Research Paper

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Lyophilization of water suspension of graphene oxide and graphene oxide-C60 and of their reduced products.

Science

KEYWORDS : graphene, fullerene C60, graphene oxide, water suspension, reduction of graphene oxide, Brassica jengea, ascorbic acid, lyophilization

Eva Zemanová	State Office for Nuclear Safety, Senovážné náměstí 9, Prague 1, Czech Republic
Karel Klouda	VŠB Technical University of Ostrava, Faculty of Safety Engineering, Lumírova 13/630, Ostrava, Czech Republic
Eliška Brabencová	The State Institute of Nuclear, Chemical and Biological Protection, Kamenná 71, Czech Republic
Stanislav Brádka	The State Institute of Nuclear, Chemical and Biological Protection, Kamenná 71, Czech Republic
Karel Lach	The Institute of Public Health in Ostrava, Partyzánské nám. 7, Czech Republic

ABSTRACT

Oxidization of graphene and joint oxidization of graphene and fullerene C60 in various weight ratios was used to produce water suspensions that allow preparation of foils with various morphologies and can be further treated, e.g. by fluorination, bromination, embedding of catalytic-active metals, preparation of composite materials with other polymers or nanofibers and also as lamellar fibers prepared by freeze drying without any additional components.

In this work we performed lyophilization of water suspensions of graphene oxide – fullerene C60 in order to obtain products with new morphology (fiber formation) and lyophilization after conversion (reduction) of oxidized suspensions. Methods employed to characterize the resulting products included microscopy, SEM imaging, FT IR, TGA and DSC analysis.

Introduction

Our previously published works [1,2] describe methods of oxidization of fullerene C_{60}, graphite and a mixture of graphite and fullerene C_{60} in various weight ratios and identification of the prepared products with a focus on their functional groups, structure and thermal stability with FT IR, DSC, TGA, XRD and mass spectroscopy.

In this work we have focused on lyophilization of prepared water suspensions of oxidized components in order to change their morphology, i.e. fiber formation. The second part of the experiments consisted in a conversion (reduction) of the oxidized suspension and also subsequent lyophilization of the reduced products.

Conversion (reduction) of graphene oxide (GO) to graphene can be performed by means of thermal decomposition, electrochemically, by traditional chemical reduction methods or biological ("green") reduction using phytoextracts or bacteria [3,4].

Examples of the applied chemical reduction procedures are the following:

- metal – acid, e.g. 2n/H_2SO_4 [5], 2n/HCl [6]
- metal – alkali, e.g. Al/NaOH [7]
- hydrides, e.g. $NaBH_4$, $LiAlH_4$ [8]
- hydrazines [9]
- hydro-halogen acids, e.g. HI [10]
- alcohols, e.g. HeOH, El-OH, glycerol [11]
- organic acids, e.g. ascorbic acid [8], caffeic acid [12]
- polyphenols, strong alkali solutions etc. [8, 12]

It has been proved that there are differences between the methods in terms of their reactivity to the oxidized groups in GO. For example, alcohols may selectively reduce epoxy groups, while –OH and –COOH groups remain unchanged [11]. Hydrazines reduce –COOH and other oxygenic groups, particularly epoxides [11]. $NaBH_4$ reacts with –C=O, and demonstrates less affinity to epoxy and carboxyl groups. There is a general agreement that hydrazines and ascorbic acid are the ideal reducing agents to reduce GO into graphene [13].

Numerous works have been published in the past 10 years which demonstrated that many biological systems, including plants, algae, blue-green algae and bacteria, have the ability to transform inorganic metal ions into metal nanoparticles [14, 15]. This is due to the reduction capacity of proteins and, particularly, metabolites (flavonoids, terpenes, polyphenols, hydroxyphenols, tannins, alkaloids, etc.) present in those organisms [16]. The most frequently used plants are herbs [14, 15, 16, 17, 18] known to the lay and traditional medicine and to the pharmaceutical industry. They have anti-diabetic, ant-inflammatory, anti-cancerous, anti-oxidizing and other therapeutic properties. In most cases the metal nanoparticles are prepared with their extracts [15, 16, 17, 19, 20] , e.g. from leaves, rhizomes, seeds, roots, tubers, bulbs, blossoms, fruit, fruit skin etc. Nanoparticles prepared by reduction with active components of plant extracts can be used in specific areas of nanomedicine and in pharmacology (e.g. antibiotics). An extract made of *Brassica jengea* seeds was used to reduce silver salts to prepare silver nanoparticles. The main components of the extract were glucose, fructose, ascorbic and citric acids and polyphenols [21, 22].

