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ARTICLE TYPE

A solid-state electrochemiluminescent sensor based on C₆₀/graphite-like carbon nitride nanosheet for detecting melamine

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Abstract: C₆₀/graphite-like carbon nitride nanosheet (C₆₀/g-C₃N₄ NS) hybrids were prepared by a simple π - π interaction, and used to construct a solid state electrochemiluminescence (ECL) sensor. Based on the fact that melamine could efficiently quench the ECL intensity of g-C₃N₄ NS, such a sensor achieved the detection of melamine. The possible quenching mechanisms were discussed. Due to the combination of the excellent ECL behavior of g-C₃N₄ NS with the outstanding electronic conductivity of C₆₀, the prepared sensor exhibited a response for melamine with a wide linear range of $5.0 \times 10^{-13} \sim 2.7 \times 10^{-11}$ M and $2.7 \times 10^{-11} \sim 1.9 \times 10^{-8}$ M. The detection limit was 1.3×10^{-13} M. In this work, the linear range of melamine was improved by six order of magnitude and the detection limit as low as sub-picomolar. Such a construction strategy provided a simple, sensitive, low-cost and green detection method for melamine.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, C₃H₆N₆) is a nitrogen-containing chemical, which is commonly used in the manufacture of filters, plastics, coatings, glues, laminates, and adhesives.¹ Unfortunately, owing to its high nitrogen level (as 66% nitrogen by mass) and low cost, melamine has been illegally adulterated in pet foods, infant formulas, milk and animal feeds to boost their total nitrogen concentration.² As is well-known, over intake of melamine above the safety limit (2.5 ppm for food and 1 ppm for infant formula in the United States and European Union) may cause various pathological diseases, including renal failure, carcinoma of urinary bladder, kidney stone, liver necrosis and even deaths.^{3,4} Therefore, the rapid and sensitive detection methods for melamine are highly desirable. Various technologies have been employed for the determination of melamine, including high-performance liquid chromatography (HPLC),⁵ enzyme-linked immunosorbent assay (ELISA),⁶ electrochemical methods,⁷ liquid chromatography/mass spectrometry (HPLC-MS),⁸ colorimetric detection,³ surface-enhanced raman spectroscopy (SERS),⁹ and fluoroimmunoassay.¹⁰ Among them, electrochemiluminescence (ECL) sensing technology has received much attention and become a valuable detection method because such a technique is characterized by high sensitivity and selectivity, wide dynamic response range, controllability and low back ground noise.¹¹ CdTe QDs, luminol and Ru(bpy)₃²⁺ have been used as luminophore for constructing the ECL sensors of melamine.¹²⁻¹⁴ Compared to solution-state ECL sensing, solid-state ECL sensing with the immobilization of the luminophore on an electrode surface would offer several advantages, such as simplifying experimental design, reducing the consumption of ECL reagent and enhancing the ECL signal.¹⁵ There are only a few solid-state ECL sensors reported to detect melamine such as Ru(bpy)₃²⁺ ECL¹⁶ and Ru(bpy)₃²⁺@SiO₂ ECL¹⁷ with a low detection limit. However, the toxicity, expensiveness, or tedious

synthetic steps of aforementioned luminophores would restrict the wide application of the sensors. Thus, it is very desirable to develop a novel, simple, low-cost, and environmentally friendly solid-state ECL sensor for melamine detection.

Since graphite-like carbon nitride (g-C₃N₄) was reported as a new type ECL luminophore, various morphologies of g-C₃N₄ were synthesized, such as g-C₃N₄ nanofibers,¹⁸ porous g-C₃N₄,¹⁹ g-C₃N₄ nanorods²⁰ and g-C₃N₄ nanosheet (g-C₃N₄ NS).²¹ Among these structures, g-C₃N₄ NS attracted extensive attention since it not only could be prepared via a simple and green liquid exfoliation method from bulk g-C₃N₄ in water, but also exhibited a stronger ECL activity, better water-dispersibility and larger surface area compared to bulk g-C₃N₄.^{22,23} Furthermore, g-C₃N₄ NS can be easily immobilized onto the electrode only by dripping the dispersion of g-C₃N₄ NS or corresponding composites onto the surface of an electrode, thus achieving a solid-state ECL sensor. Additionally, g-C₃N₄ NS can be easily functionalized to improve the properties of g-C₃N₄ NS. In our previous work, polyaniline,²⁴ phenoxy dextran,²⁵ 3,4,9,10-perylenetetracarboxylic acid²⁶ have been used to functionalize g-C₃N₄ NS to make hybrids more suitable for the applications in sensing. C₆₀ can enhance the ECL intensity of peroxydisulfate (S₂O₈²⁻) system due to its improvement in the electron and charge transfer.²⁷ Thus, the functionalization of g-C₃N₄ NS with C₆₀ would further expand the application of g-C₃N₄ in sensor field. However, the difference in water solubility between C₆₀ and g-C₃N₄ NS make their homogeneous co-interaction difficult.²⁸ Fortunately, such a limitation would be overcome by preparing the water-soluble C₆₀ via a phase transfer method,²⁹ which makes the homogeneous co-interaction between C₆₀ and g-C₃N₄ NS possible.

