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A Facile and Green Synthesis of Graphene

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Abstract: Herein, we report a simple, facile, green and cost effective strategy for the synthesis of graphene using naturally available anti-oxidants such as carotenoids present in the vegetable (carrot, sweet potato etc.) extracts. In this work, we have employed carrot extract to reduce graphene oxide to reduced graphene oxide. A red shift (in the λ_{\max} from 230 nm to 270 nm) during the course of the reduction of GO clearly indicates the effective restoration of the sp^2 graphitic carbons. In addition, we have also noticed the colour change of the reaction mixture from yellowish brown to black after 1 hour thereby indicating the reduction of GO to reduced graphene oxide (Ct-RGO). Further, an increase in the D/G ratio value of GO from 0.979 to 1.198 after the complete reduction indicated the effective restoration of the *in plane* sp^2 domains in the Ct-RGO. The morphology and conductivities of the Ct-RGO are characterized by several characterization techniques such as UV, FT-IR, Raman, XRD, XPS, SEM, TEM, AFM and EIS. The green synthesis reported in this work is expected to yield biocompatible graphene material suitable for futuristic biological applications.

Key Words: Carrot, Anti-Oxidants, Carotenoids, Green Synthesis, Bio compatible Graphene

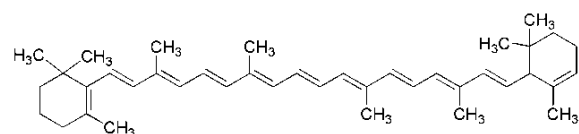
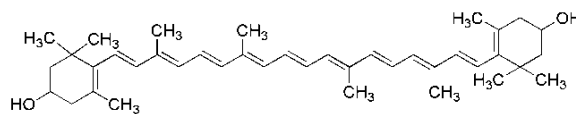


Scheme 1: Schematic representing the reduction of GO by Carrot

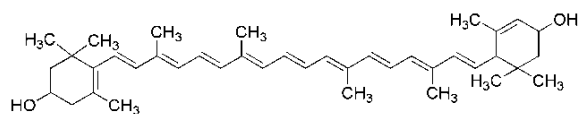
Introduction:

Graphene is a two dimensional carbon material that finds substantial applications in the area of nanoelectronic devices, biosensor, energy storage devices, solar cells, drug delivery systems and optical devices¹⁻⁴. The profound application of graphene is due to its excellent mechanical strength, electronic and thermal properties⁵. In literature, several strategies have been reported to synthesize graphene such as epitaxial growth⁵⁻⁷, cutting carbon nanotubes^{8,9}, direct sonication^{10,11}, chemical vapour deposition¹²⁻¹⁴, micro mechanical exploitation¹⁵⁻¹⁷, chemical route¹⁸, template directed method¹⁹ and thermal strategy²⁰. However, all these methods have their own advantages and disadvantages. A common problem encountered during the synthesis of graphene is to prepare a stable dispersion of biocompatible graphene and scalability of the existing methods. Though it is possible to scale-up the chemical reduction methods, a major drawback of all these methods is that they employ extremely hazardous and toxic agents such as hydrazine, dimethyl hydrazine, sodiumborohydride, etc. In order to overcome or eliminate the toxicity associated with the reduced graphene oxide (RGO) obtained using the above methods, we herein report a novel strategy to obtain eco-friendly and bio-compatible graphene by a greener route. There are reports available in the literature regarding the eco-friendly conversion of GO to RGO using non-toxic reducing agents such as vitamin C²¹, reducing sugars²² and bovine serum albumin²³. Though these existing strategies are successful in eliminating the toxic effects, the RGO obtained using these reducing agents are highly agglomerated²¹⁻²⁷. In order to overcome this issue, we developed a green and non-toxic route to reduce GO to RGO using carotenoids available in the vegetable extracts.

