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RESEARCH NOTE

Graphene Oxide Antibacterial Sheets: Synthesis and Characterization

E. Badiei[a], P. Sangpour*[a], M. Bagheri[a], M. Pazouki[b]

[a] Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center, Karaj, Iran
[b] Department of Energy, Materials and Energy Research Center, Karaj, Iran

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ABSTRACT

Graphene oxide (GO) was synthesized by oxidation of graphite powder using a time-saving modification of Hummers' method and its antibacterial activity was investigated. Different techniques were applied to characterize the synthesized GO. X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) were used to investigate the crystallinity, morphology, topography and thickness profile of the synthesized graphene oxide, respectively. Fourier Transform Infrared Spectroscopy (FT-IR) was carried out to investigate the chemical structure and purity of the synthesized sample. Optical properties were investigated by UV-vis spectroscopy. The antibacterial activity of GO against *Escherichia coli* (*E. coli*) was evaluated by the Colony Forming Count (CFU) method. The results confirmed the sample had excellent antibacterial activity against *E. coli*.

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1. INTRODUCTION

Today in spite of the fact that human life is improving, wide variety of microorganisms are proliferating and are in natural equilibrium with human body and environment. Hence, control of their harmful effects seemed to be vital [1]. To solve this problem, many ways and materials are suggested by physician, for example use of traditional antimicrobial agents such as kanamycin, spectinomycin, and penicillin. However, frequent usage of these agents causes the microbes become resistant against them [2].

Over the past decade, nanomaterials have been the subject of enormous interest [3-5]. Recently, several nanomaterials have been demonstrated to exhibit strong antimicrobial activity towards microbial agents [2,6]. These nanomaterials include metal and metal oxide nanoparticles such as silver [7,8], gold [9,10], copper and copper oxide [11, 12], TiO_2 [13-15], ZnO [16-18] and their nanocomposites [19], as well as aqueous fullerene nanoparticles (nC60) [20] and carbon nanotubes (CNT) [21].

Two dimensional structures of carbon nanomaterials are known as graphene. It exhibited considerable properties such as thermal, mechanical and electrical properties, excellent mobility of charge carriers and high surface area. Graphene has many applications in electronics, hybrid photovoltaic devices, bio-devices, composites, drug delivery systems and catalysts due to its unique properties [22-24]. Graphene oxide (GO) is one of the derivatives of graphene that is obtained from chemical exfoliation of graphene. It has similar structure to graphene including a single atom thick sheet arranged by localized sp3 defects within the sp2- bonded carbon atoms in a honey-comb (hexagonal) network [24-26]. On these sheets, hydroxyl and epoxy functional groups are located on the network of carbon atoms while carboxyl groups are placed at the edges [27].

Recently, it has been demonstrated that GO and other derivatives of graphene can be used in biotechnology due to their interactions with biomolecules and showed no cytotoxicity on human cells [23, 28, 29]. Akhavan et al. [30] studied the antibacterial activity of GO and RGO nanowalls on *E.coli* and *S.areus* bacteria as the model of Gram-negative and Gram-positive bacteria, respectively. They reported that the toxicity of RGO on bacteria was more

*Corresponding Author's Email: sangpour@merc.ac.ir (P. Sangpour)

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than GO. The antibacterial activity of GO and RGO on *E.coli* bacteria was investigated by Hu et al [31]. According to their studies, graphene and graphene oxide can effectively inhibit the growth of *E. coli* bacteria while showing minimal cytotoxicity. Moreover, they demonstrated that graphene and graphene oxide can damage bacterial membrane via direct contact and cause losing its membrane integrity. Liu et al. [32] showed that among four types of graphene family materials, namely graphite, graphite oxide, graphene oxide, and reduced graphene oxide, under similar concentration, graphene oxide dispersion has the highest antibacterial activity on *E.coli* bacteria, sequentially followed by reduced graphene oxide, graphite, and graphite oxide.

Hummer's method is a common method for synthesis of graphite oxide and graphene oxide 28. Several researchers did some modifications to this method in order to synthesize desired GO but most of the modifications are mainly long and time-consuming. In this study, a modification to Hummer's method was made in which graphene oxide was synthesized in a short period of time compared to others' work. Antibacterial activity of synthesized GO against *E. coli* as Gram-negative bacteria model was investigated. Also, synthesized graphene oxide was studied with different techniques, such as XRD, FTIR, SEM, UV-vis and AFM.

