

# A green approach for the reduction of graphene oxide nanosheets using non-aromatic amino acids



Carbon

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#### ABSTRACT

A simple and green chemistry approach for the preparation of reduced graphene oxide nanosheets through the reduction of graphene oxide (GO) using non-aromatic and thiol-free amino acids as the reductant was successfully demonstrated. L-Aspartic acid and valine amino acids were used as models to prove that the reduction method is generic to other amino acids. The significance of the proposed method is that it eliminates the use of toxic and harmful chemicals to humans and the environment, which makes it compatible for the large-scale production of graphene. Reduced GO nanosheets showed good stability in aqueous dispersions due to the strong electrostatic repulsion on the graphene surface. Structural studies confirmed the deoxygenation of GO from the loss of hydroxyl, carbonyl and epoxy groups, and preparation of graphene sheets between 20 and 70 nm suitable for broad biomedical applications.

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# 1. Introduction

Graphene is a two-dimensional nanomaterial consisting of a single layer of bonded sp<sup>2</sup> carbon atoms arranged in a hexagonal network structure [1,2]. Owning to its unique physical and chemical properties, graphene is set to revolutionize the 21st century for its wide practical uses, such as, a platform for biosensing [3], conversion or charge storage [4,5], drug delivery [6–8], and composite materials [9,10]. As these applications require high-quality graphene nanosheets many synthetic routes have been developed to mass produce graphene from the chemical reduction of graphene oxide (GO; one or few layers of bonded sp<sup>2</sup> carbon atoms heavily decorated with hydroxyl, carbonyl and epoxy functional groups). In order to obtain graphene from GO, reduction is an essential step. However, many reductants used to reduce GO, such as hydrazine [11,12], hydrazine hydrate [13,14], sodium borohy-

dride (NaBH<sub>4</sub>) [15] and metal hydrides [16,17] are either strong or highly toxic and their by-products are not environmentally benign making bulk production of graphene difficult and not suitable for further applications. Therefore, there has been a surge to find alternative approaches for the conversion of GO into single graphene sheets that are produced under mild and green conditions. Recently, new and more friendly reducing agents for GO have been reported for the substitution of hydrazine, such as, ascorbic acid (vitamin C) [18–20], alkyl amines [21–24], reducing sugars [17,25], wild carrot root [26], metal nanoparticles [27,28] and powders [29,30], phytoextract [31], green tea [32], baker's yeast [33], supercritical alcohols [34], and amino acids [19,35].

The concept of reduction of GO via amino acids has been successfully demonstrated using two specific types of amino acids including electron rich aromatic groups (L-tryptophan) and thiol based amino acids (L-cysteine) [19,35]. The aim of

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this work is to prove that other amino acids with nonaromatic structures can also be used for scalable preparation of high quality reduced GO (rGO) using natural graphite as raw material. L-Aspartic acid (L-Asp) is used as the model amino acid as it is nontoxic, biocompatible and commercially available at an industrial level. To prove that the reduction method is generic and applicable to other non-aromatic amino acids, valine amino acid was also tested. The specific objectives are to show that by controlling the time of the reduction process it is possible to control the density of oxygen groups on the basal plane of the graphene nanosheets. To have rGO with controllable oxygen groups and nanosized graphene sheets is important for broad biomedical applications including drug delivery, bioimaging and biosensing. Conversion of GO into graphene nanosheets was explored in aqueous dispersions of pH 9 via L-Asp as the carboxyl group of the amino acid is ionized under alkaline conditions allowing the dispersions to be stable due to electrostatic repulsions. To prove the mechanism of the reduction process and the transformation of GO to rGO during the different reaction times all prepared products were characterized by various surface characterization techniques including, TEM, AFM, XRD, NMR, Raman, FTIR, UV-Vis, PL, contact angle, electrical conductivity, zeta potential and particle size measurements.

