A facile green reduction of graphene oxide using *Annona squamosa* leaf extract

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Abstract

A highly facile and eco-friendly green synthesis of *Annona squamosa* (custard apple) leaf extract reduced graphene oxide (CRG) nanosheets was achieved by the reduction of graphene oxide (GO). The as-prepared CRG was characterized with X-ray diffraction (XRD), transmission electron microscope (TEM), Fourier-transform infrared spectroscopy (FT-IR), ultraviolet-visible (UV-Vis), X-ray photoelectron spectroscopy (XPS) and Raman spectroscopic techniques. Removal of oxygen containing moieties from the GO was confirmed by UV-Vis, FT-IR and XPS spectroscopic data. The XRD and Raman data further confirmed the formation of the CRG. TEM images showed the sheet structure of the synthesized CRG. These results show that the phytochemicals present in custard apple leaf extract act as excellent reducing agents. The CRG showed good dispersion in water.

Key words: graphene, reduced graphene oxide, *Annona squamosa* leaf extract, green reduction

1. Introduction

Graphene, a break-through discovery in 2004 by Novoselov et al. [1], is a perfect two-dimensional honeycomb lattice of monolayer thickness comprised of tightly packed sp\(^2\) hybridized carbons fused into six-member rings. It has several exceptional properties like high surface area (~2600 m\(^2\)/g) [2], light weight [3], high chemical stability [4], high electron mobility (200,000 cm\(^2\)/V\(-1\) s\(^{-1}\)) [5,6], good optical [7] and mechanical [8] properties, good transparency [9], impermeability [10] and enhanced photocatalysis [11]. Because of these properties, recently, in the literature, there has been a graphene-driven “gold rush” with exciting applications for graphene in various fields of research including chemistry, physics, biotechnology, material science, nanoscience and technology which will essentially lead to the development of novel and multifunctional devices. Widely accepted structures of different graphene materials are depicted in Fig. 1.

Diverse and novel synthetic techniques have been developed to synthesize graphene. These include mechanical exfoliation of natural graphite [1], chemical vapor deposition [15], thermal exfoliation of graphite oxide [16,17], epitaxial growth [18], arc discharge [19,20], unzipping CNTs [21], solution based chemical reduction of graphene oxide (GO) [22], etc. [23]. Among these, industrial scale production is highly possible by solution based chemical reduction of GO. This method includes oxidation of natural graphite to graphite oxide, then exfoliation by sonication to GO, followed by reduction to yield reduced graphene oxide (rGO). Despite its advantages, the hazardous reducing agents involved in the wet chemical method like NaBH\(_4\), [24] or hydrazine [25] are very poisonous, and trace amounts of these could lead to detrimental effects [26-36]. This prevents the use of rGO in many bio and environmental applications. Therefore, continuous endeavors are required to develop an eco-friendly synthesis of rGO using environmentally benign reducing agents.

A better solution for this problem could be the use of leaf extracts, peels, biocompounds and microbes as reducing agents which have been already used in the preparation of metal
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Fig. 1. Schematic representation of (a) graphene [12], (b) graphene oxide [13] and (c) reduced graphene oxide [14].

Fig. 2. Schematic showing the constituent chemicals of custard apple leaves.

nanoparticles by reducing their precursors. This approach is called biosynthesis or green synthesis and currently is the hottest topic in the graphene community. A literature review revealed that various natural materials such as vitamin C (L-ascorbic acid) [26], reducing sugars [27], bovine serum albumin protein [28], amino acids [29], tea polyphenols [30], wild carrot roots [31], phytochemical extracts [32], melatonin [33], bacteria [34], grapes extract [35], and fenugreek extracts [36] have been used in rGO preparations. Inspired by these approaches, herein, we report a facile and green synthesis of rGO (we labeled it as CRG for our convenience) using custard apple (Annona squamosa) leaf extract as the reducing agent.

