni Luo[a], Fangkai Du[a], Shaogang Liu[a], Heyou Han[b]

[a] School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, Guangxi Key Laboratory of Chemistry and Engineering of Forest Products, Key Laboratory of Guangxi Colleges and Universities for Food Safety and Pharmaceutical Analytical Chemistry, Nanning 530008, China
[b] State Key Laboratory of Agricultural Microbiology, College of Science, College of Food Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China

ABSTRACT

Metal-organic frameworks (MOFs) are microporous materials that consist of metal ions or clusters and organic ligands. Their luminescence activities can be improved by functionalization, and the present work describes the synthesis of a luminescence-functionalized MOF in which tris(2,2′-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3^{2+}$) was introduced into the MOF. The synthesized RuMOF was then fabricated as an ECL sensor that can be used for the rapid and sensitive detection of melamine in dairy products. The optimum detection conditions were established by single-factor analysis and surface response multivariate optimization. Under optimum conditions, the ECL intensity was proportional to log(melamine) concentration in the range 10^{-10}–10^{-4} M with a detection limit of about 3.8×10^{-11} M (signal/noise = 3). In an application to the detection of melamine in milk and infant formula powder, recoveries in the range 98–104% and 95–103%, respectively were obtained from spiked samples. Thus this novel ECL sensor represents a potentially valuable product for the analysis of melamine food samples.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Metal-organic frameworks (MOFs) are novel microporous materials that consist of a combination of metal ions or clusters with organic ligands, and which possess unique chemical and physical properties, such as large surface areas, regular pore sizes and shapes, large and permanent internal porosity, high thermal and chemical stability, and easy modification [1–3]. They have been reported to have excellent potential applications in gas storage [4,5], sensing [6–8], separation [9,10], and catalysis [11,12].

Electrochemiluminescence (ECL) involves the generation of chemical species at the surface of an electrode that then undergo electron-transfer reactions to form excited states that emit light at specific wavelengths. ECL has attracted much attention as an analytical tool, because of its high sensitivity, rapid response, wide linear range, good controllability, low background, and low detection limits [13]. However, up to now there have been few reports of the use of MOFs in ECL systems, because small organic molecules

that are traditional ligands in MOFs often lack some of the properties that are necessary for ECL, especially redox activity and luminescence [14–17]. Thus, the synthesis of MOFs that incorporate compounds with high luminescence, whilst maintaining the original structures and properties of the host, is an obvious strategy for improving both the selectivity and sensitivity of sensors. Also, functionalization may endow MOFs with high electron-transfer capacities, satisfactory charge selectivity, and good electrochemical stability.

Tris(2,2′-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3^{2+}$) is a complex with high ECL efficiency, good electrochemical stability, and a wide linear response range. However, because of problems associated with the consumption of $Ru(bpy)_3^{2+}$ in solution-phase of ECL sensors, considerable efforts have been made to produce immobilized $Ru(bpy)_3^{2+}$ on electrode surfaces in order to reduce losses of this expensive reagent and to simplify sensor design, whilst enhancing the ECL signal [18,19]. MOFs provide a platform for loading and concentrating $Ru(bpy)_3^{2+}$ to produce higher ECL intensity than other RuMOFs, $Ru(bpy)_3^{2+}$ solutions, or other immobilized $Ru(bpy)_3^{2+}$ materials [18,20,21], and MOFs doped with $Ru(bpy)_3^{2+}$ represent novel low cost and stable solid-state ECL sensors.

* Corresponding authors.
E-mail addresses: eveyeyu@163.com (Y. Wu), gxunxctan@126.com (X. Tan).

Melamine is widely used in the production of fertilizers, plastic, coatings, commercial filters, and various other products. It is also formed naturally during the roasting of various food products. However, because of its high nitrogen content (66%), melamine has been illegally used to increase the nitrogen content of foods, and thus produce "higher protein values" in analyses based on nitrogen contents [22,23]. A particular problem has been found with milk products, in which contamination with melamine can lead to reproductive and urinary system damage, urinary calculi, acute renal failure, bladder cancer, and infant death. As a result, both the United States and China have set a threshold of 1 part per million (ppm) for the melamine content of infant formula powder [24,25]. Various methods, such as capillary electrophoresis [26], fluorimetry [27], HPLC-UV [28], electroanalytical techniques [29], and colorimetry [30] have been applied to melamine analysis, but each has some disadvantage either in the form of expensive instrumentation, or time-consuming, tedious analyses. Therefore, the development of reliable, sensitive, simple and convenient melamine detection methodology is very important for product quality control and the enforcement of safety legislation.

In the present work, we describe the synthesis, properties, and ECL sensing applications of RuMOFs in which the main skeleton is the anionic bio-MOFs-1 $[Zn_8(ad)_4(BPDC)_6O\cdot2Me_2NH_2$, 8DMF, 11H$_2$O] (ad = adeninate; BPDC = biphenyldicarboxylate; DMF = dimethylformamide), which consists of infinite zinc-adeninate columnar secondary building units composed of apex-sharing zinc-adeninate octahedral cages, whilst the luminescent cationic Ru(bpy)$_3^{2+}$ could be doped into the MOF framework (displacing some biphenyl-4,4′-dicarboxylic acid BPDC) or held within pores by strong electrostatic interaction [31,32]. Ru(bpy)$_3^{2+}$ is strongly immobilized in these RuMOFs, but its original electrochemical and luminescent properties are preserved [33,34]. Since Ru(bpy)$_3^{2+}$ can react with amides, melamine (1,3,5-triazine-2,4,6-triamine), which has attracted attention as a potentially toxic additive to milk products, was used to demonstrate a potential application of the synthesized RuMOFs. The present paper reports the design of a RuMOF ECL sensor for the fast, simple, sensitive, and convenient determination of melamine over a wide concentration range with linear response and low detection limit. Its successful use for the detection of melamine in dairy products is then demonstrated.

2. Materials and methods

2.1. Materials and reagents

Tris(2,2′-bipyridyl) dichlororuthenium(II) hexahydrate (Ru(bpy)$_3$Cl$_2\cdot$6H$_2$O, >99.95%) and nafion (5 wt%) were purchased from Sigma-Ardrich (Madrid, Spain). Adenine (>98%), zinc acetate (>99.99%), melamine (>99.9%), cyanuric acid (>99.0%), anhydrous phloroglucinol (>99.0%), and ascorbic acid (>99.9%) were purchased from Aladdin (Shanghai, China). Biphenyl-4,4′-dicarboxylic acid (PBDC, >97%) was obtained from Accela ChemBio Co. (Shanghai, China). N,N-dimethylformamide (DMF) of analytical purity was purchased from Tianjin Fuyu Fine Chemical Co. (Tianjin, China). Milk and infant formula powder were bought from a supermarket. The water used for all experiments was purified by a Milli-Q Water Purification System (Millipore, America).

