



Copper ion modified graphitic C₃N₄ nanosheets enhanced luminol-H₂O₂ chemiluminescence system: Toward highly selective and sensitive bioassay of H₂S in human plasma

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ARTICLE INFO

Article history:

Received 14 October 2019

Received in revised form 31 December 2019

Accepted 5 January 2020

Available online 7 January 2020

Keywords:

Hydrogen sulfide

Chemiluminescence detection

Copper ion modified graphitic carbon nitride nanosheets

ABSTRACT

A high-efficient chemiluminescence (CL) platform for highly selective and sensitive H₂S detection was constructed on the basis of the quenching effect of S²⁻ on the copper ion modified graphitic carbon nitride nanosheets (Cu²⁺-g-C₃N₄ NSs) enhanced luminol-H₂O₂ system. Cu²⁺-g-C₃N₄ NSs with horseradish peroxidase-like catalytic activity were prepared and provide a great improvement for luminol-H₂O₂ system. The presence of S²⁻ induced the formation of CuS precipitate on g-C₃N₄ NSs surface. The precipitate can block the catalytic Cu²⁺ sites on the g-C₃N₄ NSs surface, resulting in a great CL decrease of CL system. Based on such a mechanism, a simple, highly selective and sensitive CL biosensor for H₂S detection was designed. Under the optimized conditions, luminol-H₂O₂-Cu²⁺-g-C₃N₄ NSs system gave a decrease of CL intensity with the Na₂S concentration increasing. The CL biosensor is in a linear range of 10.0 pM–50.0 nM and the detection limit for detecting Na₂S is as low as 2.0 pM. Moreover, the method here has enjoyed a successful application for determining H₂S in human plasma samples and the recovery is between 95.7% and 110.0%.

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1. Introduction

Hydrogen sulfide (H₂S) serves as a gasotransmitter molecule, the same as carbon monoxide (CO) and nitric oxide (NO), can greatly affect the living biosystems [1]. Of these, as a vital signaling molecule, H₂S participates in various physiological activities under physiological conditions, such as vasodilation, oxygen sensing, angiogenesis, apoptosis, neuromodulation and inflammation [2]. Importantly, endogenous H₂S level is correlated with various diseases like Down syndrome, liver cirrhosis, diabetes and Alzheimer's disease [3]. Previous established techniques specific to detecting H₂S mainly are methylene blue method [4], monobromobimane method [5], gas chromatography [6], and sulphide ion selective electrodes [7]. However, some problems including the complicated sample processing, high-cost instruments, as well as low selectivity and sensitivity still puzzled the researchers [8]. Therefore, the development of the selective and sensitive method for H₂S detection on simple setup is highly appealing.

The optical detection techniques can be used to detect volatile gaseous molecule effectively [9]. Fluorescence detection as one of the

most popular optical technique has proved its great potential in different biological samples from cell culture to blood serum [10,11]. The strategy by designing the fluorescence probes utilizing Cu²⁺-fluorophore complex/Cu²⁺-nanomaterials have been becoming an effective way for H₂S sensing. The mechanism is mainly based on the fluorescent change of the Cu²⁺-fluorophore complex/Cu²⁺-nanomaterials in the presence of H₂S owing to the highly affinity of sulfide ions toward Cu ions (*K*_{sp} of CuS, 1.27 × 10⁻³⁶). For example, Sasakura et al. developed an azamacrocyclic Cu²⁺-complex fluorescent probe for H₂S detection [12]. Zhuo et al. [13] prepared a fluorescent probe of Cu-doped carbon quantum dots to measure H₂S in human serum and image H₂S in living cells. However, fluorescent probes always suffer from the poor photostability and are prone to photobleaching and photodegradation, limiting their further applications. To avoid the phenomenon, chemiluminescence (CL) should be a good choice with the features of no need of excitation source, low background nature, simple equipment and high sensitivity. Inspired by the above mentioned examples, is it possible to introduce the Cu²⁺ mediated mechanism into the CL system to improve the CL performance for H₂S detection?