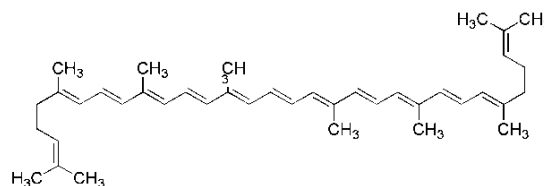
Carotenoids react with highly oxidizing species such as singlet oxygen, hydroxyl radical, superoxide, hydrogen peroxide, organic hydroperoxides and peroxy radicals generated in biological systems by any one of the following three distinct pathways *viz* electron transfer, hydrogen abstraction and radical addition²⁸. Examples of carotenoids include lycopene, lutein, β -carotene and 3-epilutein. The structures of these carotenoids are shown below.

 β -carotene

Lutein



3-Epilutein



Lycopene

Of various carotenoids mentioned above, lycopene is a highly unsaturated straight chain hydrocarbon with a total of 13 double bonds, out of which 11 are conjugated. This unique nature of the lycopene molecule makes it a very potential anti-oxidant among other carotenoids. *In vitro* studies revealed that the antioxidant property of lycopene is two times that of β -carotene, ten times that of α -tocopherol, hundred times that of Vitamin E and one hundred and twenty five times that of glutathione towards singlet oxygen quenching ability^{29, 30}. A combination of two or more carotenoids was found to be more effective than a single carotenoid in its action towards antioxidant properties. This synergistic effect was more pronounced when lycopene or lutein was present in the mixture³¹. As a result, several reactive oxygen species can be effectively reduced by carotenoids and the resultant products are physiologically friendly, relatively stable and inert. Carrot is a vegetable that contains abundant antioxidants such as β -carotene, lutein and lycopene^{28, 31, 32}. Herein, we discuss a simple, facile, green and cost effective strategy to reduce the GO into reduced graphene oxide (Ct-RGO) by using carrot extract.

Experimental Section:

Materials: Graphite powder $\leq 20 \mu\text{m}$ was purchased from Sigma Aldrich. Analytical Grade KMnO_4 , NaNO_3 , H_2SO_4 , HCl , NaOH and H_2O_2 were all purchased from Alfa Aesar and used as purchased. Fresh Carrots were purchased from Local Vegetable Market.

Methods & Instruments Employed: UV-Visible spectra were recorded using Perkin Elmer Scan Lambda 650, UV-Visible spectrophotometer. FT-IR spectra were recorded using Tensor 27 FT-

IR spectrophotometer (Bruker optics) between 4000 and 400 nm^{-1} on transmittance mode. Raman Spectra was obtained using a Reinshaw Raman system 2000 model that employ a 514 nm Ar^+ laser source. AFM images were recorded in the tapping mode at ambient conditions using Agilent technologies 5500 model AFM instrument. SEM images were obtained using Tescan SEM VEGA 3 XM and TEM analysis were performed using Tecnai G² 20 with an accelerating voltage of 220 KV (FEI make). Electrochemical impedance spectra were recorded using PGSTAT 302N model potentiostat.

Results and Discussion:

Preparation of GO: GO was obtained by Hummers Method³³. Briefly, 10g of graphite and 5g of NaNO_3 were added into a round bottom flask. To this mixture, 250 mL of con. H_2SO_4 was added drop wise and stirred for 30 min. The reaction mixture was then cooled to 20°C. Following this, 30g of KMnO_4 was gradually added to this reaction mixture with vigorous stirring. The temperature of the mixture was increased to 35°C and maintained at 35°C for 30 minutes. Following this, 450 mL of DI water was added in aliquots to the mixture and the temperature was increased to 70°C. After 20 minutes, 1.4 L of hot water and aqueous solution of 25 mL of 30% H_2O_2 were all added. This resulted in the colour change of the mixture from yellow to dark brown (Figure 1) and this suspension was repeatedly washed with dil. HCl and water and dried at 50°C under vacuum for 72 h. This resulted in the formation of a black solid.

Preparation of Carrot extract: Initially, the fresh carrots as purchased were washed in cold water for three times. Next, carrot skins were peeled and the carrots were chopped in to pieces. 20g of carrot slices were mixed with 100 mL of de-ionized water and boiled at 100°C for 50 min and filtered through Whatmann 40 filter paper. The filtrate thus obtained contains the mixtures of carotenoids namely β -carotene, lutein, and lycopene those will act as antioxidants (reducing agents) for the reduction of GO.

