Graphene Functionalization with Amines and Decorated with Metallic Nanoparticles for Antimicrobial Properties(*)

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Graphene oxide is synthesized by Hummers method from graphite. Graphene is prepared using three different methods after the chemical reduction of graphene oxide. Then covalent and noncovalent functionalization are achieved for the graphene surface by different amines as aniline and 1,6-diaminohexane. Silver nanoparticles (AgNPs) were deposited on the surface of the functionalized graphene during the synthesis process through the chemical reduction of silver nitrate ($AgNO_3$) by hydrazine hydrate. The disinfection effect was successfully tested against Total and Fecal Coliforms found in water samples from different regions in Egypt.
The changes in surface functionalities and bonding nature were studied by X-ray diffraction (XRD), Raman spectroscopy, nuclear magnetic resonance (NMR) and infrared spectroscopy (IR). The morphology and size distribution were studied by high resolution transmission electron microscopy (HRTEM) and scanning electron microscopy (SEM), where the successful immobilization of silver nanoparticles on the graphene surface was studied.

Aim of the work

The aims of this thesis are:

1- Preparation of graphene and graphene oxide using chemical methods.
2- Functionalization of the prepared graphene and graphene oxide with aliphatic amines namely 1,6-diaminohexane and aromatic amines namely aniline.
3- Deposition of silver nanoparticles over the amine functionalized graphene or the graphene oxide to form a composite.

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4- Characterization of the resulting composites using XRD, Raman, ^{1}HNMR, IR, HRTEM, SEM techniques.

5- Testing the nanocomposites for their use as antibacterial agents against Total and Fecal Coliforms found in water samples from different regions in Egypt.

Introduction

Most of infections for human and living organisms are caused through water born diseases(1,2) if it was contaminated by any of the harmful bacteria as gram positive and gram negative bacteria. Traditional methods are used in water disinfection as chlorine, chloramines, and ozone, but, many of these are carcinogenic(3) for both human and animal cells. Moreover, the resistance of the bacteria to these common chemical disinfectants is increasing. So, alternatives must be obtained to solve these problems of disinfecting water. Nanotechnology introduces some nanomaterials that have disinfection effects. Graphene, amines, and silver nanoparticles have proved excellent antibacterial effect(4-6). So the composite of these three materials is expected to be a very high antibacterial agent.

Graphene was discovered for the first time in 2004 by two Russian scientists, Andre Geim and Konstantin Novoselov, from Manshester University. They were awarded the Nobel prize in Physics for this discovery in 2010, thanks to the simple and groundbreak scotch tape method, they regarded some sheets of graphene formed on the graphite stone while cleaning it. Graphene recently received tremendous scientific activity(7). Graphene is the mother material for all graphitic materials, fullerenes and carbon nanotubes(8). It is a monolayer of sp^2 bonded carbon atoms arranged in hexagonal honeycomb lattice structure(9). It is the first two dimensional material ever known to us, besides being the lightest and thinnest material, one atom thick, it is the strongest material on earth.

Graphene has unique mechanical, thermal, electrical(10) properties, in addition it has a very high surface area around 2630 M^2/gram(11), very high elasticity, it is harder than diamond, 300 times stronger than steel, it has Young's modulus of around 1100 GB, it has a fracture strength about 125 GB[12], and thermal conductivity up to 5000 W/mol.Kelvin[13].

For its unique electronic properties, graphene is used in many applications as nanocomposites[14], solar cells[15,16], lithium ion batteries[17,18], electrochemical sensors[19], energy storage devices, nanoelectronics[20]. Graphene sheets are prepared using some methods as micro-mechanical exfoliation of graphite, epitaxial growth[21], chemical vapor deposition[22], solvothermal reduction of graphene oxide[23], the electrochemical reduction of graphene oxide[24], and the chemical reduction of graphene oxide[25].

Some nanometallics and their oxides have a broad antibacterial effect, such materials as nanoparticles of silver, copper, zinc oxide, and titanium dioxide [26,27]. But comparing with other metals, silver nanoparticles have excellent antibacterial properties, and less harmful effect toward human cells [28, 29]. So, silver nanoparticles are used to control bacterial growth in many medical applications as teeth surgical tools and in treating wounds [30,31]. This is due to the very high toxicity of silver ions and compounds against more than 12 types of bacteria such as *E. coli* [32].

