In situ growth of copper oxide-graphite carbon nitride nanocomposites with peroxidase-mimicking activity for electrocatalytic and colorimetric detection of hydrogen peroxide

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This study reported a facile approach for the preparation of copper oxide-graphitic carbon nitride nanocomposites by an in situ growth method in aqueous solution. The powder X-ray diffraction, transmission electron microscopy, X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy and atomic force microscopy characterizations of the copper oxide-graphitic carbon nitride nanocomposites suggest that we have successfully synthesized a novel nanozyme. The nano-sized of copper oxide nanoparticles embedded in graphitic carbon nitride nanoparticles were evaluated focusing on the principle of horseradish peroxidase-like activity catalyzing the oxidation of 3, 3' , 5, 5'-tetramethylbenzidine in the presence of hydrogen peroxide. Furthermore, we constructed dual sensor platforms based on copper oxide-graphitic carbon nitride nanocomposites. By constructing an electrochemical platform and a colorimetric sensor to detect hydrogen peroxide, it is shown that copper oxide-graphitic carbon nitride nanocomposites had an excellent peroxidase mimicking catalytic performance. We envision that our nanozyme have great potential in a variety of applications in catalytic field, electrochemical sensing, optical imaging, and environmental assay.

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1. Introduction

Hydrogen peroxide (H₂O₂), one of the most common small molecules, is an informative molecule in the organism. More importantly, H₂O₂ is a considerable intermediate or product of many enzyme reactions, which is vital to maintain a normal cellular physiological activity [1–3]. In addition, H₂O₂ can also directly apply to bio-pharmaceutical, medical clinical, environmental management and food manufacturing and other fields due to its excellent oxidant activity [4,5]. Therefore, electrochemical sensors [5–7], fluorescence detection [8,9] and chemiluminescence analysis [10,11] have been gradually developed for sensitive detection of H₂O₂, and electrochemical detection of H₂O₂ had the advantage of low cost, high sensitivity, portable and other excellency [12]. Up to now, many nanomaterials (such as V₂O₅–CeO₂) [13] and enzyme-based universal interface [14] have been employed to fabricate electrochemical H₂O₂ sensor, but limited successes were achieved to satisfy all of strict requirements. In particular, non-enzymatic electrochemical sensors are less affected by temperature and pH than electrochemical enzyme sensors [5], so it is necessary to develop a novel non-enzymatic electrochemical sensors.

The nanozymes, novel functional materials, are synthesis of nanomaterials that can mimic the function of natural enzymes. Considering the intrinsic drawbacks of natural enzymes, the nanoparticles with peroxidase-like activity are widely concerned and developed due to their many excellent properties [15,16]. To date, a variety of nanomaterials, such as Fe₃O₄ nanoparticles [17,18], Co₃O₄ nanoparticles [19,20], CeO₂ nanoparticles [18,21], MnO₂ nanosheets [22], CuO nanoparticles [23,24], V₂O₅ nanoparticles...
[25,26], graphene quantum dots [27–29], metal clusters [30], metal sulfides (FeS, CuS) [31,32], noble metals (Au, Pt) [33,34], metal organic frameworks [35,36], and carbon nanotubes [37] have been explored to exhibit unexpected peroxidase enzyme-like activity. Because of nanozymes have a large specific surface area and excellent catalytic activity, they possess produce or remove reactive oxygen species inherent ability. Notably, nanozymes have advantages over natural enzymes, including facile of synthesis, good stability, low cost staple, and adjustable catalytic activity. Thus, nanostructures with enzymatic activity were considered to be potential substitutes for enzyme. Graphite carbon nitride (g-C3N4), an organic semiconductor material, which presents graphene-like faveolated structure, has exhibited an extraordinary property of fluorescence [38] and photocatalysis [39] in both theoretical research and practical application. Compared with other 2D materials, biosensors based on g-C3N4 were widely used because of their advantages of stable luminescent properties, abundant and inexpensive raw materials, excellent biocompatibility [40–43]. In recent years, the development of the nanozymes which could have the catalytic function of horseradish peroxidase (HRP) has attracted more and more interest. Song and co-workers [44] prepared the g-C3N4 supported platinum nanocomposites (g-C3N4/Pt NPs) by ultrasonic cleaning with ultrapure water. Furthermore, the nanocomposites were characterized by X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), Powder X-ray diffraction (XRD) and atomic force microscopy (AFM). The ultraviolet-visible (UV–Vis) spectra were compared with the colorimetric sensor in the presence of H2O2 and 3, 3′, 5, 5′-tetramethylbenzidine (TMB). The electrochemical behavior and electrocatalytic performance of H2O2 were characterized by differential pulse voltammetry (DPV) and cyclic voltammetry (CV). The results indicated that CuO-g-C3N4 nanocomposites have strong catalytic ability and can be used as a reliable nanozyme. The nafion/CuO-g-C3N4 nanocomposites/glassy carbon electrodes have a certain potential as a sensitive and reliable electrochemical sensor for detecting H2O2 in water samples.