In many CL systems, e.g., luminol-H₂O₂ [14], N-aminobutyl-N-ethylisoluminol functionalized graphene oxide-H₂O₂ [15], nitrogen and sulfur co-doped carbon dots-H₂O₂ [16], in which horseradish

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peroxidases (HRP) are always adopted as a catalyst for the enhancement of CL intensity. With the development of nanoscience and nanotechnology, the nanomaterials with excellent catalytic effect have been synthesized to mimic the horseradish peroxidase (HRP) functions like iron oxide [17], Au [18], or Cu [19] nanoparticles. Recently, Vázquez-González et al. reported that the Cu^{2+} -modified graphitic carbon nitride nanoparticles (Cu^{2+} -g- C_3N_4 NPs) featured the HRP biocatalytic functions [20]. In this work, by integrating the unique function of Cu^{2+} -g- C_3N_4 NPs with high affinity of S^{2-} toward Cu^{2+} , a simple, highly selective and sensitive CL platform for H_2S detection was proposed in virtue of the quenching effect of H_2S on the Cu^{2+} -g- C_3N_4 nanosheets (Cu^{2+} -g- C_3N_4 NSs) enhanced luminol- H_2O_2 system. According to Scheme 1, thanks to the catalysis of Cu^{2+} -g- C_3N_4 to luminol- H_2O_2 , the system exhibits a strong CL emission. Upon the addition of Na_2S (employed as the H_2S donor), the CuS precipitate formed on the Cu^{2+} -g- C_3N_4 NSs surface and thus the CL intensity declined. The proposed method allowed for the detection of H_2S in a sensitive manner. Moreover, the CL biosensor was applied for determination H_2S in human plasma samples.

2. Experimental

2.1. Materials and Reagents

Glutathione (GSH), homocysteine (Hcy), L-cysteine (Cys) and melamine were purchased from the Aladdin Industrial Co., Ltd. (Shanghai, China). Glycine (Gly) was bought from the Seebio Biotechnology Co., Ltd. (Shanghai, China). Serine (Ser), alanine (Ala), L-lysine (Lys), sodium nonasulfate ($\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$), zinc sulfate (ZnSO_4), ferrous sulfate (FeSO_4), copper sulfate (CuSO_4), calcium chloride (CaCl_2), Ferric chloride (FeCl_3), ascorbic acid (AA), nitric acid (HNO_3) and citric acid (CA) were provided by the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1.0×10^{-2} M luminol stock solution was used to prepare the luminol working solution. Other AR-grade reagents were used directly. All aqueous solutions were prepared by pure water ($18.2 \text{ M}\Omega\cdot\text{cm}$) obtained from the ultrapure water system (Aike Water Treatment Solution Provider, China).

2.2. Apparatus

CL measurements were performed on an ultra-weak luminescence analyzer (BPCL-1-TIC, Institute of Biophysics, Chinese Academy of Science, Beijing, China). The CL emission was recorded on the F-7000 fluorescence and UV-vis absorption spectra were recorded on the U-3900H UV-vis spectrophotometer (Hitachi, Tokyo, Japan). A Tecnai G2 F20 S-TWIN microscope (FEI Co., Ltd., USA) was applied for the Transmission electron microscopy (TEM), the scanning-TEM energy dispersive X-ray spectroscopy (STEM-EDS) as well as the STEM-mapping. A KAlpha X-ray photoelectron spectrometer (Thermo Fisher Scientific Co., USA) was employed to perform X-ray photoelectron

spectroscopy (XPS) and a FTIR-8400S spectrometer (Shimadzu, Japan) helped to perform the Fourier transform infrared (FT-IR) spectroscopy.

2.3. Cu^{2+} -g- C_3N_4 NSs Preparation

The preparation of the g- C_3N_4 NSs was conducted following the reported methods [21,22] and there was a little improvement. At first, 5.0 g white melamine powders received 4 h annealing at 550°C to acquire the yellow bulk g- C_3N_4 . Second, a certain amount of the bulk g- C_3N_4 product (600 mg) were ground to a powder form and transferred to 200 mL of 5.0 M HNO_3 . Then the mixture underwent 1 h sonicate. Next, the produced solution was refluxed at 125°C for 12 h. After cooled to the room temperature, the reaction product underwent centrifugation treatment, and then water was used to wash it to near-neutral pH. Subsequently, the obtained g- C_3N_4 nanoflakes of the previous step were transferred to 60 mL of water for exfoliation, which was kept for 16 h under ultrasound. Finally, the centrifugation at 8000 rpm for 10 min was conducted for discard of the unexfoliated g- C_3N_4 . The collected suspension with g- C_3N_4 NSs was dried at 60°C for later use.