2.2. Apparatus

ECL emission and cyclic voltammetry were monitored with a electrochemiliuminescence analyzer (model MPI-B Xi'an Remax Electronic Science & Technology Co., Ltd., China) with a voltage of 800 V provided by a photomultiplier tube (PMT), and a poten-

tial scanning range from 0.2 to 1.25 V. All the experiments were carried out by a three-electrode system, which included a glassy carbon electrode (GCE, 3.0 mm in diameter) as working electrode, an Ag/AgCl (3 M KCl) reference electrode, and a platinum pole counter-electrode. UV–vis spectra were measured with a UV–vis spectrophotometer (Agilent Cary 60, Agilent Technology, America), and fluorescence spectra with a fluorescence spectrophotometer (F-4600, Hitachi, Japan). A scanning electron microscope (SEM) (SUPPRA 55 Sapphire, Carl Zeiss, Germany) was used to investigate sample morphology and to generate energy dispersive X-ray (EDX) spectra. X-ray powder diffraction patterns were collected using a Rigaku Ultima IV X-ray diffractometer operating at 40 kV, 40 mA for Cu Kα (λ = 1.5406 Å) with a scan speed of 0.1 s/step and a step size of 0.02°. Specific surface areas of the products were measured by the Brunauer-Emmett-Teller (BET) method using nitrogen gas adsorption/desorption at 77 K, and the Density Functional Theory (DFT) method was used to calculate the pore size distribution (Autosorb-IQ, Quantachrome, America). ICP-AES (iCAP 6000 Series, Thermo Fisher Scientific, America) was used to determine the Ru concentrations of the RuMOFs.

2.3. Synthesis of bio-MOF-1 and RuMOFs

Bio-MOF-1 and RuMOFs were synthesized according to reported methods [3,34]. For the preparation of RuMOF, adenine (0.125 mmol), BPDC (0.25 mmol), Ru(bpy)$_3$Cl$_2\cdot$6H$_2$O (0.00725 mmol), zinc acetate (0.375 mmol), nitric acid (1 mmol), DMF (13.5 mL), and water (1 mL) were added to a 20.0 mL vial, which was then capped and heated to 130 °C for 24 h. Rod-shaped crystals were produced, washed with DMF three times, then dried at 70 °C for 24 h in a vacuum oven. Bio-MOF-1 was prepared by the same procedures but without the addition of Ru(bpy)$_3$Cl$_2\cdot$6H$_2$O.

2.4. Preparation of the modified electrode

Prior to modification, each GCE (diameter = 3 mm) was polished successively with 1.0 μm, 0.3 μm and 0.05 μm alumina powder, and rinsed with water. The electrodes were then successively sonicated in 1:1 nitric acid, ethanol and water, and finally dried at room temperature. RuMOFs (6 mg) was dispersed in 1 mL 0.2% nafion/ethanol solution to obtain a homogeneous suspension, and then 3–7 μL of this suspension was dropped onto the surface of the pretreated GCE to complete the fabrication (Scheme 1). When not in use, the modified electrodes were stored in the dry state at room temperature.

2.5. Sample preparation

Melamine was dissolved in water to produce a 1×10^{-5} M stock solution. The standard addition method was used for melamine analysis in the milk and infant formula powder samples.

For the milk sample preparation, 4 mL 20% trichloroacetic acid was added to 20 mL milk in 50 mL centrifuge tubes, which were shaken for 10 min to promote protein coagulation. Samples were then mixed with 10 mL ethanol and acetonitrile (V:V = 1:1), vortexed for 10 min, then centrifuged at 12000 rpm for 15 min, and heated at 70 °C for 3 min to inactivate endogenous substances. The residue was further extracted with ethanol/acetonitrile solvent (8 mL × 2), the extract mixed with the supernatant, filtered through a 0.22 μm PTFE membrane, transferred to a volumetric flask, and finally diluted to 50 mL with ethanol/acetonitrile solvent [35].

The infant formula powder sample was prepared according to the literature methodology [36]. 2.00 g infant formula powder, 15 mL 1% trichloroacetic acid and 10 mL acetonitrile were added to a 50 mL centrifuge tube, ultrasonicated for 15 min, then heated for 10 min at 100 °C in a water bath to ensure protein coagulation.
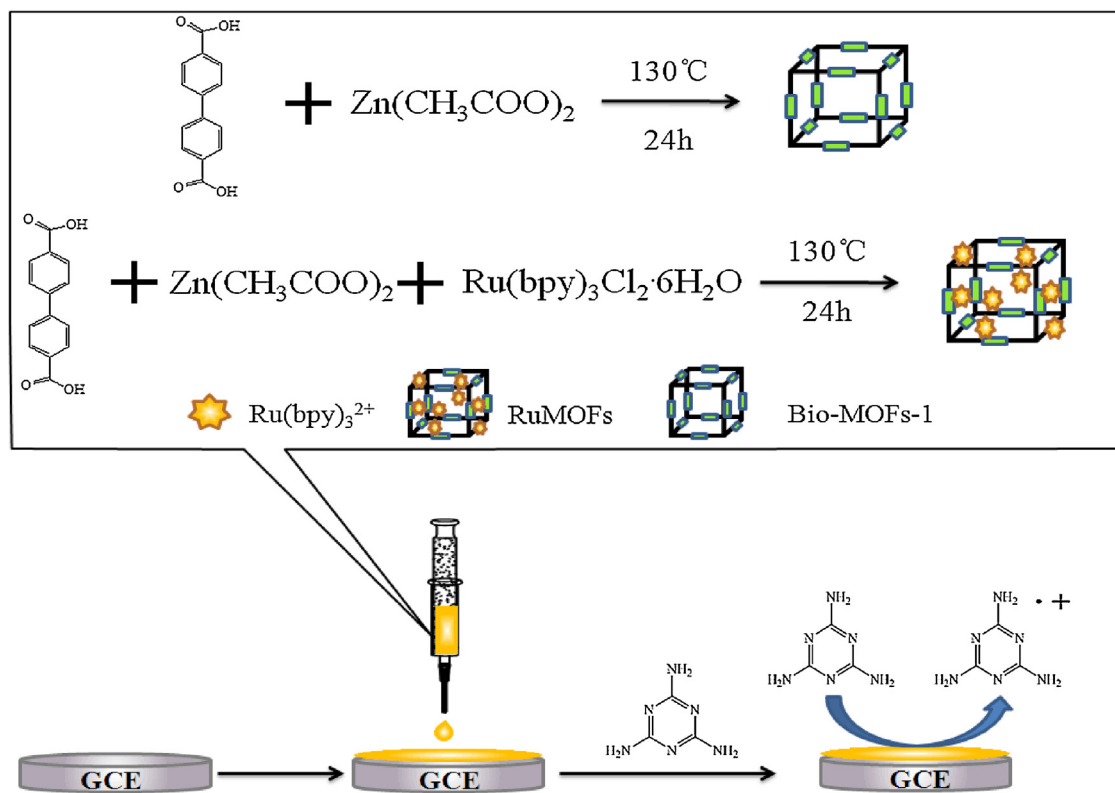
Scheme 1. Schematic representation of an ECL sensor for melamine detection.

This mixture was centrifuged at 4000 rpm for 5 min, and filtered through a 0.22 μm PTFE membrane. The filtrate was collected in a 250 mL round-bottom flask, evaporated under vacuum at 40 °C to a small volume, and the remaining solvent then removed by a rotary evaporator. The residue was dissolved with 0.1 M PBS (pH 7.0) for analysis.