Synthesis of Reduced Graphene Oxide [Ct-RGO]-A Green Approach: Initially, 2mg of GO was mixed with 2 mL of de-ionized water (1mg/mL) and sonicated for 1h. About, 20 mL of this GO suspension was added to the flask containing 200 mL of 1M NaOH and 100 mL of the carrot extract. The mixture was then stirred for 1h at 90°C. As the reaction proceeds, the yellowish

brown colour mixture slowly turns to dark black within 1hr. the mixture was kept under the same condition for one more hour to allow the GO to be reduced completely. Finally, the mixture was cooled down to room temperature and repeatedly washed with de-ionized water to remove any impurities (if present) from the product and then dried in vacuum.



Figure 1: Photograph of (a) Graphene oxide (GO) and (b) Carrot reduced graphene oxide (Ct-RGO) in de-ionized water.

Surface Characterization: Initially, the UV-visible spectra of GO and Ct-RGO has been performed to confirm the complete reduction of GO to RGO. Results indicated that the value of λ_{\max} has been shifted from 230 nm (at $t = 0$ min) to 270 nm (when $t = 60$ min) thereby confirming the effective reduction of the GO by carrot. However, we have not seen any further shift in the λ_{\max} value after sixty minutes which further confirms the total reduction has occurred within one hour. The observed behaviour is in good agreement with the Marino et al report, that the maximum red shift value can be used as the yardstick to predict the performance of the reducing agent ²⁷. Moreover, Ct-RGO depicted the absorption at the same wavelength as that of graphene obtained by the reduction of hydrazine. In addition, the absorption wavelength is higher than that of the green reduced graphene oxide ²² which suggests that carrots are efficient and effective reducing agent for the reduction of GO.

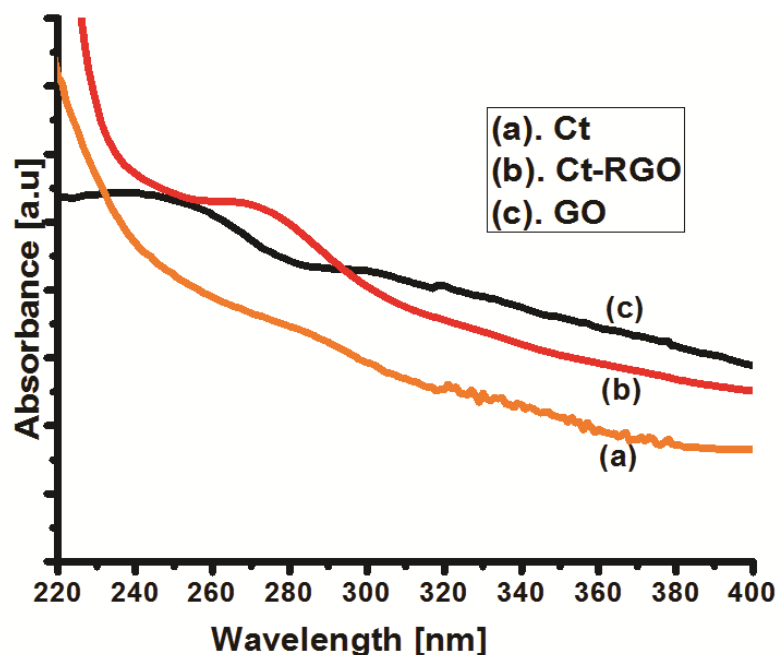


Figure 2: UV-Visible spectra of (a) Carrot extract (b) Ct-RGO and (c) GO.

