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Enhanced electrochemical biosensing efficiency of silica particles supported on partially reduced graphene oxide for sensitive detection of cholesterol

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ABSTRACT

The present work introduces partially reduced graphene oxide (pRGO)-silica (SiO_2) particles hybrid system (pRGOSHs) for sensitive and cost effective free cholesterol detection. Fabricated out of thin layers of pRGOSHs, these proposed ChOx/pRGOSHs/ITO based biosensors have a detection range of 2.6–15.5 mM with an appreciable detection limit of 1.3 mM and sensitivity of 11.1 μA/mM/cm^2. Low Michaelis–Menten constant (K_m) (4.9×10^{-4} mM) and high diffusivity constant (D) (3.2×10^{-10} cm^2/s) values clearly indicate enhanced immobilization of enzyme over the substrate. Additionally, electrochemical impedance studies indicate that the synergistic combination of SiO_2 and pRGO also results in much lower impedance values (40% and 18% decrease in comparison to SiO_2 and pRGO respectively) for an overall enhanced sensing performance. These results are further corroborated by the density functional theory based theoretical simulations indicating enhanced electron density (theoretically) in case of the proposed hybrid system. Finally, the present work also highlights the importance of Si–OH bonds formation in the proposed pRGOSHs composite system for attaining such enhanced biosensing ability.

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1. Introduction

Cholesterol, a waxy steroid metabolite, is a direct indicator for hypertension, myocardial infarction and arteriosclerosis in human beings. Less than 200 mg dL^{-1} (5.17 mM) of cholesterol (serum part) in the human blood plasma is considered normal, however, higher levels of cholesterol leads to Hypercholesterolemia, a common issue owing to the present generation's eating habits [1]. Hypercholesterolemia leads to the nucleation of deadliest cardiovascular, coronary diseases and in extreme cases culminates into transient ischemic heart attacks [2]. Thus, close monitoring of cholesterol in food and blood using efficient, accurate and economic techniques has remained an active research area. Down this line chemical/colorimetric method, spectrophotometry, thin layer chromatography, gas–liquid chromatography, fluorimetric method, polarographic method and gas chromatography/mass spectrometry and electrochemical sensing have all been used for cholesterol detection [3]. Among these, electrochemical sensing route has gained much attention owing to its high portability, sensitivity, efficiency and accuracy. Thus constant efforts are being put in to find better materials for fabricating electrochemical electrodes which have better affinity towards bioanalytes and result in higher efficiency and improved response time [4].

Nanomaterials based electrochemical electrodes have shown tremendous potential with high sensitivity, selectivity and signal to noise ratio, owing to their high surface area and unique electro-chemical nature [5,6]. Among the various metallic and non-metallic nanomaterials employed for electrode fabrication, silica nanoparticles have gained much attention in recent past. With unique properties, such as porosity, large surface areas and pore volumes allowing better loading of reactive molecules per particle, good biocompatibility and low cytotoxicity; silica nanoparticles had already made their mark for therapeutic applications [7–9]. However, the potential of these economic mesoporous nanoparticles for sensing purposes was discovered once they were produced via the sol–gel technique [10], which showcased improved response time and detection limit [11–13]. However, these wormhole-like porous structured sol–gel synthesized silica nanoparticles are limited by low conductivity

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and diffusivity, a clear motivation for combining them with a conducting base material to form a composite material.

In the near past, graphene oxide (GO) based electrodes have also shown enhanced electrochemical activities for detection of various bioanalytes including DNA, enzyme and protein [14–16]. This is owing to GO's high 2-D surface area and abundant functional groups promoting enhanced immobilization of bioanalytes. Further, the electrochemical performance of GO has been seen to improve on synergistically combining with metal and metal oxide nanoparticles. By way of examples, Au/GO [17,18] combination for H_2O_2 and uric acid detection, GO/SiO_2 for urea detection [19], Pt/GO [20,21] for H_2O_2 sensing and catalytic reduction and Pd/GO [22,23] has been used for detecting chlorophenols, hydroquinone (HQ) and catechol (CC). Although appreciated for its sensing performance, especially its high loading capacity, the efficacy of GO composites is often questioned for its unbalanced conductivity. Completely reducing GO to form planar sp^2 hybridized reduced graphene oxide with good conductivity not only makes the material insoluble but also adversely effects the loading capacity due to complete loss in of functional groups. Therefore, the solution lies in partial restoration of the sp^2 hybridized network by mild reduction of GO thereby attaining conductivity, while simultaneously retaining some functional groups crucial for electrochemical sensing [24]. New to the biosensing domain, this balanced material known as partially reduced graphene oxides (pRGO), has proven its worth in the recent times [25,26].

Thus, with a better base material at hand (pRGO) having balanced conductivity and functional groups, and a clear motivation of combining SiO_2 nanoparticles (having enough hydroxyl functional group –OH) with a conducting material, the present work investigates the pRGO-silica nanoparticles composite as a sensing platform. The composite resolves the conductivity problems associated with SiO_2 nanoparticles, while uses their high porosity, biocompatibility and catalytic activity, by synergistically combining it with pRGO for enhanced biosensing performance. Further, these pRGO-silica nanoparticles composites are fabricated using a facile, fast and reproducible synthesis route, which is both economical, owing to easily available inexpensive silica, and ecological, owing to zero hazardous by products generation.