3. Results and discussion

3.1. Characterization of RuMOFs

The bio-MOFs-1 and RuMOFs were characterized by their XRD patterns, EDX spectra, and N_2 adsorption-desorption measurements (Fig. 1). As shown in Fig. 1A, the crystallographic planes of the synthesized bio-MOF-1 and RuMOFs basically correspond with those in the simulation, although the peak form RuMOFs at about 4° 2θ is slightly smaller than in the simulation, but it does not obviously alter the MOF crystal structure. The EDX spectrum (Fig. 1B), confirms that the RuMOFs actually contained Ru. The bio-MOFs-1 had a BET surface area of 705.6 $m^2\,g^{-1}$ with a pore volume of 0.32 $cm^3\,g^{-1}$ (Fig. 1C), whereas the RuMOFs had a BET surface area of 346.1 $m^2\,g^{-1}$ with a pore volume of 0.20 $cm^3\,g^{-1}$ (Fig. 1D). DFT calculations indicated a maximum pore diameter of 1.3 nm for the bio-MOFs-1 (Fig. 1C) and 1.1 nm for the RuMOFs (Fig. 1D). A Ru content of 2.01 wt% was obtained for the RuMOFs by ICP-AES after digestion by refluxing in 0.5 M HNO_3 for 1 day. Additionally, the external morphology of the RuMOFs was investigated by SEM, which showed rod-shaped porous crystals with length <100 μm and diameter <20 μm (Fig. S1).

Both RuMOFs and $Ru(bpy)_3Cl_2\cdot 6H_2O$ had an absorption peak at 460 nm (Fig. 2A). The fluorescence spectra of bio-MOFs-1 and $Ru(bpy)_3Cl_2\cdot 6H_2O$ showed respectively excitation peaks at 235 nm and 476 nm, and emission peaks at 385 nm and 610 nm (Fig. 2B, C). When $Ru(bpy)_3^{2+}$ was doped into bio-MOFs-1, the RuMOFs showed two excitations and emissions (Fig. 2D). As a result of interaction between the conjugated π bond of $Ru(bpy)_3^{2+}$ and the bio-MOFs-1 framework, the excitation at 235 nm red shifted to 275 nm, and the excitation at 476 nm blue shifted to 455 nm.

3.2. Electrochemical and ECL responses of the modified electrode

The electrochemical behavior of GCE modified with RuMOFs in the absence and presence of 1.0×10^{-7} M melamine at pH 7.0 (0.1 M PBS) were investigated by cyclic voltammetry (Fig. 3A), which showed that melamine significantly increased the oxidation and reduction currents. The intensity of the ECL response was increased by about 3.5-times by 1.0×10^{-7} M melamine (Fig. 3B), thus indicating that the RuMOFs-modified sensor could be used for the ECL detection of melamine.

3.3. Optimization of experimental parameters

3.3.1. Single-factor analysis

Some key factors that influence the ECL response was also studied, specifically the pH, the concentration of RuMOFs, the amount of RuMOFs, and the scan rate.

As shown in Fig. 4A, the ECL intensity increased as the concentration of RuMOFs was increased from 3 to 6 mg mL^{-1}, but decreased at higher concentration. This is probably because of the limited amount of RuMOFs that can be carried by the nafion without rupturing the RuMOFs/nafion membrane. Consequently, 6 mg mL^{-1} was chosen as the optimal concentration of the RuMOFs suspension at pH 7.0 PBS; the amount of RuMOFs was 7.0 μL and the scan rate was 100 mV s^{-1}.

The relationship between the amount of RuMOFs and ECL intensity is shown in Fig. 4B. The ECL intensity increased with increasing amount of RuMOFs, up to 5.0 μL, but decreased with larger amounts. This suggests that at low amounts of RuMOFs the
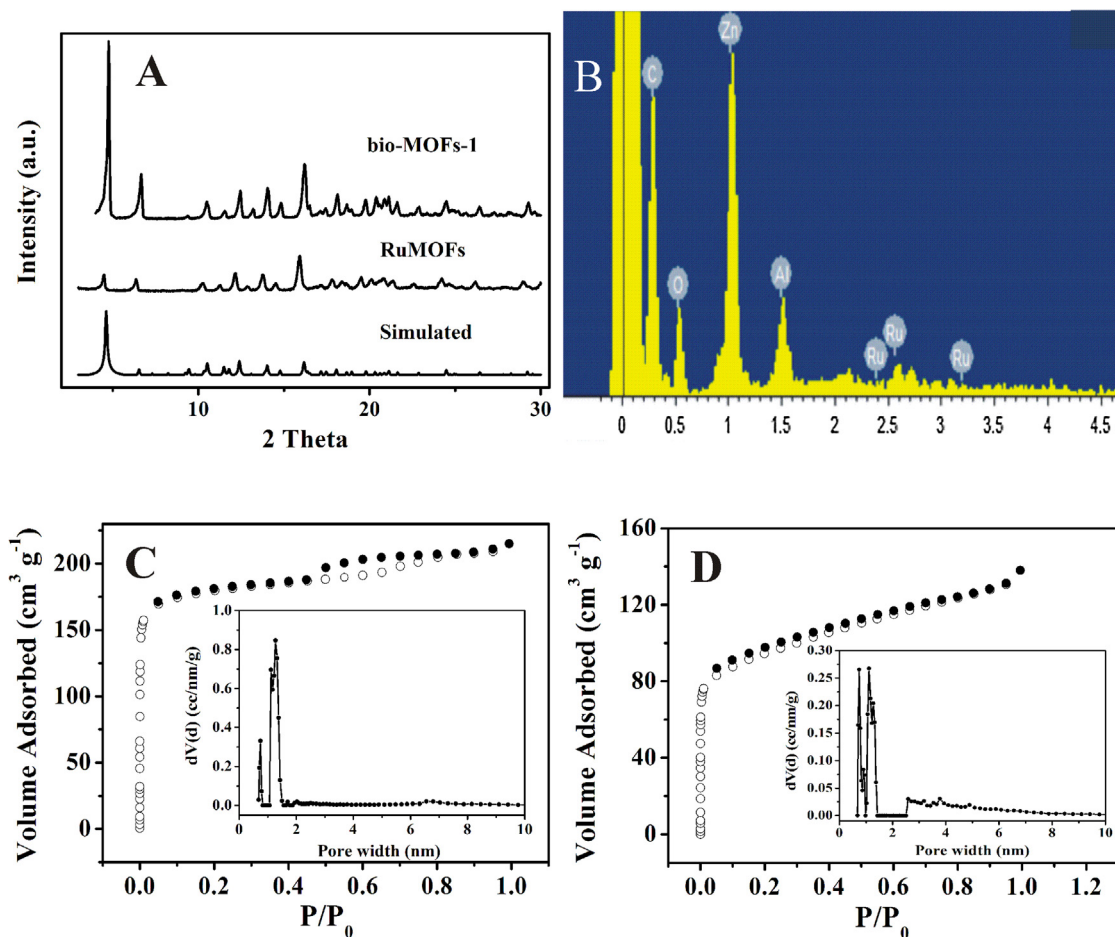
Fig. 1. (A) XRD patterns of simulated bio-MOF-1, synthesized RuMOFs and bio-MOF-1; (B) EDX spectrum of RuMOFs; (C) N$_2$ adsorption-desorption isotherms and the pore size distribution (inset) of the synthesized bio-MOFs-1; (D) N$_2$ adsorption-desorption isotherms and the pore size distribution (inset) of the synthesized RuMOFs.

number of luminophors immobilized on the surface of electrode increases with increasing RuMOFs and results in an increasing ECL response [37], but at higher amounts of RuMOFs the ECL emission may be absorbed or scattered, and the thick film tends to screen melamine from reaction with the RuMOFs. Therefore, 5.0 μL RuMOFs was chosen for sensor preparation at pH 7.0 PBS; the concentration of RuMOFs was 6 mg mL^{-1} and scan rate was 100 mV s^{-1}.