2. Materials and methods

2.1. Materials

Urea, copper acetate monohydrate, 3, 3′, 5, 5′-tetramethylbenzidine (TMB), hydrogen peroxide (H2O2) and glacial acetic acid were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Sodium hydroxide (NaOH), ethanol and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other reagents were analytical grade. The use of ultrapure water (18.25 MΩ cm) throughout the course of the experiment was from the Aquapro water purification system. Supporting electrolyte was 0.1 M sodium hydroxide. Buffer solution was 0.1 M acetate buffer saline (pH 4.2) which was prepared with CH3COOH (HAc) and CH3COONa (NaAc). All solutions were prepared with ultrapure water.

2.2. Preparation of g-C3N4 nanoparticles

The bulk g-C3N4 and g-C3N4 nanoparticles were prepared according to a classic method with slight modification [48,49]. In a typical experiment, 9 g urea was put into the porcelain boat and then shifted to the horizontal tube furnace which was equipped with a programmed temperature system, heated to 80 °C for 1 h, followed by heating at 225 °C and 350 °C for 1 h separately. Finally, the temperature was maintained at 550 °C for 4 h with a flow of nitrogen atmosphere and then naturally cooled to ambient temperature. The temperature was warmed up from room temperature with a rising speed of 5 °C min^{-1}. In addition, the g-C3N4 nanoparticles were obtained by nitric acid treatment. 0.2 g bulk g-C3N4 was ultrasonically dispersed in 20 mL 5 M HNO3 at 115 °C for 24 h. The product was centrifuged, washed five times with ultrapure water to a neutral pH, and dispersed in ultrapure water again. The resultant suspension was sonicated for 5 h and then centrifuged at 2000 rpm for 10 min to eliminate the large nanosheets and the supernatant was continued by centrifugation at 10,000 rpm for 10 min. The supernatant was lyophilized.

2.3. Preparation of CuO-g-C3N4

The CuO-g-C3N4 nanoparticles were prepared according to a method of in situ synthesis. First, 28 mL 0.02 M aqueous copper acetate and 2 mL 2 mg mL^{-1} g-C3N4 nanoparticles were added to a 150 mL Erlenmeyer flask to mix well, and sonication for 0.5 h, followed by the addition 0.1 M glacial acetic acid. The solution was heated to boiling under poignant stirring. Then, 2 mL 1.0 M NaOH aqueous solution was swiftly added to the above boiling solution, and the solution immediately became black and heated for 5 min. The precipitate was centrifuged at 12,000 rpm, washed four times with absolute ethanol and lyophilized.

2.4. Electrochemical measurements

For the modification process of the CuO-g-C3N4 material, the bare glassy carbon electrode (GCE) surface was carefully polished to get a clean surface with 0.05 μm gamma alumina slurry and ultrasonically cleansed with ultrapure water, ethanol and ultrapure water for 5 min, and then dried at room temperature.
An amount of 2 mg as-synthesized CuO-g-C3N4 was dispersed in 1 mL 0.2% Nafion solution to get a homogeneous suspension by sonication. After drying, the modified electrode was prepared by casting 10 µL of the nanoparticle composite on the GCE electrode surface and drying at the room temperature.

