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# A cholesterol biosensor based on gold nanoparticles decorated functionalized graphene nanoplatelets

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ABSTRACT

The fabrication of a cholesterol biosensor using gold nanoparticles decorated graphene nanoplatelets has been reported. Thermally exfoliated graphene nanoplatelets act as a suitable support for the deposition of Au nanoparticles. Cholesterol biosensor electrodes have been constructed with nafion solubilized functionalized graphene nanoplatelets (f-G) as well as Au nanoparticles decorated f-G, immobilized over glassy carbon electrode. f-G and Au/f-G thin film deposited glassy carbon electrodes were further functionalized with cholesterol oxidase by physical adsorption. Au nanoparticles dispersed over f-G demonstrate the ability to substantially raise the response current. The fabricated electrodes have been tested for their electrochemical performance at a potential of 0.2 V. The fabricated Au/f-G based cholesterol biosensor exhibits sensitivity of 314 nA/μM $cm^2$ for the detection of cholesterol with a linear response up to 135 μM. Furthermore, it has been observed that the biosensor exhibits a good anti-interference ability and favorable stability over a month's period.



## 1. Introduction

One dimensional carbon nanotubes (CNTs), with their high surface area and nanosize morphology, possess several unique features such as the ability to carry large current densities and fast electron transfer kinetics when used as electrodes for electrochemical sensing and supercapacitor applications [1,2]. They have been used as support materials for nanocrystalline metals for sensing applications, due to the achievement of a high degree of dispersion of these nanocrystalline metals which results in the improvement in catalytic activity [3]. Enhancement of mass transport, control over electrode micro environment catalysis, and high effective surface area are some advantages displayed by the nanoparticles when used for electroanalysis [4]. Even though CNTs are suitable candidates for biosensing applications, the high cost of CNTs is a major hurdle in its commercialization.

Two dimensional graphene, consists of a single layer of atoms tightly arranged in a honeycomb lattice, exhibits some interesting properties similar to CNTs. Graphene exhibits high surface area, unique transport performance [5], thermal conductivity [6], and it can be an efficient filler in polymer composite materials [7]. The high electrical conductivity of graphene sheets can enhance the electron transfer. In addition, it is inexpensive as compared to CNTs. Jue et al. have reported highly sensitive and selective amperometric glucose biosensor using exfoliated graphite nanoplatelets decorated with Pt and Pd nanoparticles [8]. However, the potential usage of graphene as a sensing material for cholesterol detection has been unexplored till now.

Graphene sheets prepared through the exfoliation of graphite oxide leave behind some defects and vacancies [9], and these defects can act as good anchoring sites for the deposition of metal nanoparticles which can be used for cholesterol biosensor applications. The determination of cholesterol in blood is essential in diagnosing several diseases [10]. The enzymatic reaction employing cholesterol oxidase (COx) can be described as follows:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{COx}} \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2 \rightarrow \quad (1)$$

$$H_2O_2 \xrightarrow{E} O_2 + 2H^+ + 2e^- \rightarrow \quad (2)$$

Here, the oxidation of cholesterol into cholest-4-en-3-one and $H_2O_2$ in Eq. (1) is catalyzed by the enzyme COx. The application of a suitable potential to the system, as shown in Eq. (2), enables one to find the electro oxidation current of $H_2O_2$. The overpotential necessary

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for the oxidation/reduction of $H_2O_2$ can be minimized by immobilizing COx in suitable immobilization matrix.

Conducting polymers [11], CNTs [12] nanoparticles [13] and sol–gel/hydro-gels [14] modified metals and carbon surfaces are commonly used to prepare solid electrode systems and supporting substrates. For electrochemical determination, carbon-based materials such as graphite, carbon black and carbon fiber are preferred due to their excellent electrical and mechanical properties. These materials have a high chemical inertness and provide a wide range of anode working potentials with low electrical resistivity. Noble metal nanoparticles are known to be excellent catalysts, due to their high ratio of surface atoms with free valences to the cluster of total atoms [15]. Deposition of noble metal nanoparticles over graphene nanoplatelets reduces their loading by increasing the catalyst utilization and improving the catalyst activity/performance. In the present work, we have selected nafion (NA) solubilized functionalized graphene nanoplatelets (f-G) as well as Au nanoparticles deposited f-G nanoplatelets (Au/f-G) for immobilizing the enzyme COx over glassy carbon electrode (GCE). The electrocatalytic behavior of f-G and Au/f-G modified GCE toward the electrochemical oxidation of $H_2O_2$ and cholesterol has been investigated and discussed. Besides, the anti-interference ability and the stability of the biosensors have been investigated and the results have been discussed.

## 2. Materials and methods

### 2.1. Materials

Graphene nanoplatelets were synthesized in our laboratory by thermal exfoliation of graphite oxide (GO). Graphite was purchased from Sigma Aldrich. Purity and particle sizes of graphite were respectively 99.99% and 45 μm. Cholesterol oxidase (EC1.1.3.6; $\geq 50$ U $mg^{-1}$; from brevi bacterium), cholesterol and Triton X-100 were purchased from Sigma Aldrich. $HAuCl_3 \cdot 3H_2O$ was obtained from Alfa Aesar. Phosphate buffer (PB) potassium salt (20 mM $KH_2PO_4 + 20$ mM $K_2HPO_4 + 0.1$ M KCl) was used as supporting electrolyte. A 10 mM stock solution of cholesterol was prepared by dissolving cholesterol in a flask containing Triton X-100 in a bath at 60 °C and then diluting with 0.02 M PB solution (pH 7.0). The solution was stored in a refrigerator and remained stable for 2–3 weeks (until a slight turbidity was observed).