As shown in Fig. 4C, the ECL intensity increased as the pH was increased from 5.0 to 7.0, but decreased at higher pH. Thus, further experiments were conducted at pH 7.0 using 5.0 μL RuMOFs, 6 mg mL^{-1} RuMOFs, and a scan rate of 100 mV s^{-1}. This pH effect might be caused by reduced availability of Ru(bpy)$_3$$^{3+}$ due to the competitive reaction with the OH$^-$ ion, the concentration of which increases progressively with increasing pH [38]. The scan rate can affect the ECL over a wide range, since the ECL efficiency depends significantly on the rate of generation/annihilation of the excited state [Ru(bpy)$_3$$^{2+}$]* [39]. In the present work, the highest ECL intensity was obtained with a scan rate of 100 mV s^{-1} (Fig. 4D), and this was then chosen for subsequent experiments.

In summary, the optimal experimental conditions were pH 7.0, 6 mg mL^{-1} RuMOFs, 5.0 μL RuMOFs solution, and 100 mV s^{-1} scan rate.

3.3.2. Optimizing experimental conditions by response surface analysis

The optimum experimental conditions were determined by using the statistics software package Design-Expert 8.0.6 to construct a regression model through response surface analysis. The Box-Behnken model proved to be reliable (significant F-value, P < 0.05), and Table S1 lists the ECL responses for different experimental schemes tested. 2D contour plots and 3D response surfaces are graphical representations of the regression function which reflects the influence of variables on the ECL intensity and the interplay between variables (Fig. S2). By using response surface optimization, the optimum conditions were obtained as follows: pH 7.0; RuMOFs concentration 6.0 mg mL^{-1}; scan rate 100 mV s^{-1}; amount of RuMOFs solution 5.0 μL; thus this result matches that from the single-factor analysis. A follow-up test using these modified conditions showed no significant difference between the ECL intensity (I = 5479) of the actual experiment under optimum conditions and the ECL intensity (I = 5523) predicted by the software. Hence, the modified conditions are viable and reliable. According to the significance analysis (Table S2), the primary and secondary factors that affect the experimental results are in the order: RuMOFs amount > pH > scan rate > RuMOFs concentration.

3.4. Mechanism for the ECL response in the RuMOFs − ECL system

This is an "oxidative-reductive" system [13,40,41] involving RuMOFs and melamine, in which ECL is generated from redox reactions of RuMOFs during the oxidation of melamine (Fig. 5). The putative reactions are described below in Eqs. (1)–(5), and luminescence is produced by [Ru(bpy)$_3$$^{2+}$]* as it decays to its ground state. Thus this mechanism is similar to that proposed by Richter for the Ru(bpy)$_3$$^{2+}$/tri-*n*-propylamine (TPrA) ECL system [13].
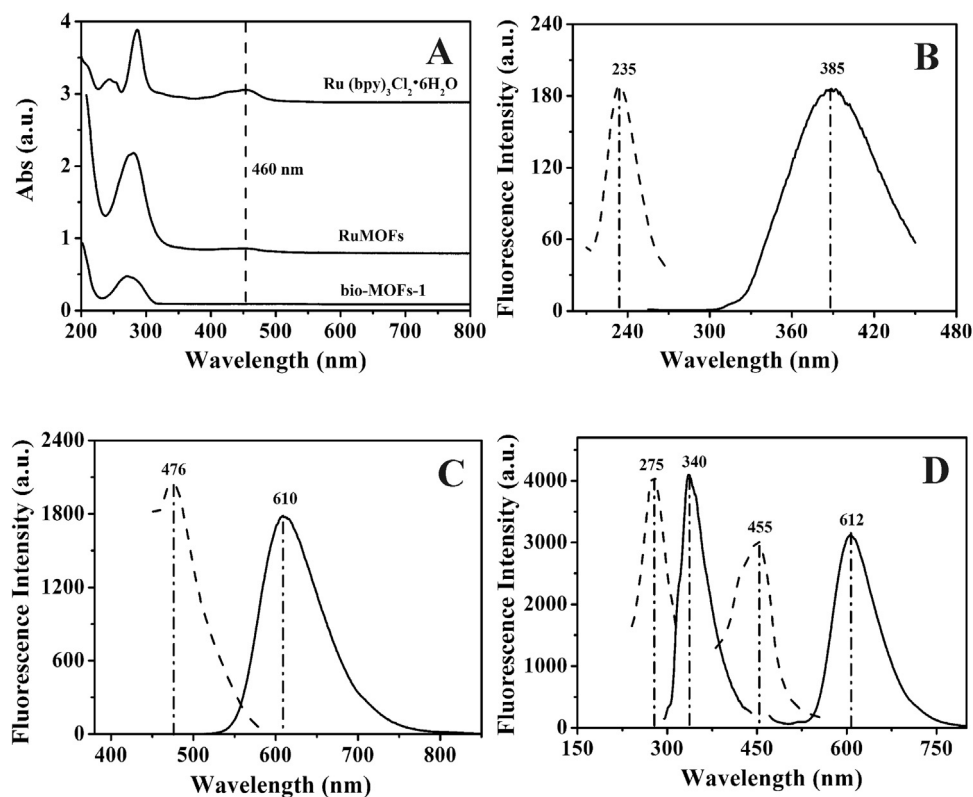
$$Ru(bpy)_3{}^{2+} \rightarrow Ru(bpy)_3{}^{3+} + e^- \tag{1}$$

Fig. 2. (A) UV-vis spectrum of bio-MOFs-1, Ru(bpy)$_3$Cl$_2$·6H$_2$O and RuMOFs; fluoresence excitation (−) and emission (−) spectra of (B) bio-MOFs-1, (C) Ru(bpy)$_3$Cl$_2$·6H$_2$O and (D) RuMOFs.
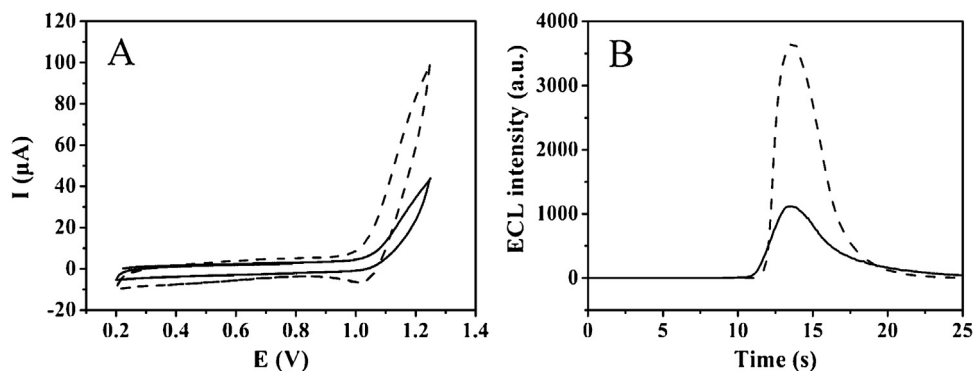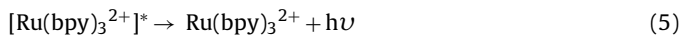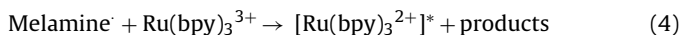

Fig. 3. (A) Cyclic voltammetry and (B) ECL-time curve for GCE modified with RuMOFs in the absence (−) and presence (−) of 1.0×10^{-7} M melamine at pH 7.0 (0.1 M PBS).

$$\text{Melamine} \rightarrow \text{Melamine}^{+\cdot} + e^- \tag{2}$$

$$\text{Melamine}^{+\cdot} \rightarrow \text{Melamine}^{\cdot} + H^+ \tag{3}$$

$$\text{Melamine}^{\cdot} + \text{Ru(bpy)}_3^{3+} \rightarrow [\text{Ru(bpy)}_3^{2+}]^* + \text{products} \tag{4}$$

$$[\text{Ru(bpy)}_3^{2+}]^* \rightarrow \text{Ru(bpy)}_3^{2+} + h\upsilon \tag{5}$$

3.5. Performance data for the ECL sensor

The ECL sensor was applied to the determination of melamine using the experimental conditions described above. The relative ECL intensity (ΔI) exhibited a linear response to the logarithmic melamine concentration in the range 10^{-10}–10^{-4} M (Fig. 6A); the correlation coefficient was 0.9918, the calibration curve $\Delta I = 933 \lg_{\text{melamine}} + 9091$; the detection limit is 3.8×10^{-11} M determined by the standard Method II of Loock [42]. Fig. 6B shows

the ECL emission of different concentrations of melamine on a RuMOFs modified GCE.