# 2. Experimental

#### 2.1. Materials

Natural graphite rocks (Uley, Eyre Peninsula, SA) were obtained and milled into a fine powder using a bench top ring mill (Rocklabs). L-Aspartic acid ( $\geq$ 98%, Fluka), valine (98%, Sigma-Aldrich), 28% ammonia solution (APS), potassium permanganate (KMnO<sub>4</sub>, Sigma-Aldrich), 98% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, Sigma-Aldrich), 85% phosphoric acid (H<sub>3</sub>PO<sub>4</sub>, Chem-Supply), 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, Chem-Supply), 35% hydrochloric acid (HCl, Merck), and ethanol (Chem-Supply) were used directly without further purification. All aqueous solutions were prepared with Milli-Q water (Purelab Option-Q) with a surface tension of 72.8 mN/m at 20 °C, a specific conductivity of 18.2 MΩ·cm at 25 °C and a pH of 5.6, unless otherwise stated.

#### 2.2. Preparation of GO

Powdered graphite (3.0 g) was used to prepare GO following the literature procedure [36]. Briefly, this method greatly improved the efficiency of the oxidation process as the reaction was performed using a 9:1 mixture of  $H_2SO_4/H_3PO_4$ (360:40 mL) and a larger amount of KMnO<sub>4</sub> (18.0 g). The mixture was heated to 50 °C and continuously stirred for 12 h. The reaction was then cooled to room temperature and poured onto ice with 30%  $H_2O_2$  (3 mL). To obtain GO, the mixture was centrifuged at 4000 rpm for 2 h and the supernatant was decanted away. The solid material was then washed multiple times in succession with distilled water, 30% HCl and ethanol. For each wash the mixture was centrifuged 4000 rpm for 2 h and again the supernatant was discarded. The final product (approx. 6.0 g) was dried overnight at 45 °C in a vacuum oven.

#### 2.3. Reduction of GO

To establish the optimal conditions required for the reduction of GO the reaction time and concentration of L-Asp with GO was initially investigated. Dispersions of GO (20 mL) at a concentration of 0.1 mg/mL were heated to 90 °C in tightly sealed glass vials with 2, 3, and 4 mM L-Asp for 3, 6, and 12 h. Dispersions were sonicated for 30 min prior to experiments and continuously stirred throughout the reaction. Homogeneous reduced GO (rGO) dispersions were obtained without aggregation. The pH of the dispersions were adjusted to approx. 9 with 28% ammonia solution (2 µL/mL of dispersion) to promote the colloidal stability of the GO sheets through electrostatic repulsion. The effect on the reduction level of GO was then monitored as a function as time and the extent of the deoxygenation of the GO sheets was characterized. Solid rGO was obtained by centrifugation at 14,500 rpm for 30 min, and then washed in successive with 5% ammonia solution, distilled water and ethanol. For each wash the solution was again centrifuged 14,500 rpm for 30 min and the supernatant was discarded. The final product was dried at 45 °C overnight in a vacuum oven.

#### 2.4. Characterization of GO and rGO

Particle size distribution (PSD) of dispersions was measured by CPS Disc Centrifuge (Model DC24000 UHR, CPS Instruments, USA), which was established from the sedimentation time of the particles injected into the center of an optically clear spinning disc. The disc is loaded with a sucrose solution that creates a density gradient as the disc spins at constant speed. CPS disc centrifuge measures particle size distribution using centrifugal sedimentation within an optically clear spinning disc that is filled with a fluid. Sedimentation is stabilised by a density gradient within the fluid as the disc spins at a constant speed. Under these conditions, the particles will separate according to differences in their size, density and shape, according to Stokes law [37]. Accuracy of the measured sizes is insured through the use of a known size calibration standard before each test. Therefore, similarly to DLS reference materials for calibration is required including density and optical properties, such as, refractive index and adsorption data. A particle calibration standard of known density and diameter is also used to determine the instrumental measurement constants [38].