The Cu^{2+} -g- C_3N_4 NSs were synthesized as follows: In brief, a certain amount of g- C_3N_4 NSs (20.0 mg) was dissolved in 20 mL pure water for 10 min by ultrasonication. Then, the formed solution was added with 20.0 mL 0.05 M CuCl_2 (The optimization of the CuCl_2 concentration, see the section of 3.2). Following 4 h sonication at 80°C , the mixture underwent centrifugation treatment and wash three times by pure water for the removal of excess CuCl_2 . Finally, the product was dried for further use.

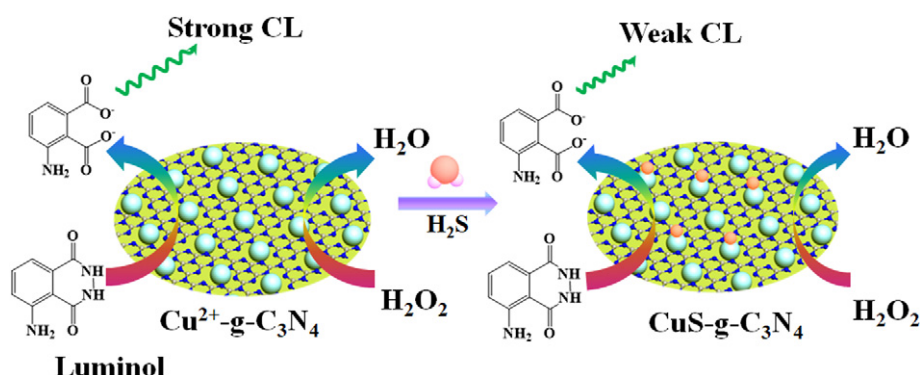
2.4. Construction of the CL Biosensor

The ingenious CL biosensor was constructed based on the probe of Cu^{2+} -g- C_3N_4 NSs-triggered strong CL response of luminol- H_2O_2 system. As displayed in Scheme 1, for H_2S detection, a series of various concentrations of Na_2S (using Na_2S as the equivalent [23]) was added into the Cu^{2+} -g- C_3N_4 NSs suspension, followed by 30 min incubation. The portion of Cu^{2+} -g- C_3N_4 NSs was first centrifuged and then washed using phosphate buffer solution, followed by being reacted with dissociated S^{2-} to form CuS -g- C_3N_4 NSs for CL assay.

3. Results and Discussion

3.1. Characterization of g- C_3N_4 NSs and Cu^{2+} -g- C_3N_4 NSs

TEM was performed to characterize the prepared Cu^{2+} -g- C_3N_4 and g- C_3N_4 NSs. According to Fig. 1A and B, pure g- C_3N_4 NSs and Cu^{2+} -g- C_3N_4 NSs present analogous laminated structure, which indicates basically no change in the basic morphology following Cu^{2+} -doping. Meanwhile, the TEM image also demonstrates that Cu^{2+} -g- C_3N_4 NSs are relatively thin with a high transparency. Then, the STEM-EDS spectrum (Fig. 1C) confirms that the Cu^{2+} -g- C_3N_4 NSs are composed of the



Scheme 1. Schematic illustration of CL assay of H_2S .

