

Graphene oxide reduction activity of seaweed polymer derivative: efficient bio-based alternative

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The present study demonstrates utilization of functionalized seaweed polysaccharide, namely agarose-gallate (Ag-GA_{Est}) for the preparation of reduced graphene oxide (rGO) under mild reaction conditions. Ag-GA_{Est} obtained with the lowest degree of substitution (degree of substitution (DS) = 0.45; with 1 : 0.5 w/w agarose : gallic acid) shows excellent performance compared to its high DS (1.1; with 1 : 2.0 w/w agarose : gallic acid) ester derivatives. Further, the formation of rGO was confirmed using UV-Vis, TEM, FTIR, Raman spectroscopy, elemental and XRD analysis. This study describes a new application of seaweed-derived polysaccharides.

Keywords: Agarose-gallate, biomaterials, green approach, graphene oxide, reduction activity.

GRAPHENE is the thinnest aromatic sheet of sp^2 hybridized carbon atom organized in the two-dimensional chicken wire pattern. It is also the strongest material known with excellent electronic properties like ballistic transport^{1,2}. Graphene and graphene-based materials are important in hydrogen storage³, organic photovoltaic devices⁴, organic solar cell⁵, lithium-ion batteries⁶, flexible field emission cathodes⁷, etc. In addition, its exceptional properties such as high surface area and flexibility have an important role in cell growth and differentiation⁸. Graphene is also used in the preparation of biomaterials for biosensors, therapeutics as well as tissue engineering applications⁸. Researchers and technologists are continuously working towards the development of an improved process for the preparation of reduced graphene oxide (rGO) through discovering novel green reducing agents. Graphene oxide (GO) is an exceptional and industrially important material with a large number of carboxylic acid, epoxy and hydroxyl functional groups^{1,9}. Nowadays rGO has great application potential due to its excellent properties, e.g. electronic, mechanical, optical and thermal properties¹⁰. Reduced graphene oxide can be

obtained using several reducing agents such as hydrazine, NaBH_4 , LiAlH_4 , hydriodic acid (HI), NaOH (ref. 11), ascorbic acid, gallic acid¹² and glucose¹³. It tends to aggregate and precipitate due to its π - π stacking interaction. To overcome this problem, various stabilizers (e.g. lysozyme, porphyrins, pyrene butyric acid) have been developed to enhance rGO dispersion. Most of the reducing agents such as chemical reductants are toxic or explosive in nature, which restricts the use of rGO in the synthesis of biomaterials. Several naturally occurring reductants such as seaweed sap, protein, carbohydrates (glucose, dextran), tannin, gallic acid, dopamine, tea and vitamin C have been reported in the literature¹²⁻¹⁴. Recently, we have successfully tested seaweed polysaccharide agarose derivatives for the preparation of eco-friendly mercury sensor, self-healing and stretchable materials¹⁵⁻¹⁸. Till date, there is no report on the reduction of GO using agarose derivatives. Hence we explored other novel applications of seaweed polysaccharides.

Here, we report a green approach for reduction of GO using a biopolymer derivative, agarose-gallate (Ag-GA_{Est}). Ethylene glycol was used as a solvent for reduction. This process is green and utilizes bio-based reducing and stabilizing agent during the reaction in a very low concentration compared to pristine gallic acid¹². The individual agarose polymer and a gallic acid equivalent to agarose-gallate (Ag-GA_{Est}) system were unable to reduce GO up to 3 h. However, Ag-GA_{Est} system having ~100-fold less gallic acid content compared to pristine gallic acid was required for the reduction of GO (ref. 12).

Experimental

Raw materials

The powdered graphite (avg. particle size ~400 nm, CAS no. 7782-42-5) was purchased from Sisco Research Lab (SRL) Chemicals Pvt Ltd, Mumbai. Other chemical reagents used in this study (e.g. AR-grade sodium nitrate,

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concentrated sulphuric acid, potassium permanganate, hydrochloric acid, methanol and hydrogen peroxide) were supplied by S.D. Fine Chemicals Ltd, Mumbai, India. Agarose used was obtained from *Gracilaria dura* as described in our previous work^{16,17}. Gallic acid (TCI Chemicals India Pvt Ltd, Chennai, India) dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), *N,N*-dimethylformamide (DMF) were purchased from Spectrochem Pvt Ltd Mumbai, and ethylene glycol used as a solvent from Merck Specialties Pvt Ltd, Mumbai.