Custard apple (A. squamosa) belongs to the annonaceae family, is native to South America and West Indies and is extensively cultivated in India for its edible fruit. The extracts from different parts of the tree have shown extraordinary medicinal properties such as anti-insecticidal, antiulcer, anti-microbial, anti-fertility and wound healing properties [37]. The major isolated phytochemicals from the leaf extract includes flavonoids, alkaloids, glycosides, and unusual lactones, and among them, germacrene D, β-elemene, α-pinene, β-pinene, bicyclogermacrene, sabi- nene, t-cadinol, and t-muurolol account for 82% of the leaf extract chemical constituents (Fig. 2) [38,39]. The reducing nature of the A. squamosa leaf extract was first revealed by Vivek et al. [40] in their green biosynthesis of silver nanomaterials.

2. Experimental

2.1. Materials

Graphite powder, H$_2$O$_2$ (30 wt%), NaNO$_3$ (99%), H$_2$SO$_4$ (98 wt%), and KMnO$_4$ (98%) were purchased from Merck (India) and used without any further purification. Fresh custard apple leaves were collected from around Acharya Nagarjuna University in June, 2016. All aqueous solutions were prepared in Milli-Q water.

2.2. Preparation of custard apple leaf extract and identification of phytochemicals

Custard apple leaf extract was prepared by adopting the general procedure reported in the literature for other leaf extracts with a small modification [31,40]. Fresh custard apple leaves (100 g) were neatly washed, finely cut into pieces, and mixed with 100 mL of Milli-Q water in a 250 mL round bottomed flask. Then, this reaction mixture was refluxed on an oil bath for 30 min. during which the chemicals in the leaves were extracted into the water. After air cooling to room temperature, the solution was filtered through Whatman filter paper (grade 1, 25 µm; GE Healthcare, UK) to remove the bulk waste and then centrifuged at 10,000 rpm for 10 min. to remove any detritus that was present in the solution. The final solution was called the custard apple leaf extract.

An examination of the leaf extract yielded positive results for phytochemicals such as glycosides by the Legal test, carbohydrates by the Molisch test, alkaloids by Dragendorff’s test, saponins by the test with vortex, tannins by the FeCl$_3$ test, flavonoids by Shinoda’s test, phytoestrogens by the conc. H$_2$SO$_4$ test, proteins by the biuret test, polyphenols by the test with FeCl$_3$ and K$_3$[Fe(CN)$_6$], and amino acids by the ninhydrin test [41,42].

2.3. Synthesis of the Custard apple leaf extract reduced graphene oxide (CRG)

Graphene oxide was prepared by adopting Hummers method [43,44] available in the literature. In short, concentrated H$_2$SO$_4$ (69 mL) was added to a mixture of graphite powder (3 g, 1 wt
3. Results and Discussion

We successfully achieved the synthesis of CRG nanosheets from its general precursor GO with a green protocol using the green and environmentally friendly reducing agent custard apple leaf extract. Preparation of the leaf extract is very easy with a low cost. This synthesized CRG was well characterized with the aid of sophisticated spectroscopic techniques. All spectroscopic investigations confirmed the formation of CRG, and our findings are presented in this report.

3.1. UV-Vis spectra analysis

Fig. 4 shows the UV-Vis absorption spectra of the GO and CRG. Absorption spectra of the GO exhibits a peak at 235 nm and a shoulder at 302 nm which can be attributed to the $\pi-\pi^*$ transitions of the aromatic C=C bonds and the n-$\pi^*$ transitions of the C=O bonds, respectively. An efficient reduction of GO should re-generate C=C double bonds in rGO thus enhancing the conjugation in the $\pi$ bonds. A red shift to 276 nm was observed in the absorption spectra of CRG which confirms the restoration of electronic conjugation after the reduction.
3.2. FT-IR analysis

The CRG was characterized by FT-IR to estimate the structural changes that occurred before and after deoxygenation. Fig. 5 shows the FT-IR pattern of the GO and CRG. The strong broad peak at 3305 cm\(^{-1}\) observed in the FT-IR of the GO can be attributed to the stretching vibrations of the hydroxyl groups. The peak observed at 1624 cm\(^{-1}\) is a characteristic of the C=C stretching. Furthermore, the peaks at 1421 cm\(^{-1}\), 1221 cm\(^{-1}\) and 1051 cm\(^{-1}\) can be assigned to alcohol, epoxy and alkoxy C-O stretching vibrations, respectively. Clearly, all these oxygen containing moiety peaks in the FT-IR pattern of CRG disappeared which reveals that the reduction of the GO was successfully achieved with the \textit{A. squamosa} leaf extract.