Next we investigated the stability of RuMOFs and the reproducibility of their use for determination of melamine, since these are important characteristics of a sensor. The relative standard deviation (RSD) for eight different electrodes constructed by the same procedure was 2.91%, and the RSD for ten successive measurements with the same sensor was 1.18% (Fig. 7), thus indicating good reproducibility. Additionally, sensor stability was assessed by determining melamine solutions after 7 days. The ECL intensity remained at 94.3-97.5% of its original response, thus showing good stability for this sensor for detecting melamine.

Selectivity experiments were also investigated with cyanuric acid and anhydrous phloroglucinol, which are structural analogs of melamine. These had little effect at a concentration of 1.0×10^{-5} M, and just resulted in an ECL intensity change of ±5% (Fig. 8).
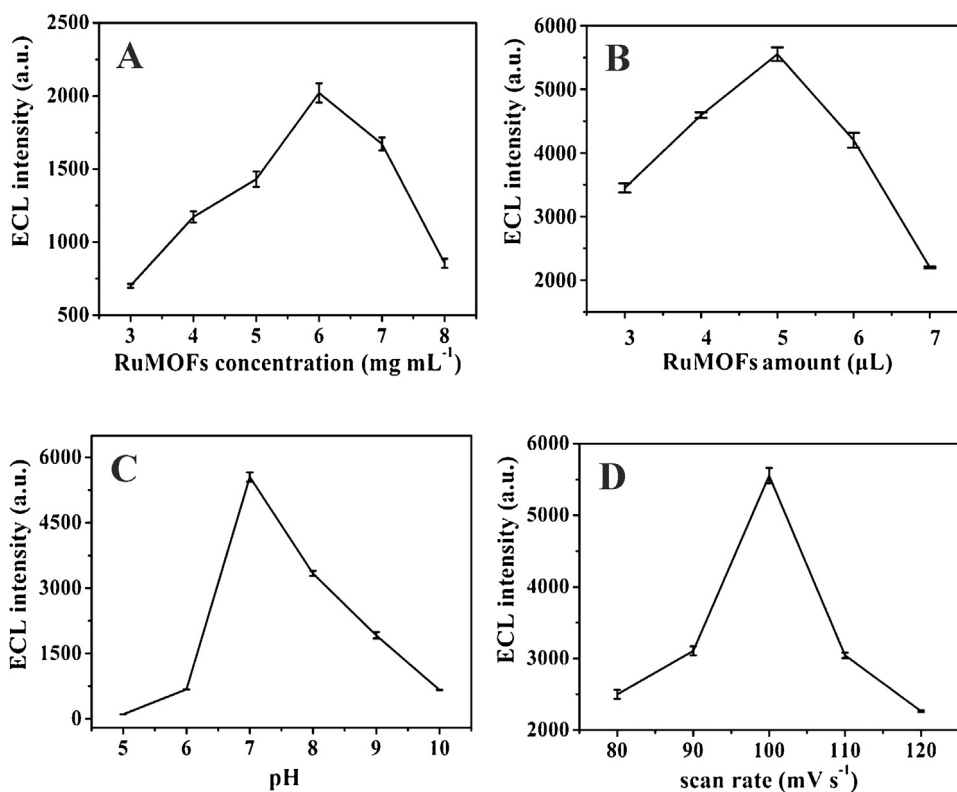
Fig. 4. Effects of (A) the concentration of RuMOFs, (B) the amount of RuMOFs, (C) pH and (D) scan rate on the ECL intensity for 1.0×10^{-5} M melamine in the potential scanning range from 0.2 to 1.25 V.

Table 1
Comparison of the methodology in the current paper with published electrochemical and electrochemiluminescence methods for the determination of melamine.

| Method	Modified	Linear range (M)	Detection limit (M)	Reference
Electrochemiluminescence	mSiO$_2$/Ru(bpy)$_3$ $^{2+}$/Nafion	$7.8 \times 10^{-9} \sim 5.0 \times 10^{-6}$	2.6×10^{-9}	[22]
Electrochemical	MOFs@XC-72-Nafion	$3.2 \times 10^{-7} \sim 7.9 \times 10^{-5}$	4.0×10^{-8}	[43]
Electrochemical	p-Mel-Glu/EPPG	$5.0 \times 10^{-7} \sim 1.5 \times 10^{-5}$	1.0×10^{-9}	[44]
Electrochemical	ZnO/AChE/Chitosan	$1.0 \times 10^{-9} \sim 2.0 \times 10^{-8}$	3.0×10^{-12}	[45]
Electrochemiluminescence	RuMOFs/nafion	$1.0 \times 10^{-10} \sim 1.0 \times 10^{-4}$	3.8×10^{-11}	This work
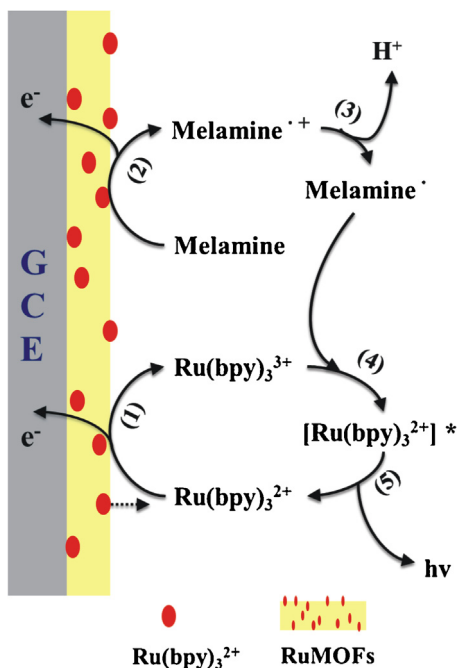
Fig. 5. Proposed chemical mechanism for reactions in the RuMOFs/melamine ECL system.

Some ions can increase the diffusibility and rate of oxidation of Ru(bpy)$_3$$^{2+}$ on the electrode leading to enhanced ECL intensity, whereas other ions could be involved in redox reactions at the electrode and reduce the efficiency of Ru(bpy)$_3$$^{2+}$. Tolerable concentration ratios for interference at the 5% level were 1000 fold (10^{-2} M) for Na$^+$, K$^+$, Cl$^-$, NH$_4$$^+$, 500 fold ($5.0 \times 10^{-2}$ M) for Ca^{2+}, Mg^{2+}, CO$_3$$^{2-}$, SO$_4$$^{2-}$, and 100 fold ($10^{-3}$ M) for ascorbic acid. Thus the Ru(bpy)$_3$$^{2+}$ − modified GCE electrode has practical applications for detecting melamine in food samples.

