



Anodic electrochemiluminescence of graphitic-phase C₃N₄ nanosheets for sensitive biosensing



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ABSTRACT

This work observed the anodic electrochemiluminescence (ECL) of C₃N₄ nanosheets (CNNS) for the first time. The ECL emission was 40 times stronger than that from bulk g-C₃N₄ in the presence of triethylamine (Et₃N) as a coreactant due to large surface-to-volume ratio, which enhanced the sensitivity for biosensing. At pH 7.0, the CNNS modified electrode prepared with 0.75 mg mL⁻¹ CNNS in 0.025% chitosan solution possesses good stability and acceptable reproducibility in the presence of 30 mM Et₃N. The ECL mechanism of CNNS/Et₃N system was proposed to be emitted from the excited CNNS, which was produced during the reaction between the electro-oxidation products of CNNS and coreactant Et₃N. Based on the annihilation between the oxidation product of dopamine (DA^{•+}) and Et₃N[•] radical, a quenching-based method was established for sensitive and specific detection of dopamine ranging from 1.0 nM to 100 nM with a detection limit of 96 pM by using the CNNS nanosheets as an ECL emitter. The proposed method showed excellent specificity, high sensitivity and low detection limit, and could be applied in analysis of real samples.

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1. Introduction

Electrochemiluminescence (ECL) detection as a well-known analytical method has many special advantages, such as low background, high sensitivity, good stability, wide linear range, and low cost, owing to the separation of extinction and detection signals [1]. A series of materials including Ru complexes [2], luminol [3], gold nanoclusters [4] and quantum dots (QDs) [5–11] have been used to design ECL sensors for the detection of heavy metal ions, small biomolecules, proteins and nucleic acids. Especially, the QDs-based ECL has become an exclusive and powerful tool for probing the signal transduction due to the unique photophysical properties, such as size-controlled fluorescence [12], high quantum yields and stability against photobleaching. However, their applications in biosensing were still limited due to the high toxicity in most QDs-based ECL systems. Thus it has become a hotspot to find new materials as luminophores with low toxicity, high efficiency, good stability and acceptable repeatability in ECL research area.

Graphite-like carbon nitride (g-C₃N₄), as a kind of metal-free semiconductor with medium band gap, has drawn growing attention in various fields due to its special structure, optical and electrical properties [13,14]. The bulk and mesoporous g-C₃N₄ materials have been widely applied in photocatalysis and degradation [15,16],

photovoltaic platform [17], photoluminescence (PL) [18] and ECL detection [19]. Compared to bulk g-C₃N₄, nanoscale g-C₃N₄ exhibits greatly improved optical and electrical behaviors in cathode ECL emission [20], PL sensing [21] and bioimaging [22]. For example, a highly efficient g-C₃N₄ nanosheet (CNNS)-based fluorosensor has been developed for the detection of Cu²⁺ by fluorescence quenching via photoinduced electron transfer [21]. Moreover, the nanosheets show enhanced photoabsorption and photoresponse for bioimaging application [22]. In this work, the anodic ECL emission of the CNNS was observed in the presence of triethylamine (Et₃N) as a coreactant. It was emitted from the excited CNNS, which was produced during the reaction between the electro-oxidation products of CNNS and coreactant Et₃N.

Dopamine (DA) as a kind of catecholamines is closely related to some diseases such as Parkinson's disease and even the HIV [23]. Therefore, the reliable detection methods of DA has been proposed based on photoelectrochemical [24], electrochemical [25–27], ECL, fluorescence [28–30] and ECL [31,32] strategies. To develop a more sensitive method for the detection of DA, the sensitive quenching effect of dopamine on the strong anodic ECL emission of CNNS, a new two-dimensional nanomaterial with large surface-to-volume ratio, by its reaction with the electro-oxidation product of CNNS was used for the design of a novel “signal-off” strategy. By simple dropping coating of the mixture of CNNS and chitosan solutions on a glassy carbon electrode (GCE), the developed ECL biosensor manifested sensitive response to DA with a detection limit down to sub-nM level. It indicated that CNNS is a promising

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candidate as the anodic ECL luminophore and opens up a new avenue to construct ECL system in biosensing.