Silver nanoparticles have unique properties as chemical stability, catalytic activity, high conductivity, and antimicrobial activity [33]. Deposited silver carbon filters were used to remove bacteria from water [34,35], moreover, graphene as well graphene nanocomposites have very high antibacterial properties [36,37]. Shen et al, reported that silver- chemically converted graphene nanocomposites showed a very high antibacterial activity toward *Colibacillus*, *S. aureus* and *C. albicans* [38]. Aniline has many applications in drug industry, polymers, dyes, pigments, rubber industry, herbicides, pesticides, fungicides [39]. Pi–Pi stacking interactions between aniline and graphene, besides, the reducing ability of the amine group toward metal ions, makes it feasible for the silver ions to be easily hybridized with the graphene functionalized by aniline molecules.

Experimental work

The following summarizes the experimental work undertaken in this thesis.

1- Preparation of graphene oxide

Graphite is converted chemically to graphene oxide following Hummers Offman method [40] according to:

Graphite + H_2SO_4 (conc, 98%) + $KMnO_4$+ $NaNO_3$

$$\xrightarrow{\text{Ultrasonication}} \text{Graphene oxide}$$

2- Preparation of graphene

Graphene is prepared chemically from graphene oxide (graphene-COOH) or graphite oxide according to the following:

Graphene oxide or graphite oxide (graphene-COOH)

$$\xrightarrow{\text{Reduction}} \text{Graphene-}CH_2OH$$

First method

Graphite oxide(graphene-COOH) + NH_4OH 30%

$$\xrightarrow{\text{Ultrasonication}} \text{Graphene-}CH_2OH$$

Second method

Graphene oxide (graphene-COOH) + $NH_2C_6H_5$+ NH_2NH_2HCl

$$\xrightarrow[\text{(II. 200°C, 30 min)}]{\text{(I. 100°C, 2h)}} \text{Graphene-}CH_2OH$$

Third method

Graphene oxide (graphene-COOH) + $NH_2NH_2.H_2O$

$$\xrightarrow[\text{(II. 200°C, 30 min)}]{\text{(I. 100°C, 2h)}} \text{Graphene-}CH_2OH$$

3- **Preparation of graphene functionalized by aniline and silver nanoparticles:**

First method: (noncovalent functionalization)

Reaction of aniline, silver nitrate and hydrazine hydrate with graphene oxide:

(Graphene-COOH) + $NH_2C_6H_5$ + $AgNO_3$ + $NH_2NH_2.H_2O$ → Graphene-COOH….. $NH_2C_6H_5$…..AgNPs

Second method: (noncovalent functionalization)

Reaction of aniline, silver nitrate, hydrazine hydrate with graphene while heating:

Graphene + $NH_2C_6H_5$ + $AgNO_3$ + $NH_2NH_2.H_2O$ $\xrightarrow{(100°C,\ 1h)}$ Graphene….. $NH_2C_6H_5$…..AgNPs

Third method: (covalent functionalization)

Reaction of graphene oxide with thionyl chloride, aniline, silver nitrate and hydrazine hydrate while heating in closed system:

* Graphene-COOH+ $SOCl_2$ $\xrightarrow{(80°C,\ 24h,\ closed\ system)}$ Graphene-COCl
* Graphene-COCl + $NH_2C_6H_5$ → Graphene-CONH-C_6H_5
* Graphene-CONH-C_6H_5 + $AgNO_3$ + $NH_2NH_2.H_2O$ → Graphene-CONH-C_6H_5…..AgNPs.

4- **Preparation of graphene functionalized by 1, 6-diaminohexane and silver nanoparticles:**

First method (noncovalent functionalization)

Reaction of graphene with hydrochloric acid, 1, 6-diaminohexane, silver nitrate and hydrazine hydrate according to:

Graphene + HCl 30 % solution + H_2NCH_2 – $(CH_2)_4$ – CH_2NH_2 + $AgNO_3$ + $NH_2NH_2.H_2O$ → Graphene….. NH_2 $(CH_2)_6$ NH_2 …..AgNPs

Second method (covalent functionalization)