Synthesis of agarose gallate

Agarose gallate was synthesized using our previously reported method¹⁸. In brief: 0.2 mol. of agarose polymer (306 g/mol molecular weight disaccharide repeating unit) was dissolved in DMF at 90°C under microwave irradiation condition. A mixture of 0.2 mol. of gallic acid (mol. wt. = 170 g/mol), 0.4 mol. of DCC (mol. wt. = 412 g/mol) and 0.05 mol. of DMAP (mol. wt. = 30.5 g/mol) was added to agarose solution under stirring conditions, and kept under microwave irradiation at 90°C for 12 min. The solid Ag-GA_{Est} product was obtained by isopropyl alcohol (IPA) treatment followed by washings with IPA and vacuum drying.

Preparation of reduced graphene oxide

GO was synthesized according to modified Hummers method from graphite nanopowder^{19,20}. Agarose-gallate, a bio-based reducing agent was used for the reduction of GO. For this, 0.25 g (containing 0.21 g agarose + 0.04 g gallic acid content) Ag-GA_{Est} was first dissolved in 50 ml ethylene glycol and 50 mg GO was added at 60°C under stirring conditions. Stirring was continued till 1 h followed by ultrasonication for 30 min to disperse the GO properly; then the whole suspension was kept at 95 ± 2°C for 3 h. Aliquots were taken at designated time intervals to check the reaction process by UV spectra. The resulting rGO was isolated using centrifugation (10,000 rpm for 5 min). The obtained rGO residue was washed with 20 ml ethylene glycol (three times) and 20 ml water (twice) to remove excess Ag-GA_{Est}, and dried under vacuum oven at 50°C. Similarly, the control experiment was carried out using 0.04 g pure gallic acid for reduction of GO under identical conditions; but no reduction was observed.

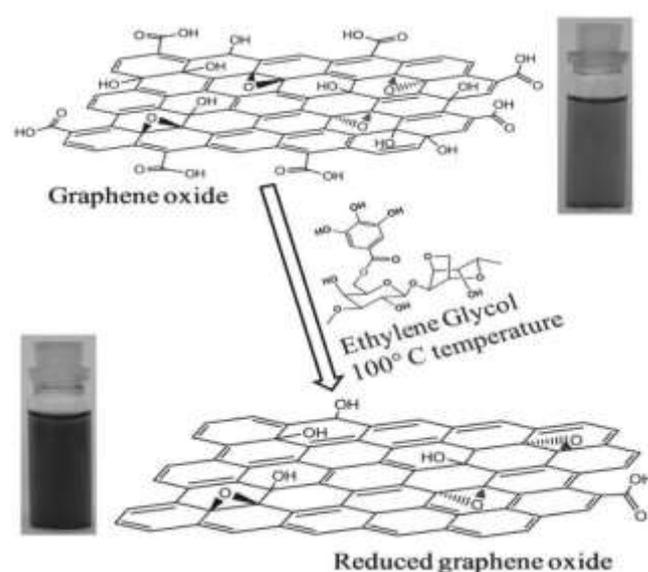
Characterization

FTIR spectra of the samples were taken using Perkin-Elmer Spectrum, GX FT-IR system; all the spectra were recorded between 4000 and 500 cm⁻¹ with 5 cm⁻¹ resolution and ten scans using KBr pellets (1 : 60 w/w ratio of

sample to KBr). UV-Vis absorption spectra were taken using Varian Cary-500 (Shimadzu UV 3600) spectrophotometer to confirm the formation of reduced graphene. X-ray diffraction (XRD) patterns were recorded on model Empyrean XRD (PAN analytical) using Cu-K α radiation. Thermal gravimetric analysis (TGA) was recorded (Mettler Toledo Thermal Analyser, GA/SDTA 851e, Switzerland). Transmission Electron Microscope (TEM) images were recorded (HR-TEM; JEOL GEM 2100) at an accelerated voltage of 200 kV. Atomic force microscope (AFM; NTEGRA, TS-150) was used to record height profile and AFM images. Raman spectroscopic analysis of GO and rGO was done using micro-Raman spectrometer (LabRAM HR 800, Horiba Jobin-Yvon) coupled with 514.5 nm argon-source laser excitation with power 10 mW between 100 and 3000 cm⁻¹, while CHNS analyzer (Perkin-Elmer) was used for elemental analysis.