2. Experimental

2.1. Materials and reagents

Melamine and chitosan were obtained from Sigma-Aldrich Inc. (USA). Et₃N was purchased from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China). Dopamine hydrochloride (DA) was purchased from Alfa Aesar China Ltd. (China). D-(+)-Glucose (Glc), glutamic acid (Glu), uric acid (UA) and citric acid (CA) were purchased from Shanghai J&K Chemical Ltd. (China). L-Ascorbic acid (AA) was purchased from Sinopharm Chemical Reagent Co., Ltd. Epinephrine (AD) and Norepinephrine (NE) were from Sigma. DA injection sample was obtained from Gulou Hospital (Nanjing). All other reagents were of analytical grade and used as received. 0.1 M phosphate buffer saline (PBS) was prepared as the detection solution by mixing stock solutions of NaH₂PO₄ and Na₂HPO₄ that contained 0.1 M KCl. Ultrapure water obtained from a Millipore water purification system (≥ 18 M Ω , Milli-Q, Millipore) was used in all assays.

2.2. Apparatus

The cyclic voltammetric (CV) and electrochemiluminescent (ECL) measurements were performed on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Ltd. Co., China). The photomultiplier tube (PMT, detection range: 300–650 nm) were employed as ECL detector biased at 800 V in pH 7.0 PBS containing different concentrations of Et₃N. A modified glassy carbon electrode (GCE, 5 mm in diameter, China), platinum wire and Ag/AgCl (saturated KCl) were employed as working, counter and reference electrodes, respectively. UV–vis

absorption spectrum was obtained with a UV-3600 UV–vis-NIR spectrophotometer (Shimadzu, Japan). Fluorescence spectrum was recorded with a RF-5301PC fluorescence spectrophotometer (Shimadzu, Japan). The scanning electron micrographs were obtained using a Hitachi S-3000N scanning electron microscope (Japan).

2.3. Synthesis of C₃N₄ nanosheets

Bulk graphitic-phase carbon nitride (g-C₃N₄) was first synthesized by polymerization of melamine according to literature [22]. The well-distributed C₃N₄ nanosheets (CNNS) were synthesized in water by a “green” liquid exfoliation route from bulk g-C₃N₄. Briefly, 100 mg of bulk g-C₃N₄ powder was dispersed in 100 mL water by ultrasonication for about 16 h. To remove the residual unexfoliated g-C₃N₄ nanoparticles and the nanosheets with large area, the obtained suspension was centrifuged at about 5000 rpm for following experiments.

2.4. Preparation of CNNS modified electrode

The GCE was polished to a mirror with 1.0 and 0.05 μm alumina slurry (Beuhler) followed by sonication in water, ethanol and water, respectively. After the electrode was rinsed with ultrapure water and dried in N₂ atmosphere, 20 μL of CNNS (0.75 mg mL⁻¹ in 0.025% chiton solution) was dropped on its surface, and allowed to dry in air at room temperature.

3. Results and discussion

3.1. Characterization of C₃N₄ nanosheets

The well-distributed CNNS was synthesized by a “green” liquid exfoliation route from bulk g-C₃N₄ [21]. The UV–vis absorption