A phytoextract has been also used for reduction of GO into graphene, in the so-called green reduction. Many reductions of GO with plant extracts have been described [8, 23], similarly as in the case of metal salt reduction to produce nanoparticles. Also in this case the sources of reducing extracts were diverse , e.g. tree leaves [24, 25], rose blossoms [26], plant roots – e.g. carrots [27] etc. In some cases the reduction of GO was not performed with a plant extract but with a commercially available pure substance which is also a part of the extracted reducing mixture, e.g. ascorbic acid – vitamin C [6, 10], caffeic acid [12], beta-carotene [18].

Experimental part

The employed chemicals and instruments

Graphite: PM – very fine crystalline powder graphite, mesh 0.025mm, Supplier: Koh-I-Noor Netolice, Czech Republic

Fullerene C_{60}: 99.5% purity, SES Research, Houston USA

Sulfuric acid, nitric acid , potassium permanganate, hydrogen peroxide,

Ascorbic acid: Supplier: Sigma –Aldrich

ATR analysis by means of FTIR spectrometry was performed using the spectrometer Brucker Alpha/FT-IR, ART crystal (identified as Platinum Diamond 1 Ref1), software OPUS 6,5, source IR SiC Globar. Number of spectrum scans 24, resolution 4 cm^{-1}, spectrum range 375-4000 cm^{-1}.

Thermal analyses TGA and DSC of the prepared product were performed on STA 1500, Instrument Specialists Incorporated-THASS, analytical scale SUMMIT, SI 234-4, at flow rate 20 ml/min., heating rate 10°C/min., ceramic crucible, diameter 5 mm and height 8 mm, degradation medium air.

Twist Digital Microscope Learning Resource, Inc. USA and

SEM Phenom FEI and SEM FEI Quanta 650 FEG (USA) were used for slides of products

Lyophilizer Free Zone 1L Benchtop, made by Labconco, model 7740030

The **BET** method was used to measure specific surface of the products. The measurement was performed in the company Pardam s.r.o.

2. Oxidization of fullerene C_{60}, graphite and a mixture of graphite-fullerene C_{60}

A detailed method of oxidization of fullerene C_{60}, graphite and a mixture of graphite-fullerene C_{60}, formation of water suspensions and preparation of foils were described in our previous publications [1, 2]. The weight ratios and representation of the individual components used for the oxidization are provided in Table 1.

Table 1. Weight representation of the individual components used for the oxidization

Ratio /weight (g)		$NaNO_3$ (g)	$KMnO_4$ (g)	H_2SO_4 (ml)	Product No.
graphite	C_{60}				
-	1/(0,7)	1	2	18	I. $C_{60\text{-}oxi}$
1/(1.5)	-	2	4,5	31	II. GO
1/(1.1)	1/(1.1)	3	7	52	III. GO-C_{60}
2/(1.5)	1/(0.7)	3	7	52	IV. GO-C_{60}
3/(1.75)	1/(0.55)	3	7	52	V. GO-C_{60}

3. Reduction (conversion) of the oxidized products GO and GO–C_{60}

Ascorbic acid was used for the reduction. The products were reduced in a suspension and also as foils prepared from suspensions of oxidized components.

The foil made of product No. IV (See Table 1) was placed into a Teflon dish and poured over with 12 ml of ascorbic acid solution (30mg/ml). Subsequently, the dish was placed into an incubator for 3 hours at 70°C. The solution was then decanted and the decantation was repeated 5 times with distilled water and with a mixture of water and : ethanol (1:1). The foil was then dried for 16 hours at 35°C and for 3 hours at 70°C. The obtained foil was brittle and missed plasticity of the foil from which it was originally made.