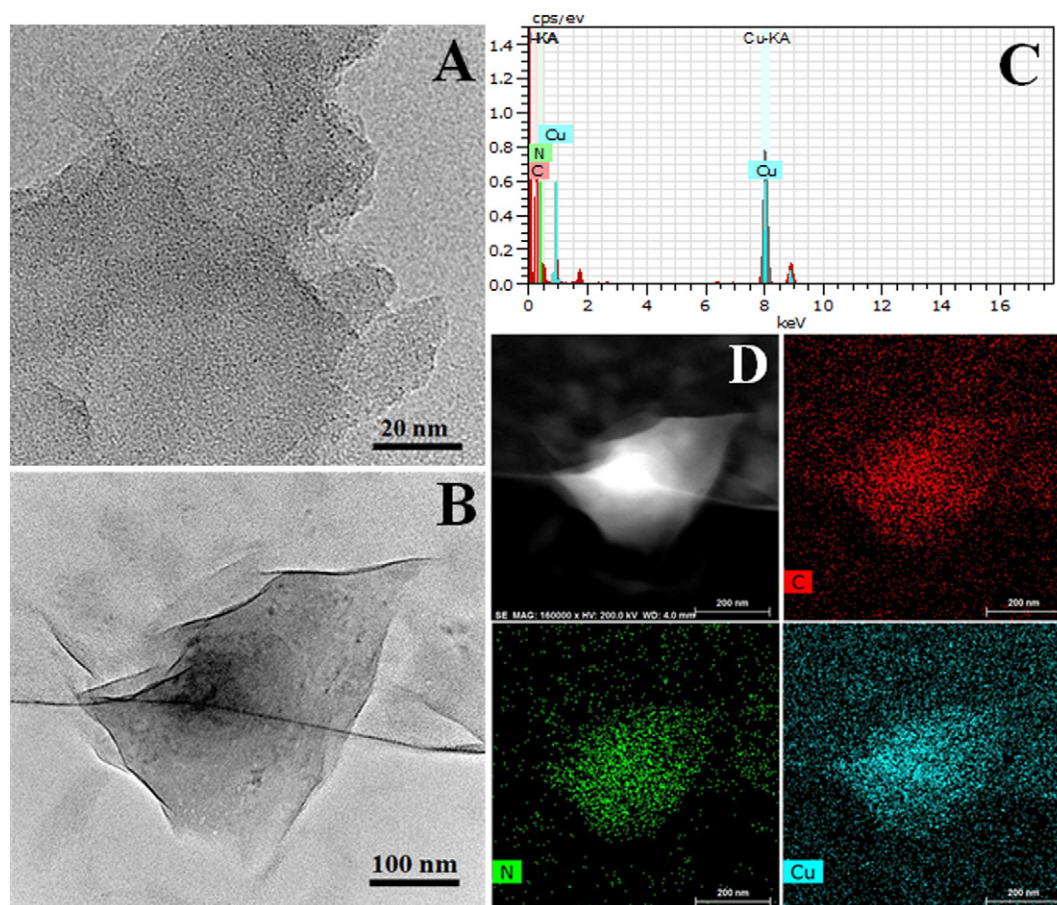


Fig. 1. TEM images of $g\text{-C}_3\text{N}_4$ NSs (A), $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs (B) and the STEM-EDS image of $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs (C) and the EDS elemental mappings of C, N and Cu elements for $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs (D).

elements C, N, and Cu. Element mapping characterizations were implemented to further explore the structure of the prepared $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs. Fig. 1D displays that, the STEM-mapping clearly exhibit the identical distribution of C, N, and Cu elements in the $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs (Fig. 1D). These data prove the successful preparation of $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs.

The surface groups, structures and components of $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs were further studied by FT-IR, UV-vis and XPS spectra. It can be seen from the FT-IR spectra in Fig. 2A that the graphitic C—N network does not change obviously after Cu^{2+} doping in $g\text{-C}_3\text{N}_4$ NSs (curves a and b). The peak at 804 cm^{-1} was caused by the breathing mode regarding the triazine units [24,25]. The peaks ranging from 1200 cm^{-1} to 1700 cm^{-1} act as typical aromatic C—N stretching modes, thereinto, the peaks at 1250 cm^{-1} is assigned as the stretching vibration of C—N, and the other two peaks at 1413 cm^{-1} , and 1635 cm^{-1} are assigned as the stretching modes of heptazine-derived units [26,27]. Broad peaks from 3000 cm^{-1} to 3500 cm^{-1} originate from the stretching vibration of the N—H and the O—H, which indicate the existence of physical adsorbed water and amino groups in both $g\text{-C}_3\text{N}_4$ NSs and $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs. A new peak appears at about 1054 cm^{-1} after Cu doping, which could be caused by the stretching vibration of the C—N in the copper phthalocyanine molecule [26]. There is an obvious absorption band ($\sim 325\text{ nm}$) in the UV-vis absorption spectra for both samples (curves a: $g\text{-C}_3\text{N}_4$ NSs, and curve b: $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs), which correspond to the electronic transition from valence band to conduction band (Fig. 2B). After being added with Cu^{2+} , absorption band shows a partial red shift ($\sim 5\text{ nm}$) because of the enhancement of the photocatalytic property in the visible area, which is related with the typical semiconductor absorption of $g\text{-C}_3\text{N}_4$ substrate.

In addition, XPS analysis was accomplished to analyze the elemental composition of the synthesized $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs in detail (Fig. 2C). The XPS survey spectra show several visible peaks at 23.2 eV (O_{2s}), 207.1 eV (Cl_{2p}), 291.6 eV (Cl_{1s}), 408.6 eV (N_{1s}), 540.6 eV (O_{1s}), 959.1 eV (Cu_{2p}) and 980.4 eV (OKLL) [28]. Furthermore, the XPS data verified the existence of the carbon (46.22%), nitrogen (45.62%), oxygen (5.53%), copper (1.34%), and chlorine (1.29%) in $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs. That is, the XPS indicated the coverage of Cu^{2+} ions corresponding to about 0.0134 mg per 1.0 mg of $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs. All the data validate that $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs were successfully synthesized.