Prior to measurements, dispersions of GO and rGO were sonicated for 30 min. The disc was loaded with a sucrose gradient (8% and 24% sucrose in water, CPS Instruments), which comprised of 10 layers, each with a total injection volume of 1.8 mL. The average density, refractive index and viscosity values of the sucrose gradient fluid were 1.02 g/mL, 1.35 and 0.95 cps, respectively. Dodecane (0.75 mL) was added as an evaporation barrier and a disc rotational frequency of 21,000 rpm was used. The sample (0.1 mL) was then injected into the disc for analysis. Measurements were performed in triplicate and before each run a 0.475  $\mu$ m polyvinyl chloride (PVC) latex calibration standard (0.1 mL) was injected. Particles were assumed to have a density of 2.267 g/mL, a refractive index of 2.42, zero absorption and a non-sphericity factor of 1.5 for cubic/irregular-shaped particles. The CPS instruments software was used to generate the PSD data with an accuracy of  $\pm 0.5\%$ .

To characterize the reduction process of GO, UV-Visible (UV-Vis) spectra were measured using a Shimadzu UV-1601 UV-Vis spectrometer (200-1100 cm<sup>-1</sup>). Fourier Transform Infrared (FTIR) spectrometer (Nicolet 6700 Thermo Fisher) and photoluminescence (PL) spectrometer (Fluoromax-4, Horiba, Jobin Yvon) were also used for comparison. Raman spectra were recorded from 800 to 2000 cm<sup>-1</sup> on a WITEC Alpha300 Raman spectroscopy with a 537 nm laser. Samples were deposited on glass slides in solid form without any solvent. All spectra shown are from raw and unprocessed data. The morphology and structure of GO and rGO sheets were obtained using a Phillips CM200 transmission electron microscopy (TEM) operating at an accelerating voltage of 160 kV. TEM samples were prepared by sonicating the dispersions for 60 min and then drying a droplet of the dispersion on a Lacey copper grid for analysis.

XRD analyses were performed on an X-ray diffractometer (Rigaku MiniFlex 600, Japan) at 40 kV and 15 mA in the range of  $2\theta = 5-75^{\circ}$  with a speed of 10 °C/min. Samples for atomic force microscopy (AFM) were prepared by drop-casting the suspensions onto clean silicon wafers, which were subsequently rinsed with ethanol and Milli-Q water and plasma-cleaned for 30 s. Imaging was accomplished under ambient conditions with a NTegra Solaris AFM (NT-MDT) in the semi-contact (tapping) mode of operation and using NSG10 AFM probes made of silicon nitride with a tip radius of 10 nm.

The electrical conductivity of the graphene was determined through measurement of their resistance by a twopoint probe method [39] where an average of six different sites was taken (See Supplementary information). Thin circular 'papers' (35 mm in diameter, 0.5 mm in thickness, see Fig. S1) of the rGO were prepared by vacuum filtration, and then dried in an oven at 70 °C for 12 h. To this end, the rGO papers (also for GO) were cut into rectangular strips about  $12 \times 5$  mm<sup>2</sup> large, taped on a clean glass microscope slide with double-sided tape and their contact angle was measured by the sessile drop method using an Attension Theta Optical Tensiometer (Finland) system. The substrate was placed on the sample stage and a drop of liquid was automatically dispensed (2 µL) on the GO (or rGO) paper from the microsyringe. The image of the water droplet was recorded right at the instant when the drop touched the surface of the paper with a FireWire camera (55 mm focus length). Curve fitting and data analysis was determined by using the OneAttension software installed on the computer. Three different positions on the film was measured and an average was taken. Measurements were performed with Milli-Q water at ambient conditions. The zeta potential of GO before and after reduction with L-Asp were also measured in triplicate with a Malvern Zetasizer (Nanoseries, Australia). Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were measured using a 600 MHz spectrometer (Oxford). Samples of solutions were prepared by dissolving 3 to 5 mg of the material in 0.7 mL of dimethyl sulfoxide- $d_6$  (DMSO- $d_6$ ) or deuterium oxide (D<sub>2</sub>O) in 5 mm NMR tubes.