The individual products No. II –V (See Table 1) in the form of water suspensions were mixed with an ascorbic acid solution (6 ml of suspension, 4.5g of ascorbic acid dissolved in 70ml of water) and the mixture was stirred for 3 hours at 70-80°C. The resulting product was repeatedly washed with distilled water and with a mixture of water and ethanol (1:1). Then it was filtered off and dried in the same way as the foil made of the product No. IV.

The wave number values of the produced IR spectrum (Fig 1) indicate that the reduced foil made of the product No. IV was reduced partly, particularly on the C-OH groups.

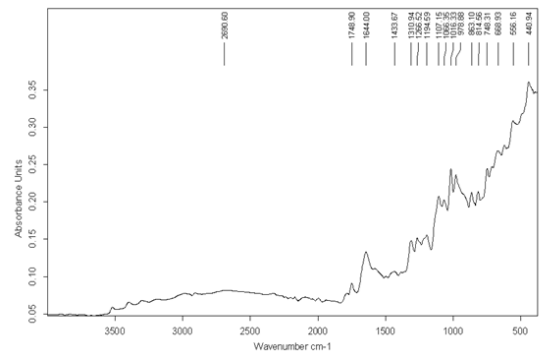

Fig 1. IR spectrum of the foil made of the product No. IV reduced with ascorbic acid

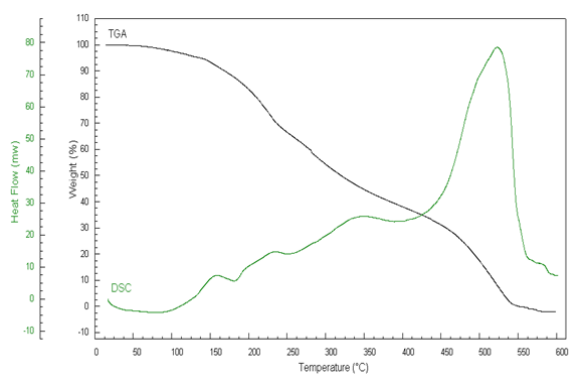

Fig 2. Thermal analyses TGA and DSC of the foil made of the product No. IV reduced with ascorbic acid (degradation medium: air, air flow 20ml/min, heating rate 10°C/min, sample weight 8.86mg)

There are two undistinguished peaks on the DSC curve corresponding to endothermic processes and one distinct peak corresponding to an exothermic process – see Fig 2. The first endothermic process occurs in the range of 14.7 – 133.2 °C with the maximum at 73.2 °C and with the peak area on the DSC curve 374.1 kJ/kg. The other endothermic process occurs in the rage of 156.4 – 194.7 °C with the peak area on the DSC curve 35.1 kJ/kg and with the maximum at 178.6 °C. The distinctive exothermic process occurs in the range of 405.9 – 567.9 °C with the maximum at 520.5 °C and with the peak area on the DSC curve -2764.4 kJ/kg, the weight decrease is practically continual.

The unreduced foil is decomposed with two exothermic effects, the first one with the maximum at 205°C (weight loss 71.1%), the other one with the maximum at 390°C (weight loss 28,1%), the total thermal effect is -1204kJ/kg. After the reduction the temperature of complete decomposition moved by 130°C and the thermal effect was smaller by one half.

A pilot test was conducted to perform the reduction with *Brassica jengea* seeds, in agreement with the OECD methodology to determine ecotoxicity based on an analysis of germinability and root length. When the germinating seeds were on a Petri dish in