Inspired by above observation, in this work, the hybrids of C₆₀/g-C₃N₄ NS were prepared by a simple π - π stacking interactions and used to construct an ECL sensor. Based on the fact that melamine could quench the ECL signal of g-C₃N₄ NS/S₂O₈²⁻

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system, the prepared ECL sensor achieved the quantitative detection of melamine with a wide linear range and low detection limit. Furthermore, the impossible mechanisms of the quenching effect were also discussed. The integration of C_{60} and $g-C_3N_4$ NS would provide a desirable strategy to detect melamine due to its simpleness, inexpensiveness, environmental friendliness and high sensitivity.

2. Experimental

2.1. Reagents and chemical

Melamine (2,4,6-triamino-1,3,5-triazine, 99%) was purchased from Aladdin Ltd. (Shanghai, China). Potassium persulfate ($K_2S_2O_8$) was provided by Shanghai Chemical Reagent Co. Shanghai, China). Fullerene (C_{60}) (99.5%) was received from YongXin Chemical Reagent Co. (Puyang, China). Phosphate buffer solutions (PBS) with various pH were prepared with the stock solution of 0.10 M KH_2PO_4 and Na_2HPO_4 . 0.10 M KCl was used as a supporting electrolyte. Doubly distilled water was used throughout this experiment.

2.2. Apparatus

The ECL emission was monitored by a model MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) with the voltage set at 600 V and the potential scan was from -1.3 V to 0 V for the determination. The UV-vis absorption spectra were recorded on a UV-2450 UV-vis spectrophotometer (Shimadzu, Japan). The FT-IR spectra were performed on Nicolet IS10 Instruments. Scanning electron microscopy (SEM) was conducted by a Hitachi Scanning Electron microscope (SEM, Hitachi, S-4800, Japan). All electrochemical experiments were equipped with a classical three-electrode system containing an Ag/AgCl as the reference electrode, a platinum wire as the counter electrode and a modified glass carbon electrode (GCE, $\Phi = 4.0$ mm) as the working electrode. All experiments were carried out at room temperature.

2.3. Synthesis of $g-C_3N_4$ NS

The $g-C_3N_4$ NS was synthesized according to the literature.²¹ Firstly, the bulk $g-C_3N_4$ was obtained in an alumina crucible with a cover by heating melamine (20.0 g) at 600 °C for 2 h with heating speed of 3 °C min^{-1} . Then, the $g-C_3N_4$ NS was prepared by liquid exfoliation of as-prepared 250 mg bulk $g-C_3N_4$ in 250 mL doubly distilled water for 10 h. Ultimately, the resultant was filtered out and dried to get the powder at room temperature.

2.4. Preparation of $C_{60}/g-C_3N_4$ NS

The $C_{60}/g-C_3N_4$ NS hybrids were prepared through a sonochemical approach. Briefly, water-soluble C_{60} was firstly obtained *via* a phase transfer method. Then, 4.0 mL deionized water was added to 4.0 mL C_{60} -toluene solution (1 mg mL^{-1}). With the aid of ultrasonic agitation, toluene in the solution would completely volatilize and C_{60} would be transferred to the water phase. The prepared $g-C_3N_4$ NS (0.010 g) was added to above solution, followed by ultrasound for 10 h. The resultant $C_{60}/g-C_3N_4$ NS hybrids were centrifuged (12000 rpm, 10 min) and washed several times with ethanol and distilled water, and finally dispersed in doubly distilled water. The preparation of the hybrids is illustrated in Scheme 1A.