## 3. Results and discussion

During the synthesis process, natural graphite flakes were oxidized by reacting with  $H_2SO_4$ ,  $H_3PO_4$  and  $KMnO_4$  to produce carbonyl, hydroxyl and epoxy groups to bond to the edges and the basal planes of the graphite structure. Therefore, produced GO formed a soluble aqueous solution due to its hydrophilicity as graphite is naturally hydrophobic in nature. Fig. 1a and b show the homogeneous, light brown dispersion of GO with nanosheets ranging between 20 and 70 nm determined by PSD measurements. Size distribution from TEM imaging show that majority of sheets range between 20 and 80 nm (maxima at 50 nm), which confirm the PSD of the GO dispersion. Slight variation in the TEM size distribution is due to agglomeration as the sheets stacked together as the dispersion was drop-casted onto the copper grid for analysis.



Fig. 1 – (a) A digital photograph and (b) PSD of a GO dispersion (0.1 mg/mL) in pH 9 measured with CPS and TEM. The inset shows the corresponding TEM images of the sheets (dotted). Scale bar is 20 nm. (A color version of this figure can be viewed online.)

# 3.1. Optimization of reduction process and rGO nanosheets

Fig. 2 shows the appearance of the GO dispersions in glass vials reduced with different molar concentrations of L-Asp (2-4 mM) and at the different reduction times (3, 6 and 12 h). In all cases, a black precipitation was obtained at the end of the reaction confirming successful reduction except for GO treated with 2 and 4 mM L-Asp at 3 h. A dark brown dispersion indicated that GO was not entirely reduced. This could be due to the fact that there wasn't sufficient amount (2 mM L-Asp) or there was too much (4 mM L-Asp) of the reducing agent within the solution and the reaction time was too short. A reduction time of 3 h with a molar concentration of 3 mM L-Asp was chosen as the optimum conditions for the reduction of GO as it was the shortest time compared to 6 h reduction of GO with 2 mM L-Asp. The dispersions were stored in ambient conditions for 3 months and no precipitation was observed, which means the graphene/L-Asp dispersion is very stable in ambient conditions.

The reduction of GO with valine (3 mM) was also tested as illustrated in Fig. 3. Similarly, homogeneous rGO dispersion was obtained without aggregation with a color change in solution from light brown to dark brown. However, further optimization of valine is required to fully reduce GO to a black solution.

For clarity, only GO dispersions reduced with 3 mM L-Asp at all reactions times will be characterized and discussed in the following sections. Similar to GO, reduced graphene nanosheets between 20 and 70 nm were observed (Fig. 2d).

Fig. 4 represents the TEM images and selected area electron diffraction (SAED) patterns (inset) of GO before reduction and rGO nanosheets at a reduction time of 3 h. The amorphous nature of the GO nanosheets before reduction can be clearly seen in Fig. 4a. Different from previous reports where GO was found to have an internal ordered crystalline structure, the diffraction rings of the SAED pattern confirmed that the GO nanosheets were amorphous. Contrarily, wrinkled and paper-like sheets were observed for rGO and the SAED pattern which indicates a crystalline structure. Strong diffraction spots are observed on the polycrystalline ring pattern illustrating the crystalline state of the rGO. The first and second ring patterns comes from the (110) and (120) planes, respectively [40]. The changes in the structural features of GO to rGO is due to the removal of the oxygen functional groups. During the reduction process the rGO is partially repaired from sp<sup>3</sup> hybridized carbon atoms dominating in GO to newly formed crystalline sp<sup>2</sup> hybridized carbons. The number of the sp<sup>2</sup> domains has increased but the sizes of the sp<sup>2</sup> domains have decreased. [13,40,41]. The AFM images and height profiles of Fig. 4c and d for GO and rGO dispersions confirmed that the thicknesses of the layers are uniform and  $\sim 1$  nm, which are attributed to single-layer sheets [10,13,18,20,42,43].

XRD patterns of raw graphite, GO and rGO are presented in Fig. 5. The XRD pattern of graphite shows an intensive peak at  $2\theta = 26.6^{\circ}$  (*d*-spacing = 0.335 nm) corresponding to (002) reflection of the graphitic structure (ICDD-PDF # 411487). Upon oxidation of graphite to GO, the (0 0 2) reflection peak shifts to a lower angle at  $2\theta = 11.1^{\circ}$  (*d*-spacing = 0.794 nm). The increase in *d*-spacing is due to the intercalation of the water molecules and the formation of oxygen moieties in the interlayer spacing of graphite. The complete transformation from GO to rGO is clearly illustrated as a straight line with no apparent diffraction peaks in the XRD pattern is obtained. This indicates that the GO is completely exfoliated to a single layer of graphene [44]. Transformation of GO to rGO is also confirmed from the contact angle measurements where the difference in the hydrophobicity of the samples is



