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Designing and Application of Graphene and Fe-Ni Nanoparticle Based Nanostructure

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Abstract: *In the present work, a novel graphene oxide (GO) modified nickel iron oxide-chitosan hybrid nanocomposite has been synthesized, which was electrophoretically deposited onto indium tin oxide (ITO) coated glass substrate, used as cathode and parallel platinum plate as counter electrode. It was observed that the incorporation of GO enhances the electrochemical properties of the nanocomposite. The size of nanocomposite was found to be 50 nm by Transmission Electron Microscopy and it was observed that nanoparticles were uniformly decorated on the graphene sheet which confirms the formation of the nanocomposite..The complete characterization of the nanocomposite electrode has been done using Scanning Electron Microscopy, Thermal Gravimetric Analysis, Contact angle, Raman spectroscopy and Fourier Transform Infrared Spectroscopy. The data supported the interaction. Further, the electrochemical response studies have been carried out using electrochemical impedance spectroscopy which reveals that this composite can be further applied for the detection of nucleic acid with high sensitivity and selectivity. Under optimal conditions, this biosensor is found to retain about 90 % of the initial activity after 8 cycles of use.*

1. INTRODUCTION

*Escherichia coli*O157:H7 is an enterohemorrhagic serotype of the bacterium. Its infection may lead to hemorrhagic diarrhoea, and to kidney failure.Therefore, rapid and accurate detection of *Escherichia coli* become urgent. Various traditional methods are available for detection of the pathogen which are either excessively time-consuming or costly and complicated [1-3].Detection of the bacteria by a rapid, sensitive and cost effective methodis therefore a subject of great importance in clinical analysis.A lot of electrochemical biosensors have been developed in detection of E. coli O157:H7 for its high selectivity and convenience[4,5]. In this study, we have synthesized agraphene oxide (GO) modified nickel iron oxide-chitosan hybrid nanocomposite for the detection of *Escherichia coli*. In recent years, graphene, the two-dimensional closely packed honeycomb carbon lattice, has been attracting much attention in the field of electrochemistry due to its intrinsic properties and merits like high current density, ballistic transport, chemical inertness, high thermal conductivity, optical transmittance and super hydrophobicity at nanometer scale and high surface area[6] Similarly, nanostructured metal oxides are also fascinating in fabrication of biosensors due to their high surface area, nontoxicity, good biocompatibility and chemical stability which make them ideal as immobilization matrices[7].Chitosan, a natural-biopolymer is used to disperse graphene and nanomaterials for fabricating biosensorsdue to its excellent capability for film formation, biocompatibility and ease of immobilization.[8]Therefore, we have taken nickel- iron oxide nanoparticles and graphene to utilize respective advantages and dispersed in chitosan to fabricate a nanocomposite. This nanocomposite was electrodeposited on ITO electrode and pDNA of *Escherichia coli*was immobilized on it for the detection. Electrochemical impedance spectroscopy(EIS) was used as detection technique. The thickness and electric insulativity of the electrical barrier on the electrode surface are related to the concentrations of target bacteria bound on the electrode surface, and could be reflected by the electron-transfer resistance of the $[Fe(CN)6]^{3-/4-}$ redox probe, which can be measured quantitatively through electrochemical impedance spectroscopy.

2. EXPERIMENTAL SECTION

2.1.1. Materials and method

Graphene powder, EDC (1 ethyl, 3 (3dimethylaminopropyl) carbodimide), glutaraldehyde, N-hydroxysuccinimide (NHS), and chitosan (MW 15000-20000), iron nitrate $[(Fe(NO_3)_3.9H_2O)]$, nickel nitrate $[(Ni(NO_3)_2.6H_2O)]$, polyethylene oxide (PEO) $[CH_2CH_2AOA]_n$ and hydrazine hydrate $(NH_2.NH_2.H_2O)$were used and these chemicals were of analytical grade and purchased from sigma –Aldrich. Two probes were used.

