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Casein mediated green synthesis and decoration of reduced graphene oxide

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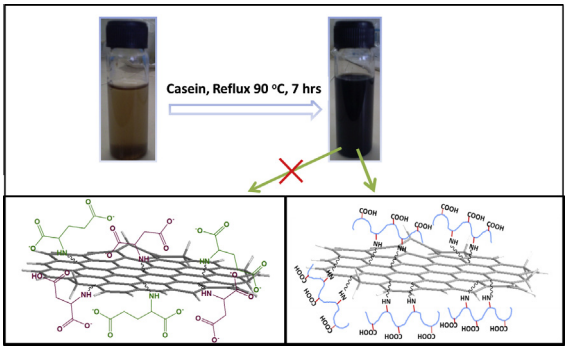
HIGHLIGHTS

- A green eco-friendly method for casein mediated reduced graphene oxide synthesis.
- Decoration of casein molecules on the surface of graphene sheets stabilizes it.
- Amino groups of aspartic, glutamic acid residues in casein cause reduction of GO.

GRAPHICAL ABSTRACT

Incorporated casein molecules via amino linkage onto the surface of graphene cause the stability of the graphene via their surface carboxylic acid groups [25,26]. Aspartic acid and glutamic acid residue of casein molecule are responsible for the graphene oxide reduction.



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ABSTRACT

This research is mainly focusing on one-step biosynthesis of graphene from graphene oxide and its stabilization using naturally occurring milk protein, casein. The synthesis of casein reduced graphene oxide (CRGO) was completed within 7 h under reflux at 90 °C with the formation of few layered fine graphene nanosheets. UV–Vis, XRD, XPS analysis data revealed the reduction process of the graphene oxide. Results of FT-IR, HPLC and TEM analysis have shown that the ensuing material consists of graphene decorated with casein molecules. Aspartic acid and glutamic acid residue present in casein molecules are responsible for the reduction of graphene oxide.

Introduction

Graphene, a two-dimensional nanomaterial of single atom thickness, have received much attention in recent years because of its outstanding physical and chemical properties [1]. Due to its exceptional electrical, mechanical, and thermal properties,

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graphene and its chemically modified forms are acting as gifted candidates in various fields such as sensors, field-effect transistors, energy-related, polymer composite and in biological applications [2–8].

From the last few years, various synthesis methods of graphene have been developed such as chemical vapor deposition [9], micromechanical exfoliation of graphite [10], chemical reduction of graphene oxide (GO) to graphene [11–13]. But, the synthesis from graphene oxide by chemical based reduction is considered to be prominent due to its low cost and large scale production. Numerous chemical reducing agents have been used for the synthesis of graphene such as $NaBH_4$ [14], hydroquinone [15], and hydrazine [16]. The bulk usage of these chemicals is dangerous because of their explosive and poisonous nature. Subsequently, the development of a new eco-friendly method for successfully converting the graphene oxide into graphene under mild conditions is necessary.

Many efforts have been made during the last few years for green synthesis of graphene using microwave, electrochemical methods and biological reducing agents such as vitamin C, reducing sugars, amino acids and protein bovine serum albumin, where they act as both reducing and stabilizing agents [17].

Casein, a proline rich globular protein which is readily available in milk has both hydrophilic and hydrophobic domains [18]. Recently, size controlled gold (Au) and silver (Ag) nanoparticles (NPs) have been synthesized by using casein as both reducing and stabilizing agent [19,20]. Herein, we report a green facile synthesis of reduced graphene oxide by using naturally occurring milk protein casein and its subsequent stabilization with casein molecules on the surface of the formed graphene sheets.

Experimental section

Materials

Graphite powder (100 mesh, 99.9995%) was purchased from Sigma–Aldrich, Bangalore and sodium nitrate ($NaNO_3$), hydrogen peroxide (H_2O_2, 30%), potassium permanganate ($KMnO_4$), concentrated sulfuric acid (98%) along with all organic solvents were purchased from Sd fine Chemicals, Mumbai.

