Advanced Science Focus
Vol. 2, pp. 34–36, 2014
(www.aspbs.com/asfo)

A Simple Route for the Synthesis of Graphene for Electrochemical Biosensing

Rajendra P. Joshi[1,2], Krishna P. Singh[1], Ashavani Kumar[2], and Subbiah Alwarappan[3,*]

[1] *Nanobiosensor Research Laboratory, Biophysics Unit, CBSH, G. B. Pant University of Agriculture and Technology, Pantnagar 263145, Uttarakhand, India*
[2] *Department of Physics, National Institute of Technology (NIT), Kurukshetra, Haryana 136118, India*
[3] *CSIR-Central Electrochemical Research Institute, Karaikudi, Tamilnadu 630006, India*

In this work, we report a very simple three-step route for the synthesis of graphene flakes. The three steps include thermal reduction, toluene-stabilization and minimal energy induction by sodium salt. Graphene flakes thus obtained were characterized by Raman spectroscopy and transmission electron microscopy and further used for electrochemical sensing.

KEYWORDS: Graphene Flakes, Biosensing, Exfoliation.

1. INTRODUCTION

Graphene has attracted great scientific interest for its excellent mechanical, electrical, thermal and optical properties.[1–7] Graphene can be produced by micromechanical exfoliation of highly ordered pyrolytic graphite,[1] epitaxial growth,[9–11] chemical vapor deposition[10,12,13] in addition to the reduction of graphene oxide (GO). First three methods provide perfectly structured graphene with excellent characteristics. While in comparison, graphene produced by the reduction of GO is inexpensive through a cost-effective chemical methods of high throughput.[14] Although there are several protocols reported to synthesize graphene[10,15–21] with extraordinary physical[2,3,22–24] and chemical[9,25–29] properties but nowadays emphasis is being given to produce few-layered[30] flake-type graphene with minimized oxidation.[30] Herein, we report a 3-step route for obtaining graphene flakes by thermal reduction, toluene-stabilization and minimal energy induction method using sodium salt. Graphene flakes thus obtained were characterized by transmission electron microscopy and Raman spectroscopy. Further electrochemical measurements were performed to assess the suitability of these flakes for any biosensing applications.

2. EXPERIMENTAL PROCEDURE

Graphite flakes and toluene were purchased from Sigma–Aldrich (USA). The crucible and other glassware used were purchased from Borosil, India. Sodium chloride salt was purchased from Fisher Scientific (USA). The deionized water was prepared in house by Millipore water purification system (Millipore Direct Q 3UV, France). Phosphate buffer saline was procured from Hi-Media, India. All the chemicals procured were used as it is without further processing.

Hummer's method[19] wasted to oxidase the graphite flakes to graphite oxide, which further vigorous ultrasonication produces grapheme oxide. Briefly, graphite was mixed in appropriate quantity of sodium nitrate and sulfuric acid at temperature below 10 °C. Potassium permanganate was added slowly to the mixture. The product was washed with water and hydrogen peroxide which was then ultra-solicited for 24 hrs to get grapheme oxide. Dark brown solution of grapheme oxide was then was then using centrifugation. A 4 ml of graphene oxide (GO) (1 mg/ml) in deionized water was taken in a crucible and heated in vacuum at 250 °C for a duration of 4 hours and treated immediately with 2 ml toluene followed by cooling at room temperature for 6 hours that resulted in a broken GO film in the crucible. This GO film was further treated with 2 mg of NaCl with a rigorous gentle stirring, resulting the formation of a high viscous paste which was kept at room temperature for 4 hours to stabilize, yielding a sticky film on the crucible. The film was separated using tweezers and washed with distilled water 5 times to remove the traces of impurities. It was further treated with 4 ml phosphate buffer saline (PBS) followed by centrifugation at 6000 rpm for 1 hr. Next, the supernatant was discarded thereby leaving tainted particles/flakes as a residue which was further mixed with excess de-ionized water and ultrasonicated. The resulting material characterized further.