Fig. 2 – Digital photographs of aqueous dispersions of GO after reduction with 2 mM, 3 mM, and 4 mM L-Asp at (a) 3, (b) 6, and (c) 12 h, respectively. All dispersions before and after reaction were at pH 9, except for GO reduced for 12 h, which decreased to pH 8. (d) PSD of rGO nanosheets with 3 mM L-Asp reduced for 3, 6, and 12 h. (A color version of this figure can be viewed online.)



Fig. 3 – Digital photograph of aqueous dispersion of GO before and after reduction with 3 mM valine at 3 h showing successful reduction conversion to rGO. (A color version of this figure can be viewed online.)

observed (Fig. 5 insert). GO is hydrophilic with a contact angle of 45° compared to rGO which is strongly hydrophobic (90°), revealing that most oxygen containing functional groups were removed after the reduction process. This result further supports the reduction of GO by L-Asp.

The electrical conductivity of rGO can reflect both the extent of the reduction and the restoration of the electronic conjugation state [45]. The average conductivity of rGO (reduced for 3 h with L-Asp) is ~700 S/m, which is comparable to the conductivity of GO reduced with hydrazine and other reductants [46]. This result showed that the electronic conjugation was re-established after the reduction of GO with L-Asp.

Typically, graphene produced by the chemical reduction method display two characteristic peaks on a Raman spectra: the D band at 1350  $\text{cm}^{-1}$  and G band at 1575  $\text{cm}^{-1}$  [47]. The D band indicates the structural defects and disorder, whereas the G band represents the graphitic component in the structure. Fig. 6 illustrates the Raman spectra of GO before (inset) and after reduction with L-Asp. The D and G bands for GO and rGO both correspond at 1360 cm<sup>-1</sup> and 1620 cm<sup>-1</sup>, respectively. Prominently, the intensity ratio of the D and G band  $(I_D/I_D)$  $I_{G}$ ) of GO after reduction with L-Asp increased gradually from 0.95 (GO) to 1.02, 1.05, and 1.11 with the increase of reduction time (3, 6, and 12 h). The step by step increase in the  $I_D/I_G$  is similar to the observations reported for the reduction of GO with L-cysteine [35] and other systems [48,49]. This change suggests an increase in sp<sup>2</sup> domains during the reduction of GO due to the presence of unrepaired defects that remain after the removal of oxygen moieties [13,50].

In order to characterize changes in the functional groups by the reduction of GO with L-Asp FTIR spectra were collected and shown in Fig. 7. For GO, several characteristic peaks are observed: the O–H stretching vibrations at 3000–3500 cm<sup>-1</sup> (broad), and 1410 cm<sup>-1</sup> (narrow), the C=O (carbonyl and carboxyl) stretching vibrations at 1745 cm<sup>-1</sup>, the C=C skeletal stretching vibrations at 1630 cm<sup>-1</sup>, the C–O (epoxy and alkoxy) stretching peaks at 1225 cm<sup>-1</sup> and 1050 cm<sup>-1</sup>, respectively. Consequently, these peaks representing oxygen functional groups are almost entirely removed in reduced



Fig. 4 – TEM images of GO before (a) and after reduction with 3 mM L-Asp (b) showing the amorphous and crystalline structures, respectively. The inset shows the corresponding selected area electron diffraction (SAED) pattern. AFM images of unreduced (c) and L-Asp-reduced (d) GO sheets onto silicon wafers from their corresponding dispersions. Lines profiles are superimposed onto each image showing that single-layer sheets are obtained in both cases. (A color version of this figure can be viewed online.)



Fig. 5 – X-ray diffraction patterns of raw graphite, GO and rGO with L-Asp. The inset shows the contact angle measurements of GO and rGO samples. (A color version of this figure can be viewed online.)