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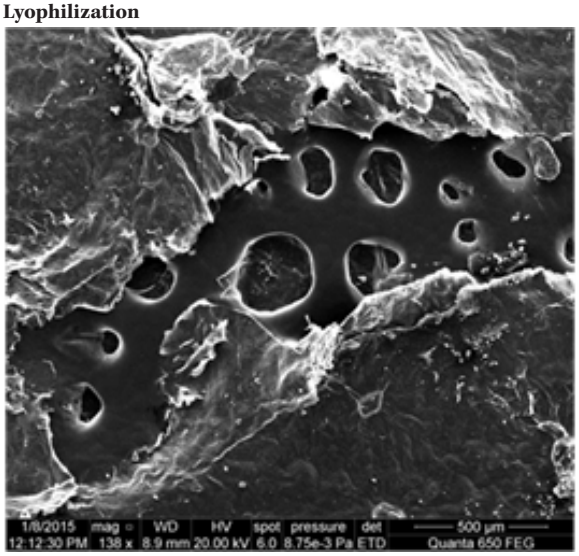
direct contact e.g. with a foil made of the No. IV. there was a visible disruption of its morphology (Fig. 3). We assume local reduction, effects of myrosinase enzymes or reactions with products of sinigrin and sinalbin decomposition.

Fig 3. Disrupted morphology of the GO-C60 (2:1) foil at the place of contact with a germinating seed of *Brassica jengea*

Lyophilization





Lyophilization of water suspension of oxidized products of GO and GO–C_{60} and their forms reduced by ascorbic acid was conducted using a modified procedure described in a Czech patent [29]. The possibility of fiber formation (change of morphology) with this method was tested for the products GO (No. II.) to GO-C_{60} (No. V) by means of controlled vacuum freeze drying (vacuum sublimation) from their water dispersion. Lyophilization of water suspension was performed at -40°C, under pressure 0.153 mbar, for 18 hours, both for the oxidized and reduced forms.

Results

The lyophilization of water suspensions of GO and GO–C_{60} produced slightly opalescent fibers (thick fibrous wadding), particularly in case of the samples GO–C_{60} No. III and IV. A similar result was obtained also for GO No. II, as shown in Fig 4. Fig. 5 shows SEM images of a freeze dried fiber of the sample No. IV.

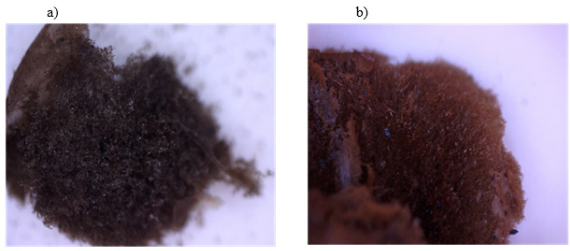


Fig 4. Microscopic image of lyophilized samples
a) No II. (GO); b) No IV. (GO–C_{60} 2:1).

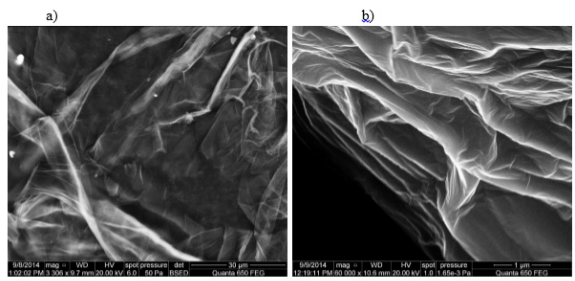


Fig 5. SEM images of fibers produced by lyophilization of the product No. IV (GO–C_{60} 2:1) **suspension**
a) line segment = 30 µm; b) line segment = 1 µm)

Specific surface of lyophilized samples:
Specific surface of initial samples:

No. V (GO–C60 3:1) - 777.5 m2/g
No. IV (GO–C60 2:1) – 504.4 m2/g
No. III (GO–C60 1:1) – 188.6 m2/g

The lyophilization of water suspensions of GO and GO–C60 reduced with ascorbic acid produced very fine powdery substances with increased specific volumes. The most compact of them was lyophilized reduced product No. IV. The microscopic images from a digital microscope (resolution 1280-960) are shown in Figs. 6, 7, 8, 9. Fig. 10 shows SEM images of lyophilized reduced product No. IV.

