



Research Paper

Hemoglobin immobilized in g-C₃N₄ nanoparticle decorated 3D graphene-LDH network: Direct electrochemistry and electrocatalysis to trichloroacetic acid

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ABSTRACT

A 3D hybrid of graphitic C₃N₄ nanoparticle decorated in the assembly of graphene and Co₂Al layered double hydroxide nanosheets (GR-LDH-C₃N₄) was prepared by simple hydrothermal treatment and subsequent calcination-recovery process. X-ray diffraction, scanning electron microscopy, transmission electron microscopy and X-ray photoelectron spectroscopy clearly demonstrated that the g-C₃N₄ nanoparticle was successful incorporated into the porous GR-LDH nano-scaffold. Then the conductive composite and chitosan (CTS) were used to immobilize hemoglobin (Hb) on a carbon ionic liquid electrode (CILE) for fabrication of a trichloroacetic acid (TCA) biosensor. The spectroscopic results revealed that the immobilized Hb retained the native catalytic activity and conformation. Electrochemical results indicated that the GR-LDH-C₃N₄ film possessed the large electroactive surface area and fast heterogeneous electron transfer rate. Due to synergistic effects of three components, CTS/GR-LDH-C₃N₄-Hb/CILE gave rise to the excellent direct electrochemistry and electrocatalytic activity toward TCA. This bioelectrode exhibited a wide linear detection range from 0.2 to 36.0 mM with a low detection limit of 0.05 mM (3σ). The Michaelis-Menten constant of the developed bioelectrode was obtained as 3.30 mM. Thus, the 3D porous GR-LDH-C₃N₄ architecture is a promising material for construction of the third-generation biosensors.

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1. Introduction

Layered double hydroxides (LDH), also named anionic clays, are defined as a kind of lamellar hydrotalcite-like compounds with a generic formula of [M_{1-x}^{II}M_x^{III}(OH)₂]^{x+}[(Aⁿ⁻)_{x/n}·mH₂O]. Part M^{II} coordinated octahedrally by hydroxyl groups are uniformly substituted by M^{III}, which results in the positively charged host layers with exchangeable charge-balancing interlayer anions. Due to the flexible ion-exchange property and positive charges, LDH have been broadly applied to immobilize biomolecules for electrocatalysis and biosensor [1–4]. Recently, exfoliation of LDH into single- or several-layer hydrotalcite-like nanosheets has attracted much attention. In this way, LDH nanosheets can afford larger surface area, more electroactive sites and a convenient access to the inner surfaces of the host layer [5,6]. However, LDH nanosheets cannot

fully exert their performance due to their poor stability after removing polar organic solvents. The intrinsic non-conductivity is another shortcoming for LDH sheets, therefore such carbon materials as graphene (GR) or carbon nanotube (CNT) have been used as hierarchical spacers to simultaneously improve the chemical stability and conductivity and electrocatalytic activity [7,8].

GR is an atomic-plane-thick nanosheet by employing the sp² hybrid carbon atoms, which pack into a 2D honeycomb network. Because of conspicuous thermal and mechanical properties, good flexibility, larger surface areas (2630 m²/g) and high electron transfer rate, GR has attracted considerable attention since 2004 [9]. However, many outstanding properties of GR can perform only when it occurs in single or several nanosheets in complex assembly [10]. Therefore, GR-based hybrids have become more fascinating in sensing field [11]. The idiomatic synthesis route of GR-based material is chemical reduction of exfoliated graphene oxide (GO). Unfortunately, this method not only leads to many framework defects due to irreversible aggregation, but also introduces contaminants from the excess reducing agents, which greatly impairs the

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full performance of the individual 2D carbon lattice [12]. An effective approach to fabricate GR-based composite is firstly to assemble GR nanosheets with other 2D ones through electrostatic interactions. Particularly for the electrostatic assembling of exfoliated LDH nanosheets with GO, this well-controlled technology is prone to maximize the direct contact of active metals of LDH nanosheets with sp^2 carbon atoms of GO ones for their perfect electrochemical performance. It is affirmable that this assembling method is obviously superior to the in situ growth/crystallization of LDH on GR or GO substrate, because the latter can cause a densely stacked, large LDH phases [13]. This face-to-face construction manner can compensate for the respective defects of GR and LDH nanosheets, which can guarantee the excellent electrochemical activity.

Additionally, gelation of GO is a powerful tool to incorporate individual GO nanosheets into monoliths with 3D porous architectures, which can also prevent GR sheets from irreversible agglomerating or restacking to graphitic structure. Among numerous GR gels, GR hydrogel has several native merits including unique structures, large specific surface areas, and potential applications [14]. Particularly its classical 3D networks can provide faster electron transfer, large surface area and superb stability. More importantly, this 3D framework is a desirable for preparation of various functional nanocomposites. For example, the GR-based hydrogels containing noble-metal was prepared at 120 °C and showed the high conductivity and catalytic activity [15]. The self-assembly of GR-Au nanoparticles was produced in 3D architecture with high adsorption activity and fast electron transfer [16]. These results have proved that the hydrothermal method is an effective way to prepare GR-based composite hydrogels containing other components such as metal species [16] and metal hydroxide [17].