Fig. 6 – Raman spectra of rGO with 3 mM L-Asp at different reduction times. The inset is the Raman spectrum of GO.



Fig. 7 – FTIR spectra of GO and rGO with l-Asp at different reduction times from 500 to 4000 cm<sup>-1</sup>.

GO with L-Asp after 3, 6 and 12 h as the reduction of GO is mainly aimed at eliminating epoxy and hydroxyl groups on the plane of the graphitic surface.

#### 3.2. Controlling the deoxygenation of the GO nanosheets

The effect on the reduction level of GO was monitored as a function of time to study the extent of deoxygenation of the GO nanosheets. Fig. 8a represents the zeta potential of the aqueous dispersions of GO before and after reduction with L-Asp at the different time intervals. The reaction was performed under basic conditions (pH 9), thus the measured zeta potential of GO and rGO was -52 mV and -40 mV, respectively. The zeta potential of GO is high due to the presence of negatively charged OH and COOH groups compared to reduced GO, as its oxygen functional groups are removed during the reaction. Evidence of the chemical reduction taking place is confirmed by the gradual color change from light brown to black as shown in the inset of Fig. 8a. Homogeneous rGO dispersions with L-Asp with no particle agglomeration was obtained as the PSD were consistent with that of GO (Fig. 8b).

The reduction progress was monitored by UV–Vis spectroscopy by taking a series of spectra that are presented in Fig. 9. As shown in Fig. 9, with the increase of reduction time the intensity of the UV–Vis absorption peak at 250 nm for GO increases. The adsorption peaks of the reduced GO dispersions red shifted into wavelengths around 260 nm, corresponding to deoxygenation of the GO nanosheets during the reduction progress.

Further comparative characterization of GO before and after reduction with L-Asp during 10-180 min was carried out with FTIR spectroscopy and PL spectroscopy. The oxygen functional groups in GO by L-Asp reduction decay rapidly even with a reduction time of 10 min (see Supplementary information, Fig. S2). However, the C-C stretching peak at 1630 cm<sup>-1</sup> still exists. This suggests that the sp<sup>2</sup> carbon atoms on the graphene sheet after reduction by L-Asp are retained well, as before. GO is known to have photoluminescence properties owing to the presence of oxygen-containing functional groups attached on both sides of the graphene sheet [51]. Fig. 10 represents the PL spectra of GO and rGO nanosheets recorded as a function of reduction time (10-180 min) using an excitation wavelength of 325 nm. A sharp intensity peak at 640 nm is observed for GO. However, during the reduction reaction, the hydroxyl, carbonyl and epoxy groups in GO are effectively reduced by L-Asp as the intensity peak decreases, and disappears after 3 h. It has been reported that this is due to the blue shift and quenching in PL emission arising from the structural ordering and newly formed crystalline sp<sup>2</sup> clusters in rGO, which clearly confirm the transition of GO to rGO [51,52].

In previous studies two specific types of amino acids including electron rich aromatic groups (L-tryptophan) and thiol based amino acids (L-cysteine) were used for the reduction of GO [19,35]. It was shown that oxidation of indole and the thiol (SH) group are involved in this process. In this work we demonstrated for the first time that other amino acids can also be used, but mechanism of the oxido-reduction process still remains unclear at this present stage. We postulated that



Fig. 8 – (a) Zeta potential of aqueous dispersions of GO before and after reduction with 3 mM L-Asp as a function of time (pH 9). The insets are digital photographs of rGO at different time intervals from 10 to 180 min (3 h) showing the color change in solution during the reaction from brown to a black precipitation. (b) PSD of rGO with 3 mM L-Asp for the different time intervals at pH 9. For clarity, only selected reduction times are shown. (A color version of this figure can be viewed online.)