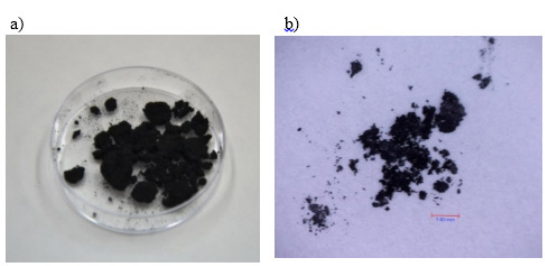


Fig 6. Lyophilizate of the sample No. II (GO) reduced with ascorbic acid.
a) without magnification; b) magnification 28x

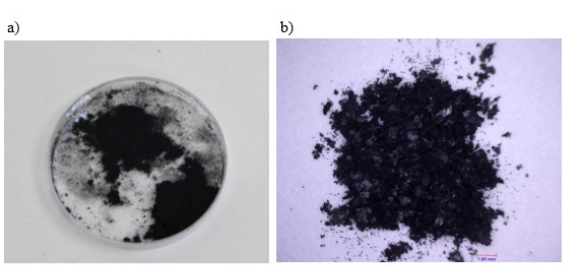


Fig 7. Lyophilizate of the sample No. III (GO-C_{60}, 1:1) reduced with ascorbic acid.
a) without magnification; b) magnification 23x

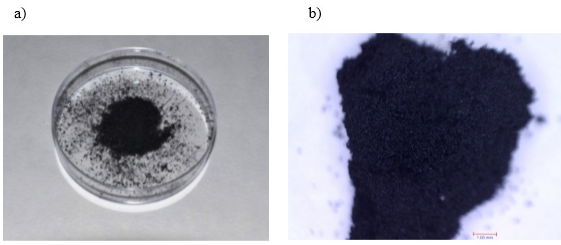


Fig 8. Lyophilizate of the sample No. IV. (GO-C_{60}, 2:1) reduced with ascorbic acid.

a) without magnification; b) magnification 23x

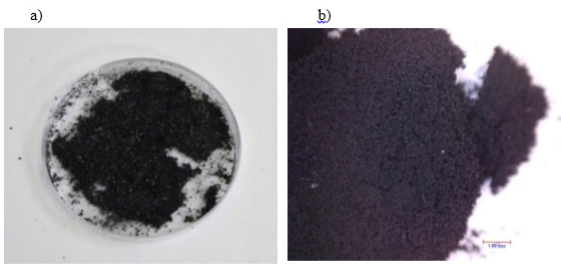


Fig 9. Lyophilizate of the sample No. V. (GO-C_{60}, 3:1) reduced with ascorbic acid.
a) without magnification; b) magnification 23x

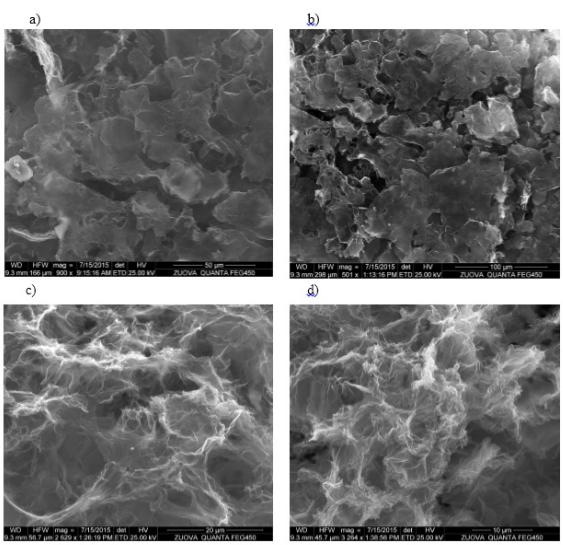


Fig 10. SEM images of lyophilizate of the reduced product No. IV. (GO-C_{60}, 2:1)

a) line segment = 50 μm; b) line segment = 100 μm; c) line segment = 20 μm; d) line segment = 10 μm

The product No. IV is not only more compact but it has a different IR spectrum (Fig. 11). In this spectrum we have identified valence and deformation vibrations characteristic for crystal water (3338-3253cm^{-1}, 1634cm^{-1}).