Electrochemical experiments were carried out under nitrogen atmosphere by differential pulse voltammetry (DPV) and cyclic voltammetry (CV) in a cell containing 4.0 mL 0.1 M NaOH with the modified electrode and all the electrochemical experiments were carried out with three electrode systems, in which the GCE was applied as working electrode, a platinum wire was served as the counter electrode and a calomel electrode used as the reference electrode. The CV was registered by cycling the potential between −0.8 V and 0 V at a scan rate of 100 mV s⁻¹. DPV measurements were carried out in potential range from −0.6 V to −0.2 V. In this experiment, the current-potential data was recorded after successive addition different concentrations of H2O2 into the supporting electrolyte at room temperature.

2.5. Characterization

Fluorescence spectra were performed on a RF-5301PC fluorescence spectrophotometer which was purchased from Shimadzu Ltd., (Japan). Transmission electron microscopy (TEM) images were employed to HITACHI H-7000FA transmission electron microscope at an acceleration voltage of 100 kV. Atomic force microscopy (AFM) images were taken on a commercial atomic force microscope instrument (Dimension Edge with NanoDrive V8 controller, Bruker, USA). X-ray photoelectron spectroscopy (XPS) measurements were performed on an ESCALAB 250Xi instrument. Powder X-ray diffraction (XRD) patterns were obtained on a Shimadzu (Japan) XRD 6100 powder X-ray diffractometer, using Cu Kα radiation (λ = 1.5418 Å). Ultraviolet-visible (UV-Vis) absorption spectra were recorded on a UV-Vis spectrophotometer (UV-2700, Shimadzu, Japan). Fourier transform infrared spectroscopy was measured on FT-IR NICOLET iS10 spectrophotometer (Thermo scientific, USA) from 4000 cm⁻¹ to 650 cm⁻¹.

All electrochemical measurements were carried out with CHI660E electrochemical workstation (Shanghai Chenhua Instruments Co. Ltd., China). A three-electrode electrochemical system which consists of nafion/CuO-g-C3N4 modified glass carbon electrode as working electrode, saturated calomel electrode (SCE) as reference electrode and platinum electrode as the auxiliary electrode was used in this experiment. Before the measurements, nitrogen was continuously fed into 4.0 mL 0.1 M NaOH for 30 min to remove oxygen. Branson 2000 ultrasonic cleaner (USA) was used to clean the glassy carbon electrode (GCE, 3 mm in diameter).

3. Results and discussion

3.1. Characterization of nanoparticles

The freshly prepared Cu²⁺ and g-C3N4 were taken as a precursor for the preparation of CuO-g-C3N4 nanocomposites by using in situ growth method (Fig. 1). In order to bind with Cu²⁺, g-C3N4 and Cu²⁺ were mixed and subjected to sonication in advance. Thus, Cu²⁺ could be closely embedded on the surface of the g-C3N4 nanoparticles by coordination interactions. g-C3N4 is a kind of like-graphene sheet bearing triazine and oxygen-containing functional groups on their basal planes and edges. These functional groups can act as anchor sites and facilitates the in-situ growth of metal nanoparticles on the surface and edge of g-C3N4. Then, with the addition of NaOH, Cu(OH)₂ was formed gradually and deposited on the surface of the g-C3N4 [24]. At last, decoration of g-C3N4 with CuO was achieved by coprecipitation. Due to Cu(OH)₂ was a metastable phase and prone to transform into stable CuO via thermal dehydration in aqueous phase [52]. This preparation method is easy and fast and will be a better candidate for large-scale manufacturing.

