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Diastase induced green synthesis of bilayered reduced graphene oxide and its decoration with gold nanoparticles

Sireesh babu Maddinedi [a,b], Badal Kumar Mandal [b,*], Sagar Hindurao Patil [c], Vaibhav Vilas Andhalkar [d], Shivendu Ranjan [e], Nandita Dasgupta [e]

[a] The Key laboratory of Advanced Textile Materials and Manufacturing Technology of Ministry of Education, National Engineering Lab for Textile Fiber Materials and Processing Technology (Zhejiang), College of Materials and Textiles, Zhejiang Sci-Tech University, Hangzhou 310018, China
[b] Trace Elements Speciation Research Laboratory, Department of Chemistry, School of Advanced Sciences, VIT University, Vellore 632014, India
[c] Center for Material Characterization (CMC), National Chemical Laboratory, Pune 411008, India
[d] Chemistry Technology Centre of Catalonia, Universitat Rovira i Virgili, Tarragona, Spain
[e] Nano-food Research Group, Instrumental and Food Analysis Laboratory, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India

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ABSTRACT

In this paper, we report an enzyme dependent, green one-pot deoxygenation cum decoration method to synthesize diastase-conjugated reduced graphene oxide (DRG) nanosheets, DRG/gold nanoparticles (DRG/Au) composite. The DRG synthesis was completed in 7 h under heating at 90 °C on water bath. Selected area electron diffraction (SAED) and Atomic force microscopy (AFM) study has revealed the formation of bilayered reduced graphene oxide sheets. Transmission electron microscopy (TEM) images of DRG/Au composite have shown the uniform decoration of gold nanoparticles (AuNPs) onto the DRG nanosheet surface. Fourier transform infrared spectroscopy (FTIR) and Raman results additionally have shown the functionalization of enzyme molecules onto the DRG nanosheet surface after reduction making it as an effective platform towards the efficient binding of gold nanoparticles. In vitro cytotoxicity studies by MTT assay on A549 and HCT116 cell lines exhibited that the cytotoxicity of the prepared graphene oxide (GO), DRG and DRG/Au is dose dependant. These results have shown that this synthetic method is effective for the production of large scale graphene in a low cost, simple and green method. Since this process avoids the use of hazardous and toxic substances, the produced DRG/Au composites are likely to offer various potential applications in biology and medicine.

1. Introduction

Graphene, one carbon atom thickness 2D nanosheet, has paid great attention in recent times due to its excellent chemical and physical properties [1]. The outstanding thermal, mechanical, optical and electrical features of functionalized graphene materials have made them as exceptional candidates in the field of nanoscience and nanotechnology. For instance, the surface modified graphene materials have a wide scope of applications in biosensors, energy materials, Li-ion batteries, polymer nanocomposites and in biology [2–8].

Several synthetic strategies for graphene production have been reported in the past few years such as chemical vapor deposition (CVD) [9], deoxygenation of graphite oxide (GO) to reduced graphene oxide [10–12] and micromechanical exfoliation of graphite [13]. However, the graphite oxide reduction is important due to its simplicity and economy in the large scale production. Different chemical substances have been used as reducing agents for the reduced graphene oxide synthesis such as hydrazine [10], hydroquinone [14] and $NaBH_4$ [15]. However, the produced graphene materials have lost its scope of applications in biology due to the toxic and explosive nature of the chemicals used. Consequently, the introduction of new green and eco-friendly reagents for the successful reduction of GO to RGO is highly desirable and challenging.

Recently, many bioreductants have been used for graphene production such as glucose [16], ascorbic acid [17], amino acids [18] and protein bovine serum albumin [19], which play role as both reducing and capping/stabilizing agents. Recently, our group has shown the ability of casein [20] and *T. chebula* polyphenols [21] as reducing and decorating agent for the deoxygenation of graphene oxide.

Herein, we report the use of diastase, a thiol group containing enzyme has been used as reducing and stabilizing agent for the one pot eco-friendly synthesis of graphene. We further have shown the decoration of AuNPs onto the surface of diastase stabilized graphene sheets

* Corresponding author.
E-mail addresses: badalkmandal@hotmail.com, badalmandal@vit.ac.in (B.K. Mandal).

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herein. We also studied the in vitro cytotoxicity of GO, DRG and DRG/Au towards A549 and HCT116 cell lines.

2. Experimental Section

2.1. Materials

Graphite powder (100 mesh, 99.9%), potassium permanganate ($KMnO_4$), concentrated sulfuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2, 30%), chloroauric acid ($HAuCl_4$), sodium nitrate ($NaNO_3$), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Dulbecco's modified Eagle's Medium (DMEM), Foetal bovine serum (FBS) and all other solvents were purchased from Sd Fine Chemical-Mumbai.

2.2. Preparation of Reduced Graphene Oxide

Graphene oxide, a precursor for the synthesis of graphene was prepared by following the reported procedure [22]. About 20 mg of prepared GO was dispersed in 20 mL water and ultrasonicated for about 2 h to obtain the exfoliated graphene oxide. Then 200 mg of diastase enzyme powder was added to the sonicated GO solution and thoroughly mixed by shaking. The pH of the subsequent mixture was adjusted to 12 using NH_4OH, which then refluxed at 90 °C for about 6 h on a water bath. The complete conversion of GO to DRG is known by the color change of reaction mixture from yellowish brown to black (also confirmed by UV–Vis analysis).