2. EXPERIMENTAL

2. 1. Raw Materials To synthesize graphene oxide, graphite fine powder, sodium nitrate and KMnO4 were purchased from Merck and used without further purification.

2. 2. Equipment X-ray diffraction was carried out to investigate the structure of samples using Philips PW 3710 x-ray Diffractometer by CoKα radiation with wavelength of 1.789A◦. FT-IR was performed to confirm formation of graphene oxide and determines presence of impurities using Perkin Elmer, Spectrum RXI. Scanning Electron Microscopy (SEM) images and Atomic Force Microscopy (AFM) test were used for investigation of morphology and the number of GO layers, respectively. SEM images were acquired using Oxford Stereo Scan S360 and AFM results were obtained using Veeco Autoprobe CP-Research with a silicon tip of 10 nm radius.

2. 3. Synthesis Graphene oxide was prepared from graphite fine powder based on modified Hummers' method. In brief, 0.5 g graphite powder was mixed with 0.3 g sodium nitrate. Then, 16.5 ml cold and concentrated H_2SO_4 was added to the mixed powder. After 15 min stirring, 1.5 g $KMnO_4$ was slowly added to the mixture while the temperature was maintained at 0°C using ice-bath. Then, the ice-bath replaced with an oil-bath and the mixture was stirred for 40 min at 35°C. 23 ml of DI water was added to the mixture; it caused the temperature to increase to 98°C. The mixture was stirred for 20 min. The procedure was followed with the addition of 70 ml of DI water and 1.75 ml of hydrogen peroxide, successively. Then the resulted suspension was centrifuged several times and alternatively washed with HCl solution (10%) to remove residual salts and with ethanol to remove residual HCl. The resulted dark brown paste was dried in a vacuum oven for 24 h at 60°C.

3. RESULTS AND DISCUSSIONS

The crystalline structure of synthesized sample was studied using X-ray powder diffraction analysis. As it can be seen in Figure 1, a sharp peak located at 11.34° and another one at 49.34° indicates that GO was formed by the modified Hummers' method.

The first peak is attributed to the diffraction of (002) facet of GO that corresponds to the interlayer distance of 0.90 nm. It is longer than interlayer distance of natural graphite (0.34 nm), which proves exfoliation of graphene layers by the oxygen-containing groups. There is no extra peak that indicates that prepared GO includes no impurity.

The GO sample was also characterized by FT-IR spectroscopy in order to investigate chemical structure and the degree of purity of the synthesized sample. FT-IR spectrum of the prepared GO sample is shown in Figure 2. As it can be seen in this figure, a broad band at 3356 cm^{-1} can be attributed to the stretching vibration of hydroxyl groups (O-H). The bands at 1053, 1225 and 1412 cm^{-1} are due to stretching vibrations of oxygen-containing groups of C-O of alkoxy and epoxy, respectively. The absorption band at 1618 cm^{-1} originates from aromatic C=C which is assigned to the skeletal vibrations of unoxidized graphitic domains. Also, the C=O stretching vibration mode at 1720 cm^{-1} belongs to COOH groups. The band at 891 cm^{-1} is attributed to bending of C-H. All of the mentioned peaks confirm that the graphene oxide was formed. Similar to XRD results, no additional peaks are seen which indicates that the sample is pure.

Figure 3 shows UV-visible absorption spectrum of the GO sample dispersed in ethanol. As can be seen in Figure 3, a strong absorption peak is seen at about 238 nm. It can be attributed to the $\pi \rightarrow \pi^*$ transitions for aromatic C–C bonds[29]. This maximum wavelength is an indication of degree of conjugation. The higher conjugate systems needs less energy for excitation, so the peak wavelength shifts to higher wavelengths. Also there is a shoulder around 300 nm at the spectrum of GO which can be ascribed to $n \rightarrow \pi^*$ transitions of the

carbonyl groups (C=O bonds) that can be observed from the FT-IR results [30, 31].

SEM analysis was performed to study the morphology of the graphene sample. SEM image of the sample is shown in Figure 4. As it can be seen, this image exhibits a layered structure that is formed by stacking of graphene oxide sheets. The GO sheets with highly sharp edges are folded onto themselves resulting in a wrinkled surface.