To investigate the properties of materials, the g-C3N4 and nanocomposites were also characterized by X-ray diffraction (XRD). Fig. 2A showed the XRD pattern of bulk g-C3N4 and g-C3N4 nanoparticles without growth of CuO. It is implied that the successful synthesis of bulk g-C3N4 and the g-C3N4 nanoparticles has been modified. For CuO-g-C3N4 nanocomposites (B), XRD analysis showed the presence of CuO monoclinic phase with a refractive index of (110), (002), (111/200), (-202), (020), (202), (-113), (022/-311), (113/220), (311) and (004/-222) (JCPDS-PDF 89-2530) in the nanocomposites [50]. The results showed a series of excellent peaks, which were associated with high crystallinity and the high purity of CuO product in the as-prepared nanocomposites. It could be seen from Fig. 2B that the diffraction peaks of g-C3N4 in the nanocomposites were not obviously, which can be attributed to the large amount of CuO grown on g-C3N4 nanoparticles according to CuO-g-C3N4 nanocomposites and pure g-C3N4 nanoparticles masked its diffraction peak.

The TEM diagram of the g-C3N4 nanoparticles (C) and CuO-g-C3N4 nanocomposites (D), as shown in Fig. 2, it could be seen that the final formation of CuO-g-C3N4 nanocomposites consisted of CuO and g-C3N4 nanoparticles, which clearly indicated that the formation of the nanocomposites structure was in situ growth of CuO on g-C3N4 nanoparticles, respectively. In order to further investigate the formation of nanocomposites, the atomic force microscope (AFM) was used. It could be seen from Fig. 3 that the particle size of g-C3N4 nanoparticles were about 50 nm and not very uniform. However, it could be seen that the surface of g-C3N4 nanoparticles grown copper oxide nanoparticles (CuO NPs) and...
exhibited irregular spherical shape, which was consistent with TEM diagram. In addition, FI-IR spectra were used to pinpoint the functional groups of the as-prepared g-C3N4 and CuO-g-C3N4 nanocomposites (Fig. S2). In the FI-IR spectrum of g-C3N4 nanoparticles, the strong band near 3214 cm\(^{-1}\) was credited to the vibration absorption of \(\equiv\text{OH}\) or \(\equiv\text{COOH}\). The characteristic peaks of the skeleton vibration attributable to the g-C3N4 triazine ring at 809 cm\(^{-1}\) and the absorption band between 1200 and 1650 cm\(^{-1}\) belong to the vibrational characteristic peaks of C\(-\equiv\text{N}\) and C\(-\equiv\text{N}\) in the heterocyclic ring of g-C3N4. According to the comparison of two infrared spectra, the surface functionalities of the g-C3N4 NPs were changed upon growing the CuO NPs, we suspected that the triazine functionalities associated with the g-C3N4 NPs act as carrier for CuO growth. Then, the fluorescence spectra of g-C3N4 nanoparticles were characterized (Fig. S3). From the fluorescence spectrum of g-C3N4 nanoparticles, when excited at 320 nm, the g-C3N4 nanoparticles exhibited an energetic emission peak at 430 nm, which was similar to the reported g-C3N4 nanoparticles [49]. When the 365 nm UV beam was used, the g-C3N4 nanoparticles solution emitted blue light (430 nm). In order to research the oxidation performance and chemical bonding