2. Experimental

2.1. Materials

Graphite flakes (NGS Naturgraphit GmbH, Germany), TEOS {Si$(OC_2H_5)_4$}, (Aldrich, purity ≥99% with trace metal basis), H_2SO_4, H_3PO_4, $KMnO_4$, H_2O_2, 1-ethyl-3-(−3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), hydrazine hydrate, ammonia solution, ethanol, etc. used were of analytical reagent grade. All the other chemicals employed for the fabrication of cholesterol biosensor, namely, cholesterol oxidase (ChOx), cholesterol, etc. were procured from Sigma-Aldrich.

2.2. Synthesis of GO and silica particles

Specifically, GO was prepared by the improved method proposed by Marcano et al. [27]. Briefly, a 9:1 combination of concentrated H_2SO_4/H_3PO_4 (240/26.7 mL) was added to 2 g of graphite flakes and 12 g of $KMnO_4$. The reaction mixture was then stirred for 12 h at a constant temperature of 50 °C. Finally this reaction was quenched by addition of ~270 mL of ice along with 2 mL of 30% H_2O_2. The as obtained yellowish slurry mixture was then shifted, centrifuged and filtered. The filtrate thus obtained was washed with 30% HCl and distilled water several times, until pH ~7 was achieved. At the end it was dried at 70 °C to procure the required solid GO.

The well-known Stober's method was employed for the production of uniform silica particles [28]. In short, in a round bottom flask, 75 mL of ethanol (98%) was taken and 7 mL of ammonia solution was added to it. The pH of the solution was adjusted ~12. This solution was then stirred for about 20 min. Under continuous stirring, the sol–gel reaction was initiated by adding 1.5 mL of TEOS to the above solution. The temperature was maintained at ~55 °C for 1 h and the stirring was continued for additional 3 h. The as obtained solution was then centrifuged at a speed of 3000 rpm for 15 min, followed by washing with ethanol and then drying in an oven at 80 °C. Finally, the white solid product obtained was used for further measurements.

2.3. Production of pRGO and SiO_2 decorated pRGO hybrid system (pRGOSHs)

100 mg of as-synthesized GO was well dispersed in 100 mL of distilled water (DW) by ultrasonication. This was done in order to restrict the GO sheets to single or few layers, with increased interlayer spacing. Further, pH of above dispersion was crucially adjusted to 10, by adding few drops of ammonia solution. To this dispersion, 300 μL of hydrazine hydrate (10 mg of silica particles dispersion was additionally added for the production of pRGOSHs) was added for the partial reduction while maintaining the temperature at 75 °C. Ultrasonication was done for an hour, followed by subsequent stirring for 3 h at 80 °C. The corollary was the partial reduction of GO to form pRGO (pRGOSHs). As the final step, this solution was washed several times and dried overnight at 70 °C.

2.4. Fabrication of pRGO and pRGOSHs thin film electrodes

The formation of both pRGO as well as pRGOSHs thin films over indium tin oxide (ITO) electrodes was achieved by electrophoretic deposition (EPD) technique {Fig. 3(a)}. In this, 10 mL colloidal solutions of pRGO and pRGOSHs (3 mg dL^{-1}) in acetonitrile were taken in two-electrode glass cell. A platinum foil of 1 × 2 cm was used as the counter electrode, while a well cleaned ITO-coated glass substrate of recorded sheet resistance of 30 Ω cm^{-1} was taken as the working electrode. These electrodes were positioned parallel to each other, separated by a distance of 1 cm. Film deposition over ITO-coated glass plate (0.25 cm^2) was accomplished by applying a DC voltage of 150 V for 45 s, in the case of pRGO and 50 V for 2 min, in the case of pRGOSHs. Further, about 10^{-5}–10^{-4} M of Mg$(NO_3)_2 \cdot 6H_2O$ was added as an electrolyte into the colloidal suspension, in order to create surface charge on both pRGO as well as pRGOSHs, which is essential for successful EPD. Finally, these electrodes were removed from the suspension, followed by thorough washing with deionized water and subsequent drying.

2.5. Solution preparation and fabrication of bioelectrodes

600 mg dL^{-1} (15.54 mM) of cholesterol stock solution was prepared in a heat bath maintained at 60 °C. Typically, cholesterol was dissolved in a flask (kept on heat bath) containing Triton X-100. This solution was further diluted with 0.02 M PBS solution (pH 7.0) for making different cholesterol concentrations (50 to 600 mg dL^{-1}). The EDC-NHS chemistry was applied on both pRGO and pRGOSHs electrodes to activate the COOH groups, prior to the immobilization of ChOx. For the immobilization of ChOx on pRGO/ITO and pRGOSHs/ITO electrodes, 5 μL of ChOx (1 mg dL^{-1}) (Cholesterol oxidase (EC1.1.3.6 ≥50 U mg^{-1})) was uniformly spread over these electrodes. After 4 h, the ChOx-pRGOSHs/ITO and ChOx-pRGO/ITO bioelectrodes were rinsed with PBS to remove any unbound ChOx and stored at 4°°C when not in use.

2.6. Characterization

pRGO, SiO_2 and pRGOSHs composites were characterized by X-ray diffraction (XRD) technique (Rigaku miniflex-II diffractometer at 30 kV, 15 mA). The wavelength of Cu-Kα1 radiation (λ = 1.5405 Å) was used for obtaining the XRD pattern. Surface morphology was investigated using scanning electron microscopy (SEM), employing JEOL — Model JSM6300F-SEM. Transmission electron microscopic (TEM) characterization was done using FEI Tecnai-G2 electron microscope. Further,

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