Reaction of graphene oxide with thionyl chloride, 1,6-diaminohexane, silver nitrate and hydrazine hydrate while heating in closed system:

$$\text{* Graphene-COOH} + SOCl_2 \xrightarrow{(80^\circ C,\ 24h,\ \text{closed system})} \text{Graphene-COCl}$$

$$\text{* Graphene-COCl} + NH_2CH_2CH_2CH_2CH_2CH_2CH_2NH_2 \rightarrow \text{Graphene-CONH}-(CH_2)_6-\text{NHCO-Graphene}$$

$$\text{* Graphene-CONH-}(CH_2)_6\text{-NHCO-Graphene} + AgNO_3 + NH_2NH_2.\ H_2O \rightarrow \text{Graphene-CONH-}(CH_2)_6\text{-NHCO-Graphene.....AgNPs}$$

5- Bacterial infection test

Three "untreated" infected water samples from three different regions in Benisweef governorate were treated according to: 0.1 g graphene aniline /silver nanocomposite /100 mL " untreated " water. This results in complete disinfection for all samples. Also, 0.1 g graphene 1,6-diaminohexane /silver nanocomposite /100 mL " untreated " water succeeded in disinfecting the water samples from Bahr El-Bakar and Kochainer sewages. Bacterial infection tests were conducted in the central laboratories of the Nile Research Institute of the National Water Research Center in Egypt according to the following procedure:

Fecal Coliforms density was determined using seven hours Fecal Coliforms test and applying membrane filter technique according to standard method (APHA, 1989). M-7h/FC ingredients were heated in boiling water bath then cooled to 55°C and pH value was adjusted to 7.3 before pouring in Petri dishes. After incubation at 41.5°C for 7 hours, yellow colonies were counted as Fecal Coliform bacteria.

Stock solutions were prepared using the appropriate solvent as indicated or distilled water. Polluted (infected) water samples were collected according to environmental procedures following environmental protection agency regulations using sterilized glass containers. The samples were kept at low temperatures in ice bath and delivered to the laboratory for immediate testing. Infected water samples were collected from the Khour, mixing zone and the river, all from Benisweef governorate (Elwasta area, five kilometers along Nile river from the 82.5 to 87.5 kilometers of the Nile river) and the samples collected from Bahr El-Bakar and Kochainer sewage water.

Results and Discussion

One of the major goals of this thesis is to explore the efficiency of the synthesized composites towards anti-bacterial activities.

Therefore, Aniline-functionalized graphene and decorated with silver nanoparticles was tested for its antibacterial activity where, three water samples were collected according to the regular protocols for water sampling from three locations of El-Wasta zone in Beni-sweef governorate. The water samples were taken from the following locations: Khour, Mixing Zone and Nile.

The analyses for the anti-bacterial activities were performed in cooperation with the Nile Research Institute at the National Water Research Center of Egypt. The results were compared to the control samples before treatment with the composite material.

First, the results of using graphene functionalized with aniline and silver nanoparticles for disinfecting water against Total Coliform and Fecal Coliform Bacteria are presented. Before treatment, the analyses showed that Total Coliforms were 3400, 4400, and 30 CFU/100 mL for Khour, Mixing Zone and Nile, respectively. While those for Fecal Coliforms were 1600, 1500, and 20 CFU/ 100 mL for the same samples, respectively.

The treatment was achieved by using 0.1 gram of the graphene functionalized by aniline and AgNPs in 100 mL infected water sample. The analyses showed that full disinfection of all samples from Total and Fecal Coliforms were reached. A zero value was obtained as can be noticed from the data given in Table 1.

	Parameters	Unit	Khour	Mixing Zone	Nile
Before treatment	Total Coliform	CFU/100mL	3400	4400	30
	Fecal Coliform	CFU/100 mL	1600	1500	20
After treatment	Total Coliform	CFU/100 mL	0	0	0
	Fecal Coliform	CFU/100 mL	0	0	0

Table 1. (Showing the complete disinfection of the samples after treating with graphene functionalized by aniline and AgNPs).

Also, graphene functionalized by 1,6- diaminohexane and silver nanoparticles composite was tested for its antibacterial activity, therefore, four water samples were collected according to the regular protocols for water sampling from two locations around water sewage around Bahr Elbakar and Kochaner regions. The results were compared to the control samples before treatment with the composite material.

The results of using graphene functionalized by 1,6-diaminohexane with silver nanoparticles for disinfecting water against Total Coliform and Fecal Coliform Bacteria are presented.