3.2. Optimization of CL Working Conditions

To achieve excellent analytical performance, the concentrations of luminol, H_2O_2 , $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs, and CuCl_2 used for $g\text{-C}_3\text{N}_4$ NSs modification needed to be optimized. The effect of luminol concentration ranged from 0.25 to 5.0 mM on the CL signal response was tested. The luminol concentration increase from 0.25 to 1.5 mM helped to significantly enhance the CL intensity (Fig. S1A). However, as the luminol concentration further increased, the CL intensity decreased. Therefore, 1.5 mM luminol was chosen. As the coreactant of luminol, H_2O_2 concentration was also screened. As depicted in Fig. S1B, the CL intensity increased as the H_2O_2 concentration increased and reached a plateau at 2.0 mM . Hence, 2.0 mM H_2O_2 was selected.

As a catalyst mimicking the HRP, $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs possessed the biocatalytic function to luminol- H_2O_2 system. To screen the appropriate concentration of $\text{Cu}^{2+}\text{-}g\text{-C}_3\text{N}_4$ NSs, the concentration from 0.05 to 4.0 mg mL^{-1} was tested. As shown in Fig. S1C, the CL intensity increased to a maximum value at 0.5 mg mL^{-1} . Therefore, 0.5 mg mL^{-1} was used.

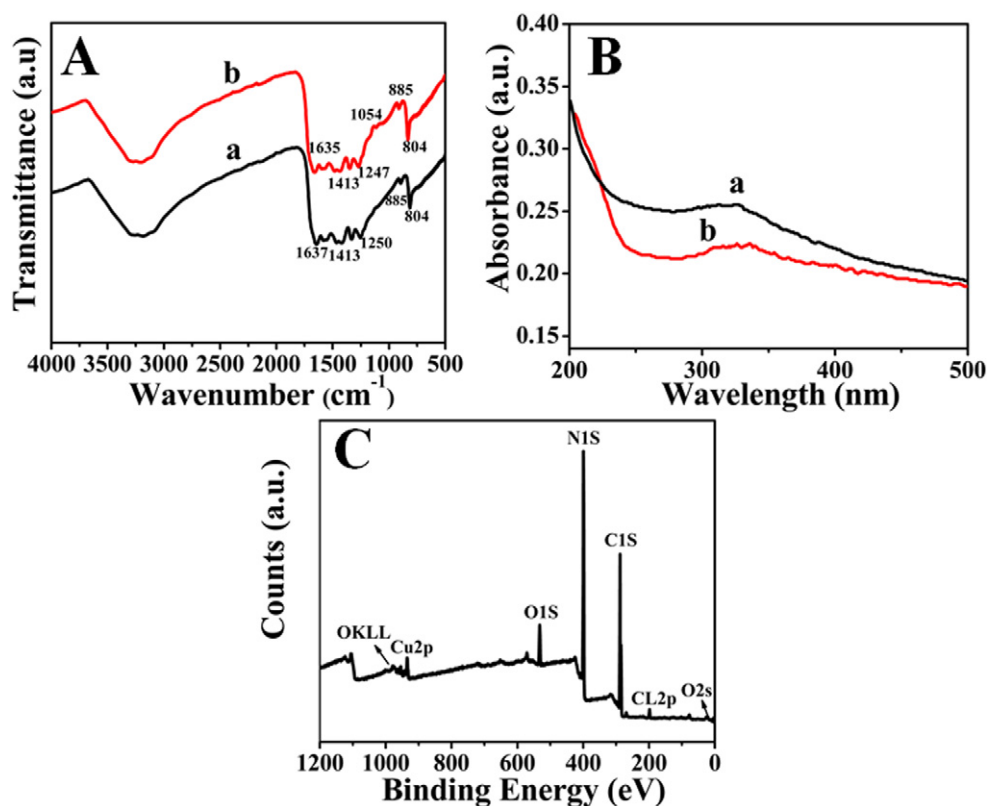


Fig. 2. FT-IR (A) and UV-vis spectra (B) obtained for g-C₃N₄ NSs (a), and Cu²⁺-g-C₃N₄ NSs (b). (C) XPS spectra of Cu²⁺-g-C₃N₄ NSs.