Probe I: probe DNA (pDNA): Amine-5′-GGT CCG CTT GCT CTC GC-3′ Probe II: Complementary DNA (cDNA): 5′-GCG AGA GCA AGC GGA CC-3′

Graphite oxide was prepared by chemical oxidation and exfoliation of natural graphite according to a modified Hummers method [9]. Polyethylene oxide (PEO) capped nickel iron oxide nanoparticle was synthesized according to

the reported literature[10]. GO solution was prepared by mixing 1 mg GO in 1 ml water.This was ultra sonicated for about 15 minutes. Chitosan solution was prepared by mixing 0.1 g chitosan powder in 20 mL, 0.2 M AcOHsolution andultrasonicated it for 30 minutes. 1 mg of nickel iron oxide/GO composite was added in the chitosan solution. Then 0.5 ml (0.5% w/v) EDC was added in this solution followed by ultrasonication of the solution for 30 minutes and the required composite synthesized.

The prepared GNiFch composite was deposited electrophoretically onto a pre-hydrolyzed ITO electrodeat a DC potential of 10 V for 60 s using a two-electrode system, with parallel-placed platinum as the counter electrode.The composite is positively charged due to the presence of primary amino groups in chitosan, so it moves towards cathode leading to the deposition of the nanocomposite onto the ITO electrode.Now, 20 μL of pDNA was spread onto the modified electrode surface using glutaraldehyde as a cross-linker (0.1% v/v; 4 h). The prepared pDNA/GNiFch/ITO bioelectrodes were utilized for detection of *Escherichia coli*by treating them to various concentrations of complementary target DNA for 30 min and the corresponding difference in R_{ct} value was measured by EIS.

3. RESULTS AND DISCUSSION

3.1. Characterization

The morphological investigations of GNiF/ch have been carried out bythe scanning electron microscopy (SEM) images using a JEOLJSM-6700F field-emitting scanning electron microscope (FESEM, 15 kV). Fourier transform infra-red (FT-IR) spectroscopy measurements have been carried out using Perkin-Elmer spectrometer (Model Spectrum BX) at 25°C. Electrochemical analysis has been conducted on an Auto lab potentiostat/galvanostat (Eco Chemie, Netherlands) using a three-electrode cell using ITO as working electrode, platinum as auxiliary electrode and Ag/AgCl as reference electrode in phosphate buffer (PBS, 100 mM, pH 7.4, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$.

3.2 FT-IR studies

The formation of GNiFchnanocomposite was further investigated by FTIR spectra .Fig. 1 shows the FT-IR spectra of nickel iron oxide nanoparticles.A wide peak can be observed at 3346 cm^{-1} which may be assigned to –a OH vibration which shifts to higher wave number in composite[4]. Bands at 1659 cm^{-1} and 1459 cm^{-1}can be assigned to asymmetric and symmetric stretching vibration of COO^-. A peak at 623 cm^{-1} may be due to Fe-O absorption is obtainedwhich shifts slightly to higher wave number during composite formation.A peak at 489 $cm^{-1}$is observed which may be due to Ni-O absorption [11]which disappears in the composite.A band at 975cm^{-1}which may be due toO–H bending ofcarboxylic acids is observed.Theband observed at about 1000 cm^{-1} may be assigned to C-H stretching out of the plane.