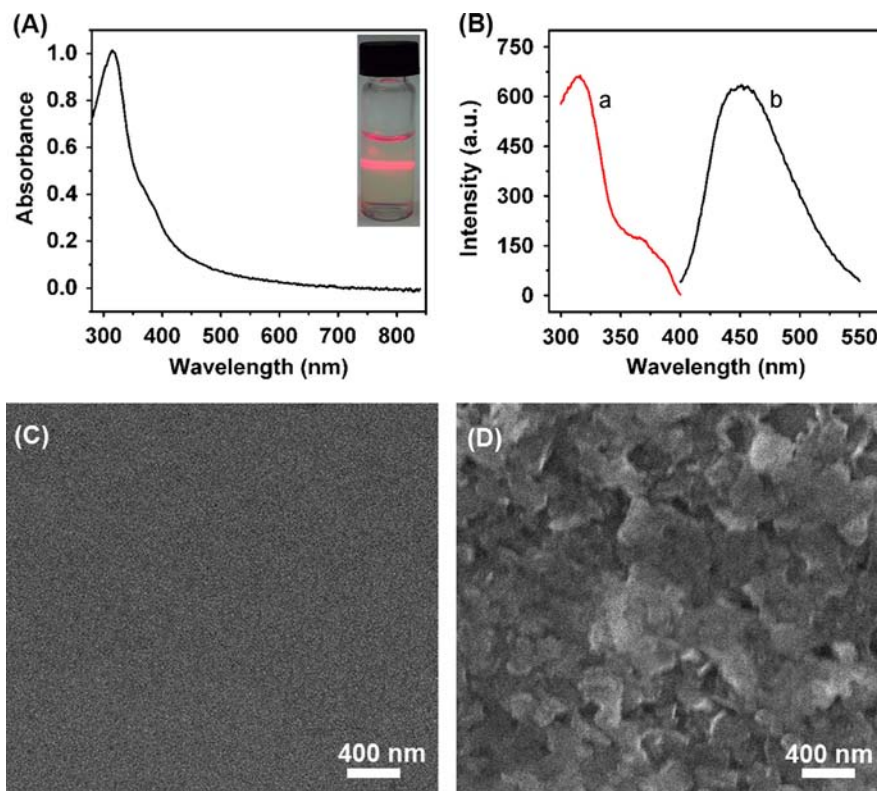


Fig. 1. (A) UV–vis absorption, (B) fluorescence excitation (a) and emission (b) spectra of CNNS, and SEM images of (C) bare GCE and (D) CNNS modified GCE, Inset in A: Tyndall effect of CNNS dispersion.

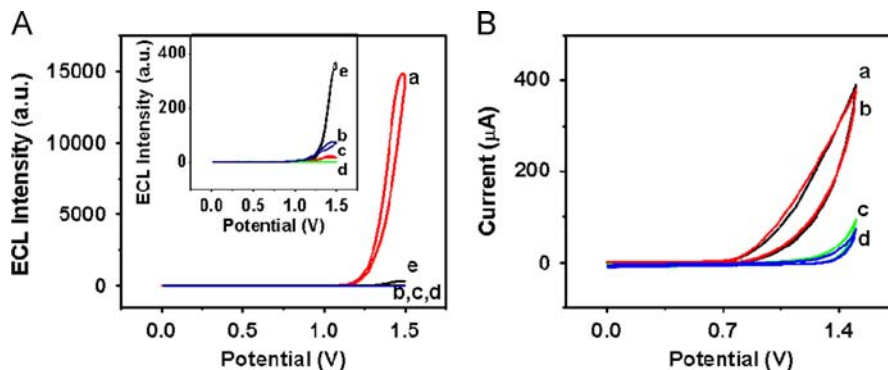


Fig. 2. (A) Cyclic ECL curves of CNNS modified GCE (a, b), bare GCE (c, d) and bulk $g\text{-C}_3\text{N}_4$ modified GCE (e) in the presence (a, c, e) and absence (b, d) of 30 mM Et_3N in 0.1 M pH 7.0 PBS. Inset: The enlarged ECL curves b–e. (B) CVs of CNNS modified (a, c) and bare GCE (b, d) in the presence (a, b) and absence (c, d) of 30 mM Et_3N . Scan rate: 0.1 V s^{-1} .

band of the prepared CNNS centered at 315 nm (Fig. 1A), which referred to the intrinsic absorption edge of ultrathin $g\text{-C}_3\text{N}_4$ nanosheets, and its maximum PL excitation and emission wavelength was 316 nm and 451 nm (Fig. 1B), respectively, which were similar to those previously reported data [22], and identified the successful formation of CNNS. The scanning electron micrographs (SEM) of bare GCE (Fig. 1C) and CNNS modified GCE (Fig. 1D) showed obvious packing of the CNNS on the electrode with a porous structure, which should significantly facilitate the ECL emission. Besides, the occurrence of the Tyndall effect of the diluted dispersion revealed the colloidal nature of the dispersed CNNS (inset in Fig. 1A), indicating a well dispersion in water. In fact, the prepared CNNS could stabilize in water for several weeks without precipitation.