---

[*] Author to whom correspondence should be addressed.
Email: salwarap@gmail.com

Received: 24 January 2014
Accepted: 4 March 2014

2330-0760/2014/2/034/003 doi:10.1166/asfo.2014.1073

For FTIR stuffy, A 200 mg of spectroscopic grade KBr (Fisher Scientific) was completely dried prior to formation of a palette in die (PCI, India) and background was collected for the salt after mounting it in cell holder in FTIR spectrometer (Nicolet 6700) with sensitivity < 0.001 cm^{-1}. An amount ~ 5 μl of GO solution and graphene flakes were subjected in desiccators equipped with silica bag one by one to record the spectra followed by subtraction of water peaks by water correction option of OMNIC software.

Raman spectroscopy was carried out under 514-nm excitation at low power levels to avoid the laser heating. To separate the effect of the substrate from the spatial variations in the graphene layer properties we accumulated 10–20 spectra in different location for each of the examined samples. The 2D-band sensitivity to the number of layers is explained by the double resonant model adopted for graphene.

In order to check the electrochemical properties of the graphene flakes we have conducted the cyclic voltammteric (CV) studies. In this work, we have performed the CV of graphene flake electrodes using 1 mM $[Fe(CN)_6]^{3-/4-}$ in 1 M KCl (Ag/AgCl as the Reference electrode and Pt wire is the Aux Electrode). In this work we witnessed a reversible voltammogram with a ΔE_p of 57 mV indicating the reversible nature of our electrodes. This characterization confirmed the usability of the graphene flakes synthesized in our work for bio-sensing applications.

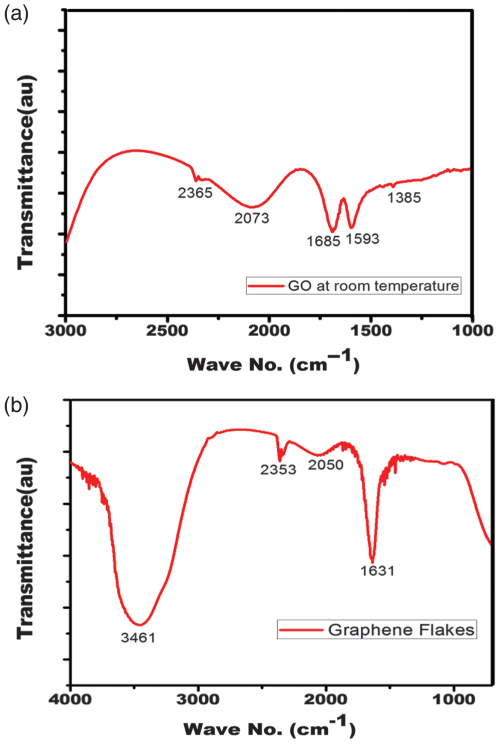

Fig. 1. FTIR spectra of graphene oxide (a) and graphene (b) at 25 °C.

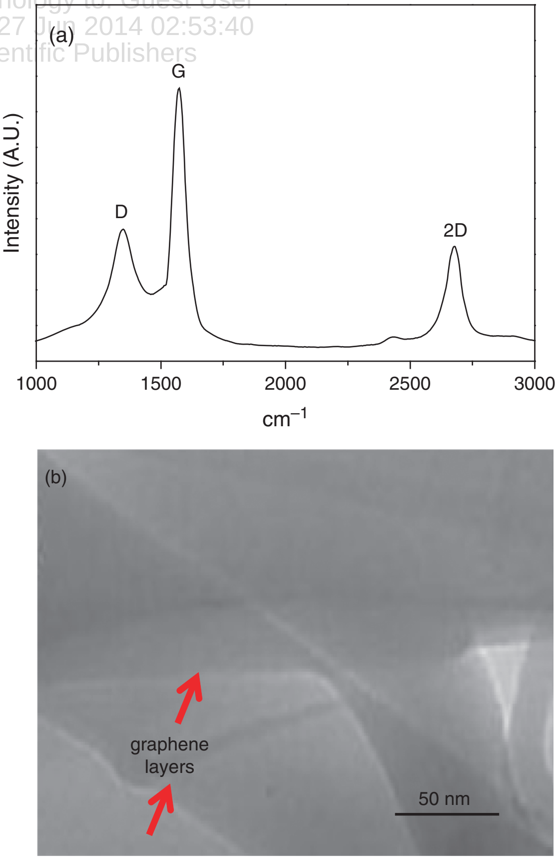

Fig. 2. Raman spectra of graphene flakes (a) and TEM image of graphene (b).