Preparation of GO

Chemical synthesis of graphene oxide was carried out from graphite flakes by the modified Hummers method [21]. Briefly, 0.5 g of graphite flakes and 0.5 g of sodium nitrate were added to 23 mL of 12.1 M H_2SO_4 (98%) and the mixture was magnetically stirred for 15 min in an ice bath. Then, the reaction was continued by the slow addition of 4 g of $KMnO_4$ maintaining the temperature below 20 °C. Further, the mixture was constantly stirred at 40 °C for 90 min in water bath followed by the addition of 50 mL of double distilled water. The subsequent dark brown suspension formed was treated slowly with 6 mL of 30% H_2O_2 solution followed by dilution with 50 mL double distilled water. The resulting GO suspension was washed several times with 5% HCl followed by deionized water to remove excess of manganese salt until neutrality was reached. Finally, the purified GO was dried in oven at 60 °C for one hour. GO dispersion (1 mg GO/mL) was prepared by ultrasonication in an ultrasonic bath for about 2 h. The resulting stable dispersion was used for further experiments.

Preparation of reduced graphene oxide

About 2 g of casein protein powder was added to 200 mL GO solution (1 mg/mL) and mixed well by manual shaking. pH of the above solution was adjusted to 12 using NH_4OH solution. The resulting suspension was refluxed on a water bath at 90 °C for about 7 h. The completion of reduction of GO was confirmed by change in color of GO from yellowish brown to black (also confirmed by UV–Vis analysis).

Characterization

Ultraviolet–Visible (UV–Vis) spectroscopy

Initial characterization of synthesized CRGO was done by Jasco V-670 UV–Vis double beam spectrophotometer after dispersing the dried CRGO sample in double distilled water. λ_{max} was determined by recording spectra between 200 and 800 nm against double distilled water as blank. The obtained data was plotted using Origin 6.1. A simultaneous measurement of bandgap by diffuse reflectance spectroscopic (DRS) study was also carried out.

Transmission electron microscopy (TEM)

In order to determine the morphology of casein reduced graphene oxide, samples were prepared by dispersing dried CRGO in water (1 mg mL^{-1}) by sonicating under ultrasonic conditions, which further placed a drop of it on a Lacey Carbon Coated Copper Grid and then allowed to dry in vacuum. CRGO were visualized using JEOL-2100F electron microscope at an operating voltage of 200 kV.

X-ray diffraction (XRD)

The X-ray diffraction (XRD) study for the purified CRGO was conducted at room temperature by a Bruker D8 Advance diffractometer with Cu Kα radiation (λ = 1.54 Å) over the range of 2θ from 3° to 80° with a scanning rate of 4°/min and a step size of 0.02°. The instrument was calibrated with lanthanum hexaboride (LaB6) before to the analysis.

Fourier transformed infrared spectroscopy (FTIR)

Purified CRGO was analysed by using FT-IR spectroscopy. For FTIR analysis, CRGO pellet was prepared by mixing the dried CRGO powder with KBr, the spectra was recorded by using JASCO FT-IR 4100 instrument in the diffuse transmittance mode at a resolution of 4 cm^{-1}. To study the reduction process, GO was pelletized and used as control.

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) images for the prepared CRGO were taken by using a digital Nanosurf Atomic Force Microscope in contact mode. Samples for AFM analysis were prepared by coating a thin film on the surface of the glass slide with the dispersion of CRGO in DMSO and subsequent drying in oven.

Dynamic light scattering (DLS)

Dynamic light scattering (DLS) analysis for the casein reduced graphene oxide was done by using Malvern Instruments. DLS analysis was carried out after the prolonged sonication of the CRGO powder in water (1 mg mL^{-1}).

X-ray photoelectron spectroscopy (XPS)

PHI Quantera SXM, scanning X-ray microprobe (ULVac-PHI Inc) instrument was used for the High resolution X-ray photoelectron spectroscopy (HRXPS) analysis. Sample measurements were