3.3. XRD analysis

XRD patterns of the GO and CRG are shown in Fig. 6. The XRD reflection peak at \(2\theta = 11.2^\circ\) can be attributed to the (002) plane of the GO. In the XRD of the CRG, this peak disappeared, and a new broad peak at \(2\theta = 23^\circ\) appeared which indicates the reduction of the GO and the formation of few-layered graphene (CRG).

3.4. TEM analysis

High-resolution transmission electron microscopy (HR-TEM) analysis was performed to understand the morphology of the obtained CRG nanosheets. Fig. 7a and b shows clear pictures of transparent, lengthy, and few-layer thin CRG nanosheets. Fig. 7c shows a selected area of the electron diffraction pattern for the CRG nanosheets which confirms its crystal nature.

3.5. XPS analysis

The XPS spectra of the CRG are shown in (Fig. 8). Fig. 8b shows typical raw and deconvoluted peaks at 284.68, 285.98, 286.88, and 287.58 eV. The peak at 284.68 eV corresponds to the C 1s peak of C=C/C-C in the aromatic rings and is a major peak compared to the other peaks at 285.98, 286.88, and 287.58 eV. These three peaks were
3.7. Confirmation of the green synthesis of CRG

The reduction of GO to CRG was primarily confirmed by the color change of the reaction mixture from brownish yellow (GO) to black (CRG). This indicates that the phytochemicals-glycosides, carbohydrates, alkaloids, saponins, flavonoids, tannins, phytosterols, proteins, phenolic compounds and amino acids in the custard apple leaf extract [40] as good reducing agents. The presence of these constituents in our extract was also confirmed by their respective test protocols described in the experimental section. Among them, individual chemicals such as sugars, proteins, amino acids and tea polyphenols were reported to be good reducing as well as stabilizing agents used in the green synthesis of rGO, and their reduction mechanisms have been well documented [27-37].

In general, reducing chemicals either with –OH, –NH or –SH nucleophilic moieties involve in the epoxide ring opening of the GO through nucleophilic substitution. Then, a subsequent redox reaction converts the GO into rGO, and the reducing agents undergo oxidation.

The major chemical constituents of the custard apple leaf extract (Fig. 2) contain an alkene functional group in their structure. To the best of our knowledge, there have been no reports on the reduction mechanism of GO using chemicals that contain an alkene functional group. Therefore, we propose the following plausible mechanism to explain the reduction mechanism (Scheme 1). In the first step, protonation activates the epoxide of the GO which then opens up with the nucleophilic substitution of an alkene to generate a diol after the addition of a water molecule. A thermal redox reaction now forms the double bond by expelling the water molecule (Route I). In a similar fashion, GO undergoes reduction by transferring its oxygen to the alkene functional groups and is converted to a graphene by re-attaining the conjugation (Route II).

The synthesized CRG showed good dispersibility in water, and the solution is stable for 1 day. This indicates that the phytochemicals in the custard apple leaf extract act as good reducing...
as well as capping agents. This capping nature prevents the re-agglomeration of the obtained graphene sheets. The electrostatic repulsions that exist between these phytochemicals further stabilize the CRG dispersed solution.

4. Conclusions

In this study, an eco-friendly green reduction of GO was achieved with Annona squamosa leaf extract. The as-prepared CRG has a better dispersibility compared to chemically reduced GO. This confirms that the phytochemicals present in the custard apple leaf extract act as better capping agents as well as reducing agents. UV-Vis, FT-IR and XRD techniques primarily confirmed the removal of oxygen-containing functional groups from the surface of the GO. XPS and Raman data further supported the formation of the CRG. TEM confirmed the sheet structure of CRG nanosheets. The major advantages of our eco-friendly green reduction of GO are as follows: high yields, non-toxic, efficient, cost effective and simple procedure. Hence, we conclude that this green method is effective and could be used for large scale production of GO.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

References


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