properties of CuO-g-C3N4 layered structure, many features had been tested. The corresponding XPS images of g-C3N4 nanoparticles and CuO-g-C3N4 intermediates were shown in Fig. 3. Only the C1s peak (284 eV, 288 eV), N1s peak (399 eV) and the O1s peak (532 eV) in the full range XPS of the acidified prepared g-C3N4 nanoparticles (Fig. 3A). Fig. 3A showed the CuO-g-C3N4 nanocomposites in which the peaks of C1s and O1s were clearly visible and the weak peaks of N1s. The C1s and O1s peaks may be caused by the absorbed \(\equiv\text{COOH}\) from solution. Except for the C1s, N1s and O1s peaks, the peaks at 77.0 eV and 124.1 eV were contributed by Cu3p and Cu3s, respectively. The Cu LMM Auger peaks consisted of two peaks of 571.1 eV and 650.1 eV. It is known that Cu (0) (metallic copper) (LMM) had a binding energy of about 567 eV and the binding energy of oxidized Cu (I) and Cu (II) (LMM) was about 569 eV [24]. Therefore, it could be inferred that the copper of the composite species did not exist in the form of monatomic copper but in the form of oxides. In addition, the peak of 942.8 eV corresponding to Cu2p\(^3\) could be clearly observed in the wide scan XPS pattern compared to the value of the metallic copper standard 932.7 eV [51], which was more convinced that copper is present in the complex as an oxide. The peaks at the core level 2p\(_{3/2}\) and 2p\(_{1/2}\) transitions were 933.8 eV and 953.5 eV, respectively, by high-resolution Cu 2p spectral analysis of the CuO-g-C3N4 nanocomposites (Fig. 3B). The gap between these levels was 20 eV, which was consistent with the standard specifications of CuO [51]. Fig. 3B, where two peaks located at 933.8 eV and 953.5 eV can be assigned to the binding energy of Cu 2p\(_{3/2}\) and Cu 2p\(_{1/2}\) respectively, indicating the presence of the Cu\(^{2+}\) on the sample. In addition, two extra shake-up satellite peaks for Cu 2p\(_{3/2}\) and Cu 2p\(_{1/2}\) at 942.1 eV and 962.2 eV were also observed at higher binding energy side, implying the presence of an unfilled Cu 3d\(^0\) shell and thus further confirmed the presence of Cu (II), which was consistent with the results of XRD [53]. The high resolution XPS spectra of C1s are shown in Fig. 3C and F. The peaks locating at 284.8 eV, 286.4 eV and 288.1 eV can be assigned to C\(-\equiv\text{C}\), C\(-\equiv\text{O}\) and C\(-\equiv\text{N}\) respectively. In Fig. 3D and G, the deconvoluted spectrum of N1s in g-C3N4 nanoparticles and CuO-g-C3N4 nanocomposites contains the pyridinic nitride at 398.4 eV, pyrrolic nitride at 399.3 eV, graphitic nitride at 400.9 eV and oxidized nitride at 406.5 eV separately. While the high resolution XPS spectra of O1s were shown in Fig. 3E and H. The peaks locating at 531.5 eV, 532.1 eV and 533.5 eV belong to the C\(-\equiv\text{OH}\), C\(-\equiv\text{O}\) and C\(-\equiv\text{O}\) respectively. In the middle of the peak locating at 529.9 eV was attributed to Cu\(-\equiv\text{O}\) [54,55]. Therefore, we could suggest that we have prepared CuO-g-C3N4 nanocomposites, as illustrate in Fig. 1.
3.2. Kinetic analysis of CuO-g-C$_3$N$_4$ nanocomposites as peroxidase mimetics