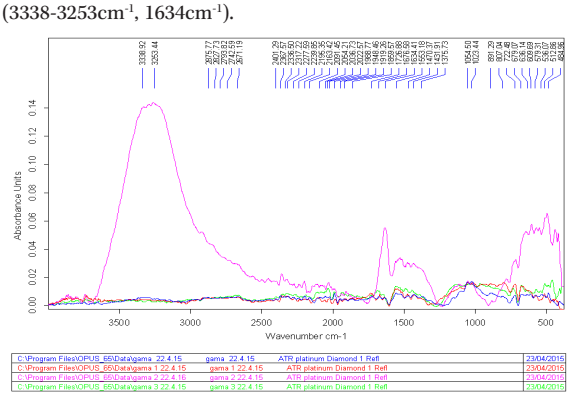


Fig 11. IR spectrum of lyophilizates of the reduced products No. II – V.

(No. II – blue curve, No. III – orange curve, No. IV – violet curve, No. V – green curve)

A certain difference of the lyophilized sample No. IV is also visible on the DSC curve (Fig. 12) , in the height of the H_{fi} peak, which is characteristic for the reaction rate.

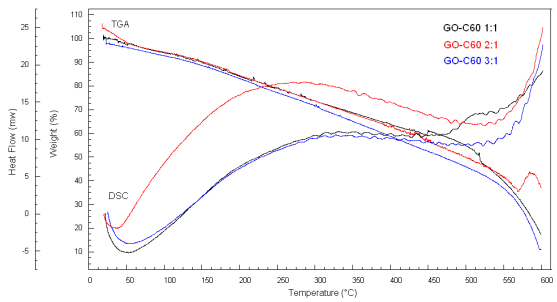


Fig 12. Comparison of TGA and DSC curves of lyophilizates of the reduced products

(No III. – black curve, No IV. – red curve, No V. – blue curve)

The TGA curves of the samples No. III – V can be divided into several sections with different gradients, i.e. the weight loss rates. This division, including the temperatures and weight loss values, is shown in Table 2. DSC curves for all the products No. III – V demonstrated one peak corresponding to an endothermic process. The temperature interval of the mentioned process (the minimum temperature and the thermal effect) is provided in Table 3.

Table 2 Division of the TGA curve into temperature intervals

Product No.	Interval No.	Temperature range (°C)	Weight loss (%)
III	1	19.7 – 110.5	7.0
	2	110.5 – 446.7	34.2
	3	446.7 – 519.4	11.2
	4	519.4 – 600.0	31.1
IV	1	41.4 – 79.7	4.4
	2	79.7 – 185.1	9.1
	3	185.1 – 522.5	39.7
	4	522.5 – 568.9	11.3
V	1	23.0 – 143.3	8.6
	2	143.3 – 416.9	32.7
	3	416.9 – 551.6	20.1
	4	551.6 – 600.0	25.2

Legend: ^{x} – outlined with intersections of tangents on the respective arc of the TGA curve

Table 3 Parameters of the ongoing thermal processes (DSC)

Product No.	Process No.	Temperature range (°C)	ΔH (kJ/kg)	H_{fi} (mW)	Tmin (°C)
III	1	19.7 – 133.4	1739.1	5.2	50.2
IV	1	18.2 – 92.8	845.4	1.9	34.4
V	1	22.6 – 122.6	1522.3	4.1	55.1

Legend: ΔH = thermal effect of the process according to DSC curves

(ΔH > 0: endothermic process , ΔH < 0: exothermic process)

H_{fi} = absolute value of the height of a thermal process peak on the DSC curve, related to the point in which the thermal process starts

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The minimum temperatures are relatively low to reflect only humidity. We believe that they represent desorption of other components from the atmosphere. After increasing the temperature the DSC curves do not demonstrate any significant maximums or minimums. The individual curves have similar shapes (Fig. 8) and they can be divided into increase, mild decrease and steep increase of exothermic effect of the reaction. The temperature ranges of those shape changes of DSC curves are indicated in Table 4.