To ensure that the Cu²⁺-g-C₃N₄ NSs exhibit a highly catalytic function toward luminol-H₂O₂ system, the CL of the Cu²⁺-g-C₃N₄ NSs with the variable concentrations of CuCl₂ (0–0.2 M) at fixed amount of g-C₃N₄ NSs (0.1 mg mL⁻¹ g-C₃N₄ suspension) were recorded. As shown in Fig. S1D, the modification of Cu²⁺ on the g-C₃N₄ NSs surface (curve b–g) could remarkably improve the system's CL intensity compared with the pure g-C₃N₄ NSs (curve a), indicating the vital catalytic role of coordinated Cu²⁺ in Cu²⁺-g-C₃N₄ NSs [25]. The CL intensity increased with the CuCl₂ concentration increasing until 0.05 M (curve a–e). The concentration beyond 0.05 M gives no obvious CL change, which could be ascribed to the saturated loading of Cu²⁺ on g-C₃N₄ NSs surface. Based on the above analysis, the 0.05 M concentration of CuCl₂ was selected for probe synthesis.

3.3. Possible Mechanism

To investigate the catalytic property of Cu²⁺-g-C₃N₄ NSs on luminol-H₂O₂ system, a series of experiments were designed. Fig. 3A depicts the CL kinetics curves of different CL systems. As shown, the CL signal of the luminol-H₂O₂ system is very weak (curve a), and the separated addition of Cu²⁺ (1.05 × 10⁻⁴ M) or g-C₃N₄ NSs could promote CL signals (curves b and c), suggesting the Cu²⁺ and g-C₃N₄ NSs have certain catalytic activity toward the luminol-H₂O₂ system, respectively. Upon the introduction of Cu²⁺-g-C₃N₄ NSs into luminol-H₂O₂ system, a sharp and strong CL response was observed (curve d).

To further explore the CL mechanism of the luminol-H₂O₂-Cu²⁺-g-C₃N₄ NSs system, the CL spectra of different systems were measured by the fluorescence spectrometer by taking off the light source. As

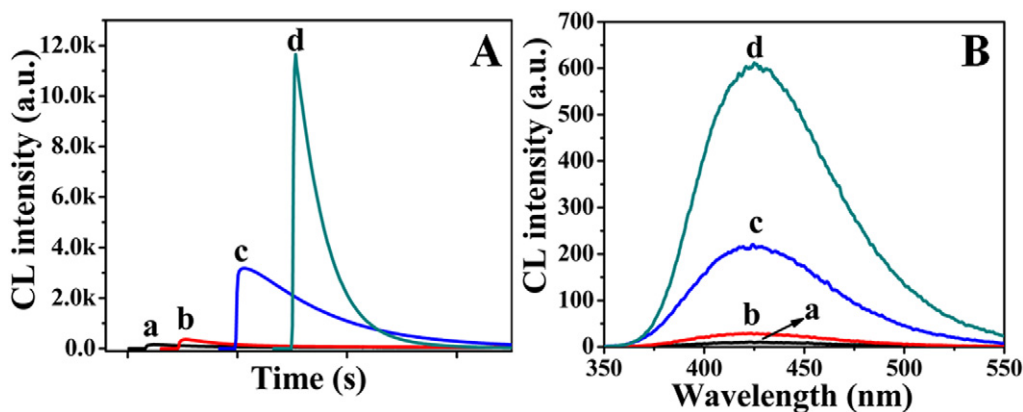
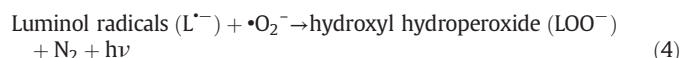
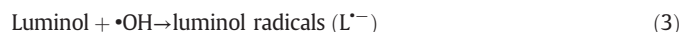
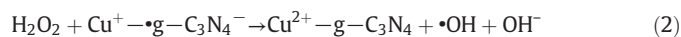
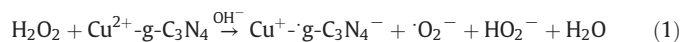


Fig. 3. CL kinetics curves (A) of and CL spectra (B) of luminol-H₂O₂ (a), luminol-H₂O₂-Cu²⁺ (C_{Cu2+} = 1.05 × 10⁻⁴ M) (b), luminol-H₂O₂-g-C₃N₄ NSs (c), and luminol-H₂O₂-Cu²⁺-g-C₃N₄ NSs (d).