Results and discussion

In continuation with our previous study, we have successfully tested agarose derivative (agarose-gallate) in the reduction of GO¹⁸. Hence, this study provides an example of a sustainable process for the synthesis of rGO through a green approach using seaweed polysaccharide-based reducing agent (Ag-GA_{Est}). Scheme 1 shows the synthetic protocol for reduction of GO using bio-based reducing agent (Ag-GA_{Est}). The brown colour of GO dispersion turns black with 0.5% Ag-GA_{Est} treatment within 3 h at 100°C under stirring conditions. The appearance of black colour of suspension suggests the effective reduction of GO indicating removal of oxygen functionality of GO^{21,22}.



Scheme 1. Schematic outlay for reduction of graphene oxide using agarose-gallate.

UV-Vis analysis

After the preliminary confirmation of colour change, the samples were subjected to UV-Vis spectroscopy to confirm the reduction of GO in the presence of Ag-GA_{Est} (Figure 1). UV-Vis absorbance spectra of pure GO showed λ max at \sim 230 nm due to aromatic π - π^* transitions of carbon-carbon bond, but the appearance of absorption maximum at 274 nm after treatment with Ag-GA_{Est} confirmed the formation of rGO^{23,24}. A control experiment was also carried out using 0.04 g pure gallic acid (equal amount of gallic acid is present in 0.25 g Ag-GA_{Est}) as reductant under identical conditions. However, appearance of the absorption maximum at \sim 232 nm in UV-Vis spectroscopy revealed that there was no reduction of GO with 0.04 g pure gallic acid (Supplementary Figure 1). Reduction of GO was further confirmed by measuring carbon-oxygen (C/O) ratio using elemental analysis. The C/O ratios were 0.67 and 0.78 for

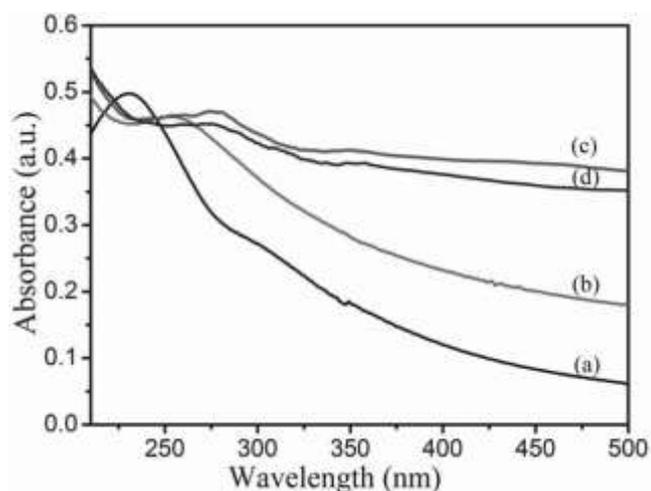


Figure 1. UV-Vis spectra of (a) graphene oxide (GO), (b) Ag-GO, (c) reduced graphene oxide (rGO) 3 h and (d) rGO - 6 h.

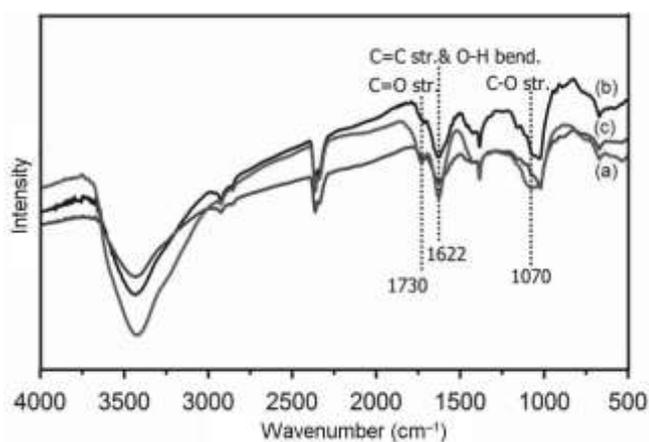


Figure 2. Fourier-transform infrared spectroscopy spectrum of (a) GO; (b) rGO-3 h and (c) rGO - 6 h.