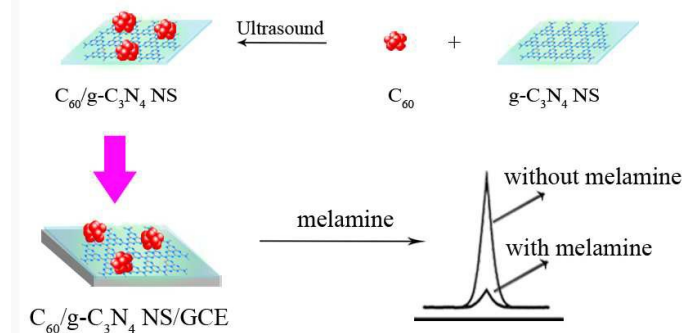
2.5. Construction of ECL sensor

Prior to the fabrication, the GCE was polished with 0.3 and 0.05 μm alumina slurry, and then washed ultrasonically in water and alcohol, respectively. 10 μL of the suspension of $C_{60}/g-C_3N_4$ NS was dropped onto the surface of pretreated GCE and dried at

the room temperature to achieve the sensor $C_{60}/g-C_3N_4$ NS/GCE (as shown in Scheme 1A). For a comparison, $g-C_3N_4$ NS/GCE was also prepared using the similar procedure of $C_{60}/g-C_3N_4$ NS/GCE by replacing $C_{60}/g-C_3N_4$ NS with $g-C_3N_4$ NS.

2.6. Measurement procedure

The ECL behavior of the sensor was investigated in PBS (3.0 mL, 0.10 M) containing 0.010 M $K_2S_2O_8$. The voltage of the photomultiplier tube (PMT) was set at 600 V and the applied potential was from -1.3 V to 0 V (vs. Ag/AgCl) with a scan rate of 100 $mV s^{-1}$. With increasing melamine concentration, the ECL signal decreased. Therefore, the changes of ECL intensity ($\Delta I = I_0 - I_t$) directly reflected the concentration changes of melamine. Here, I_0 and I_t are the ECL intensity without and with melamine, respectively.



Scheme 1. Schematic illustration of preparing $C_{60}/g-C_3N_4$ NS and fabricating procedures of proposed ECL sensor.

3. Results and discussion

3.1. Characterization of the materials

The morphologies of C_{60} , $g-C_3N_4$ NS and $C_{60}/g-C_3N_4$ NS hybrids were monitored by SEM. As shown in Fig. 1A, the SEM image exhibited the globular structures of C_{60} . Fig. 1B illustrates the layered structure of $g-C_3N_4$ NS. For $C_{60}/g-C_3N_4$ NS (Fig. 1C), globular C_{60} was evenly adhered to the layered structure of $g-C_3N_4$ NS, suggesting the successful preparation of $C_{60}/g-C_3N_4$ NS hybrids.

The UV-visible absorption spectra of C_{60} , $g-C_3N_4$ NS and $C_{60}/g-C_3N_4$ NS hybrids provide more effective information on the successful assembly of C_{60} onto the $g-C_3N_4$ NS (Fig. S1A, ESI†). For C_{60} (Fig. S1A, curve a), three strong absorption peaks ascribed to the dipole-allowed transitions in pristine C_{60} were observed at 206, 289, and 370 nm, respectively, which was in agreement with the literature.³⁰ The UV-vis absorption spectrum of $g-C_3N_4$ NS (Fig. S1A, curve b) showed a strong peak at 319 nm, which is in line with the literature.³¹ The characteristic absorption peak of $g-C_3N_4$ NS at 319 nm red shifted to 324 nm in the absorption spectrum of $C_{60}/g-C_3N_4$ NS (Fig. S1A, curve c), indicating the π - π interactions between C_{60} and $g-C_3N_4$ NS. This result indicated that $C_{60}/g-C_3N_4$ NS hybrids were successfully prepared.

FT-IR spectra were used to confirm the combination of C_{60} with $g-C_3N_4$ NS, and the results are shown in Fig. S1B, ESI†. As seen from Fig. S1B curve a, two characteristic bands of C_{60} were observed at 525 and 575 cm^{-1} , respectively, which consistently matched the literature.³² For $g-C_3N_4$ NS (Fig. S1B, curve b), the sharp peak at around 810 cm^{-1} was contributed to the heptazine ring system.²¹ The bands between 1000 cm^{-1} and 1800 cm^{-1} were assigned to the stretching vibration of connected units of C-NH-C (partial condensation) or C-N(-C)-C (full condensation). The peak between 3000 cm^{-1} and 3600 cm^{-1} was related to the N-H

stretching. When C_{60} was stacked onto $g-C_3N_4$ NS (Fig. S1B, curve c), $C_{60}/g-C_3N_4$ NS presented characteristic peaks of both $g-C_3N_4$ NS and C_{60} , suggesting the successful preparation of $C_{60}/g-C_3N_4$ NS hybrids.