Fig. 9 – UV–Vis spectra of GO and rGO as a function of time. The highlighted red shading block indicates the shift in wavelength of GO to rGO. (A color version of this figure can be viewed online.)

this process occurred by oxidation of L-Asp into polymeric imides with two possible product formation of cyclic polysuccinimide (PSI) and linear polyimide (PI). Fig. 11 illustrates the mechanism for the chemical reduction of GO with L-Asp. It is speculated that a two-step  $S_N^2$  nucleophilic substitution reaction followed by a thermal elimination is occurring. L-Asp is a non-aromatic amino acid containing only an amine and carboxylic acid group. Therefore, under basic conditions the amine functions as a nucleophile attacking the electrophilic carbon of the C-O bond causing it to break, resulting in ring opening reaction of the epoxy group [53]. The leaving group is the oxygen atom of the epoxide in the form of the alkoxide, which is converted to the alcohol to form water molecules. Finally, upon heating this product thermally decomposed into PSI leading to the formation of rGO sheets. L-Asp does not polymerized into linear PI during the reduction process as additional reagents and reactions are required [54-56].



Fig. 10 – PL spectra of GO and rGO as a function of time. (A color version of this figure can be viewed online.)

During washing and preparation of the rGO product after the reduction process the presence of a yellow powder is observed, which we assumed is the product of the proposed polymerization process. To confirm that the observed product is the polymer proposed in our mechanism we synthesized PSI and performed comparative FTIR and NMR characterizations (see Supplementary information for synthesis of PSI). FTIR graphs of the observed yellow product, compared with FTIR of L-Asp and synthetic PSI, which is also light-yellow is presented in Fig. 12a. FTIR spectrum of L-Asp shows multiple absorption bands of amide and carboxylate groups at 1200-1700 cm<sup>-1</sup> [57,58]. Spectrum of the synthetic PSI at around  $1700 \text{ cm}^{-1}$  shows a large absorption band of high intensity which corresponds to the C=O vibrations of cyclic imides [55,56,59]. A strong and broad absorption band at 3500 cm<sup>-1</sup> and  $2950 \text{ cm}^{-1}$  indicated hydroxyl and alkyl groups (NH stretching). The presented FTIR of the yellow powder sample showing imide functional groups (1800  $cm^{-1}$  and 1500  $cm^{-1}$ )



Fig. 11 – Possible mechanism for the reduction of GO with L-Asp.



Fig. 12 – Comparative (a) FTIR and (b) <sup>1</sup>H NMR spectra of L-Asp (DMSO- $d_6$ ), synthetic PSI (D<sub>2</sub>O), and yellow powder (D<sub>2</sub>O) formed during GO reduction process. (A color version of this figure can be viewed online.)

supports the above hypothesis mechanism for the reduction of GO as the imide ring, NH vibrations and C=O absorption band are also presence.

The proposed mechanism was further confirmed with NMR study as shown in Fig. 12b. The <sup>1</sup>H NMR spectrum of L-Asp shows signals at 2.1–3.2 ppm and 3.8–4.0 ppm, which were assigned to the methylene (a) and methane (b) protons, respectively. The NMR spectrum of the synthetic PSI showed signals at 2.5–3.5 ppm also assigned to the methylene proton and at 5.0–5.5 ppm for the methane proton [54,59–61]. The presented NMR spectra of the yellow powder supports the proposed mechanism for the reduction of GO with L-Asp (Fig. 11) as similar methylene (2.6–3.0 ppm) and methane (5.0–5.5 ppm) peak signals were also observed, therefore confirming that the polymer produced in the reduction process is PSI.

#### 4. Conclusions

In summary, rGO nanosheets were successfully produced by chemical reduction of GO using non-aromatic and thiol-free amino acids (L-Asp, valine) was characterized using various physical and chemical techniques. The use of these amino acids as a reductant provides a green method for the reduction of GO replacing the use of toxic chemicals that is appropriate for bulk production of graphene from natural graphite. It was shown that by controlling the reduction time it is possible to achieve rGO with different densities of oxygen groups. The mechanism of GO reduction process is proposed to involve the polymerization of amino acid (L-Asp into PSI), which presence is confirmed by synthesis of PSI and comparative FTIR and NMR characterizations. This process and prepared graphene nanosheets from natural raw graphite showing nanoscale dimensions and controllable functionalities are excellent biomaterial for many biomedical applications, including nanocarriers for targeting and localized drug delivery, bioimaging and biosensing.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbon. 2014.04.067.

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