3. RESULTS AND DISCUSSION

FTIR spectra for graphene oxide (Fig. 1(a)) exhibit peaks at 1385 cm^{-1} (C—O, carboxy), 1593 cm^{-1} (C=C, aromatic), 1685 cm^{-1} (C=O (carbonyl/carboxy). FTIR spectra of grapheme (Fig. 1(b)) flakes presents that O—H stretching vibrations observed at 3400 cm^{-1} due to deoxygenation. However, stretching vibrations from C=O at 1631 cm^{-1} were observed and minor C—O stretching vibrations at 1060 cm^{-1} are visible, which were caused by remaining negligible carboxyl groups even after thermal reduction.

The number of layers in graphene flakes sample varies from 1–4 and depends on the nature of toluene and its stabilization time. Raman spectra show a strong enough 2D band at 2700 cm^{-1} ensures the nature of graphene in the form of flakes. The Observed *G* band in Raman analysis is related to electronic collisions in sp^2 carbon material and a very strong *G* band resolution shows quality graphene flakes. Typical Raman spectrum is shown in Figures 2(a) while (b) shows the typical transmission electron microscopy image of the graphene flakes.

In order to test the applicability of the graphene synthesized using this method, cyclic voltammetry of graphene electrodes has been performed in 1 mM $[Fe(CN)_6]^{3-/4-}$ in 1 M KCl. As shown in Figure 3. The electrodes exhibited a reversible voltammogram corresponding to the reduction of $[Fe(CN)_6]^{3-}$ at 280 mV indicating a totally reversible

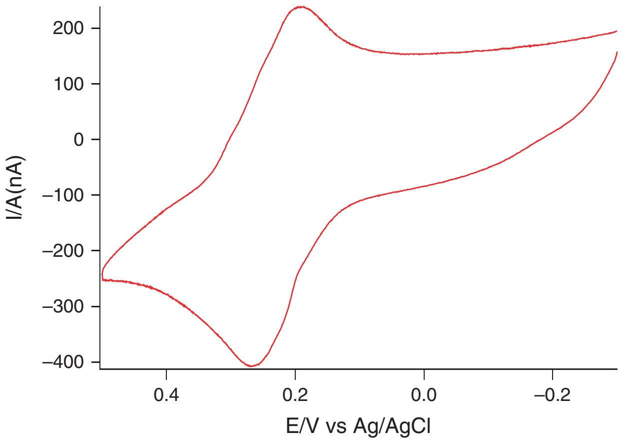

Fig. 3. CV of the graphene flake electrodes in 1mM $[Fe(CN)_6]^{3-}$ in 1 M KCl.

system. The voltammograms indicated the suitability of the graphene material obtained in this work for application in the electrochemical biosensors.

4. CONCLUSION

The present investigational work demonstrates a 3-step way of synthesis of graphene flakes by using sodium chloride an intercalation agent. Preparation includes thermal reduction, toluene-stabilization and minimal energy induction by sodium salt. Synthetically procured graphene flakes were of good quality. The ionic thermal energy, induced by interpolation of sodium chloride into thermally reduced graphene oxide not only provides outstanding electrochemical properties of synthesized graphene flakes but also economically acceptable and cost effective. The proposed protocol of synthesis may pave an alternate way to graphite redox processes.

Acknowledgment: This work was supported by Department of Science & Technology, Govt. of India.