Steady-state kinetics of the TMB-H$_2$O$_2$ reaction system was further investigated to better understand the effect of the catalytic performance of peroxidase-like CuO-g-C$_3$N$_4$ nanocomposites. The apparent steady-state kinetic parameters of the TMB discoloration reaction were determined by varying the concentration of TMB or H$_2$O$_2$ in the reaction mixture. The kinetic experiments were carried out using 10 μg/mL CuO-g-C$_3$N$_4$ nanocomposites in 2 mL 0.1 M NaAc-HAc buffer solution (pH 4.2) containing the concentration of TMB or H$_2$O$_2$ in the reaction mixture. Michaelis-Menten kinetics equation,

$$\frac{1}{V} = \left(\frac{K_m}{V_{max}}\right) \cdot \left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

is used to describe the rate of enzymatic reactions. Where $V$ is the initial velocity, $K_m$ is the Michaelis-Menten constant, $V_{max}$ is the
maximal reaction velocity and \([S]\) is the concentration of the substrate. As a specific constant of the enzyme, the Michaelis-Menten constant \(K_m\) represents the substrate concentration at half the reaction rate of \(V_{max}\), which could reflect the affinity of the given enzyme to the substrate. Fig. 4 showed the substrate concentration-dependent response rate and the corresponding fitting curve for the Michaelis-Menten equation. The \(K_m\) value of CuO-g-C3N4 nanocomposites with TMB as the substrate was 0.9 mM, and the corresponding \(K_m\) value was 1.05 mM with \(H_2O_2\) as the substrate. The results obtained from Lineweaver-Burk plots indicated that the \(K_m\) value of CuO-g-C3N4 nanocomposites with \(H_2O_2\) as the substrate was much lower than that of HRP [17], and the \(K_m\) value of CuO-g-C3N4 nanocomposites with TMB as the substrate was much higher than that of HRP. It is worth to mention that the \(K_m\) (\(H_2O_2\)) value of CuO-g-C3N4 nanocomposites is much lower than HRP (3.7 mM), suggesting that the CuO-g-C3N4 nanocomposites have a considerably higher binding affinity toward \(H_2O_2\).

### 3.3. Determination of \(H_2O_2\) by colorimetry

Because of natural enzymes had some serious drawbacks, such as their catalytic activity can be easily inhibited and they could be digested by proteases, so the modeling of nanzyme modeling was the key in the present. In order to confirm the peroxidase-like activity of the synthesized CuO-g-C3N4 nanocomposites, catalytic oxidations of the substrate TMB were tested in the presence or absence of \(H_2O_2\).

As shown in Fig. 5, the CuO-g-C3N4 nanocomposites-TMB system was colorless and the TMB-\(H_2O_2\) system without CuO-g-C3N4 nanocomposites exhibited negligible color changes under experimental conditions. In contrast, the CuO-g-C3N4 nanocomposites-\(H_2O_2\) system produced a dark blue solution. These results clearly suggested that the obtained CuO-g-C3N4 nanocomposites have excellent peroxidase-like activity. Furthermore, the catalytic activity of CuO-g-C3N4 nanocomposites was dependent on the concentration of \(H_2O_2\), which could be used to detect \(H_2O_2\).

As shown in Fig. 6, CuO-g-C3N4 nanocomposites catalyzed the TMB redox reaction in the presence of \(H_2O_2\), led to a blue product with strong absorption at 652 nm. Since the catalytic activity of the CuO-g-C3N4 nanocomposites depended on the concentration of \(H_2O_2\), it could be used as a colorimetric assay for \(H_2O_2\) by monitoring the colored products at 652 nm by spectroscopy or visual observation. The initial TMB concentration was 1.0 mM and the CuO-g-C3N4 nanocomposites concentration was 10 \(\mu\)g mL\(^{-1}\) when the initial \(H_2O_2\) concentration varied from 2 \(\mu\)M to 150 \(\mu\)M, an increased amount of the oxidation product was produced, as observed by increasing the absorbance peak. Where the linear regression equation in Fig. 6B was \(A = 0.014 + 0.0021C\) (\(\mu\)M), where \(A\) was the absorbance intensity at 652 nm and \(C\) was the concentration of \(H_2O_2\). The detection limit (3\(\sigma/k\) = 3) of \(H_2O_2\) was found to be 1.2 \(\mu\)M. Compared with those previously reported biosensors of \(H_2O_2\) (Table S1 in the Supporting information), the proposed biosensor has a desirable linear range and detection limit because of excellent properties of CuO-g-C3N4 nanocomposites. This result indicates that CuO-g-C3N4 nanocomposites could indeed be used to simulate horseradish peroxidase catalyzed oxidation of TMB oxidation in the presence of \(H_2O_2\).
3.4. Electrochemical detection of $\text{H}_2\text{O}_2$ by CuO-g-C$_3$N$_4$ nanocomposites