3.2. ECL behavior of the CNNS modified GCE

The CNNS modified GCE showed an efficient anodic ECL emission peaking at +1.48 V (vs. Ag/AgCl) with an initial potential of ca. +1.15 V in the presence of Et_3N as the coreactant (Fig. 2A, curve a). As comparison, in the absence of Et_3N , the ECL curve showed only a very weak ECL emission (Fig. 2A, curve b), while no ECL emission was observed at the bare GCE even in the presence of Et_3N (Fig. 2A, curves c and d). These results indicated that the ECL signal originated from the CNNS as the emitter. Under the same conditions, the anodic ECL intensity of bulk $g\text{-C}_3\text{N}_4$ was 1/40 times lower than that of CNNS (Fig. 2A, curve e). Moreover, the ECL peak potential of CNNS modified GCE was at +1.48 V, much more negative than that of +2.0 V for bulk $g\text{-C}_3\text{N}_4$ [33]. These results indicated that CNNS was an excellent nano-sized luminophore with strong anodic ECL emission, providing a novel emitter for sensitive ECL biosensing.

To probe the role of Et_3N in the ECL process, cyclic voltammetric experiments were performed. In the presence of coreactant Et_3N , the CNNS modified electrode showed a broad anodic wave with the initial potential of ca. +0.75 V (Fig. 2B, curve a), which was also observed at the bare GCE (Fig. 2B, curve b). Thus, the broad anodic wave should be attributed to the electro-oxidation of Et_3N to produce $\text{Et}_3\text{N}^{\bullet+}$, which then released a proton to generate $\text{Et}_3\text{N}^{\bullet}$ radical (Eqs. (1) and (2)), similar to the electro-oxidation process of triethanolamine as the ECL co-reactant [33]. In the absence of Et_3N , the CNNS modified GCE showed a small oxidative current that started at ca. +1.15 V (Fig. 2B, curve c) and was obviously greater than that observed at bare GCE (Fig. 2B, curve d). The starting potential was similar to the initial ECL potential, and thus could be attributed to the electro-oxidation of CNNS (Eq. (3)). The electro-oxidation product $g\text{-C}_3\text{N}_4^{\bullet+}$ reacted with $\text{Et}_3\text{N}^{\bullet}$ radical formed the excited state $g\text{-C}_3\text{N}_4^*$, leading to intense ECL emission (Eqs. (4) and (5)). The anodic ECL mechanism of the CNNS

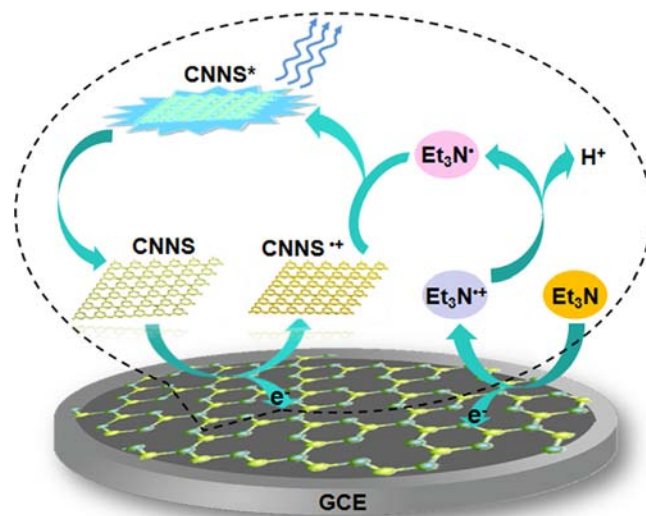


Fig. 3. Schematic illustration of anodic ECL mechanism at CNNS modified GCE.

modified GCE in the presence of Et_3N could be concluded in Fig. 3.