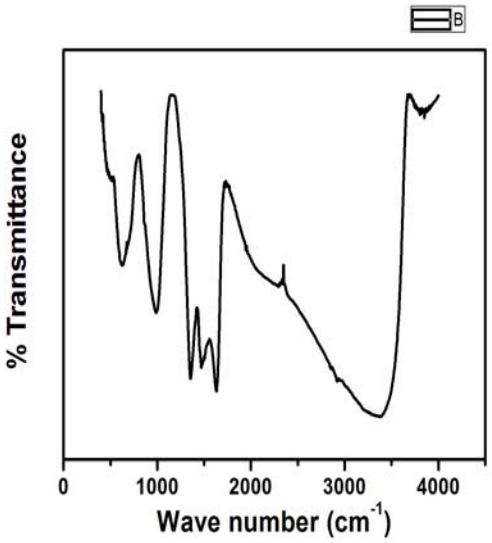

Fig. 1: FTIR spectra of GNiFchnanocomposite

3.3Scanning electron microscopic (SEM) studies

SEM analysis was done to study the surface morphology of the composite. Fig. 2 shows the SEM image of the composite deposited on to the ITO electrode, which shows glossy and crimpled shapes of graphene[12].It can be observed that the graphene is well dispersed on the surface of ITO electrode.A large number of spherical shaped structures can be observed in the SEM image which implies that graphene provide large surface area for the spread of nickel- iron nanoparticles.

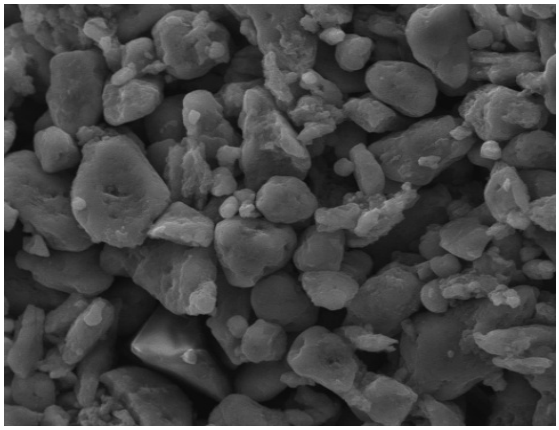

Fig. 2: SEM image of the prepared nancomposite.

3.4 Contact angle (CA) studies

The CA measurements were done to investigate pDNA/GNiFch/ITO bioelectrode fabrication. CA of ITO was

found to be 84.6° (Figure3i) .It was observed that after the deposition of the nanocomposite on ITO,contact angle decreased (Fig. 3 ii) which may be due to the introduction of graphene oxide having polar groups –COO- increasing the hydrophilicity of the electrode surface.When DNA was immobilized on the GNiFch/ITO [13]electrode, there was further decrease in CA observed(Figure3 iii) which may be attributedto the negatively charged phosphodiester backbone of DNA causing increase in hydrophilicity.