Next, FT-IR spectroscopy is used to investigate the extent of GO reduction (See Figure 3). From Figure 3, it is evident that GO has its characteristic bands at 1734 cm^{-1} , 1179 cm^{-1} , 1070 and 1011 cm^{-1} , 1629 cm^{-1} corresponding to COOH, -COOR, C-O-C, and C=C stretching respectively. After the reduction of GO by carrot, the FT-IR spectra of Ct-RGO exhibited bands at 1094 cm^{-1} , 1641 cm^{-1} corresponding to aromatic (C=C stretching), ether linkage (C-O-C stretching). The disappearance of bands between 1179 cm^{-1} and 1734 cm^{-1} indicated that the reduction resulted in the significant removal of -COOH and -COOR moieties. Further, appearance of a new band at 1538 cm^{-1} is attributed to the restoration of aromatic rings. Upon comparison, it is evident that carrot extract is an effective reducing agent to convert GO to RGO. Raman spectroscopy of GO and Ct-RGO was then performed and is shown in Figure 4. As evident from Figure 4, both GO and Ct-RGO has shown the characteristic peaks at 1336 cm^{-1} and 1593 cm^{-1} corresponding to D and G band respectively. Further, the D/G ratio was calculated as 0.979 for GO and 1.198 for Ct-RGO. The increase in the D/G ratio in the Ct-RGO is due to the restoration of sp^2 network and the formation of the unrepaired defects after the removal of plenty of oxygen functionalities³⁰. A significant increase in the D/G ratio from 0.979 to 1.198 has clearly indicated the reduction of GO by carrot extract. It is evident that the increase in D/G ratio is an indication of the effective reduction of GO by carrot extract. Further, the appearance of a

sharp 2D band in the Ct-RGO indicated the complete reduction of GO to RGO. Another evidence to support the formation of RGO was the appearance of the characteristic peak at 26.5° , which is absent in GO (see Figure 5).