2.3. Preparation of DRG/Au Composite

About 10 mg of DRG was dispersed in 10 mL of 0.5 mM $HAuCl_4$ solution by ultrasonication. To this solution 3 mL of 1% aqueous diastase solution was added, and heated at 60 °C for 10 min under ultrasonication conditions to obtain the DRG/Au composite.

2.4. Cytotoxicity Evaluation

The cytotoxic study of the GO, DRG and DRG/Au on HCT116 and A549 cells was performed by MTT assay. Cells were cultured in DMEM supplemented with streptomycin (100 U mL^{-1}), 10% heat-inactivated FBS and penicillin (100 mg mL^{-1}) in tissue culture flasks (T-25) at 37 °C in a humidified atmosphere and 5% CO_2. Cancer cells were sowed one day before their exposure to sample materials. Later, the actively growing HCT116 and A549 cells were maintained at 1×10^4/well followed by incubation in DMEM/1% FBS with test materials at different volumes (0.5, 1.0, 1.5 and 2.0 mL) in a relative humidity of >80% and 5% CO_2 at 37 °C for about 24 h. On the other hand, a control experiment was performed without test material.

To evaluate the cytotoxicity of the test materials, the medium was thrown after incubation of 24 h and a 20 μL of MTT solution (0.5 mg mL^{-1}. An MTT reagent diluted in phenol red-free DME without FBS) was added followed by incubation at 37 °C, 5% CO_2 for 1 h. Later, the MTT solution was removed and a 20 μL of DMSO was added to each well. The optical absorbance for each well is measured at 550 nm by using an ELISA plate reader against a reference at 655 nm. On the other hand, the cytotoxicity of test materials was compared with Cisplatin (CDDP) as standard drug.

2.5. Characterization

Preliminary characterization of prepared DRG was carried out by using Jasco V-670 UV–Vis double beam spectrophotometer. Sample for UV–Vis analysis was prepared by dispersing the dried DRG in double distilled water and the spectral measurements were recorded between 200 and 800 nm, while double distilled water was used for blank measurements. The morphology of the prepared DRG was determined by using TEM analysis. TEM samples were prepared by dispersing the purified dried DRG in double distilled water (0.5 mg mL^{-1}) under ultrasonic conditions. A drop of the prepared DRG dispersion was placed on a copper grid, which further left for drying in vacuum. TEM images for DRG were visualized at different magnifications using JEOL-2100 F electron microscope at an operating voltage of 200 kV. Atomic Force Microscopic (AFM) images and thickness of DRG were obtained by using Multimode Scanning Probe Microscope (NTMDTNTEGRA, Russia) after transferring onto Si (100) wafer substrates. A Bruker D8 Advance diffractometer was used to take XRD measurements for the prepared DRG. Measurements were taken at room temperature over the range of 2θ from 3° to 80° with a scanning rate of 4°/min with Cu Kα radiation (λ = 1.54A°) and

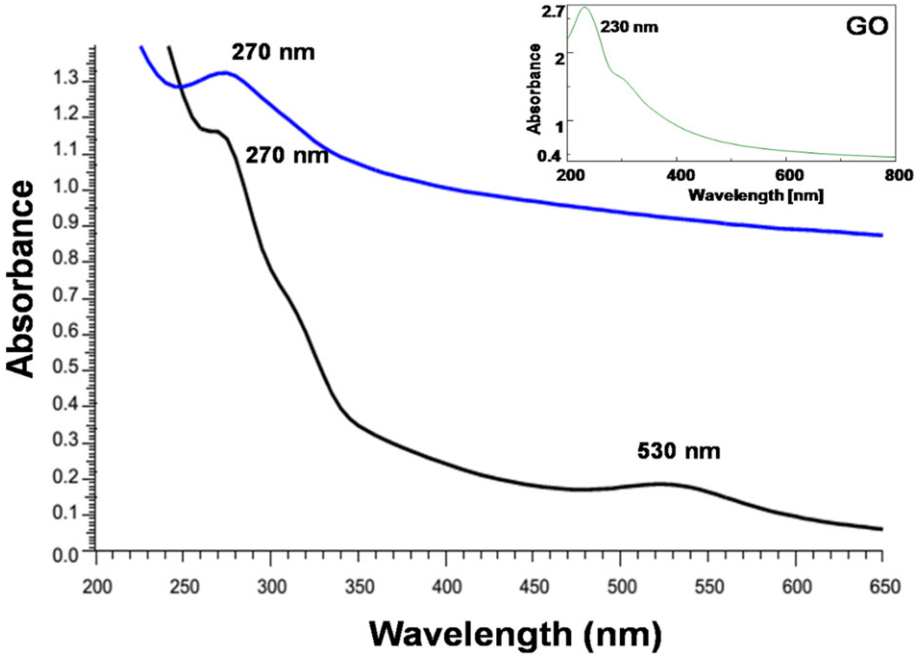

Fig. 1. UV–Vis spectra of GO (inset), DRG (blue) and DRG/Au (black).