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Development of Nano Biosensor for Cholesterol Detection

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ABSTRACT

The aim of this study is to develop cholesterol biosensor using synthesized reduced Graphene Oxide (rGO) by modified Hummer's method and reduced Graphene Oxide-Silver (rGO-Ag) nanocomposite had prepared by a simple chemical method. The rGO-Ag nanocomposite was synthesized in the presence of Silver Nitrate (AgNo3) and Sodium borohydride (NaBH4). The synthesized reduced Graphene Oxide (rGO) and reduced Graphene Oxide–silver rGO-Ag nanocomposite were characterized by Ultraviolet-Visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR) and Dynamic Light Scattering (DLS). Then the Cholesterol Oxidase (ChOx) enzyme is to be immobilized on rGO-Ag poly-pyrrole network. The rGO-Ag poly-pyrrole/ChOx enzyme is coated on electrode and its electrochemical studies are to be accomplished. The bioenzyme integrated nanostructure platform is very sensitive toward cholesterol and it has a fast response time.

Keywords : Cholesterol, Enzyme, Silver Nanoparticles, rGO.

I. INTRODUCTION

A biosensor is an analytical device, used for the detection of a chemical substance, that combines a biological component with a physicochemical detector. The transducer or the detector element, which transforms one signal into another resulting from the interaction of the analyte with the biological element, to easily measure and quantify the substance [1].

Graphene is a two-dimensional honeycomb crystalline single layer of carbon lattice [2]. It has extraordinary electrical and thermal conductivities [3][4], high mechanical stiffness [5], good biocompatibility [6] and low cost [7].

Silver is extremely soft, ductile and malleable transition metal, though it is slightly less malleable than gold. It has very high electrical and thermal conductivity. The interaction of silver with other material takes place with lower electron mobility. The electrical conductivity of silver is the greatest of all metals [8].

Cholesterol is an essential structural component of nerve cells and plasma membrane. Cholesterol is an organic molecule. It is a sterol type of lipid. Cholesterol is carried in the blood by lipoproteins. Cholesterol serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D [9].
Nowadays cardiovascular disease and cardiac arrest are the most important cause of death due to the increased concentration of cholesterol level in blood

(hypercholesterolemia). It is important to develop the existing cholesterol biosensor by improving its sensitivity. Detection and determination of biomolecules are clinically highly significant for diagnosis and treatment of various diseases.

Enzymatic biosensor is a very well accepted system for sensing biomolecules based on their electrochemical reaction (oxidation or reduction) with the enzyme, which is immobilized on the electrode surface. The electrochemical output signal corresponds to the concentration of the analyte molecule. The analytical performance of a biosensor depends on the electron transfer between the metal active site of the enzyme and the electrode surface.

Enzyme can be directly immobilized on the electrode surface to achieve direct electron transfer between the electrode and the enzyme. However, it might result in the denaturation of the enzyme and hence affect the biosensor response. In order to improve enzyme adsorption, improve stability and enhance the direct electron transfer, nanomaterials have been widely used as immobilization matrix, a mediator between the enzyme and the electrode. The biosensor selectively detects cholesterol even in the presence of interfering agents found in clinical serum samples. It is to be designed for rapid and sensitive detection of cholesterol.

II. EXPERIMENTAL METHOD

A. Materials

The chemicals such as Graphite and Hydrogen peroxide (H_2O_2) were purchased from LOBA CHEMIE PVT LTD, Sodium nitrate ($NaNo_3$) and Potassium permanganate ($KMnO_4$) were purchased from SRL chem, Conc. Sulphuric acid (H_2SO_4) was purchased from Isochem Laboratories, Silver nitrate ($AgNO_3$) was purchased from HIMEDIA, Sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$) and Sodium borohydride ($NaBH_4$) were purchased from MERCK. Double distilled (DD) water was used for all the solution preparation.

B. Preparation of reduced Graphene Oxide (rGO)

The rGO was synthesized by modified Hummer's method. The Graphite powder and $NaNO_3$ mixture were added to 100 ml of DD. The mixture was subjected to magnetic stirring for 15 mins. 115 ml of Conc. H_2SO_4 was added slowly to the solution placed in an Ice bath. Then the solution was stirred for 15 min. 15 gm of $KMnO_4$ was added to the solution and stirred for 15 mins. 250 ml of DD water was added to the solution and stirred for 15 mins. 10 ml of H_2O_2 was added to the solution for the completion of the reaction. The above solution was added with distilled water to make up the solution as 400 ml. The solution was subjected to stir for 7 days. Then the prepared solution was dried in hot plate in order to obtain powder form.