As demonstrated above, CuO-g-C$_3$N$_4$ displayed excellent catalytic activity and could facilitate electron-transfer process. The cyclic voltammetry was recorded to study the electrocatalytic behavior of $\text{H}_2\text{O}_2$ by modified electrode. Fig. 7A and B showed the CVs of the electrode in 0.1 M NaOH at a scanning rate of 100 mV s$^{-1}$ in the absence (Fig. 7A, curve a, b, c, d) and presence (Fig. 7B, curve a, b, c, d) of 0.5 mM $\text{H}_2\text{O}_2$. Dissolved oxygen was removed from the N$_2$ saturated solution because the electrolytic reduction of dissolved oxygen would interfere with the current response for $\text{H}_2\text{O}_2$. It could be seen that bare GCE (Fig. 7A, curve a), nafton/GCE (Fig. 7A, curve b), nafton/g-C$_3$N$_4$ nanoparticles/GCE (Fig. 7A, curve c) and nafton/CuO-g-C$_3$N$_4$ nanocomposites/GCE (Fig. 7A, curve d) displayed almost no electrochemical response in the absence of $\text{H}_2\text{O}_2$. CuO-g-C$_3$N$_4$ nanocomposites/GCE indicated a high catalytic current of about 16.37 mA, indicating that CuO NPs attached to the g-C$_3$N$_4$ nanoparticles surface exhibited significant catalytic properties for $\text{H}_2\text{O}_2$. The large amount of catalytic current obtained could be attributed to the rough surface and conductivity of g-C$_3$N$_4$ as CuO NPs carrier, CuO NPs provided a number of anchor sites, and remedied for the defects of g-C$_3$N$_4$, thus retaining the CuO NPs excellent catalytic activity.

To examine the catalytic activity of nafton/CuO-g-C$_3$N$_4$ nanocomposites/GCE toward $\text{H}_2\text{O}_2$, the effect of $\text{H}_2\text{O}_2$ concentration on the current response was investigated. In Fig. 7C, the reduction current was linearly related to the increase of $\text{H}_2\text{O}_2$ concentration. Fig. 7D showed the linearity of the sensor response through the CVs test. The linear equation was $I_p (\mu\text{A}) = 8.94 + 14.05 C (\text{mM})$ and the correlation coefficient was 0.9988. Furthermore, as shown in Fig. 8, detection of $\text{H}_2\text{O}_2$ was performed by differential pulse voltammetry (DPV). Fig. 8A showed the DPV detection of different concentrations of hydrogen peroxide, Fig. 8B showed the relevant linear relationship, catalytic $\text{H}_2\text{O}_2$ linear regression equation expressed as $I_p (\mu\text{A}) = 1.40 + 2.24 C (\text{mM})$. The detection limit ($3\sigma/k = 3$) of $\text{H}_2\text{O}_2$ was found to be 7.7 $\mu\text{M}$. With the increase of $\text{H}_2\text{O}_2$ concentration, the reduction current increased gradually, suggesting that the nafton/CuO-g-C$_3$N$_4$ nanocomposites/GCE showed excellent catalytic performance. Thus, g-C$_3$N$_4$ with roughened surface and conductivity could be supported as CuO NPs. CuO-g-C$_3$N$_4$ nanocomposites retained a higher surface volume ratio and remedied for the defects of g-C$_3$N$_4$, with larger surface areas and more electroactive sites for the adsorption and reaction of $\text{H}_2\text{O}_2$ molecules.

4. Conclusion

In summary, CuO-g-C$_3$N$_4$ nanocomposites had been successfully prepared by an in situ growth method, which had the function of horseradish peroxidase. It could be used to construct colorimetric/ electrochemical detection platform for $\text{H}_2\text{O}_2$. Taking into account the ease of preparation and good experimental results, CuO-g-C$_3$N$_4$ nanocomposites could be used as a promising electrocatalytic...
material for the construction of biosensors and for bio-mimicking horseradish peroxidase.

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at https://doi.org/10.1016/j.carbon.2017.11.096.

**References**