3.3. Optimal conditions for ECL emission

To get the best ECL emission, the CNNS concentration for preparation of CNNS modified GCE, pH value of detection solution and the coreactant concentration were optimized. The effect of CNNS concentration in the 0.025% chitosan solution on the ECL intensity was shown in Fig. 4A. With the increasing concentration of CNNS, the ECL intensity increased and reached the plateau value at 0.75 mg mL^{-1} . Therefore, 0.75 mg mL^{-1} CNNS was selected. The ECL intensity increased with the increasing concentration of Et_3N in detection solution and reached the maximum value at 30 mM (Fig. 4B). So 30 mM Et_3N was chosen as the optimal concentration of the coreactant. The ECL intensity intensely depended on solution pH (Fig. 4C), as the case of the pH-dependent PL behavior of CNNS due to the presence of free zigzag

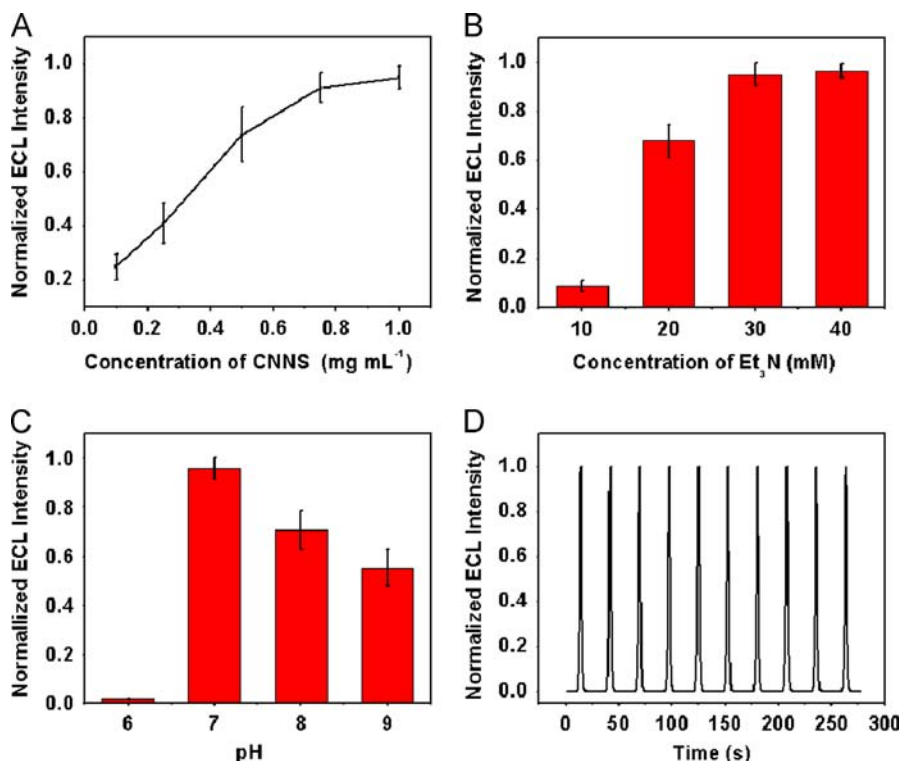


Fig. 4. Effects of (A) CNNS concentration, (B) Et₃N concentration and (C) pH on the ECL intensity of the CNNS modified electrode. (D) Continuous cyclic scans of the modified electrode in pH 7.0 containing 30 mM Et₃N.

sites on the surface [22]. Thus pH 7.0 was applied in the ECL detection, which was suitable for biological system.

The stability of the CNNS modified GCE was examined by 10 repeated cyclic scans in the potential range from 0 to 1.50 V (Fig. 4D). The relative standard deviation (RSD) of the ECL emission for each scan was 1.9%, showing a good fabrication stability of the modified electrode and the stability against photobleaching [34]. The RSD for ECL emission measured at five modified electrodes was 3.7%, indicating an acceptable reproducibility of this method.

3.4. ECL quenching by dopamine and its ECL detection

In dopamine solution, dopamine could be oxidized at the modified surface during anodic sweep (Eq. (6)), which led to an anodic peak at +0.23 V (not shown). Different from the hydrogen atom-transfer fluorescence quenching mechanism [35], the oxidation product (DA^{•+}) would annihilate Et₃N[•] radical (Eq. (7)) to decrease the ECL emission. Taking the advantage of the efficient ECL behavior of CNNS, a facile “signal-off” strategy could be proposed for the detection of dopamine based on the consumption of electro-oxidation product of coreactant.