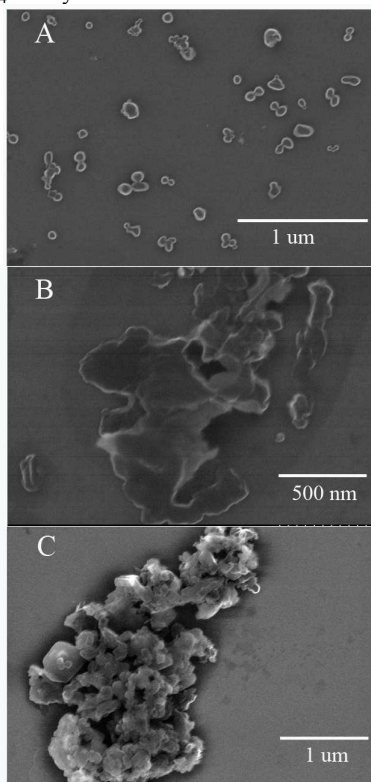


Fig. 1 SEM images of (A) C_{60} , (B) $g-C_3N_4$ NS, and (C) $C_{60}/g-C_3N_4$ NS.

3.2. ECL behavior of $g-C_3N_4$ NS and $C_{60}/g-C_3N_4$ NS

The comparison of ECL intensity between $g-C_3N_4$ NS/GCE and $C_{60}/g-C_3N_4$ NS/GCE was performed. As shown in Fig. S2 ESI†, the ECL intensity from $C_{60}/g-C_3N_4$ NS modified GCE was obviously larger than that from alone $g-C_3N_4$ NS modified GCE. The reasonable explanation may be as following. C_{60} would be beneficial to the improvement in ECL intensity of $g-C_3N_4$ NS with peroxydisulfate ($S_2O_8^{2-}$) as the coreactant. Besides, such a non-covalent preparation method for the hybrids would be an effective strategy for enhancing the chemical properties of carbon nanomaterials while their structure and properties could be well retained.³³ Thus, the $C_{60}/g-C_3N_4$ NS hybrids were very promising for the construction of the ECL sensor.

3.3. Detection of melamine with the sensor

Before performing the detection of melamine with the sensor, the experimental parameters concluding the pH of PBS, the concentration of $K_2S_2O_8$ and the scan rate were optimized and the results are shown in Fig. S3, ESI†. Under the optimal experimental conditions, the ECL response of the sensor towards the melamine was investigated. As shown in Fig. 2A, with the increase in the concentrations of melamine, the ECL peak intensity decreased. A good linear relationship between ΔI and the logarithmic value of melamine concentration ($\lg c$) was obtained in two ranges, from 5.0×10^{-13} M to 2.7×10^{-11} M and 2.7×10^{-11} M to 1.9×10^{-8} M, as shown in Fig. 2B. The linearization equations were $\Delta I = 4198.1 \lg c + 52210.2$ ($R = 0.990$) and $\Delta I = 689.0 \lg c + 14639.0$ ($R = 0.992$), respectively. Here, ΔI is the change of ECL intensity (ΔI), c is the concentration of melamine,

and R is the correlation coefficient of the linear fitting curve. A relatively low detection limit (LOD) of 1.3×10^{-13} M was obtained. The performances of the sensor in this work were compared with other melamine sensors previously reported and the results are shown in Table S1. As seen, although a solution-state based ECL sensor³⁴ showed a lower detection limit as compared to our sensor, it would lead to the consumption of a large amount of expensive ECL reagent and environmental pollution. A solid-state ECL sensor¹⁷ exhibited a similar linear range as our sensor, but the toxicity, expensiveness, or tedious synthetic steps of the sensor would restrict its wide application. Our sensor exhibits a broader linear range with lower detection limit owing to the combination of the excellent ECL behavior of $g-C_3N_4$ NS and the outstanding electronic conductivity of C_{60} comparing to the previous sensors (Table S1).