References and Notes

1. K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva, and A. A. Firsov, *Science* 306, 666 (**2004**).
2. A. K. Geim, *Science* 324, 1530 (**2009**).
3. A. K. Geim and K. S. Novoselov, *Nat. Mater.* 6, 183 (**2007**).
4. S. Mouras, Y. Lin, and R. Saito, *Revue de Chimie Minerale* 24, 572 (**1987**).
5. C. G. Lee, X. D. Wei, J. W. Kysar, and J. Hone, *Science* 321, 385 (**2008**).
6. A. A. Balandin, S. Ghosh, W. Bao, I. Calizo, D. Teweldebrhan, F. Miao, and C. N. Lau, *Nano Lett.* 8, 902 (**2008**).
7. M. Orlita, C. Faugeras, P. Plochocka, P. Neugebauer, G. Martinez, D. K. Maude, A. L. Barra, M. Sprinkle, C. Berger, W. A. de Heer, and M. Potemski, *Phys. Rev. Lett.* 101, 267601 (**2008**).
8. D. R. Dreyer, S. Park, C. W. Bielawski, and R. S. Ruoff, *Chem. Soc. Rev.* 39, 228 (**2010**).
9. C. Berger, Z. Song, X. Li, X. Wu, N. Brown, C. Naud, D. Mayou, T. Li, and J. Hass, *Science* 312, 1191 (**2006**).
10. J. Wintterlin and M. L. Bocquet, *Surf Sci.* 603, 1841 (**2009**).
11. T. A. Land, T. Michely, R. J. Behm, J. C. Hemminger, and G. Comsa, *Surf. Sci.* 264, 261 (**1992**).
12. M. Eizenberg and J. M. Blakely, *Surf. Sci.* 82, 228 (**1979**).
13. K. S. Kim, Y. Zhao, H. Jang, S. Y. Lee , J. M. Kim, K. S. Kim, J. H. Ahn, and P. Kim, *Nature* 457, 706 (**2009**).
14. S. Pei and H.-M. Cheng, *Carbon* 50, 3210 (**2012**).
15. J. Sakamoto, J. van Heijst, O. Lukin, and A. D. Schlu¨ter, *Angew. Chem. Int. Ed.* 48, 1030 (**2009**).
16. Y. N. G. Regis, K. Spyrou, and P. Rudolf, *J. Phys. D* 43, 374015 (**2010**).
17. O. C. Compton and S. T. Nguyen, *Small* 6, 711 (**2010**).
18. S. Park and R. S. Ruoff, *Nat. Nanotechnol.* 4, 217 (**2009**).
19. Y. Zhu, S. Murali, W. Cai, X. Li, J. W. Suk, J. R. Potts, and R. S. Ruoff, *Adv. Mater.* 22, 3906 (**2010**).
20. X. Huang, Z. Yin, S. Wu, X. Qi, Q. He, Q. Zhang, Q. Yan, F. Boey, and H. Zhang, *Small* 7, 1876 (**2011**).
21. S. Guo and S. Dong, *Chem. Soc. Rev.* 40, 2644 (**2011**).
22. A. H. C. Neto, F. Guinea, N. M. R. Peres, K. S. Novoselov, and A. K. Geim, *Rev. Mod. Phys.* 81, 109 (**2009**).
23. A.K. Geim and A. H. MacDonald, *Phys. Today* 60, 35 (**2007**).
24. M. I. Katsnelson and K. S. Novoselov, *Solid State Commun.* 143, 3 (**2007**).
25. C. N. R. Rao, A. K. Sood, K. S. Subrahmanyam, and A. Govindaraj, *Angew. Chem. Int. Ed.* 48, 7752 (**2009**).
26. K. P. Loh, Q. Bao, P. K. Ang, and J. Yang, *J. Mater. Chem.* 20, 2277 (**2010**).
27. D. W. Boukhvalov and M. I. Katsnelson, *J. Phys.: Condens. Matter* 21, 344205 (**2009**).
28. M. J. Allen, V. C. Tung, and R. B. Kaner, *Chem. Rev.* 110, 132 (**2009**).
29. X. Huang, X. Qi, F. Boey, and H. Zhang, *Chem. Soc. Rev.* 41, 666 (**2012**).
30. J. Kwon, S. H. Lee, K.-H. Park, D.-H. Seo, J. Lee, B.-S. Kong, K. Kang, and S. Jeon, *Small* 7, 864 (**2011**).