Based on the quenching mechanism, the ECL peak intensity decreased with the increasing dopamine concentration (Fig. 5). The calibration plot showed a good linear relationship between the ECL intensity and logarithmic value of dopamine concentration ranging from 1.0 nM to 100 nM, with a correlation coefficient of 0.992 (Fig. 5, inset). The detection limit was estimated to be 96 pM at $S/N=3$, which was much lower than those of electrochemical (7.8 nM) [26] and fluorescence (33.5 nM) methods [30], indicating a high sensitivity of this ECL strategy.

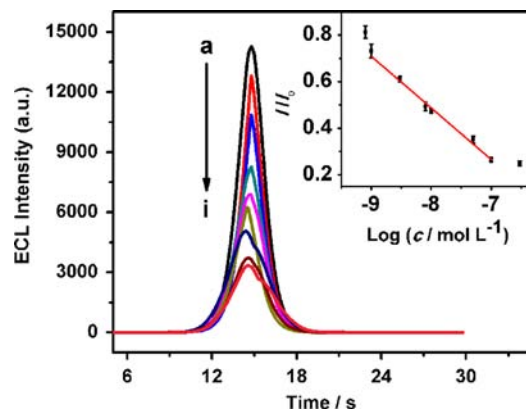


Fig. 5. ECL responses of the CNNS modified GCE in presence of 30 mM Et₃N to 0, 0.8, 1.0, 3.0, 8.0, 10, 50, 100 and 300 nM DA (from top to bottom). Inset: calibration curve.

3.5. Selectivity of ECL biosensor for dopamine

The specificity was investigated with GLc, Glu, CA, UA, AA, AD and NE as interferences. Comparing with the quenching efficiency of 63% for dopamine, these interferences at 10-fold concentration showed weak quenching efficiency up to 19% (Fig. 6). Even though epinephrine and norepinephrine structurally close to dopamine showed weak quenching effect on the ECL responses, considering the similar concentrations of these interferences to dopamine in real samples, they did not interfere with the detection of dopamine.

3.6. Detection of real samples

The analytical reliability and application potential of the proposed method was evaluated by detecting the dopamine hydrochloride injection sample. The dopamine concentration was

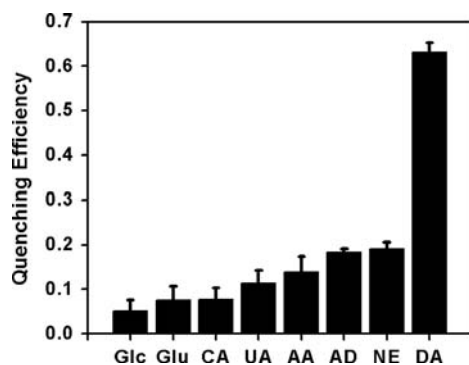


Fig. 6. Comparison of quenching efficiency of 3×10^{-8} M DA and 10-fold concentration of other reagents on the ECL of ultrathin CNNS modified GCE in the presence of Et_3N . The quenching efficiency refers to $(I_0 - I)/I_0$, I_0 is the initial ECL intensity, I is the ECL intensity in the presence of individual analyte.

determined to be 2.86 ± 0.02 nM, which was consistent with the referred value of 2.65 nM. Thus, the present method satisfied the need for the detection of dopamine in practice.

4. Conclusions

The $\text{g-C}_3\text{N}_4$ nanosheets with excellent stability can be conveniently prepared in aqueous solution. They show much stronger anodic ECL emission than bulk $\text{g-C}_3\text{N}_4$ in the presence of Et_3N coreactant due to the high ratio of surface area-to-volume and high exposure of the coordination sites. The ECL mechanism has been identified. The CNNS modified electrode possesses good stability and acceptable reproducibility. With a quenching process of dopamine on the strong ECL emission, which results from the reaction between the electro-oxidation products of coreactant and dopamine, an analytical method has been proposed for the detection of dopamine. This method shows excellent specificity, high sensitivity and low detection limit down to sub-picomole, and has been applied in analysis of real samples. The metal-free $\text{g-C}_3\text{N}_4$ nanosheets as a biocompatible material could extend the promising application of ECL techniques in biosensing.

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