To investigate the size and thickness of GO sheets in detail, AFM was performed in tapping mode. This mode was used in order to prevent dislocation of GO sheets on surface. Figure 5 shows the results of the AFM image and the height profile of GO. Samples were prepared from dispersion of GO in ethanol and then dried on mica substrate. As it can be seen, GO sheets, similar to SEM images have highly sharp edges. The AFM images revealed that GO sheets have lateral dimensions of about one hundred nanometers to several micrometers with a thickness of approximately 1.5 nm indicating prepared GO sheets are two-layer.

In order to investigate the antibacterial properties of the synthesized GO, antibacterial test of sample was performed against *E. coli* bacteria as Gram-negative model by CFU (Colony Forming Count) method. For this purpose *E. coli* PTCC 1399 was used as bacterial agent.

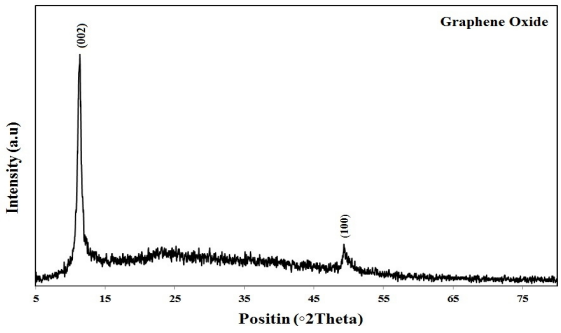

Figure 1. X-Ray pattern of the synthesized GO (CoKα)

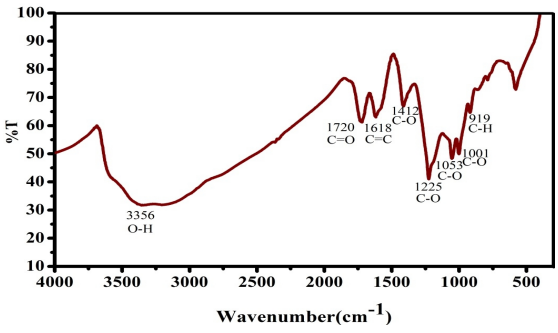

Figure 2. FT-IR spectrum of the synthesized GO

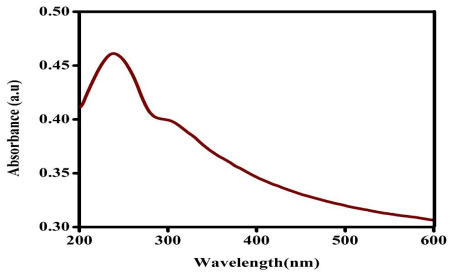

Figure 3. UV–vis absorption spectrum of GO

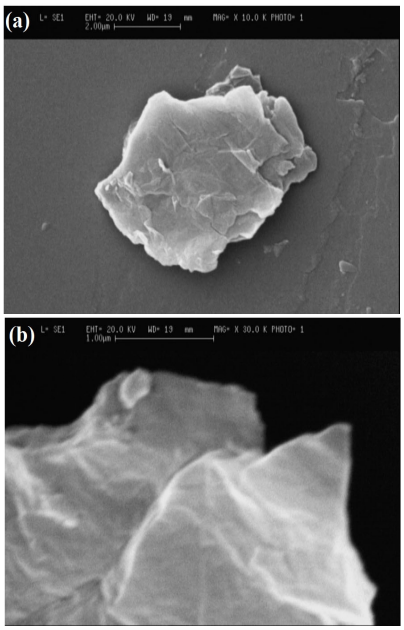

Figure 4. SEM images of GO sheets at (a) low-magnification and (b) high-magnification

Before each microbiological experiment, all the used lab wares, plates and nutrient agar were sterilized by autoclaving at 120°C for 15 min. For the antibacterial test, all the test tubes contained 9 ml growth medium were seeded with fresh *E. coli* at a concentration of 105 colony forming units per ml (cfu/ml). 0.03g GO was added to each tube and a blank sample was prepared at the same condition without GO as a control. Then the tubes were incubated at 37°C for 12 h in a shaker incubator. After incubation, media were diluted 10^{-3}, 10^{-4} and 10^{-5} times. Afterward, 50 µL of each bacterial suspension (blank and containing GO) was cultured on a nutrient agar plate and incubated at 37°C for 12 h to count the surviving bacterial colonies. Counting of colonies was performed by Sana SL-902 and the reported data are the average value of three separate similar trials. Nutrient agar plate after incubation at 37°C for 12 h can be seen in Figure 6. The results

revealed that the antibacterial ability of the sample is about 80%.