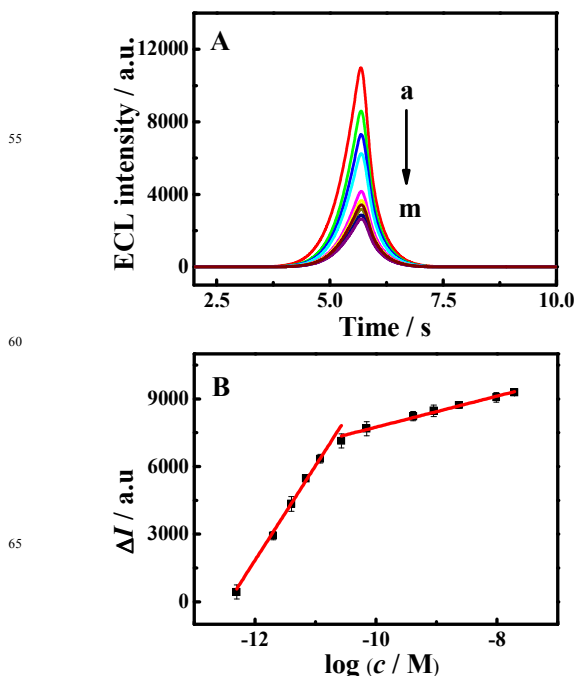
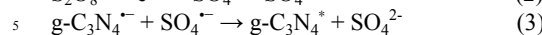
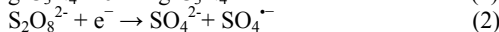
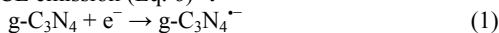


Fig. 2 (A) ECL response of the sensor in 0.10 M PBS (pH 8.0) containing 0.010 M $K_2S_2O_8$ for different concentrations of melamine: (a) 0, (b) 5.0×10^{-13} , (c) 2.0×10^{-12} , (d) 4.0×10^{-12} , (e) 7.0×10^{-12} , (f) 1.2×10^{-11} , (g) 2.7×10^{-11} , (h) 7.0×10^{-11} , (i) 4.1×10^{-10} , (j) 9.0×10^{-10} , (k) 2.3×10^{-9} , (l) 9.7×10^{-9} , and (m) 1.9×10^{-8} M. (B) The calibration curve for melamine detection.

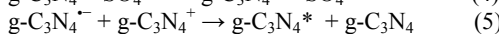
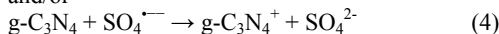
3.4. Mechanism of the ECL quenching of $C_{60}/g-C_3N_4$ NS by melamine

In the presence of $S_2O_8^{2-}$ as coreactant, $g-C_3N_4$ NS gives a strong ECL emission with onset and maximum potential at -0.95 and -1.27 V, respectively. Based on this fact, the applied potential from -1.3 V to 0 V (vs. Ag/AgCl) was set when performing the ECL measurements. The possible ECL emission mechanisms of $g-C_3N_4$ NS/ $S_2O_8^{2-}$ system were proposed and illustrated in Scheme 2.³⁷ Firstly, $g-C_3N_4$ was reduced to $g-C_3N_4^{\cdot-}$ (Eq. 1). Meanwhile, $S_2O_8^{2-}$ was reduced to the strong oxidant $SO_4^{\cdot-}$ (Eq. 2), which reacts with $g-C_3N_4^{\cdot-}$ to form the excited state $g-C_3N_4^*$ (Eq. 3). Finally, an intense emission is obtained when $g-C_3N_4^*$ decays back to the ground state $g-C_3N_4$ (Eq. 6). The possible mechanisms were shown in scheme 2A. Another possible way to generate $g-C_3N_4^*$ is illustrated in scheme 2B. $g-C_3N_4$ and $S_2O_8^{2-}$ was reduced to $g-C_3N_4^{\cdot-}$ (Eq. 1) and the strong oxidant $SO_4^{\cdot-}$ (Eq. 2), respectively. Since $SO_4^{\cdot-}$ is strong oxidant, it may oxidize $g-C_3N_4$ to $g-C_3N_4^+$ (Eq. 4), and $g-C_3N_4^+$ further

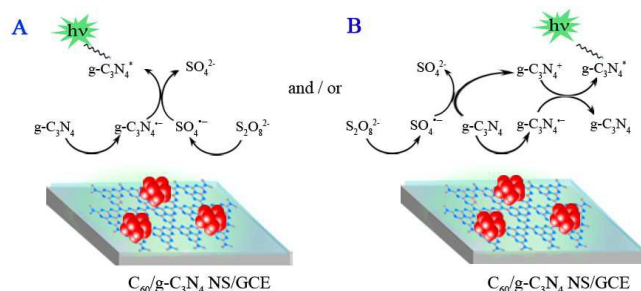
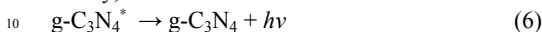
reacts with $g\text{-C}_3\text{N}_4^-$ to generate $g\text{-C}_3\text{N}_4^*$ (Eq. 5), then giving the ECL emission (Eq. 6)³⁷.