C. Instrumentation

The UV analysis were carried out in Agilent Technologies Cary 8454 UV-Vis Spectrophotometer. The Functional group measurements were performed with a Perkin-Elmer FTIR spectrophotometer. The particle size distribution of the prepared powder was analysed in NANOPHOX.

III. RESULTS AND DISCUSSION

Thus the rGO prepared by modified Hummer's method was obtained in black colour. The Characterization of rGO such as UV, FTIR, DLS are discussed below.

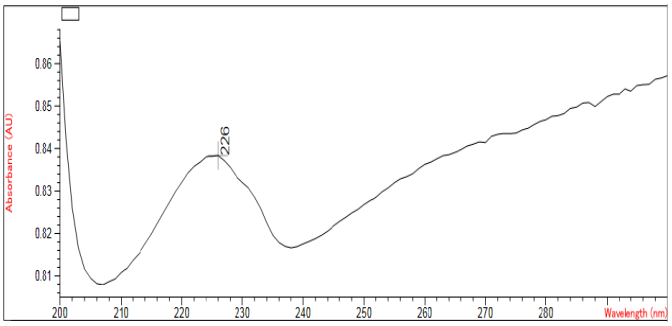

Fig. 1 UV-Visible (A) spectra of rGO.

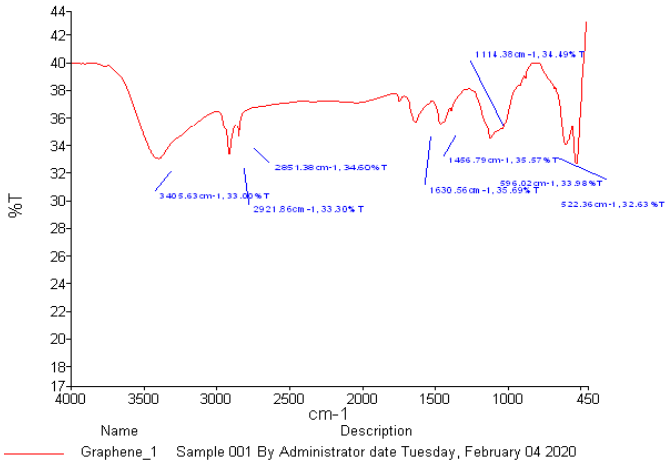

Fig. 2 FTIR (B) spectra of rGO

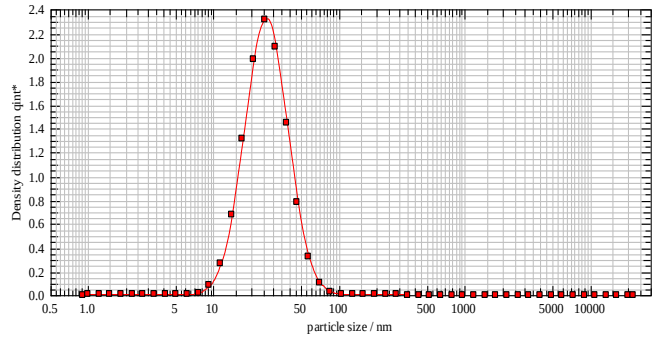

Fig. 3 DLS (C) of rGO

UV spectra analysis as shown in Figure 1 (A) is a quantitative technique used to measure how much a chemical substance absorbs light. It measures the intensity of light that passes through sample with respect to the intensity of light through a reference sample. The UV-visible absorption spectrum of rGO shows a main band at 226 nm corresponding to the π-π* transition of aromatic C-C and carboxyl C-O bonds respectively.

FTIR spectra analysis was performed to investigate the different types of functional groups of the nanoparticles, as shown in Figure 1 (B). Firstly, the broad peak at 3405 cm^{-1} originates from stretching vibrations of hydroxyl (-OH) group, then an aromatic (C=C) peak appears at 1630 cm^{-1} and carboxyl peak (C-O) appears at 1114 cm^{-1}.

Dynamic Light Scattering (DLS) of rGO as shown in Figure 1 (C) measures the Brownian motion of molecules and particles to determine size and size distributions. The average particle size distribution rGO was obtained as 13 nm (D_{90}).

IV. CONCLUSION

In this way the reduced Graphene oxide was successfully synthesized by modified Hummers method. The particle size was obtained as 13 nm in DLS. Then the Graphene Oxide functional groups were analysed using FTIR. The UV absorption spectrum of rGO was obtained in a successful manner. The prepared rGO is used as sensing platform for biological applications.

V. ACKNOWLEDGMENT

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VI. FUTURE WORK

Further characterization such as SEM and XRD analysis for rGO will be carried out. The rGO - Ag nanocomposite will be synthesised and their characterization will be done. The above composite will be used as sensing platform for detecting cholesterol.

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