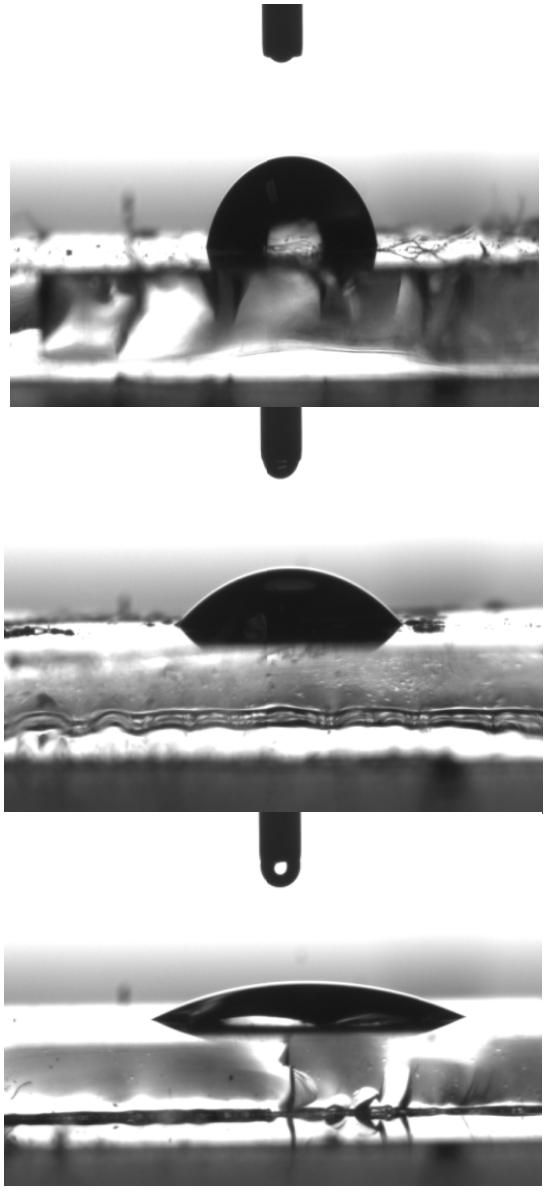

Fig. 3: Contact angle images of (i) ITO electrode and (ii) GINiFch/ITO electrode and (iii) pDNA/GINiFch/ITO bioelectrode

3.5 Electrochemical studies

Electrochemical characterisation was done by performing cyclic voltammetry as a function of scan rate (10-300 mV/s).It was found that, anodic and cathodic peak increase with increase in scan rate and I_{pa}/I_{pc}› 1, revealing that the process is quasireversible[14].It was observed that both the anodic and cathodic peak current decreases after the immobilization of DNA.(Fig. 4ii).This may be due to repulsion of $Fe(CN)_6]^{3-/4}$with negatively charged phosphate backbone of DNA.With the introduction of cDNA,there was further decrease in current due to more repulsion cause by DNA(data not shown).

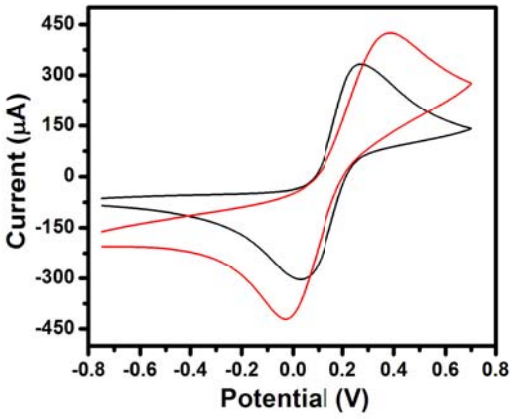

Fig. 4.Comparison of cyclic voltammogram of GNiFch/ITO electrode and pDNA/GNiFch/ITO electrode

3.6Response of the bioelectrode

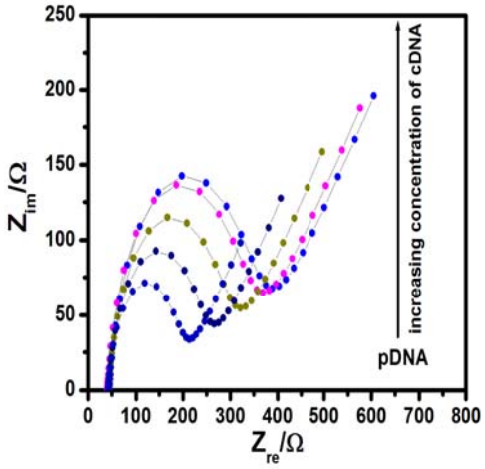

Fig. 5: EIS analysis showing the variation of R_{ct} with change in cDNA concentration

Electrochemical impedance spectroscopy(EIS) was used for measuring sensitivity of the bioelectrode by varying the concentration of cDNA from 10^{-6}M to 10^{-13} M(Fig. 5).It was found that there was increase inRct value with increase in cDNA concentration which reveals that the hybridization has

taken placecausing more accumulation of negatively charged phosphate backbones, hence increase inR_{ct} value. The difference between the value of the pDNA immobilized electrode and that after hybridization with cDNA (ΔRct = Rct(cDNA)-Rct(pDNA) has been used as the measurement signal and it was found that change inR_{ct} value varies linearly as log[cDNA].Detection limit was calculated using formula 3б/sensitivity and found to be 1 x 10^{-13}M.

4. ACKNOWLEDGEMENTS

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