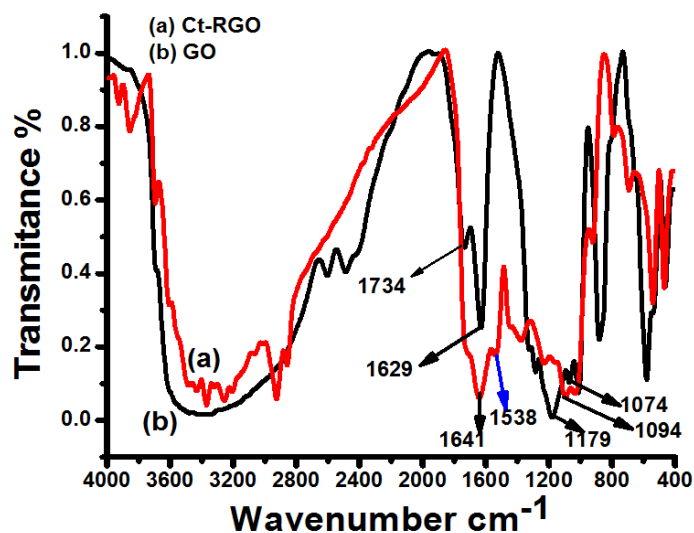


Figure 3: FT-IR spectrum of (a) GO and (b) Ct-RGO

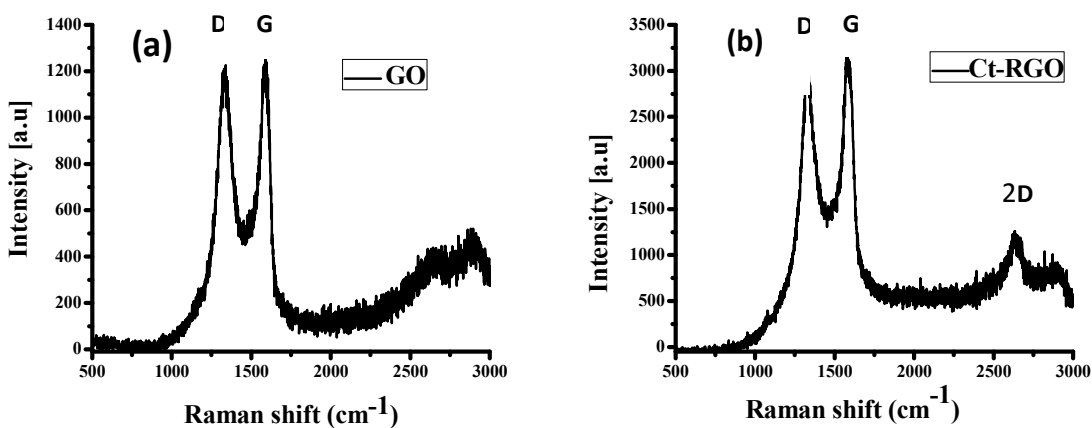


Figure 4: Raman spectrum of (a) GO and (b) Ct-RGO

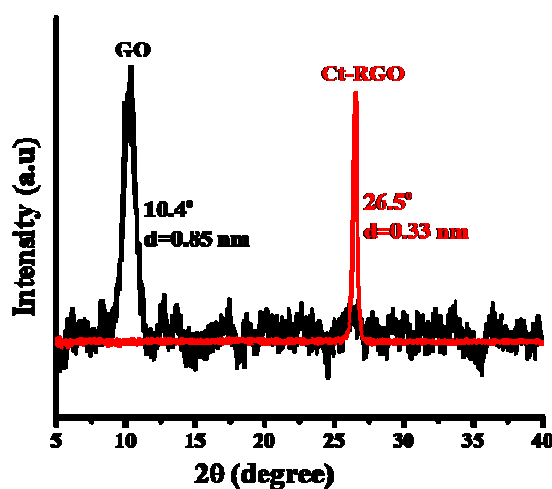


Figure 5. X-ray diffraction Pattern of GO and RGO

Figure 6(a) is the representative image of GO in which we noticed a clear layer by layer stacking of GO. On the other hand, Figure 6(b) represents the SEM image of Ct-RGO where we can clearly see the isolation of the graphene layers.