displayed in Fig. 3B, the emission peaks of the luminol-H₂O₂, luminol-H₂O₂-Cu²⁺, luminol-H₂O₂-g-C₃N₄ NSs, and luminol-H₂O₂-Cu²⁺-g-C₃N₄ NSs are all around 425 nm. These data reveals that the luminescent species in these systems are excited luminol [29,30], and the signal enhancement trend of CL spectrum is consistent with the CL kinetic behavior. Based on the above analysis, combining with the previously reported catalytic mechanism of Cu²⁺ on luminol-H₂O₂ system [31,32] and the CL reaction process of g-C₃N₄ NSs-based system [33,34]. The possible luminescent mechanism of the luminol-H₂O₂-Cu²⁺-g-C₃N₄ NSs system could be described by the following equations:



3.4. Analytical Performance

To demonstrate the feasibility of Cu²⁺-g-C₃N₄ NSs as probes for H₂S sensing, Na₂S with varied concentrations were tested. The results showed that CL signal changed proportionally with Na₂S concentration in logarithmic scale in the range of 10.0 pM–50.0 nM (Fig. 4A). The reason for this is that the enhanced concentration of Na₂S could result in the increased amount of CuS formed on g-C₃N₄ NSs surface, blocking catalytic Cu²⁺ sites on the surface of the g-C₃N₄ NSs. Fig. 4B depicts that the CL intensity is linearly related to the logarithm of Na₂S concentration, which follows the regression equation $I = -3378.02 - 1286.92 \log C$ (C represents the concentration of S²⁻, $R^2 = 0.997$). The detection limit is found to be 2.0 pM ($S/N = 3$). Moreover, the proposed method was compared with the previously reported method for detecting H₂S. For the well-designed biosensor, the linear range was relatively wide and the detection limit was low for H₂S detection (Table 1).

3.5. Specificity, Stability, and Precision

For evaluating the selectivity of the CL biosensor, some possible interfering species such as metal ions, amino acids, biothiols and CA, glucose and AA were examined. As shown in Fig. S2A, the existence of 100 μM of Na⁺, K⁺, Mg²⁺, Gly, Ser, Lys, CA, Glucose, Ala and AA, led to scarcely change of the CL signal, whereas 50.0 nM Na₂S could result in an obvious CL decrease. The other interferences such as Fe³⁺, Cu²⁺,

Table 1

Comparison of the proposed method and the referenced methods for H₂S detection.

Methods	Linear ranges	LODs	References
PEC method	10.0 nM–1.0 mM	0.31 nM	[35]
FL probe	1.0 μM–10.0 μM	0.12 μM	[36]
Multicolor colorimetric assay	0.05 μM–50.0 μM	19.0 nM	[37]
Smart colorimetric probe	0.7 μM–10.0 μM	0.2 μM	[38]
Ultrasensitive PEC sensor	1.0 pM–10.0 nM	0.36 pM	[39]
CL biosensor	10.0 pM–50.0 nM	2.0 pM	This work

Zn²⁺, Fe²⁺, Hcy, Cys, and GSH (each of 100 μM) caused a slight change of CL response, and the corresponding signal fluctuation rate of CL was calculated as $\Delta I/I_{\text{blank}}$ values, i.e., $(I_{\text{Fe}^{3+}} - I_{\text{blank}})/I_{\text{blank}} = 3.2\%$, $(I_{\text{Cu}^{2+}} - I_{\text{blank}})/I_{\text{blank}} = 3.3\%$, $(I_{\text{Zn}^{2+}} - I_{\text{blank}})/I_{\text{blank}} = 2.8\%$, $(I_{\text{Fe}^{2+}} - I_{\text{blank}})/I_{\text{blank}} = 2.9\%$, $(I_{\text{Hcy}} - I_{\text{blank}})/I_{\text{blank}} = -2.6\%$, $(I_{\text{Cys}} - I_{\text{blank}})/I_{\text{blank}} = -3.5\%$, $(I_{\text{GSH}} - I_{\text{blank}})/I_{\text{blank}} = -4.4\%$ are all less than $\pm 5\%$. Therefore, the results exhibited that the method has good selectivity for H₂S detection.

The CL response of the Cu²⁺-g-C₃N₄ NSs probe with various storage times (0 week, 1 week, 2 weeks and 3 weeks) for 50.0 nM Na₂S was recorded to examine the stability of the system. As demonstrated in Fig. S2B, the difference in CL intensity is negligible within 3 weeks, indicating that the system presents an acceptable stability.