Table 4. Temperature intervals on the DSC curves

Product	Temperature increase (°C)	Mild decrease (°C)	Steep increase (°C)
No III.	50.2 – 328.1	328.1 – 475.5	475.5 – 600.0
No IV.	34.4 – 283.5	283.5 – 536.2	536.0 – 600.0
No V.	55.1 – 324.2	324.2 – 534.0	534.0 – 600.0

Conclusion

The work focuses on verification of other methods to process water suspensions of GO and hybrid compounds based on GO-C60. The results follow up on previous results stated in the quoted literature when we combined the foils in various mutual ratios, including a combination with nanofibers. In this work we have focused on treatment of the foils by reduction and on processing of suspensions with lyophilization, both before and after the reduction. We intentionally used ascorbic acid, which is one of the so-called "green reduction" agents, as we believe that this method of reduction is the most suitable one for further utilization of the product in biomedicine. After the lyophilization we obtained products with such composition, aerogel (sponge) morphology and increased thermal stability after reduction that makes them more suitable even for general utilization of graphene and graphene oxide. Moreover, there is the added value of the fullerene molecule. The advantages include particularly the sorption capacity, application in biomedicine (biological activity, immobilization of biomolecules, biosensor, biocatalyst etc.), including other applications in the so-called Energy Storage.

