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Electrochemical genosensor based on carboxylated graphene for detection of water-borne pathogen

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ABSTRACT

This work reports the application of newly synthesized carboxylated graphene nanoflakes (Cx-Gnfs). The Cx-Gnfs were synthesized by wet chemical method in sulfuric acid/nitric acid mixture and was further electrophoretically deposited on indium tin oxide (ITO) coated glass substrates using Mg^{2+} ions which provides an overall charge to the materials for deposition onto the anode. The materials were characterized using SEM, TEM, contact angle, UV–vis spectroscopy, FT-IR, XRD and electrochemically characterized by cyclic voltammetry, chronocoulometry and electrochemical impedance spectroscopy. The sensitive quantitative determination of nucleic acid *Escherichia coli* O157: H7 (*E. coli*) has been achieved using Cx-Gnfs and r-GO as the sensing layer using electrochemical impedance spectroscopy. The electrochemical results reveal that the Cx-Gnfs based genosensor exhibits a linear response to complementary DNA (10^{-6} M to 10^{-17} M) with a detection limit of 1×10^{-17} M while the rGO based genosensor shows a detection limit of 1×10^{-15} M. Under optimal conditions, this Cx-Gnfs based genosensor was found to retain about 85% of its initial activity after being used for 6 times.

1. Introduction

Pathogenic bacteria are considered to be serious threat to human health mainly due to food borne and water borne illnesses [1,2]. The consumption of less processed products such as fruits, vegetables and ready to eat foods are the major sources of food borne outbreaks which has created a major concern for their immediate monitoring to ensure the safety of food products for consumption. In spite of the availability of regulations, good manufacturing practices, hazard analysis and critical control points for keeping a check on the presence of *E.coli* in food it has not been possible to achieve the desired result.

The most commonly practiced techniques in the area of food and safety for detection and enumeration of *E. coli* O157:H7 are conventional culture and molecular based methods. The culture-based method includes enumeration of bacterial colonies by growing bacteria in nutrient rich medium. While in the molecular based method enumeration are done by polymerase chain reaction (PCR) technique which is highly specific and sensitive for the detection of specific sequences of nucleic acid for the targeted bacteria using the hybridization methods. The molecular based method is known to be more rapid as compared to the conventional culture method [3–5].

The conventional methods such as culturing, PCR, flow cytometry, solid-phase cytometry and ATP bioluminescence etc. are used in the public health laboratories all over the world for the detection of *E.coli* O157:H7. Inspite of some advantages these conventional methods have drawbacks such as need of bulky instrumentations, large amount of medium which creates biological waste, time for culture and enumeration, interference by other substances of food matrices, requirement of proper training for careful handling of the instrument and a certain level of expertise for interpretation of results [6–9]. Hence, there is a need for the development of method for rapid and low cost detection of *E.coli* with improved specificity, reliability, feasibility, miniaturization, and enumeration. In this respect, biosensors are one of the most helpful and applied devices for monitoring the chemical and

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biological analytes. With the advent of nanotechnology, the construction of biosensors has become more fascinating. The use of nanoscale material helps to achieve a direct wiring of electrode with the biomolecules, providing a larger surface area for electrochemical reactions, and also creates a nano-encryption for biomaterials so that the sensitive and selective signal of biological recognition element can be amplified. The nanostructured materials such as nanoparticles, nanotubes, nanowires etc. of carbon or metal oxides are used for recognition of biological events in electrochemical biosensors due to their enhanced properties [10–13]. Recently, graphene as a true 2-dimensional material, has received much attention due to its unique physicochemical properties like high mechanical strength, excellent conductivity, high surface area, ease of functionalization and mass production [14,15]. The unique structure of graphene provides a remarkable electronic, optical, mechanical, thermal, and electrochemical properties; and makes them a potential candidate for next generation biosensing materials. In comparison to CNTs, graphene shows potential advantages of cost-effectiveness, high surface area and ease of processing [16,17]. Further, the presence of oxygen-containing groups at the edges/surface of graphene influence the electrochemical performance in terms of the heterogeneous electron transfer rate. These functional groups provide a conducive environment for controlled functionalization and immobilization of biomolecules on its surface.

Many reports for detection of *E.coli* using different nanomaterials and electrodes are available [18–45]. Biosensors based on different biological recognition entities such as antigen/antibody [18–21], single strain DNA [22–42], whole cell [43] and aptamer [44,45] have been reported for *E. coli* detection. Among these nucleic acid biosensors have been widely used for *E. coli* detection due to their enhanced sensitivity, reproducibility, and selectivity. In this context, Xu et al. fabricated a simple and sensitive electrochemical DNA biosensor based on graphene oxide/chitosan hybrid nanocomposites modified glassy carbon electrode for detection of *E.coli* O157:H7 [24]. Tiwari et al. have used graphene oxide modified iron oxide–chitosan hybrid nanocomposite film for *E. coli* O157:H7 detection and obtained a detection limit of 1×10^{-14} M [26]. Zainudin et al. have reported a label-free impedimetric *E. coli* genosensor based on graphene nanosheets displaying a wide range of linear response (1.0×10^{-10} M to 1.0×10^{-14} M) and low detection limit (0.7×10^{-15} M) [32]. Although numerous work has been done towards the detection of *E. coli* DNA using different platforms but still the sensitivity, selectivity, detection time and reusability of the sensor is a challenge which curbs its transfer to a technology. Table 1 consists of detailed discussion of non-labeled and labeled *E. coli* genosensor reports available in literature for a decade.