pure GO and rGO respectively. Increment in C/O value from 0.67 (in GO) to 0.78 (in rGO) after reduction with Ag-GA_{Est} may be due to the reduction of oxygen functionality in GO, further indicating the formation of rGO. As reported in the literature; the C/O ratios obtained from elemental analysis and X-ray photoelectron spectroscopy (XPS) are consistent^{14,25,26}.

The results of the present study reveal that very low amounts (0.08% w/w) of gallic acid in combination with seaweed polysaccharide agarose is suitable to achieve GO reduction compared to individual 4% w/w gallic acid reported in the literature¹², while individual agarose does not work up to 0.5% w/w. Therefore, this study provides a simple, green and eco-friendly solution and also opens new areas for seaweed polysaccharides and their derivatives for the development of sustainable materials. The study also reveals that the concentrations of individual reducing agents could be decreased significantly by aggregation with polymers through chemical modification.

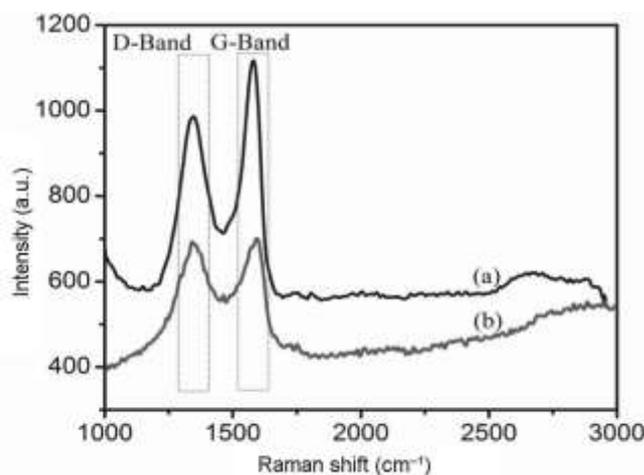


Figure 3. Raman spectra of (a) GO and (b) rGO.

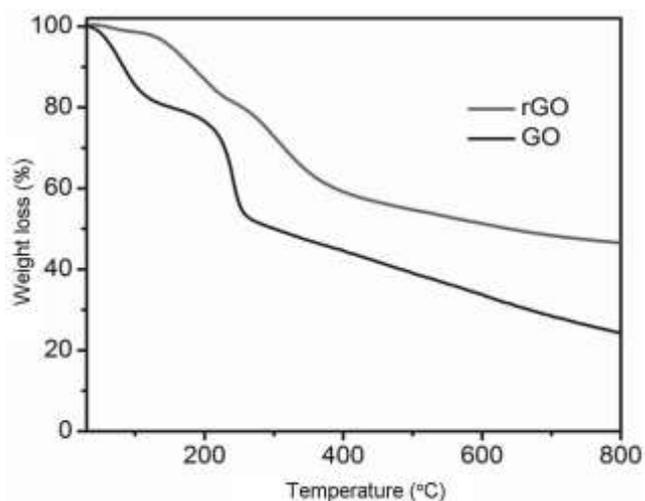


Figure 4. Thermal gravimetric analysis of (a) GO and (b) rGO oxide.