and/or



finally,



Scheme 2. Two possible ECL emission mechanisms of $g\text{-C}_3\text{N}_4$ NS/ $\text{S}_2\text{O}_8^{2-}$ system. (A) One possible ECL emission mechanisms (Eq.1, 2, 3 and 6) (B) another possible mechanisms (Eq.1, 2, 3 and 6)

To investigate the possible ECL inhibition mechanism of melamine to $g\text{-C}_3\text{N}_4$ NS/ $\text{S}_2\text{O}_8^{2-}$ system, on the one hand, UV–visible absorption spectra were tested. As shown in Fig. 3A, the UV–vis spectrum of $\text{C}_{60}/g\text{-C}_3\text{N}_4$ NS/ $\text{S}_2\text{O}_8^{2-}$ –melamine system was the simple sum of the individuals of $\text{C}_{60}/g\text{-C}_3\text{N}_4$ NS, $\text{S}_2\text{O}_8^{2-}$ and melamine. Such a fact indicated that no new compounds were produced when simple mixing $\text{C}_{60}/g\text{-C}_3\text{N}_4$ NS, $\text{S}_2\text{O}_8^{2-}$ and melamine. On the other hand, in order to investigate whether the ECL quenching of $g\text{-C}_3\text{N}_4$ NS/ $\text{S}_2\text{O}_8^{2-}$ system by melamine was resulted from the consumption of intermediate species of $g\text{-C}_3\text{N}_4$ NS by melamine, thus inhibiting the formation pathway of excited-state $g\text{-C}_3\text{N}_4$ NS, the ECL behaviors of $g\text{-C}_3\text{N}_4$ NS modified GCE were investigated in PBS containing melamine but without $\text{K}_2\text{S}_2\text{O}_8$. As seen in Fig. 3B, the ECL signal at $g\text{-C}_3\text{N}_4$ NS/GCE decreased with increasing the amount of melamine in absence of $\text{K}_2\text{S}_2\text{O}_8$. As comparison, $\text{C}_{60}/g\text{-C}_3\text{N}_4$ NS modified GCE with C_{60} as ECL signal enhancer was also studied in PBS containing melamine but without $\text{K}_2\text{S}_2\text{O}_8$. It was found that the similar results were observed. Namely, ECL signal decreased upon the addition of melamine in absence of $\text{K}_2\text{S}_2\text{O}_8$ (Fig. 3C). Such a fact suggested that the decreased ECL intensity would be related to the interaction between melamine or the electrochemical reaction product of melamine and the intermediate species of $g\text{-C}_3\text{N}_4$ NS. Similar ECL inhibition mechanisms have been reported. For example, the oxidation product of dopamine would annihilate $\text{Et}_3\text{N}^{\cdot}$ radical produced in the ECL processes of $g\text{-C}_3\text{N}_4$ nanosheets/ Et_3N system³⁸ and the oxidation product of methylene blue will react with 2-(dibutylamino)ethanol radical (DBAE $^{\cdot}$)³⁹, leading to a decrease in ECL signal. In our system, melamine or the electrochemical reaction product of melamine would react with the intermediate species produced in the ECL processes of $g\text{-C}_3\text{N}_4$ NS/ $\text{S}_2\text{O}_8^{2-}$. The more melamine was added into the solution, the more intermediate species of $g\text{-C}_3\text{N}_4$ was consumed, thus resulting in a decrease in the ECL intensity of $g\text{-C}_3\text{N}_4$ NS/ $\text{S}_2\text{O}_8^{2-}$ system.