REFERENCE

[1] Klouda Karel, Zemanová Eva, Brabencová Eliška, Brádka Stanislav, Dvorský Richard, 2014. Joint Oxidation of Fullerene C60 and Graphite. International Journal of Emerging Technology and Advanced Engineering, 4 (10), 504-522. [2] Klouda Karel, Zemanová Eva, Friedrichová Romana, Brádka Stanislav, Gembalová Lucie, 2014. Thermal Stability of Foils Made of Graphene-Oxide and Graphene-Oxide with Fullerene and their Composites with Methylcarboxy Cellulose and With Beta 1,3/1,6 – D – Glucan. International Journal of Emerging Technology and Advanced Engineering, 4 (10), 26-48. [3] Agharkar, M., Kochrekar, S., Hidouri, S., Azeez, M.A., 2014. Trends is green reduction of graphene oxides, issues and challenges: A review. Materials Research Bulletin, 59, 323-328. [4] Wang, G., Qian, F., Saltikov, Ch.W., Jiao, Y., Li, Y., 2011. Microbial Reduction of Graphene Oxide by Shewanella. Nano Res., 4(6), 563-570. [5] Dey, R.S., Hajra, S., Sahu, R.K., Raj, C.R., Panigrahi, M.K., 2011. A rapid room temperature chemical route for the synthesis of graphene: metal-mediated reduction of graphene oxide. Chem. Commun, 48, 1787-1789. [6] Chen, J., Shepherd, R.L., Razal, J.M., Huang, X., Zhang, W., Zhao, J., Harris, A.T., Wang, S., Minett, A.I., Zhang, H., 2013. Scalable Solid-Template Reduction for Designed Reduced Graphene Oxide Architectures. Applied Materials & Interfaces, 5, 7676-7681. [7] Fan, Z., Wang, K., Wei, T., Yan, J., Song, L., Shao, B., 2010. An environmentally friendly and efficient route for the reduction of graphene oxide by aluminum powder. Carbon, 48, 1670-1692. [8] Chua, Ch.K., Pumera, M., 2014. Chemical reduction of graphene oxide: a synthetic chemistry viewpoint. Chem. Soc. Rev., 43, 291-312. [9] Ramesha, G.K., Kumara, A.V., Muralidhara, H.B., Sampath, S., 2011. Graphene and graphene oxide as effective adsorbents toward anionic and cationic dyes. Journal of Colloid and Interface Science, 361, 270-277. [10] Su, Y., Kravets, V.G., Wong, S.L., Waters, J., Geim, A.K., Nair, R.R., 2014. Impermeable barrier films and protective coatings based on reduced graphene oxide. Nature Communications, 5. [11] Xu, Ch., Yuan, E., Wang, X., 2014. Selective reduction of graphene oxide. New Carbon Materials, 29(1), 61-66. [12] Bo, Z., Shuai, X., Mao, S., Yang, H., Qian, J., Chen, J., Yan, J., Cen, K., 2014. Green preparation of reduced graphene oxide for sensing and energy storage applications. Scientific reports, 4:4684, 1-8. [13] Pei, S., Cheng, H-M., 2012. The reduction of graphene oxide. Carbon, 50, 3210-3228. [14] Makarov, V.V., Love, A.J., Sinitsyna, O.V., Makarova, S.S., Yaminsky, M.E., Kalinina, N.O., 2014. „Green" Nanotechnologies: Synthesis of Metal Nanoparticles Using Plants. Acta Naturaei, 6(1), 35-44. [15] Kharissova, O.V., Rasika Dias, H.V., Kharisov, B.I., Olvera Pérez, B., Jiménez Pérez, V.M., 2013. The greener synthesis of nanoparticles. Trends in Biotechnology, 31(4), 240-248. [16] Park, Y., 2014. A New Paradigm Shift for the Green Synthesis of Antibacterial Silver Nanoparticles Utilizing Plant Extracts. Toxicological Research, 30(3), 169-178. [17] Hasna Abdul Salam, Rajiv, P., Kamaraj, M., Jagadeeswaran, P., Gunalan, S., Sivaraj, R., 2012. Plants: Green Route for Nanoparticle Synthesis. International Research Journal of Biological Sciences, 1(5), 85-90. [18] Vadlapudi, V., Kaladhar, D.S.V.G.K., Behara, M., Sujatha, B., Naidu, G.K., 2013. Synthesis of Green Metallic Nanoparticles (NPs) and Applications. Oriental Journal of Chemistry – An International Journal of Pure & Applied Chemistry, 29(4). [19] Rodríguez-León, E., Iñiguez-Palomares, R., Navarro, R.E., Herrera-Urbina, R., Tánori, J., Iñiguez-Palomares, C. and Maldonado, A., 2013. Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts). Nanoscale Research Letters, 8, 318. [20] Mubayi, A., Chatterji, S., Rai., P.M., Watal, G., 2012. Evidence based green synthesis of nanoparticles. Advanced Materials Letters, 3(6), 519-525. [21] Haverkamp, R.G., Marschall, A.T., 2009. The mechanism of metal nanoparcticle formation in plants: limits on accumulation. J. Nanopart Res, 11, 1453-1463. [22] Marshall, A.T., Haverkamp, R.G., Davies, C.E., Parsons, J.G., Gardea, J.L., Agterveld, D. 2007. Accumulation of Gold Nanoparticles in Brassic Juncea. International Journal of Phytofemediation, 9, 197-206. [23] Agharkar, M., Kochrekar, S., Hidouri, S., Azeez, M.A., 2014. Trends is green reduction of graphene oxides, issues and challenges: A review. Materials Research Bulletin, 59, 323-328 [24] Gurunathan, S., Han, J.W., Park, J.H., Eppakyala, V., Kim. J., 2014. Ginkgo biloba: a natural reducing agent for the synthesis of cytocompatible graphene. Int. J. Nanomedicine, 9, 363-377. [25] Lee, G., Kim, BS., 2014. Biological reduction of graphene oxide using plant leaf extracts. Biotechnol. Progress, 30, 463-469 [26] Haghighi, B., Tabrizi, M.,A., 2013. Green-synthesis of reduced graphene oxide nanosheets using rose water and a survey on their characteristics and applications. RSC Adv, 3, 133365-13371. [27] Kuila, T., Bose, S., Kharna, P., Mishra, A.K., Kim, N.H., Lee, J.H., 2012. A green approach for the reduction of graphene oxide by wild carrot root. Carbon, 50, 914- 921. [28] Zaid, R. M., Chong, F.Ch., Teo, E. Y.L., Ng, E.P., Chong, K.F., 2014. Reduction of graphene oxide nanosheets by natural beta carotene and its potential use as upercapacitor electrode. Arabian Journal of Chemistry. 1-10. [29] Dvorský R., 2012. Method of preparation of fibrous and lamellar microstructure controlled by vacuum freeze drying of liquid dispersion of nanoparticles, Patent CZ, 303513/2012.