3.6. Comparison with other methods and modified electrodes for the determination of melamine

The analytical performance of this ECL sensor for detection of melamine was compared with that from other recent studies using electrochemical and electrochemiluminescence methods (Table 1). The ECL sensor in the present work exhibited a wider linear range and lower detection limit than was seen with other methods. RuMOFs are based on the anionic bio-MOFs-1 structure doped with the luminescent cationic Ru(bpy)$_3$$^{2+}$, which could be doped into the MOF framework or held within pores by strong electrostatic interaction. It high capacity of RuMOFs for Ru(bpy)$_3$$^{2+}$ provides high sensitivity, whilst the large surface area facilitates the reaction between Ru(bpy)$_3$$^{2+}$ and melamine.
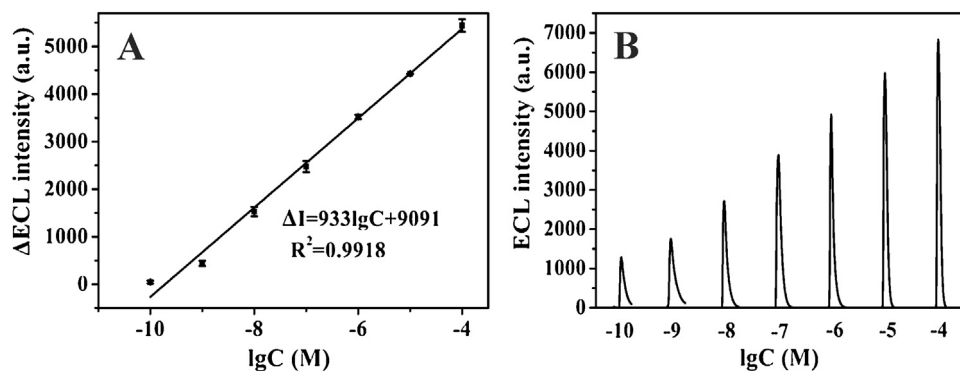
Fig. 6. (A) Calibration curve of ECL intensity versus logarithmic melamine concentration and (B) the ECL emission of different concentrations of melamine on a RuMOFs modified GCE at pH 7.0 (0.1 M PBS), the potential scanned from 0.2 to 1.25 V and scan rate was 100 mV s^{-1}.

Table 2
Analytical results obtained for the detection of melamine in food samples.

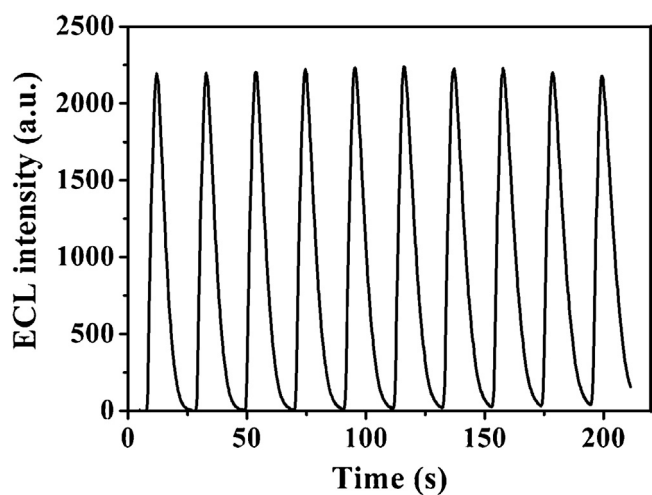| Sample	Added (M)	Detected (M)	Recovery (%)	RSD (%)
Milk	1.00×10^{-6}	$(1.01 \pm 0.03) \times 10^{-6}$	101 ± 3	3.01
	1.00×10^{-7}	$(1.02 \pm 0.01) \times 10^{-7}$	102 ± 1	1.14
	1.00×10^{-8}	$(0.99 \pm 0.02) \times 10^{-8}$	99 ± 12	1.54
Infant formula powder	1.00×10^{-6}	$(0.99 \pm 0.03) \times 10^{-6}$	99 ± 3	2.67
	1.00×10^{-7}	$(1.02 \pm 0.02) \times 10^{-7}$	102 ± 2	1.50
	1.00×10^{-8}	$(1.00 \pm 0.02) \times 10^{-8}$	100 ± 2	2.07

Fig. 7. ECL emission for 10 continuous cycles for 1.0×10^{-7} M melamine at pH 7.0 (0.1 M PBS); the potential scanned from 0.2 to 1.25 V and scan rate was 100 mV s^{-1}.

3.7. Application of the RuMOFs-based ECL sensor for determining melamine in food samples

In order to test the practicality of using the ECL sensor with actual food products, its application to the determination of melamine in milk and infant formula powder was investigated. Using the sample pretreatment methods described in Section 2.5, the recoveries of melamine from spiked milk samples were in the range 98–104%, and 95–103% for infant formula powder (Table 2). The good recoveries from these samples demonstrate the broader potential applicability of this RuMOFs ECL sensor for the determination of melamine in food samples.

4. Conclusions

We have constructed a sensitive and convenient electrochemiluminescence sensor based on RuMOFs for determination of melamine by immobilizing Ru(bpy)$_3$$^{2+}$ in the bio-MOF-1 framework. This sensor was stable to leaching, and its response to melamine was linear over a wide range of concentrations; it also had a low detection limit, and exhibited good reproducibility. The practical application of this ECL sensor was demonstrated by its successful use to determine melamine in milk and infant formula powder. This represents the first report of the fabrication of RuMOFs as an ECL sensor for the determination of an amide, and its excellent properties suggest the development of more applications.
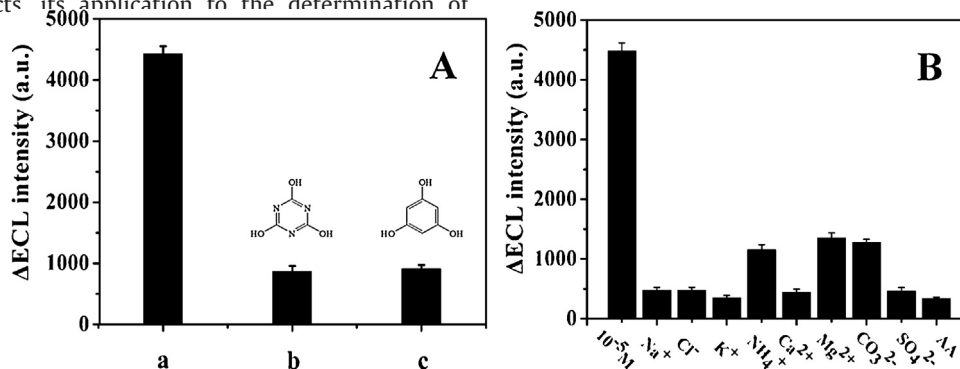

Fig. 8. (A) Selective tests for the ECL response of RuMOFs modified GCE to melamine (a), cyanuric acid (b) and anhydrous phloroglucinol (c), and (B) test for interference from various ions in the potential scanning ranged from 0.2 to 1.25 V and scan rate was 100 mV s^{-1}, the pH of all solutions were 7.0 (0.1 M PBS).

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.snb.2018.03.046.

References

[1] M. Eddaoudi, J. Kim, N. Rosi, D. Vodak, J. Wachter, M. O'Keeffe, O.M. Yaghi, Systematic design of pore size and functionality in isoreticular MOFs and their application in methane storage, Science 295 (2002) 469–472.

[2] H. Li, M. Eddaoudi, M. O'Keeffe, O.M. Yaghi, Design and synthesis of an exceptionally stable and highly porous metal-organic frameworks, Nature 402 (1999) 276–279.

