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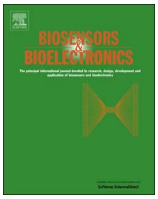

In situ synthesis of cylindrical spongy polypyrrole doped protonated graphitic carbon nitride for cholesterol sensing application

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ABSTRACT

Herein, we demonstrate the exfoliation of bulk graphitic carbon nitrides (g-C_3N_4) into ultra-thin (~3.4 nm) two-dimensional (2D) nanosheets and their functionalization with proton (g-$C_3N_4H^+$). The layered semiconductor g-$C_3N_4H^+$ nanosheets were doped with cylindrical spongy shaped polypyrrole (CSPPy-g-$C_3N_4H^+$) using chemical polymerization method. The as-prepared nanohybrid composite was utilized to fabricate cholesterol biosensors after immobilization of cholesterol oxidase (ChOx) at physiological pH. Large specific surface area and positive charge nature of CSPPy-g-$C_3N_4H^+$ composite has tendency to generate strong electrostatic attraction with negatively charged ChOx, and as a result they formed stable bionanohybrid composite with high enzyme loading. A detailed electrochemical characterization of as-fabricated biosensor electrode (ChOx-CSPPy-g-$C_3N_4H^+$/GCE) exhibited high-sensitivity (645.7 μAmM^{-1} cm^{-2}) in wide-linear range of 0.02–5.0 mM, low detection limit (8.0 μM), fast response time (~3 s), long-term stability, and good selectivity during cholesterol detection. To the best of our knowledge, this novel nanocomposite was utilized for the first time for cholesterol biosensor fabrication that resulted in high sensing performance. Hence, this approach opens a new prospective to utilize CSPPy-g-$C_3N_4H^+$ composite as cost-effective, biocompatible, eco-friendly, and superior electrocatalytic as well as electroconductive having great application potentials that could pave the ways to explore many other new sensors fabrication and biomedical applications.

1. Introduction

Tremendous approaches have been endorsed to prevent, reduce and treat the life-threatening dyslipidemia and cardiovascular diseases such as hypertension, atherosclerosis and cardiopathy (Baigent et al., 2010; Arsenault and Puri, 2016). The undesirable accumulation of cholesterol is the main cause of such disease, which is due to excessive use of cholesterol-containing food, lack of regular physical activities, and tobacco smoking. At present, there is substantial increase in number of patients suffering from heart and stroke related diseases that attracted more attention and challenges in global health for the coming decades (Labarthe and Dunbar, 2012). Thus, constant monitoring of blood cholesterol level is required for early clinical diagnostic and treatment.

Development and utilization of highly specific, low-cost, scalable, biocompatible, and eco-friendly cholesterol biosensors are required as promising biomedical devices to replace the commonly used time consuming expensive equipment's (Saxena and Das, 2016). In this context, an extensive research work has been performed for the fabrication of effective biosensor architecture to monitor cholesterol level with fast response time (Komathi et al., 2016). Among variety of reported techniques and strategies for cholesterol detection, an electrochemical based method has received significant interest as an easy, high sensitivity, fast response time, cost-effective, and highly accurate during measurement (Ahmadalinezhad and Chen, 2011; Fang et al., 2006; Mansano et al., 2010; Ahmad et al., 2013, 2014; Labib et al., 2016). Enzyme based electrochemical cholesterol biosensors are specific, accurate and significantly enhance biocatalytic reaction during analyte detection (Wilson and Hu, 2000). However lack of appropriate micro/nanostructures and properties of composite matrix, the redox active sites of immobilized enzyme lower its performance and hinder the electrode efficiency for direct and fast electron transport. To

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enhance the performance of enzyme based biosensors, various nanomaterials including metals and metal oxides, graphene, chitosan, and carbon nanotubes, semiconductors nanocrystals and quantum dot (QDs) have been employed as nanocomposite materials (Tan et al., 2005; Hahn et al., 2012). Despite intensive research efforts on extensive exploitation of metal and metal oxide nanoparticles, remarkable challenges still remain such as dispersion, less economical, hard to modify their surface, and ecological concerns while being used in biological applications (Medintz et al., 2005; Somers et al., 2007).

Taking all these facts into consideration, organic conducting polymers (OCPs) have been paid noteworthy attention for various applications as a consequence of their highly conductive, biocompatible, cost-effective, easily-copolymerization, and environment friendliness properties (Aydemir et al., 2016; Malhotra et al., 2006; Quigley et al., 2009; Yuan et al., 2013). Additionally, π-conjugated backbone in OCPs enhance electrical conductive pathway which would facilitate the charges transport process (Ahuja et al., 2007). Among many OCPs, polypyrrole (PPy) as an intrinsic OCP has been widely recognized electron transfer material for unconditional grafting into hybrid materials and spineless bipolarons state, which demonstrated the interesting application for biosensors (Bredas and Street, 1985). Additionally, varied surface morphologies (tubular, nanowires, globular, and cylindrical) of oxidized PPy inherits pivotal roles in redox activities, strong biocompatibility, and non-toxic towards biomolecules (Sadki et al., 2000; Qian et al., 2014; Shrestha et al., 2016; Brahim et al., 2001). Previously, oxidized PPy nanostructures integrated with ChOx has been reported for cholesterol detection. The bioengineered composite materials of PPy can be obtained by chemical and electrochemical polymerization, which are regarded as a fast, effective and ideal strategy for biosensor fabrication to detect cholesterol. Despite its attractive sensing applications, none of the studies have been explored the utilization of PPy nanostructures with graphitic carbon nitride (g-C_3N_4) to make nanohybrid composite material.