Before treatment, the analysis showed that Total Coliforms were 5000000, 2600000 CFU/100 mL for samples 1, 2 respectively of the first zone and 3000000, 1200000 CFU/100 mL for samples 3, 4 respectively of the second zone. While those for Fecal Coliforms were 700000, 500000 CFU/100 mL for samples 1, 2 respectively of the first zone and 1200000, 300000 CFU/100 mL for samples 3, 4 respectively of the second zone.

The treatment was achieved by using 0.1 gram of the 1,6-diaminohexane-silver nanoparticles-functionalized graphene in 100 mL infected water sample. The analyses according to the protocols explained in the experimental section showed that full disinfection of all samples from Total and Fecal Coliforms were reached except the first sample where the disinfection therein was few colonies above zero value, about 10 and 3 colonies remaining after treatment for Total and Fecal Coliforms respectively. A zero value was obtained for the other three samples as can be noticed from the data given in Table 2.

	Parameters	Unit	Zone 1		Zone 2	
			Sample1	Sample2	Sample3	Sample4
Before Treatment	Total Coliform	CFU/100 mL	5000000	2600000	3000000	1200000
	Fecal Coliform	CFU/100mL	700000	500000	1200000	300000
After Treatment	Total Coliform	CFU/100 mL	10	0	0	0
	Fecal Coliform	CFU/100mL	3	0	0	0

Table 2. Disinfection treatment data from Total Coliform and Fecal Coliform using 1,6-diaminohexane -silver nanoparticles-functionalized graphene. Zone 1 for Bahr El-Bakar sewerage and Zone 2 for Kochainer sewerage.

References

1 - Li, Q. L. Mahendra, S. Lyon, D. Y. Brunet, L. Liga, M. V. Li, D. Alvarez, P.J.J. 2008, Water Res. 42, p. 4591.

2 - Gangadharan, D., K. Harshvardan, G. Gnanasekar, D. Dixit, K. M. Popat, P. S. Anand, 2010, Water Res. 44, p. 5481.

3 - Kratschmer, W. Lamb, L. D. Fostiropoulos, K. Huffman, D. R. 1990, Nature. 347, p. 354.

4 - Kim, K. Park, H.-J. Woo, B. Kim, K. Kim, G. Yun, W.-S. 2008, Nano Lett. 8, pp. 3092-3096.

5 - Lv, W. Tang, D.-M. He, Y.-B. You, C.-H. Shi, Z.-Q. Chen, X.-C. Chen, C.-M. Hou, P.-X. Liu, C. Yang, Q.-H. 2009, ACS Nano. 3, pp. 3730–3736.

6 - Aymonier, C. Schlotterbeck, U. Antonietti, L. Zacharias, P. Thomann, R. Tiller, J. C. and Mecking. S. Chem. Commun. 2002, (Camb). 24, pp. 3018-3019.

7 - Novoselov, K. S. Geim, A. K. Morozov, S. V. Jiang, D. Zhang, Y. Dubonos, S. V. I. V. Grigorieva, A. A. Firsov, Science. 306, 2004, pp. 666–669.

8 - Geim, A. K., K. S. Novoselov. Nat. Mater. 6, 2007, pp. 183-191.

9 - Li, F. H., Y. Bao, J. Chai, Q. X. Zhang, D. X. Han, L. Niu, Langmuir. 26, 2010, pp. 12314–12320.

10 - Orlita, M., C. Faugeras, P. Plochocka, P. Neugebauer, G. Martinez, D. K. Maude, A.-L. Barra, M. Sprinkle, C. Berger, W. A. de Heer, M. Potemski, Phys. Rev. Lett. 101, 2008, pp. 267-601.

11 - Stoller, M. D., S. Park, Y. Zhu, J. An, R. S. Ruoff. Nano Lett. 8, 2008, pp. 3498–3502.

12 - Lee, C. G., X. D. Wei, J. W. Kysar, J. Hone, Science. 321, 2008, pp. 385–388.

13 - Balandin, A. A. Ghosh, S. Bao, W. Calizo, I. Teweldebrhan, D. Miao, F. 2008, Nano Lett., 8, pp. 902–907.

14 - Li, F. H., Yang, C. Shan, Q. Zhang, D. Han, A. Ivaska, L. Niu, J. Mater. Chem. 19, 2009, pp. 4022–4025.