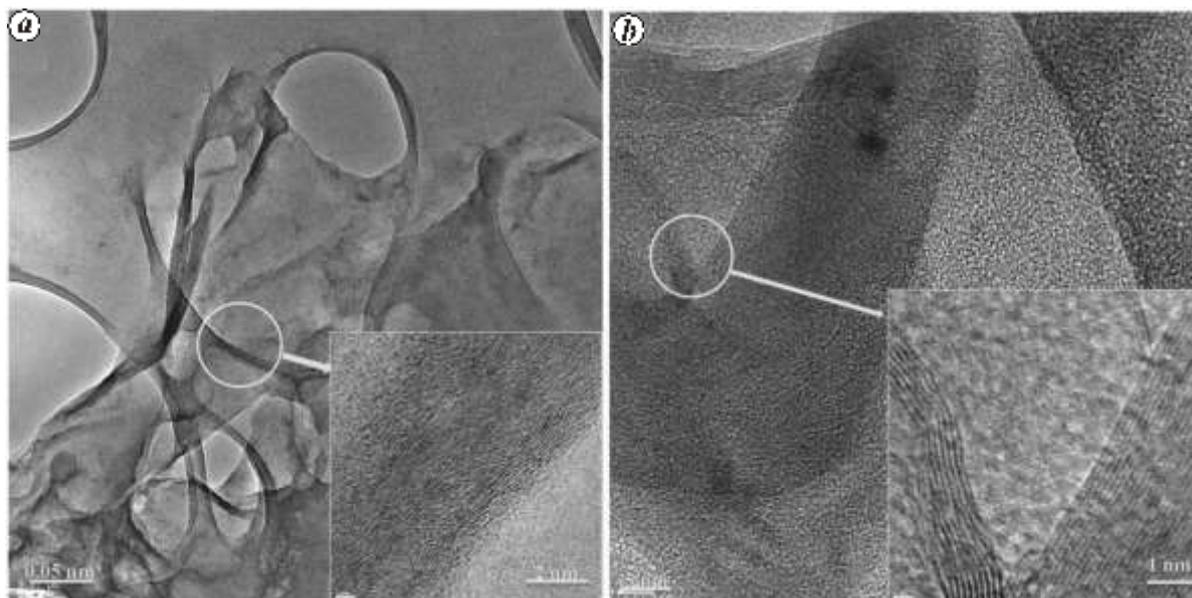


Figure 5. TEM images of GO (a) before and (b) after reduction.

FTIR and Raman analysis

The formation of rGO was also confirmed by Fourier-transform infrared spectroscopy (FTIR) (Figure 2). Significant changes were observed in the carbonyl and epoxide functional groups present in GO before and after reduction. Figure 2 shows the IR spectrum of GO before and after reduction with Ag-GA_{Est}, in which the appearance of absorption bands between ~ 1000 and ~ 1740 cm^{-1} (C=O stretching vibration band at 1730 cm^{-1} ; C=C stretching vibration and O-H bending vibrations bands at 1622 cm^{-1} ; phenolic groups and C-O in epoxy groups bands at 1360 cm^{-1} and 1224 cm^{-1} ; and C-O in alkoxy groups bands at 1070 cm^{-1}) indicates the presence of various functional groups on GO sheets such as hydroxyl, keto, carboxylic, etc.^{27,28}. The absorption band intensity at 1730 cm^{-1} (carbonyl) and 1070 cm^{-1} (alkoxy) groups decreased remarkably after reduction of GO, further indicating removal of oxygen functionality¹². According to FTIR results of this study, the intensities after 3 h are almost identical with those obtained after 6 h of reduction with Ag-GA_{Est} indicating that 3 h reaction with Ag-GA_{Est} reagent is sufficient to reduce a large number of carboxylic acid and alkoxy groups in GO.

The effect of Ag-GA_{Est} on pristine GO was also analyzed using Raman spectroscopy (Figure 3). Figure 3 shows the G and D bands of pristine GO at 1580 and 1346 cm^{-1} respectively. However, the G band is shifted by 13 cm^{-1} (1580 – 1593 cm^{-1}) showing the reduction of GO while the D band is at almost identical position (1341 cm^{-1}). In addition, GO and rGO exhibit D/G intensity ratio of 0.88 and 0.983 respectively. The D/G ratio is found to be higher for rGO compared to GO showing the more crystalline nature of rGO (ref. 29).