In recent few years, metal-free, bio-functional 2D materials have opened up exciting possibilities for the development of amperometric cholesterol biosensors due to their unique electrical, optical, mechanical, chemical and topographical properties (Ramendra and Raj, 2010). Polymeric g-C_3N_4, a 2D nanosheet analogous of graphene has triggered keen interest because of biocompatibility, less expensive, easy to synthesize, semiconductor behavior with appropriate band gap energy and large surface area to volume ratio. Thus, their potential application as catalyst, and energy transducer employed as best ideal substrate in biosensor platforms (Wang et al., 2009; Lu et al., 2017; Xiong et al., 2017). Owing to the nitrogen-doped, the g-C_3N_4 showed strong mechanical and good electrical properties and also exhibited robust thermal and chemical stability, which ensured key interest for sensing applications (Lu et al., 2015; Zhang et al., 2010). The presence of large number of tri-s-triazine units in g-C_3N_4 also confirms the high thermal stability. Even though, their inter layers interactions hold g-C_3N_4 insoluble in most water and organic solvents (Shi et al., 2016). Recently, Niu et al. synthesized ultra-thin nanosheets of mesoporous g-C_3N_4 with high surface area by chemical and thermal oxidation etching and liquid exfoliation in polar solvent for improved photocatalytic activities (Niu et al., 2012; Bai et al., 2014). Zhang et al. and Wang et al. reported improvement in homogenous dispersibility through surface functionalization of g-C_3N_4 by iodine and sulfur doping, respectively (Zhang et al., 2014; Wang et al., 2015). Furthermore, Fang et al. used Pd/Au bimetallic nanoparticles to decorate the g-C_3N_4 nanosheets to enhance the catalytic performance (Fang et al., 2016). Importantly, there are no reports offering cholesterol biosensor fabrication utilizing CSPPy modified ultra-thin 2D g-C_3N_4 nanosheets for cholesterol detection.

In this work, we report, for the first time, synthesis of highly efficient and desirable nanohybrid composite of CS shaped PPy doped protonated ultra-thin g-$C_3N_4H^+$ nanosheets. Subsequently, ChOx was immobilized onto nanohybrid composite (CSPPy-g-$C_3N_4H^+$) to construct cholesterol biosensor (ChOx-CSPPy-g-$C_3N_4H^+$/GCE) electrode. As a consequence, our designed cholesterol biosensor exhibited improved electrochemical sensing performance. We believe that our results will lead a guiding role to design a metal-free, label-free, non-toxic, cost-effective and electrochemically active bio-sensing electrode.

2. Experimental section

2.1. Proton functionalization of g-C_3N_4 (g-$C_3N_4H^+$)

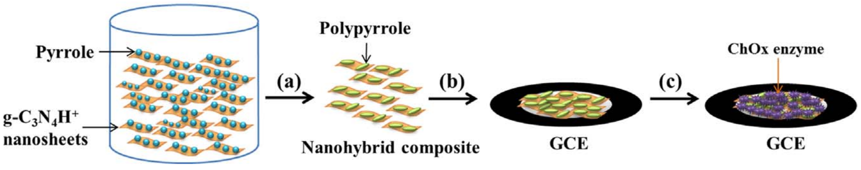
Bulk g-C_3N_4 in heterogeneous aqueous suspension was employed in probe sonication for 4 h to obtain fine exfoliated ultra-thin nanosheets. The complete protonation was performed under reflux heating at 70 °C for 4 h with constant stirring. Prior to reflux in condenser, 0.5 g g-C_3N_4 ultra-thin nanosheets were dispersed in 100 mL of concentrated HCl and sonicated for 30 min. Then, the product was allowed to cool down to room temperature followed by dilution and continuous washing with distilled water and anhydrous ethanol during filtration. Finally, the obtained light yellow residue of proton functionalized g-C_3N_4 (g-$C_3N_4H^+$) was dried overnight at 80 °C (Ong et al., 2015).

2.2. Preparation of CSPPy doped g-$C_3N_4H^+$(CSPPy-g-$C_3N_4H^+$) nanohybrid composite

To dope the g-$C_3N_4H^+$ with CSPPy, first, 0.25 g of engineered g-$C_3N_4H^+$ nanosheets were added into 100 mL of 1 M aqueous acetonitrile solution containing 0. 01 M pyrrole and sonicated for 30 min. Then, the suspension was treated with 0.01 M ammonium persulfate in flask at 5 °C for 24 h, while stirring at constant speed (Scheme 1a). Complete oxidative chemical polymerization of pyrrole produces bipolaron state of PPy in cylindrical shape deposited on the surface of g-$C_3N_4H^+$ nanosheets. Finally, as-prepared CSPPy-g-$C_3N_4H^+$ nanohybrid composite was filtered and washed several time with anhydrous ethanol and distilled water, respectively, and dried overnight at 70 °C.

2.3. Fabrication of cholesterol biosensor (ChOx-CSPPy-g-$C_3N_4H^+$/GCE) electrode

To fabricate cholesterol biosensor, first, bare glassy carbon electrode (GCE; 3 mm in diameter) was polished successively with 0.3 µm and 0.05 µm particle sizes of alumina suspensions followed by 1 µm particle size of diamond suspension on rayon polishing pad to obtain mirror finishing of GCE surface. All polishing steps were followed by sonication in ethanol for 15 min and extensive rinsing with anhydrous ethanol and deionized water. The cleaned GCEs were treated by CV technique in the potential range from −0.2 to 1.0 V (vs. SCE) using 0.5 M H_2SO_4 as



Scheme 1. Schematic illustration of (a) one-step in situ chemical polymerization of PPy on g-$C_3N_4H^+$ nanosheets, (b) deposition of hybrid composite on GEC, and (c) ChOx enzyme immobilization by physical adsorption method to fabricate cholesterol biosensor electrode.