15 - Wang, X., L. Zhi, K. Mullen. Nano Lett. 8, 2007, pp. 323–327.

16 - Xu, Y., G. Long, L. Huang, Y. Huang, X. Wan, Y. Ma, Y. Chen, Carbon. 48, 2010, pp. 3308–3311.

17 - Yoo, E., J. Kim, E. Hosono, H.-S. Zhou, T. Kudo, I. Honma, Nano Lett. 8, 2008, pp. 2277–2282.

18 - Wang, G., X. Shen, J. Yao, J. Park, Carbon. 47, 2009, pp. 2049–2053.

19 - Zhu, C., S. Guo, Y. Zhai, S. Dong, Langmuir. 26, 2010, pp. 7614–7618.

20 - Areshkin, D. A., C. T. White, Nano Lett. 7, 2007, pp. 3253–3259.

21 - Berger, C., Z. Song, X. Li, X. Wu, N. Brown, C. Naud, D. Mayou, T. Li, J. Hass, A. N. Marchenkov, E. H. Conrad, P. N. First, W. A. deHeer, Science. 312, 2006, pp.1191–1196.

22 - Wang, J. J., M. Y. Zhu, R. A. Outlaw, X. Zhao, D. M. Manos, B. C. Holloway, Carbon. 42, 2004, pp. 2867–2872.
23 - Nethravathi, C., M. Rajamathi, Carbon. 46, 2008, pp. 1994–1998.
24 - Wang, Z., X. Zhou, J. Zhang, F. Boey, H. Zhang, J. Phys. Chem. C. 113, 2009, pp. 14071–14075.
25 - Wang, G., J. Yang, J. Park, X. Gou, B. Wang, H. Liu, J. Yao, J. Phys. Chem. C. 112, 2008, pp. 8192–8195.
26 - Zhang, H. Y., G. H. Chen, Environ. Sci. Technol. 43, 2009, p. 2905.
27 - Ruparelia, J. P., A. K. Chatteriee, S. P. Duttagupta, S. Mukherji, Acta Biomater. 4, 2008, p. 707.
28 - Jain, J., S. Arora, J. M. Rajwade, P. Omray, S. Khandelwal, K. M. Paknikar, Mol. Pharm. 6, 2009, p. 1388.
29 - Liu, J. Y., R. H. Hurt, Environ. Sci. Technol. 44, 2010, p. 2169.
30 - Catauro, M., M. G. Raucci, F. D. De Gaetano, A. Marotta, J. Mater. Sci. Mater. Med. 15, 2004, pp. 831 - 837.
31 - Crabtree, J. H., R. J. Burchette, R. A. Siddiqi, I. T. Huen, L. L. Handott, A. Fishman, Perit Dial Int. 23, 2003, pp. 368- 374.
32 - Zhao, G., S. E. Stevens, J. Biometals. 11, 1998, pp. 27- 32.
33 - Frattini, A., N. Pellegri, D. Nicastro, O. de Sanctis, Mater. Chem. Phys. 94, 2005, p. 148.
34 - Lepape, H., F. Solano-Serena, P. Contini, C. Devillers, A. Maftah, P. Leprat, Carbon. 40, 2002, p. 2947.
35 - Ortiz-Ibarra, H., N. Casillas, V. Soto, M. Barcena-Soto, R. Torres-Vitela, W. de la Cruz, S. Gomez-Salazar, J. Colloid Interface Sci. 314, 2007, pp. 562.
36 - Hu, W., C. Peng, W. Luo, M. Lv, X. Li, D. Li, Q. Huang, C. Fan, ACS Nano. 7, 2010, p. 4317.
37 - Akhavan, O., E. Ghaderi, J. Phys. Chem. C. 113, 2009, p. 20214.
38 - Shen, J., M. Shi, N. Li, H. Ma, M. Ye, Nano Res. 3, 2010, p. 339.
39 - United States Environmental Protection Agency, OPPT Chemical Fact Sheets, Aniline Fact Sheet, Support Document (CAS No. 62-53-3), December 1994.
40 - Pham, T. A., B. C. Choi, K. T. Lim, Y. T. Jeong, Appl. Surf. Sci. 257, 2011, pp. 3350–3357.