Here in this work, efforts have been made to fabricate a genosensor based on carboxylated graphene nanoflakes (Cx-Gnfs) for *E.coli* O157:H7 detection. A new protocol has been used for the synthesis of graphene nanoflakes in which graphene edges have been modified with carboxylate groups [46,47]. The main purpose of choosing this material as the electrode modifier is due to the presence of carboxylated groups which facilitate the targeted attachment of the nucleic acid. The results were compared with chemically reduced graphene oxide (r-GO) using electrochemical impedance spectroscopy technique. The sensor performance of the Cx-Gnfs was found to be better than r-GO and most of the reported works [Table 1].

2. Chemicals and material required

Graphite powder, multiwall carbon nanotubes (MWCNT), 1-ethyl-(dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) of analytical grade were purchased from Sigma-Aldrich. The specific sequence of *E. coli* probe (17 mer.) has been identified from the 16 s rRNA coding region of the *E. coli* genome. The complementary, non-complementary and one-base mismatch target sequences were obtained from Sigma Aldrich, Milwaukee, USA. Sequences of oligonucleotide probes are listed as below:

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′.

Probe III: non-complementary DNA: 5′-CTA GTC GTA TAG TAG GC-3′.

Probe IV: one-base mismatch DNA: 5′-GCG AGA GAA AGC GGA CC-3′.

The oligonucleotide solution was prepared in Tris–EDTA buffer (1 M Tris − HCl, 0.5 M EDTA) of pH 8.0 and is stored at -20 °C prior to use.

3. Experimental section

3.1. Synthesis of Cx-Gnfs

MWCNT (1.00 g, Bayer Materials Science) were dispersed in 100 mL of a 3:1vol% mixture of H_2SO_4 (97 w/w) and HNO_3 (70 w/w) via ultrasonication for 30 min. The mixture was heated at 100 °C for 2 h, then cooled, and diluted three-times with deionized water. The resulting black dispersion was filtered (0.2 µm Whatman track-etched polycarbonate membrane) to remove any unreacted carbon material on the membrane. Finally the black filtrate was neutralized by the addition of KOH pellets under external ice bath cooling. After neutralization a large amount of white precipitates (consisting of K_2SO_4 and some KNO_3) were formed, which were separated from the black filtrate by filtration. The dispersion was re-acidified by the addition of diluted formic acid and then dialyzed against high purity *Milli-Q* deionized water using a pre-treated Spectra Por 3 regenerated cellulose dialysis membrane (MWCO 3.5 kDa, Spectrum laboratories). The dialysis procedure was considered complete when the conductivity of the water surrounding the membrane decreased below 5 $\mu S.cm^{-1}$, which was determined using a Mettler Toledo conductivity meter using a Mettler Toledo LE703 conductivity sensor. The pure brown-black dispersion underwent lyophilization to obtain 222 mg of a pure brown-black material, identified as Cx-Gnfs [46].

3.2. Synthesis of r-GO

The reduction of graphene oxide (GO) has been conducted using the two-step reduction procedure. Upon dispersion in deionized water, the pH of the solution was increased to 10.0 using 5 wt% sodium carbonate solution. Sodium borohydride (800 mg) was directly added to a 400 mL GO suspension (1 mg mL^{-1}) under magnetic stirring, and the mixture was kept at 80 °C for 1 h with constant stirring, leading to a change in the color from brown to black. The solid product was purified using water and washed with ethanol, followed by drying. Further, the obtained product was re-dispersed in H_2SO_4 at 120 °C and filtered through Millipore filter paper to yield reduced graphene oxide (r-GO) [48].

3.3. Fabrication of electrodes

For electrophoretic deposition (EPD), a very well dispersed stock solution of Cx-Gnfs (20 mg dL^{-1}) in acetonitrile was ultrasonicated (80 W, 0.25 A) for about 2 h. Then, 100 µL of this stock solution was dispersed in 10 mL of acetonitrile to make the colloidal suspension of Cx-Gnfs. The film was electrophoretically deposited using two-electrode cell by applying a DC voltage (140 V, 2 min), where platinum foil was used as the cathode and a precleaned ITO-coated glass substrate as anode. To create surface charge on the Cx-Gnfs, 10^{-5} M of $Mg(NO_3)_2.6H_2O$ was added into the suspension as electrolyte [49,50]. The deposited film (Cx-Gnfs/ITO electrode) was then removed from the suspension followed by washing with deionized water and drying. The schematic view of electrodeposition and fabrication of Cx-Gnfs/ITO electrode and pDNA/Cx-Gnfs/ITO bioelectrode has been illustrated in Fig. 1.