Thermal analysis

The mass loss patterns of pristine GO and rGO were recorded up to 800°C (Figure 4). As shown in Figure 4, mass loss for GO was $\sim 18\%$ below 120°C as a result of loss of absorbed water. Significantly higher mass loss $\sim 76\%$ was observed at 800°C , which may be due to decomposition of thermally labile oxygen functionality present in pure GO in the form of CO, CO₂ and steam. Consequently, a mass loss of $\sim 13\%$ was obtained in rGO at $\sim 200^\circ\text{C}$. Furthermore, significantly low ($\sim 54\%$) mass loss was obtained in rGO up to 800°C , which indicates the reduction of GO and presence of low oxygen functionality in rGO. Therefore, significantly higher 46% residual mass was obtained for rGO compared to GO (24%) at 800°C . In this study, TGA results confirm that oxygen-containing functional groups in rGO are significantly low compared to GO, which may be due to the reduction of GO with Ag-GA_{Est} (ref. 24).

TEM and AFM analysis

Figure 5 shows TEM images of pure GO and rGO. Figure 5a clearly exhibits layered and sheet-like arrangement in pure GO²⁴. TEM images of rGO show few layered GO sheets (Figure 5b). The average size of the sheets is about 2 nm having an average interlayer distance of ~ 3.4 nm. Previous studies have also reported that the thickness of pristine GO sheets decreased after reduction due to the removal of oxygen group³⁰.

The height profile measurements of pure GO and rGO were also done using AFM to confirm the variation in thickness in pristine GO sheets after reduction with

Ag-GA_{Est} (Supplementary Figure 2). As shown in Figure S2, the height of pristine GO sheets was found to significantly decrease from ~24 to ~3.5 nm after reduction. The results of AFM confirm that modification of GO sheets has taken place^{24,31}. Further, powder XRD analysis was performed to obtain the atomic structure and interlayer spacing of pure GO and rGO with Ag-GA_{Est} (Supplementary Figure 3). The pristine GO exhibited sharp diffraction peak at ~10.5° with an interlayer spacing of 7.60 Å, indicating oxygen functionality after oxidation of graphite²⁴. The characteristic peak of GO at ~10.5° disappeared after reduction of GO, which may be due to the removal of large oxygen functionality after reduction with Ag-GA_{Est} (refs 23, 24). The appearance of a new broad diffraction peak at ~26° with very less interlayer spacing of ~3.4 Å in rGO may be the result of the restacked graphene particles, and this also confirmed the reduction of GO by Ag-GA_{Est} (refs 24, 32). In addition, the interlayer spacing of GO was significantly larger compared to that of rGO, revealing the elimination of oxygen functionality³³.

Zeta potential measurement

The zeta potential of the pristine GO and rGO suspensions was measured to show the suspension stability of the product. The zeta potential of pristine GO and rGO was -7.71 and -38.38 mV respectively. To form a stable suspension, the particles should have greater negative surface charge which mainly generates the electrostatic repulsion between particles¹². Hence, suspension of rGO with -38.38 mV zeta potential is stable for a longer time.

Conclusion

In this study, we have described the reduction of GO using agarose ester (agarose-gallate) working as reductant as well as stabilizer during the reaction. Ag-GA_{Est} with low gallic acid content was found to be better for reduction of GO. The raw materials agarose and gallic acid used in this study to synthesize Ag-GA_{Est} were produced naturally, and successfully used to develop an eco-friendly reducing agent for GO. Ag-GA_{Est} contains very low ~0.04% w/w gallic acid to reduce GO compared to individual gallic acid (4% w/w). The synthesized rGO exhibited good dispersion in water up to 1.1 mg/ml. This study provides a green route towards large-scale production of rGO with excellent dispersion, enhancing its applications in the fields of biomaterials, composites and energy.

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ACKNOWLEDGEMENTS. CSIR-CSMCRI, Bhavnagar registration No. CSMCRI-123/2015. We thank the Analytical Discipline and Centralized Instrument Facility at CSIR-Central Salt and Marine Chemicals Research Institute for analytical data, and DST-SERB and CSIR, New Delhi for financial assistance under a project (No. SB/EMEQ-052/2013 and EMR/2016/004944). N.V. thanks AcSIR for Ph D enrollment.

Received 16 February 2017; accepted 10 April 2017

doi: 10.18520/cs/v113/i07/1361-1366