According to AFM and SEM images, graphene oxide sheets have sharp edges which can cause degradation of cell membrane of bacteria and finally missing whole of its membrane. In fact, these materials cause the bacteria death based on a three-step antibacterial mechanism via membrane and oxidation stress. Based on this mechanism, deposition of bacteria cell on the graphene oxide could cause initial degradation of the cells due to direct contact with GO sheets and creation of membrane stress. Then, the ultimate degradation is created through glutathione oxidization, which is independent of Reactive Oxygen Species (ROS) production, resulted in complete degradation of bacteria as reported by others [24-26].

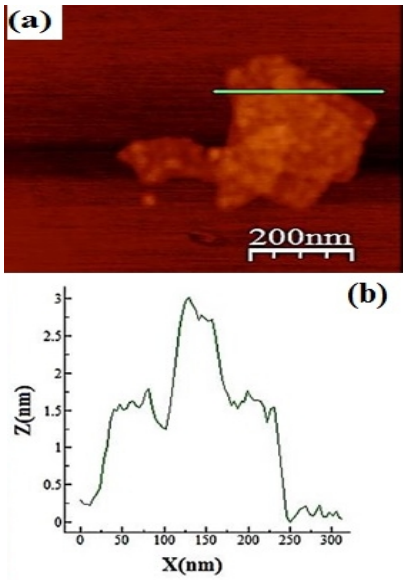

Figure 5. AFM images of GO sheets: (a) topography and (b) height profile

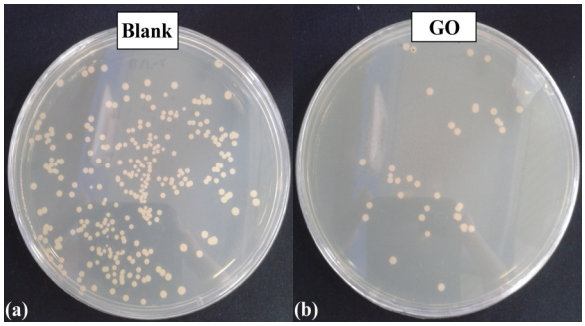

Figure 6. Nutrient agar plate after incubation at 37°C for 12 h for the detection of the inhibitory effect of graphene oxide on *E.coli*; a) blank and b) graphene containing medium

4. CONCLUSION

Graphene oxide was successfully synthesized by modified Hummers' method in short time. XRD results showed that GO sheets were synthesized without any impurity. XRD result was confirmed by FTIR in which the absorption bands from aromatic C=C, C=O indicated formation of GO. In addition, we have found a strong absorption peak at 238 nm in UV-vis spectrum of GO which attributed to C-C bonds. The SEM analysis revealed that GO sheets with highly sharp edges were formed which can improve its antibacterial activity. Size and thickness of these sheets were measured by AFM results. We have found that synthesized GO sheets were in two-layer. Finally, antibacterial activity of sheets were investigated against *E. coli* bacteria and a suitable mechanism was presented for high antibacterial activity of GO sheets.

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E. Badiei[a], P. Sangpour[a], M. Bagheri[a], M. Pazouki[b]

[a] *Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center, Karaj, Iran*
[b] *Department of Energy, Materials and Energy Research Center, Karaj, Iran*

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چکیده

اکسیدگرافن بوسیله اکسیداسیون پودر گرافیت از طریق روش اصلاح شده هامر در زمان کوتاه سنتز شد و خواص ضد میکروبی آن بررسی شد. تکنیکهای مختلفی برای مشخصه یابی اکسیدگرافن سنتز شده بکارگرفته شد. پراش پرتوی ایکس (XRD)، میکروسکوپ الکترونی روبشی(SEM) و میکروسکوپ نیروی اتمی (AFM) به ترتیب برای بررسی ساختار بلوری، مورفولوژی، توپوگرافی و پروفایل ضخامت اکسیدگرافن سنتز شده مورد استفاده قرارگرفت. تبدیل فوریه طیف مادون قرمز (FTIR) به منظور بررسی ساختار شیمیایی و خلوص نمونه انجام گرفت. خواص نوری بوسیله طیف سنجی UV-Vis بررسی شد. فعالیت ضدمیکروبی اکسیدگرافن در مقابل باکتری اشرشیاکلی با استفاده از روش واحد کلونی ساز (CFU) تخمین زده شد. نتایج نشان داد که نمونه خاصیت ضدمیکربی بالایی در مقابل این باکتری دارد.

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