[3] N.L. Rosi, J. Kim, M. Eddaoudi, B.L. Chen, M. O'Keeffe, O.M. Yaghi, Rod packings and metal-organic frameworks constructed from rod-shaped secondary building units, J. Am. Chem. Soc. 127 (2005) 1504–1518.

[4] M. Anbia, M. Faryadras, In situ Na·Cu$_3$(BTC)$_2$ and Li·Cu$_3$(BTC)$_2$ nanoporous MOFs synthesis for enhancing H$_2$ storage at ambient temperature, J. Nanostruct. Chem. 5 (2015) 357–364.

[5] I. Spanopoulos, C. Tsangarakis, E. Klontzas, E. Tylianakis, G. Froudakis, K. Adil, Y. Belmabkhout, M. Eddaoudi, P.N. Trikalitis, Reticular synthesis of HKUST-like tbo-MOFs with enhanced CH$_4$ storage, J. Am. Chem. Soc. 138 (2016) 1568–1574.

[6] Z.L. Wu, J. Dong, W.Y. Ni, B.W. Zhang, J.Z. Cui, B. Zhao, Unique chiral interpenetrating d-f heterometallic MOFs as luminescent sensors, Inorg. Chem. 54 (2015) 5266–5272.

[7] M.G. Campbell, S.F. Liu, T.M. Swager, M. Dincǎ, Chemiresistive sensor arrays from conductive 2D metal-organic frameworks, J. Am. Chem. Soc. 137 (2015) 13780–13783.

[8] X. Lian, B. Yan, A postsynthetic modified MOF hybrid as heterogeneous photocatalyst for α-phenethyl alcohol and reusable fluorescence sensor, Inorg. Chem. 55 (2016) 11831–11838.

[9] R. Lin, L. Ge, H. Diao, V. Rudolph, Z. Zhu, Ionic liquids as the MOFs/polymer interfacial binder for efficient membrane separation, ACS Appl. Mater. Interfaces 8 (2016) 32041–32049.

[10] X.H. Zhang, S.M. Xie, A.H. Duan, B.H. Wang, L.M. Yuan, Separation performance of MOFs Zn(ISN)$_2$·2H$_2$O as stationary phase for high-resolution GC, Chromatographia 76 (2013) 831–836.

[11] S.H. Doan, K.D. Nguyen, T.T. Nguyen, N.T.S. Phan, Direct arylation of benzoazoles with aldehydes utilizing metal-organic framework Fe$_3$O(BDC)$_3$ as a recyclable heterogeneous catalyst, RSC Adv. 7 (2017) 1423–1431.

[12] B. Tang, W.C. Song, E.C. Yang, X.J. Zhao, MOF-derived Ni-based nanocomposites as robust catalysts for chemoselective hydrogenation of functionalized nitro compounds, RSC Adv. 7 (2017) 1531–1539.

[13] M.M. Richte, Electrochemiluminescence (ECL), Chem. Rev. 104 (2004) 3003–3036.

[14] C.Y. Xiong, H.J. Wang, W.B. Liang, Y.L. Yuan, R. Yuan, Y.Q. Chai, Luminescence-functionalized metal-organic frameworks based on a Ruthenium(II) complex: a signal amplification strategy for electrogenerated chemiluminescence immunosensors, Chem. Eur. J. 21 (2015) 9825–9832.

[15] S. Das, S. Karmakar, D. Saha, S. Baitalik, A combined experimental and DFT/TD-DFT investigation of structural electronic, and cation-induced switching of photophysical properties of bimetallic Ru(II) and Os(II) complexes derived from imidazole-4,5-dicarboxylic acid and 2,2′-Bipyridine, Inorg. Chem. 52 (2013) 6860–6879.

[16] C.A. Kent, D. Liu, T.J. Meyer, W. Lin, Amplified luminescence quenching of phosphorescent metal-organic frameworks, J. Am. Chem. Soc 134 (2012) 3991–3994.

[17] C. Wang, W. Lin, Diffusion-controlled luminescence quenching in metal-organic frameworks, J. Am. Chem. Soc. 133 (2011) 4232–4235.

[18] X.Y. Li, X.C. Tan, J. Yan, Q. Hu, J.W. Wu, H. Zhang, X. Chen, A sensitive electrochemiluminescence folic acid sensor based on a 3D graphene/CdSeTe/Ru(bpy)$_3$$^{2+}$-doped silica nanocomposite modified electrode, Electrochim. Acta 187 (2016) 433–441.

[19] H. Wei, E. Wang, Solid-state electrochemiluminescence of tris(2,2′-bipyridyl) ruthenium, TrAC-Trends Anal. Chem. 27 (2008) 447–459.

[20] X.M. Fan, S.M. Wang, Z.J. Li, P. Liu, Y. Yu, L.L. Chang, K.B. Li, X.W. Zheng, Electrochemiluminescence sensor for the detection of tiapride hydrochloride with Chitosan-Ru(bpy)$_3$$^{2+}$-SiO$_2$NPs/MCNT/Nafion modifed electrode, J. Braz. Chem. Soc. 28 (2017) 277–284.

[21] W. Zhang, H.W. Xiong, M.M. Chen, X.H. Zhang, S.F. Wang, Surface-enhanced molecularly imprinted electrochemiluminescence sensor based on Ru@SiO$_2$ for ultrasensitive detection of fumonisin B1, Biosens. Bioelectron. 96 (2017) 55–61.

[22] H.M. Cao, X.Q. Hu, C.U. Hu, Y. Zhang, N.Q. Jia, A novel solid-state electrochemiluminescence sensor for melamine with Ru(bpy)$_3$$^{2+}$/mesoporous silica nanospheres/Nafion composite modified electrode, Biosens. Bioelectron. 41 (2013) 911–915.

[23] S. Lian, Z. Huang, Z. Lin, X. Chen, M. Oyama, X. Chen, A highly selective melamine sensor relying on intensified electrochemiluminescence of the silica nanoparticles doped with [Ru(bpy)$_3$]$^{2+}$/molecularly imprinted polymer modified electrode, Sens. Actuators B-Chem. 236 (2016) 614–620.

[24] World Health Organization Melamine and Cyanuric Acid: Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food; October 3, 2008.

[25] A. Kim, S.J. Barcelo, R.S. Williams, Z. Li, Melamine sensing in milk products by using surface enhanced Raman scattering, Anal. Chem. 84 (2012) 9303–9309.

[26] Z.J. Chen, X.M. Yan, Simultaneous determination of melamine and 5-hydroxymethylfurfural in milk by capillary electrophoresis with diode array detection, J. Agric. Food Chem. 57 (2009) 8742–8747.

[27] X. Jiang, F. Tian, F. Yang, X. Dou, J. Wang, Y. Song, Design and synthesis of a novel lanthanide fluorescent probe (Eu III −dtpa-(bis)melamine) and application in melamine detection in milk products, Sens. Actuators B-Chem. 238 (2017) 605–612.

[28] A.S. Yazdi, S.R. Yazdinezhad, T. Heidari, Determination of melamine in soil samples using surfactant-enhanced hollow fiber liquid phase microextraction followed by HPLC-UV using experimental design, J. Adv. Res. 6 (2015) 957–966.

[29] C. Fu, C. Liu, Y. Li, Y. Guo, F. Luo, P. Wang, L. Guo, B. Qiu, Z. Lin, Homogeneous electrochemical biosensor for melamine based on DNA triplex structure and exonuclease III-assisted recycling amplification, Anal. Chem. 88 (2016) 10176–10182.