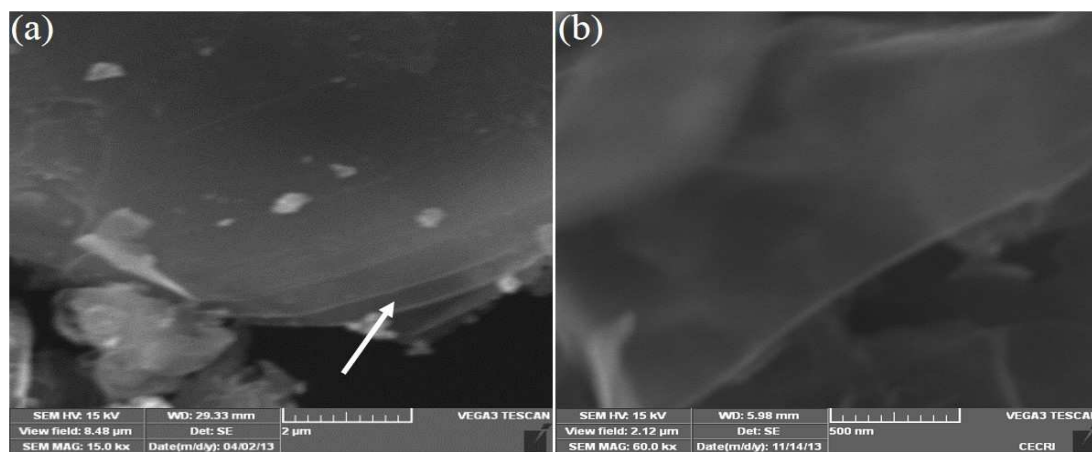


Figure 6: SEM images of (a) GO and (b) Ct-RGO

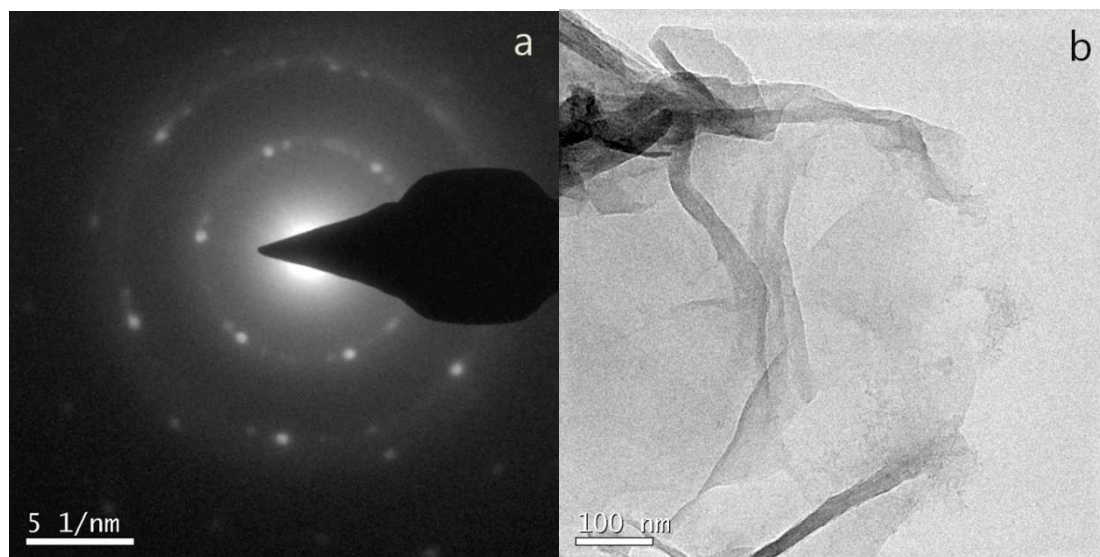


Figure 7: (a) SAED pattern of Ct-RGO and (b) TEM image of Ct-RGO

Figure 7(a) is the TEM of Ct-RGO indicating a typical silky, transparent and rippled morphology due to its high aspect ratio. Figure 7(b) is the SAED patterns of the Ct-RGO showing only one set of hexagonal diffraction pattern and a single crystalline lattice structure which is in agreement with the Wang et-al report²⁹. Following this a more detailed C/O ratio analysis was performed using X-ray photoelectron spectroscopy. In this analysis, we witnessed a C/O ratio of 5.38 in GO whereas the C/O ratio in RGO has tremendously increased to 18.8 (See Figure 8). A tremendous increase in this C/O ratio is due to the effective decrease in the oxygen content as a result of successful reduction of carboxyl and ketonic groups by the anti-oxidants present in the juice.