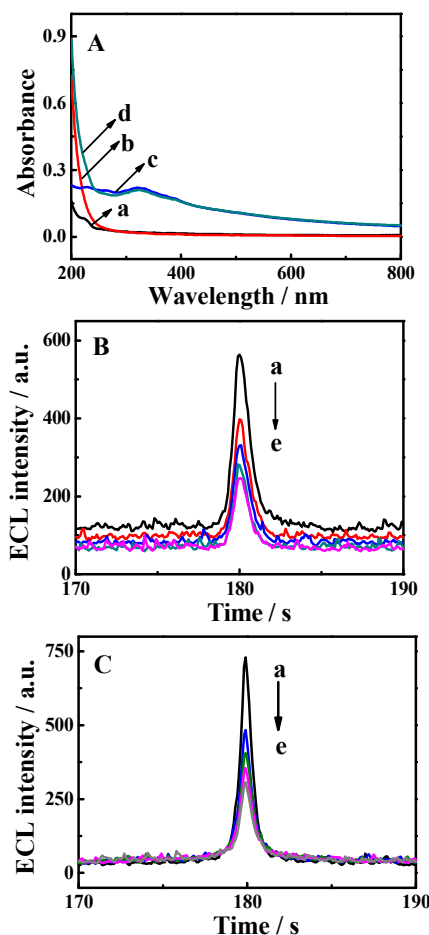


Fig. 3 (A) UV–visible absorption spectra of (a) 1.2×10^{-6} M melamine, (b) 7.5×10^{-5} M $\text{K}_2\text{S}_2\text{O}_8$, (c) 0.01 mg mL^{-1} $\text{C}_{60}/g\text{-C}_3\text{N}_4$ NS and (d) the mixture of (a), (b) and (c). The ECL response of (B) $g\text{-C}_3\text{N}_4$ NS/GCE and (C) $\text{C}_{60}/g\text{-C}_3\text{N}_4$ NS/GCE to different concentration melamine in 0.10 M PBS (pH 8.0) without $\text{K}_2\text{S}_2\text{O}_8$: (a) 0, (b) 9.0×10^{-12} , (c) 7.0×10^{-11} , (d) 9.0×10^{-10} , and (e) 3.9×10^{-9} M.

3.5. Selectivity and stability of the proposed sensor

The selectivity of the sensor was evaluated by adding the 65 potential interfering substances in detection cell, including 1.0×10^{-8} M magnesium ion, glucose, lactose, ascorbic acid and uric acid. As shown in Fig. 4A, compared with the higher signal change for the target melamine, a negligible signal change from above interferences was observed, suggesting an acceptable selectivity 70 of the sensor.

Fig. 4B displays the ECL signal of the sensor for 8 cycles of continuous potential scans between -1.3 V and 0 V (vs. SCE) in 0.10 M PBS containing 0.010 M $\text{K}_2\text{S}_2\text{O}_8$. The stable ECL signals were obtained with relative standard deviations (RSD) of 1.70% 75 for 3.3×10^{-9} M melamine. On the other hand, the long-term storage stability was investigated by monitoring the ECL response of the sensor. As shown in Fig. 4C, the ECL response of the sensor maintained 92.9% of the initial response after the sensor was stored in refrigerator at 4 °C for 2 week, implying that 80 our proposed sensor had an acceptable stability.

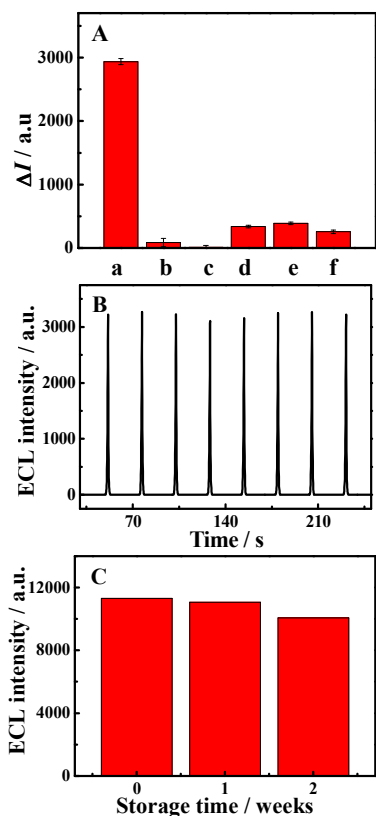


Fig. 4 (A) The ECL intensity obtained at the sensor to (a) 2.0×10^{-12} M melamine, and 1.0×10^{-8} M interfering substances concluding (b) MgCl_2 , (c) lactose, (d) glucose, (e) ascorbic acid, and (f) uric acid. (B) Stability of the sensor under consecutive cyclic potential scans for 8 cycles in 0.10 M PBS (pH 8.0) containing 3.3×10^{-9} M melamine and 0.010 M $\text{K}_2\text{S}_2\text{O}_8$. Scan rate: 100 mV s^{-1} . (C) Storing stability of the sensor for 0 week, 1 week, and 2 weeks, respectively.