Diluted cholesterol solutions were freshly prepared from the stock solution using 0.02 M PB solution containing 1% Triton X-100. Doubly distilled water was used for the dilution of stock solutions. All other reagents used were of analytical grade.

### 2.2. Instruments

The morphology and structure of the samples were characterized by field emission scanning electron microscope (FESEM) (FEI QUANTA 3D Scanning Electron Microscope) and transmission electron microscope, (TEM, JEOL JEM-2010F) at acceleration voltages of 30 kV and 200 kV respectively. The functional groups were identified by taking Fourier Transform Infrared (FTIR) spectra, using PERKIN ELMER Spectrum One FTIR spectrometer in the range of 100 to 4000 $cm^{-1}$. The electrochemical measurements were performed with CH Instruments CHI 608C Electrochemical Analyzer/Workstation connected to a computer. The three electrode system consisted of a platinum wire counter electrode, Ag/AgCl (3 M KCl) reference electrode and Au/f-G or f-G based glassy carbon working electrode (diameter 3 mm). The electrodes were inserted into a modified 5–10 ml glass cell (Model CHI-222) for the measurement. All potentials are referred to the Ag/AgCl reference electrode. For amperometric measurements, the working electrodes were operated at the desired potentials and current was measured for successive additions of analyte.

### 2.3. Preparation of functionalized graphene nanoplatelets

Graphene nanoplatelets were prepared by thermal exfoliation of GO. First, GO was prepared by treating graphite with an oxidizing solution of sulfuric acid and nitric acid for 4 days and dried at 80 °C for 6 h. Thermal exfoliation was done in quartz tube at 1050 °C for 0.5 min [16]. Functionalization of graphene nanoplatelets was done by sonicating graphene nanoplatelets with a solution of conc. sulfuric acid and conc. nitric acid for nearly 30 min.

### 2.4. Preparation of Au/f-G nanocomposite

The f-G prepared by the above method was then decorated with 10 wt.% of Au nanoparticles by chemical reduction method. Briefly, 0.1 g of f-G was dispersed in de-ionized water and subsequently treated with salt of Au ($HAuCl_4 \cdot 3H_2O$). The solution was then stirred magnetically for 12 h. The nanocrystalline Au particles were deposited over graphene nanoplatelets using a reducing solution containing 0.1 M $NaBH_4$ and 1 M NaOH. After the reaction is complete, the solution was washed, filtered and dried at 80 °C.

### 2.5. Fabrication of f-G or (Au/f-G) based bioelectrode

1 mg/ml graphene nanoplatelet solution was prepared by ultrasonicating 1 mg of f-G in 1 ml of 0.5% nafion solution. Nafion gives better dispersion as well as stability to the graphene nanoplatelets. 8 μl of the solution was dropped over the cleaned GCE. 5 μl of COx was film cast over the dried f-G film. Finally, another 5 μl layer of NA was dropped and allowed to dry at 4 °C in a refrigerator. All the enzyme electrodes were washed thoroughly with PB solution (pH 7) before use and stored at 4 °C in dry state unless specified otherwise. The similar procedure was used for fabricating Au/f-G electrode. The fabricated electrodes will be termed as f-G/COx/NA/GCE and Au/f-G/COx/NA/GCE from here onwards.

## 3. Results and discussion

### 3.1. Characterization of f-G and Au/f-G

A schematic of the modification of GCE with Au/f-G, COx and NA for efficient detection of cholesterol is shown in Fig. 1. The characterization of the prepared samples: f-G and Au/f-G were done by FESEM, TEM, EDX and FTIR spectroscopy.

Dispersion of f-G in solvents can be obtained by their surface modification with oxygen containing functional groups. The

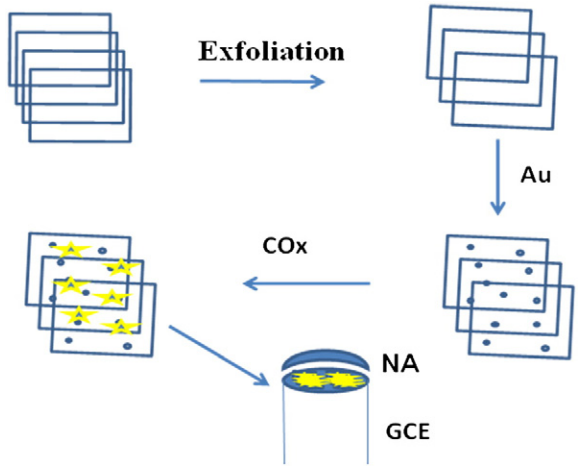


**Fig. 1.** Schematic diagram showing the fabrication procedure of Au/f-G/COx/NA/GCE.