The assay of 50.0 nM Na₂S for 6 parallel measurements within one day and three days was carried out to estimate the intra-assay precision and inter-assay precision. The relative standard deviations (RSDs) for intra-assay were no more than 3.5%, while the RSDs for inter-assay were less than 6.3%.

3.6. Analytical Applications

To verify the applicability of the system, the content of sulphide in healthy human plasma (provided by Xinyang Central Hospital) were determined. Prior to analysis, the fresh human plasma samples are properly diluted and pretreated as the follows: briefly, acetonitrile was added into human plasma (7:1 v/v plasma/acetonitrile) to remove the proteins [40]; After centrifugated for 10 min at 10000 rpm, the resultant supernatant was immediately used for analysis. As listed in Table S1, the sulphide concentration of the plasma averaged at $0.47 \pm 0.04 \mu\text{M}$ from three healthy individuals, which were basically consistent with the reported methods [8,40]. In addition, the recovery experiments were executed by spiking Na₂S (0.2 and 0.4 μM) into three human plasma samples, respectively. The recoveries vary from 95.7% to 110.0%, and the RSDs are no more than 6.1%, indicating that the method has a good accuracy for the detection of H₂S in the biological samples.

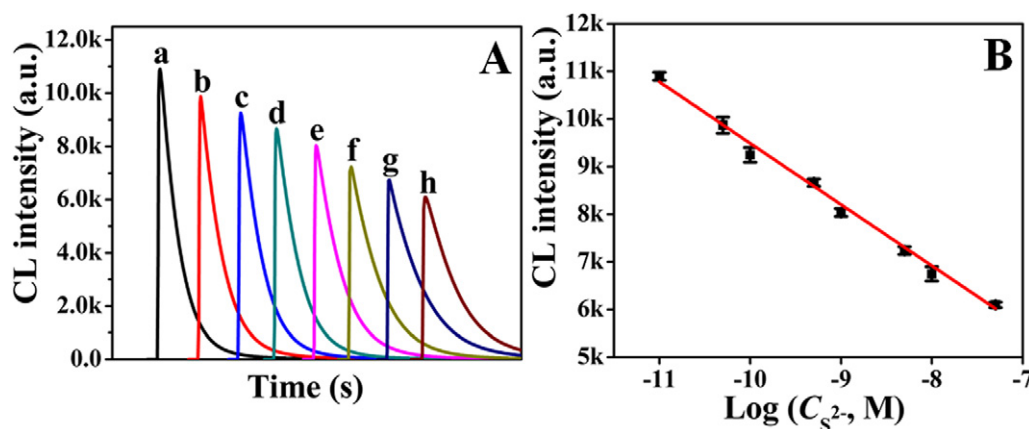


Fig. 4. (A) CL signal responses of different concentrations of Na₂S: (a) 10.0 pM, (b) 50.0 pM, (c) 100.0 pM, (d) 500.0 pM, (e) 1.0 nM, (f) 5.0 nM, (g) 10.0 nM and 50.0 nM. (B) The linear plot between CL intensity and the logarithm of Na₂S concentration.

4. Conclusions

In summary, the study developed a CL biosensor for detecting H₂S on the basis of the quenching effects of H₂S on Cu²⁺-g-C₃N₄ NSs enhanced luminol-H₂O₂ system. Cu²⁺-g-C₃N₄ NSs exhibit excellent catalytic activity to luminol-based system. By virtue of the blocking effects of S²⁻ to catalytic Cu²⁺ sites on the surface of the g-C₃N₄ NSs, the presence of H₂S produce a great signal decline of the Cu²⁺-g-C₃N₄ NSs-luminol-H₂O₂ system. Hence, the constructed CL system presents a high sensitivity and good selectivity for detecting H₂S. The CL biosensor has been applied to determining H₂S in human plasma samples, providing a new promising method for sensitive detection of H₂S in biofluids.

Credit Author Statement

Jun-Tao Cao: Conceptualization, Methodology, Supervision, Project administration, Writing-original draft, Writing-review & editing. **Wen-Sheng Zhang:** Conceptualization, Methodology, Investigation, Writing-original draft. **Xiao-Long Fu:** Investigation. **Hui Wang:** Supervision. **Shu-Hui Ma:** Validation. **Yan-Ming Liu:** Conceptualization, Methodology, Supervision, Project administration, Writing-original draft, Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 21675136 and 21874115), Science & Technology Innovation Talents in Universities of Henan Province (18HASTIT003), and Nanhu Scholars Program for Young Scholars of XYNUn.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.saa.2020.118040>.

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