3.6 The accuracy evaluation on the sensor using the high performance liquid chromatography-mass spectrometry (HPLC-MS)

In order to evaluate the accuracy of our proposed method, we compared the results between our prepared sensor and the HPLC-MS for melamine detection in milk samples. Milk powder sample and corresponding HPLC-MS data were obtained from Chongqing entry-exit inspection and quarantine bureau (Chongqing, China). Firstly, HPLC-MS detection was performed to make sure that no melamine was contained in milk powder sample. Then, recovery experiments were performed using HPLC-MS by standard addition methods and the recoveries for 8.0×10^{-8} M and 1.6×10^{-7} M melamine were 93.0% and 90.0% in milk samples, respectively. According to Chinese National Standards GB/T 22388-2008, the limit of quantitation of HPLC-MS is 0.01 mg kg^{-1} ,⁴⁰ which is beyond the linear range of our ECL sensor, thus the samples for HPLC-MS detection have to be diluted with trichloroacetic acid to satisfy the dynamic response range of our sensor. Three milk samples with the melamine concentration of 1.06×10^{-8} M, 7.44×10^{-9} M and 7.44×10^{-10} M were prepared, and the detection results obtained using our sensors were compared with those using HPLC-MS, respectively. As shown in Table 1, relative errors in the range of 7.1%~9.5% were obtained. The errors might be attributed to different conditions and assay procedures. These results indicated that the prepared ECL sensor had acceptable accuracy and a great potential for practical applications.

Table 1 Comparison between our proposed methods with HPLC-MS testing

Sample	C_{melamine} of HPLC-MS testing (nM)	C_{melamine} of the proposed method (nM) ^a	Relative error (%)
1	10.06	10.87 ± 0.05	8.0
2	7.44	7.97 ± 0.03	7.1
3	0.74	0.81 ± 0.03	9.5

⁴⁰ ^a Mean \pm SD, $n = 3$.

4. Conclusions

$\text{C}_{60}/\text{g-C}_3\text{N}_4$ NS hybrids were prepared to construct a solid state ECL sensor for melamine. The integration of the excellent ECL behavior of $\text{g-C}_3\text{N}_4$ NS and the good electronic conductivity of C_{60} achieved a highly sensitive detection of melamine with a linear range wide as six orders of magnitude and a detection limit low as the sub-picomolar level. Besides, our proposed method has been compared with HPLC-MS for milk sample analysis, and it was found that the prepared ECL sensor had acceptable accuracy. Furthermore, with low LOD and high sensitivity of our proposed sensor, it maybe has potential applications for detecting a lower concentrations melamine residue in human urine, animal tissues and body fluids due to the presence of low concentration melamine in these cases.

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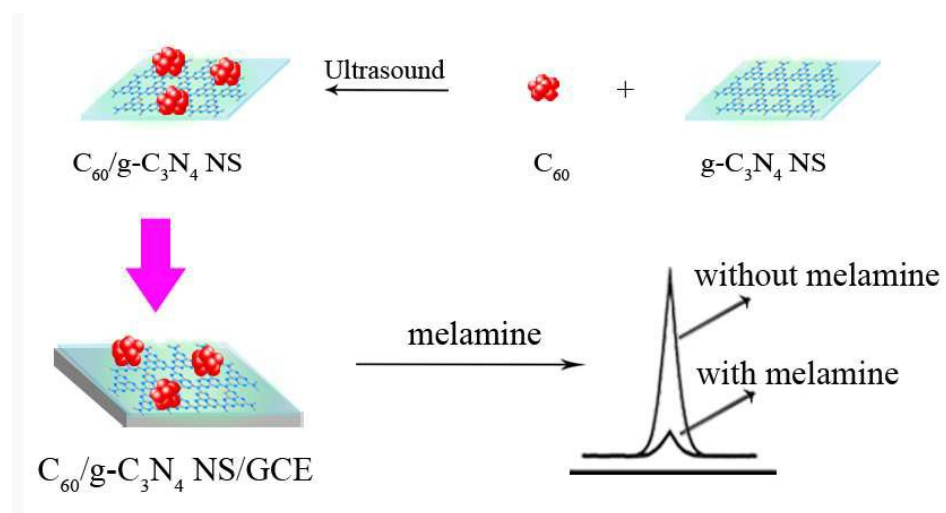
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Notes and references

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Scheme 1. Schematic illustration of preparing C₆₀/g-C₃N₄ NS and fabricating procedures of proposed ECL sensor.