Graphitic carbon nitride ($g-C_3N_4$), the most stable allotrope of carbon nitride, has presented a prosperous application in photo/electro-catalysis [18], bioimaging [19], and energy conversion processes [20]. It is mainly due to its peculiar thermal stability, high in-plane nitrogen content, appealing electronic structure and favorable biocompatibility [21]. Given that the intrinsic poor conductivity ($\sigma < 10^{-2} \text{ S cm}^{-1}$), $g-C_3N_4$ are generally used to prepare the nitrogen doped carbon-based composites to improve the electron-donor property of carbon matrix. As a consequence, $g-C_3N_4$, as nitrogen species, can improve the dispersity, stability and durability of composite due to the enhanced electronic coupling between $g-C_3N_4$ and enthetic carbon matrix. Moreover, nitrogen doping can increase the surface charge density and active sites for the enhanced electrochemical responses [22]. On the other hand, carbon ingredient in composite can promote the electron transportation and increase the conductivity of C-N material. For example, the proper amount of $g-C_3N_4$ in situ grown on GR has efficiently enhanced the redox signals and accelerated electron transfer of biomolecules [23]. Additionally, the low-cost preparation, excellent biocompatibility and nontoxicity also make $g-C_3N_4$ serve as an ideal nitrogen-doping agent to enhance the electrocatalytic ability of composite.

It is well known that direct electrochemistry of redox proteins can afford the foundation for the electron transfer mechanism in biological process [24], biosensors [4], bioreactors [25] and biomedical devices [26] without adding any mediator and promoter. However, thanks to adsorptive denaturation, deep embedment of electroactive centers and unsatisfactory mass-transfer rate, the unrealizable direct electron transfer of protein on the bare electrode casts a shadow over its promising prospect. In order to improve the heterogeneous electron transfer rate and maintain the native secondary structure of the redox protein, various nanostructures including layered, sphere, nanorod-like and porous nanocomposites are employed to modify the working electrode [3,27–30].

Basing on the above studies, it is well believed that the decoration of $g-C_3N_4$ nanoparticle in GR-LDH network can fully play the synergistic electrochemical performance of three components. Especially, gelation treatment of the mixed GO and LDH nanosheets suspension can provide the porous nanostructure comprising the well dispersed nanosheets building blocks. The doping of $g-C_3N_4$ can increase the catalytic active sites and stability. Hence, in this work the 3D GR-LDH- C_3N_4 nanocomposite was synthesized by hydrothermal treatment following calcination-recovery procedure. The resultant porous 3D nanocomposite is desirable for the enzyme capture and the direct electron transfer. Then hemoglobin (Hb) was immobilized on this matrix with assistance of film-forming agent chitosan (CTS). Additionally, previous studies have demonstrated that carbon ionic liquid electrode (CILE) can enhance resistance against electrode-fouling, electron transfer rate, electrochemical stability and sensitivity [3,28,29]. Therefore, 3D GR-LDH- C_3N_4 was deposited on CILE surface for immobilization of Hb owing to the large surface area, active sites, and biocompatibility. Consequently, this porous composite maximized the synergistic effects of the three components for the faster electron transportation and the better electrochemical performance. The direct electrochemistry and electrocatalysis of the bioelectrode to trichloroacetic acid (TCA) were investigated for a potential unmediated third-generation electrochemical biosensor.

2. Experimental

2.1. Regents

The bovine hemoglobin (Hb, MW. 64 500, Sinopharm Chemical Reagent Co., China), 1-butylpyridinium hexafluorophosphate (BPPF₆, Lanzhou Green chem ILS. LICP. CAS.), graphite powder (average particle size 30 μm , Colloid Chemical Co. Shanghai, China) and trichloroacetic acid (TCA, Tianjin Kemiou Chemical Co. Ltd., China) were used as received. All the other chemicals were analytical grade and used without further purification.

2.2. Apparatus

UV-vis absorption and FTIR spectra were executed on a TU-1901 spectrophotometer (Beijing Puxi, China) and a Nicolet iS10 spectrometer (Thermoelectron co.), respectively. X-ray diffraction patterns (XRD) were characterized on a Rigaku powder diffractometer with Ni-filtered Cu K α radiation (λ 1.54050 Å, 40 kV and 100 mA). The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were obtained with JSM-6700F and JEOL JEM-2000EX microscopes, respectively. High resolution transmission electron microscopy (HRTEM) images were recorded on the FEI Tecnai G2 F20 S-TWIN microscope. X-ray photoelectron spectroscopy (XPS) was conducted on an HP 5950A ESCA spectrometer with an MgK α source.

2.3. Preparation and exfoliation of LDH

According to the procedure [31], the mixed aqueous solution were prepared by adding $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and urea with their concentrations as 10, 5, and 35 mM, respectively. After 2 h stirring at room temperature, the mixed solution was further stirred for 48 h at 97 °C. The pink LDH was obtained through centrifugation following thoroughly washing with DI water and ethanol. The obtained 100 mg LDH was dispersed in 100 mL aqueous solution (1 M NaCl + 3.3 mM HCl), which was agitated for 12 h under N_2 atmosphere for complete decarbonation. The resultant Cl-LDH was added to 100 mL 0.1 M NaNO_3 aqueous solution. The mixed suspension was also stirred for 12 h under N_2 atmosphere. Subsequently,

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