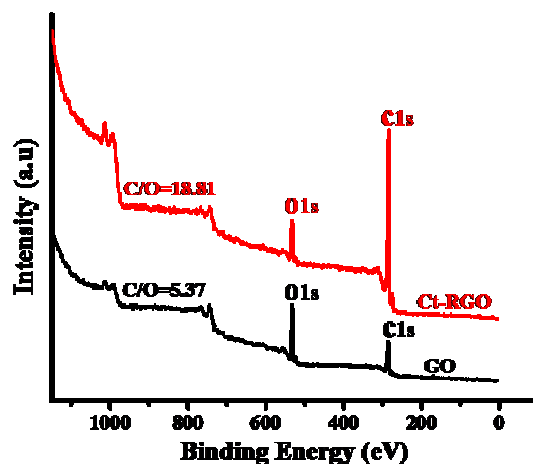


Figure 8. XPS of GO and ct-RGO

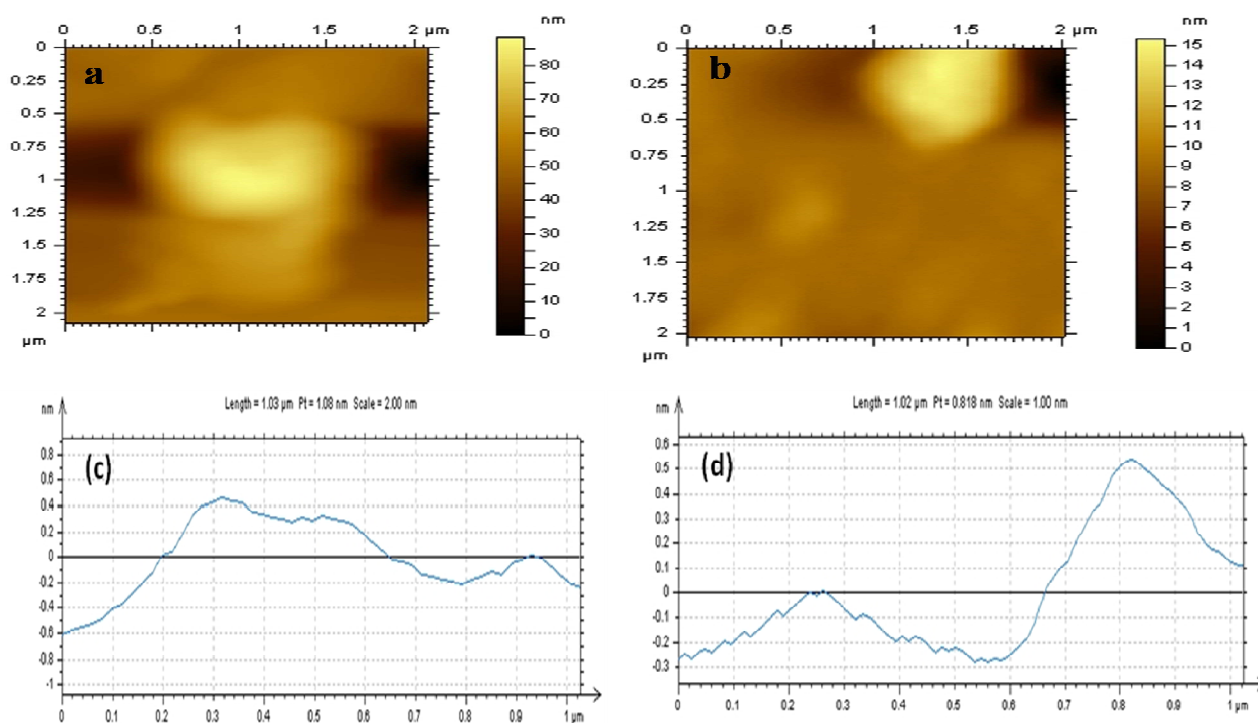


Figure 9: AFM images of (a) GO and (b) Ct-RGO and Surface profiles of (c) GO and (d) Ct-RGO

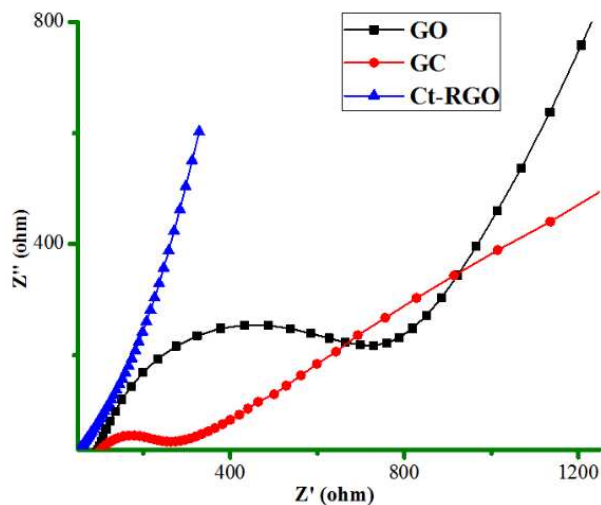


Figure 10: Electrochemical impedance spectra (EIS) of bare GC, GC/GO and GC/Ct-RGO in 1:1 ratio of 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in 0.1M KCl.

AFM measurements were then conducted to calculate the thickness of the GO and Ct-RGO. Based on our experiments, we have calculated the thickness of GO and Ct-RGO to be 1.1 nm and 0.8 nm respectively (Figure 9). Following this, the electrochemical impedance spectroscopy (EIS) was conducted at GC, GC/GO and GC/Ct-RGO in 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in

0.1 M KCl and the corresponding Nyquist plot was constructed (Figure 10). From the Nyquist plot it is evident that the semicircle of the GO is greater than GC thereby indicating the difficulty experienced by the interfacial charge transfer due to the semi-conducting property of GO. On the other hand, the Nyquist plot corresponding to Ct-RGO has no semicircle portion thereby facilitates the electron transfer between the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and electrode. From the comparison, it is evident that the conductivity of the Ct-RGO is greater than GO and is due to the restoration of the graphitic nature (i.e sp^2 bonds) in Ct-RGO.

Conclusion

In summary, for the first time we report a green, simple, facile and cost effective strategy for the direct reduction of GO by a vegetable extract. A stable dispersion of graphene can be readily prepared by the reduction of GO by carrot extract. Ct-RGO obtained in this work is expected to be a biocompatible product and will find several potential applications in various fields such as drug delivery, bio composite and in biosensors.

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