[30] Q. Cao, H. Zhao, Y.J. He, X.J. Li, L.X. Zeng, N. Ding, J. Wang, J. Yang, G.W. Wang, Hydrogen-bonding-induced colorimetric detection of melamine by nonaggregation-based Au-NPs as a probe, Biosens. Bioelectron. 25 (2010) 2680–2685.

[31] J.Y. An, S.J. Geib, N.L. Rosi, Cation-triggered drug release from a porous zinc-adeninate metal-organic framework, J. Am. Chem. Soc. 131 (2009) 8376–8377.

[32] J. An, C.M. Shade, D.A. Chengelis-Czegan, S.P. Petoud, N.L. Rosi, Zinc-adeninate metal-organic framework for aqueous encapsulation and sensitization of near-infrared and visible emitting lanthanide cations, J. Am. Chem. Soc. 133 (2011) 1220–1223.

[33] J.Y. An, N.L. Rosi, Tuning MOF CO$_2$ adsorption properties via cation exchange, J. Am. Chem. Soc. 132 (2010) 5578–5579.

[34] X. Lin, F. Luo, L. Zheng, G. Gao, Y. Chi, Fast, sensitive, and selective ion-triggered disassembly and release based on tris(bipyridine) ruthenium(II)-functionalized metal-organic frameworks, Anal. Chem. 87 (2015) 4864–4870.

[35] F. Liu, Y. Gao, W. Li, J. Shao, Determination of melamine based on electrochemiluminescence of Ru(bpy)$_3$$^{2+}$ at chemically converted graphene-modified glassy carbon electrode, RSC Adv. 4 (2014) 34003–34007.

[36] Q. Xu, H.P. Wei, S. Du, H.B. Li, Z.P. Ji, X.Y. Hu, Detection of subnanomolar melamine based on electrochemical accumulation coupled with enzyme colorimetric assay, J. Agric. Food Chem. 61 (2013) 1810–1817.

[37] L. Zhou, J. Huang, L. Yang, L. Li, T. You, Enhanced electrochemiluminescence based on Ru(bpy)$_3$$^{2+}$-doped silica nanoparticles and graphene composite for analysis of melamine in milk, Anal. Chim. Acta 824 (2014) 57–63.

[38] M. Zorzi, P. Pastore, F. Magno, A single calibration graph for the direct determination of ascorbic and dehydroascorbic acids by electrogenerated luminescence based on Ru(bpy)$_3$$^{2+}$ in aqueous solution, Anal. Chem. 72 (2000) 4934–4939.

[39] F. Liu, X. Yang, S. Sun, Determination of melamine based on electrochemiluminescence of Ru(bpy)$_3$$^{2+}$ at bare and single-wall carbon nanotube modified glassy carbon electrodes, Analyst 136 (2011) 374–378.

[40] J.K. Leland, M.J. Powell, Electrogenerated chemiluminescence Part 54. Electrogenerated chemiluminescence of ruthenium(II) 4,4'-diphenyl -2,2'-bipyridine and ruthenium(II) 4,7-diphenyl-1, 10-phenanthroline systems in aqueous and acetonitrile solutions, J. Electroanal. Chem. 318 (1991) 91–99.

[41] E.M. Gross, J.D. Anderson, A.F. Slaterbeck, S. Thayumanavan, S. Barlow, Y. Zhang, S.R. Marder, H.K. Hall, M.F. Nabor, J.F. Wang, E.A. Mash, N.R. Armstrong, R.M. Wightman, Electrogenerated chemiluminescence from derivatives of aluminum quinolate and quinacridones: cross-reactions with triarylamines lead to singlet emission through triplet-triplet annihilation pathways, J. Am. Chem. Soc. 122 (2000) 4972–4979.

[42] H.P. Loock, P.D. Wentzell, Detection limits of chemical sensors: applications and misapplications, Sens. Actuators B 173 (2012) 157–163.

[43] W. Zhang, G. Xu, R. Liu, J. Chen, X. Li, Y. Zhang, Y. Zhang, Novel MOFs@XC-72-Nafion nanohybrid modified glassy carbon electrode for the sensitive determination of melamine, Electrochim. Acta 211 (2016) 689–696.

[44] M. Raj, R.N. Goyal, A poly-(melamine)/poly-(glutamic acid) based electrochemical sensor for sensitive determination of 2-Thioxanthine, Sens. Actuators B-Chem. 250 (2017) 552–562.
[45] M. Ezhilan, M.B. Gumpu, B.L. Ramachandra, N. Nesakumar, K.J. Babu, U.M. Krishnan, J.B.B. Rayappan, Design and development of electrochemical biosensor for the simultaneous detection of melamine and urea in adulterated milk samples, Sens. Actuators B-Chem. 238 (2017) 1283–1292.

Biographies

DeFen Feng is currently an MS student, School of Chemistry and Chemical Engineering at Guangxi University for Nationalities under the direction of Professor Xuecai Tan. Her research interests focus on electroanalytical chemistry and biosensors.

Yeyu Wu is a teacher of Guangxi University of Nationalities, China. Her main research interests are sample pretreatment techniques, chromatographic techniques and metal organic framework materials in analytical chemistry.

Xuecai Tan is currently a Professor of Chemistry in the School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, China. He was born in Badong County, Hubei Province, China, in 1965. He received his B.Sc. in 1988 from Hubei University, his M.S. in 1995 from Beijing Normal University and his Ph.D. in 2005 from Sun Yat-sen University. He worked at Hubei University for Nationalities from 1988 to 2000 and then moved to the Guangxi University for Nationalities in august 2000. His current research interests include electroanalytical chemistry, bio-electrochemistry, sensors, photoelectrochemistry, food safety and pharmaceutical analytical chemistry. He has published over 80 papers in peer-reviewed journals.

Quanyou Chen is currently an MS student, School of Chemistry and Chemical Engineering at Guangxi University for Nationalities under the direction of Professor Xuecai Tan. His research interests focus on electroanalytical chemistry and biosensors.

Jun Yan is a teacher of Guangxi University of Nationalities, China. He received a Ph.D. degree from Central South University, China. His research work has been mainly focused on chemometrics.

Min Liu is a teacher of Guangxi University of Nationalities, China. She received her M.S. degrees from Guangxi University of Nationalities. Her main research interests are sample pretreatment techniques and chromatographic techniques.

Chenhao Ai is currently an MS student, School of Chemistry and Chemical Engineering at Guangxi University for Nationalities under the direction of Professor Xuecai Tan. Her research interests focus on electroanalytical chemistry and biosensors.

Yanni Luo is currently an MS student, School of Chemistry and Chemical Engineering at Guangxi University for Nationalities under the direction of Professor Xuecai Tan. Her research interests focus on electroanalytical chemistry and biosensors.

Fangkai Du is a teacher of Guangxi University of Nationalities. He received his Ph.D. degree and he was a postdoctor in South China University of Technology. His research interests focus on fluorescent nanomaterials, bioanalysis, biosensing and drug delivery.

Shaogang Liu is a teacher of Guangxi University of Nationalities. He received his Ph.D. degree in Tongji University. His research work has been mainly focused on environmental analysis.

Heyou Han was born in Anhui Province, China, in 1962. He received his Ph.D. degree in Wuhan University in 2000 and he was a postdoctor in Jeckson State University (America) from 2000 to 2004. He has been a Professor of Huazhong Agricultural University since 2004. He has published over 100 papers in international journals. His research interests focus on functionalized nanomaterials for bioanalysis, food safety and energy applications.