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Research paper

Structural, electronic structure and antibacterial properties of graphene-oxide nano-sheets

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ABSTRACT

Correlation between the structural/electronic structure properties and bio-activity of graphene-based materials need to be thoroughly evaluated before their commercial implementation in the health and environment precincts. To better investigate the local hybridization of sp^2/sp^3 orbitals of the functional groups of graphene-oxide (GO) and their execution in the antimicrobial mechanism, we exemplify the antibacterial activity of GO sheets towards the Escherichia coli bacteria (*E. coli*) by applying the field-emission scanning electron microscopy (FESEM), near edge X-ray absorption fine structure (NEXAFS) and scanning transmission X-ray microscope (STXM) techniques. C K-edge and O K-edge NEXAFS spectra have revealed lesser sp^2 carbon atoms in the aromatic ring and attachment of functional oxygen groups at GO sheets. Entrapment of *E. coli* bacteria by GO sheets is evidenced by FESEM investigations and has also been corroborated by nano-scale imaging of bacteria using the STXM. Spectroscopy evidence of functional oxygen moieties with GO sheets and physiochemical entrapment of *E. coli* bacteria have assisted us to elaborate the mechanism of cellular oxidative stress-induced disruption of bacterial membrane.

1. Introduction

Over the last decade, synthesis and characterization of graphene-based materials (i.e., 2D graphene sheets, graphene oxide (GO) and reduced graphene oxide (rGO)) have evoked much attention because of their diversifying electronic, thermal, optical, mechanical and magnetic properties [1–5]. The aromatic-structure of GO is often assumed to be a graphene sheet but the diverse sp^2 hybridization of carbon atoms and attachment of oxygen species; hydroxyl, carboxyl or epoxy groups, make the GO exciting candidate for the catalyst and biological/cellular applications [6–9]. Although GO is an insulator, the concept of zero-band gap energy is possible via complete removal of C—O bonds and/or tuning the content of fourfold sp^3 and threefold sp^2 states of carbon atoms via controlled oxidation-reduction processes [10].

More recently, besides the large area synthesis of GO/rGO and their elementary characterization, much attention has been paid to explore the GO nano-structures as an antibacterial agent [11–20]. This is because of the fact that the sharp edges of GO can amalgamate with the bacteria membranes and may obey the synergy of physical and chemical effects [11–14]. Additionally, the smaller sized GO sheets have been reported for the vigorous rupturing of the bacterial membrane via physicochemical processes [15]. Experimental evidence of the destruction of *E. coli* bacteria, by GO nano-sheets, has been demonstrated by transmission electron microscopy (TEM) and theoretical simulations [12]. Likewise, field-emission scanning electron microscopy (FESEM) has also been extensively applied to elucidate the bursting of the outer cell membrane by GO nano-structures [13–20]. However, the detailed dynamic process and underlying molecular mechanism of bacterial membrane devastation by the GO nano-sheets remain unclear.

Besides the electron microscopy evidence of bacterial killing by GO, Nanda et al. [16] have reported the spectroscopy evidence of bacteria cell damage by applying the Raman spectroscopy. In correspondence to the electron microscope and Raman spectroscopy techniques, the soft X-ray based scanning transmission X-ray microscope (STXM) can also be the choice for antimicrobial investigations of GO. This is because of the fact that this technique operates at a low energy of X-ray photons and moderate vacuum conditions. STXM is known as the class of spectroscopy technique which not only has imaging capability with a spatial resolution of

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few tens of nanometers but also offers chemical characterization of constituent elements in the biological, polymer and solid state samples [21–26]. There are a few reports available on the application of STXM towards electronic structure and morphology investigations of GO/rGO [23,24]. The STXM investigations may offer direct evidence of GO interaction induced cellular damage of bacterial cells because of its nano-scale space resolution; which, however, has not been explicitly reported. This may be because of the delicate sample preparation of bacterial cells on Si_3N_4 membrane and limited synchrotron facilities. In this manuscript, we provide structural, morphological and local electronic structure characterization of GO by XRD, Raman, TEM, NEXAFS, FESEM, and STXM. The clear evidence of *E. coli* bacteria entrapment is accomplished by FESEM along with the STXM images.

2. Experimental

2.1. Synthesis of GO

A sonication based approach is applied to prepare the GO sheets by keeping in mind that the sonication may provide vigorous reactions among the graphite flakes (2 g, 99.99% pure from Alfa Aesar), concentrated H_2SO_4 (50 mL, 98% Sigma Aldrich) and $KMnO_4$ (3 g, 99% Sigma Aldrich) [27]. By considering the Hummer's method [28], addition of H_2SO_4 into graphite flakes was done in the round bottom flask which was placed in the ice-vessel. $KMnO_4$ was slowly added into the above mixture while maintaining the reaction temperature ~10 °C–12 °C. This step is presented in Fig. 1(a). The solution was subjected to 30 min stirring and 10 min sonication for several times and is presented in Fig. 1(b). The reaction was quenched by adding the 250 mL DI water followed by sonication for 2 h. The quenching step is shown in Fig. 1(c). After this stage, the exfoliated graphite layers were treated with H_2O_2 for their oxidation [27,28]. As shown in Fig. 1(d), for synthesizing the GO sheets, 20 mL H_2O_2 (40%, Alfa Aesar) was added into the exfoliated graphite solution and then stirred for 30 min. The product was washed with 1 M HCl and DI water for several times and then dried in air at room temperature. The final powdered form of the as prepared GO is shown in Fig. 1(e). Fig. 1(f) shows the schematic of separated GO sheets structures, originated from the exfoliated graphite under the vigorous oxidation conditions. Attachment of various oxygen moieties and formation of carbon related defects are expected in the GO sheets under the exfoliation and oxidation reactions.

2.2. Characterization of samples

XRD measurements were performed by applying the Rigaku (DMax2500) diffractometer, operated at 40 kV accelerating voltage and 200 mA tube current. The radiation produced from the Cu target was *Cu* Kα of a wavelength of 1.5418 Å. The Raman spectra were collected by using the Renishaw: InVia Raman Microscope with Nd:YAG (neodymium-doped yttrium aluminum garnet) laser, which produces 532 nm wavelength of the photon beam. Transmission electron microscopy (TEM) measurements were performed using the FEI (Titan:80–300) transmission electron

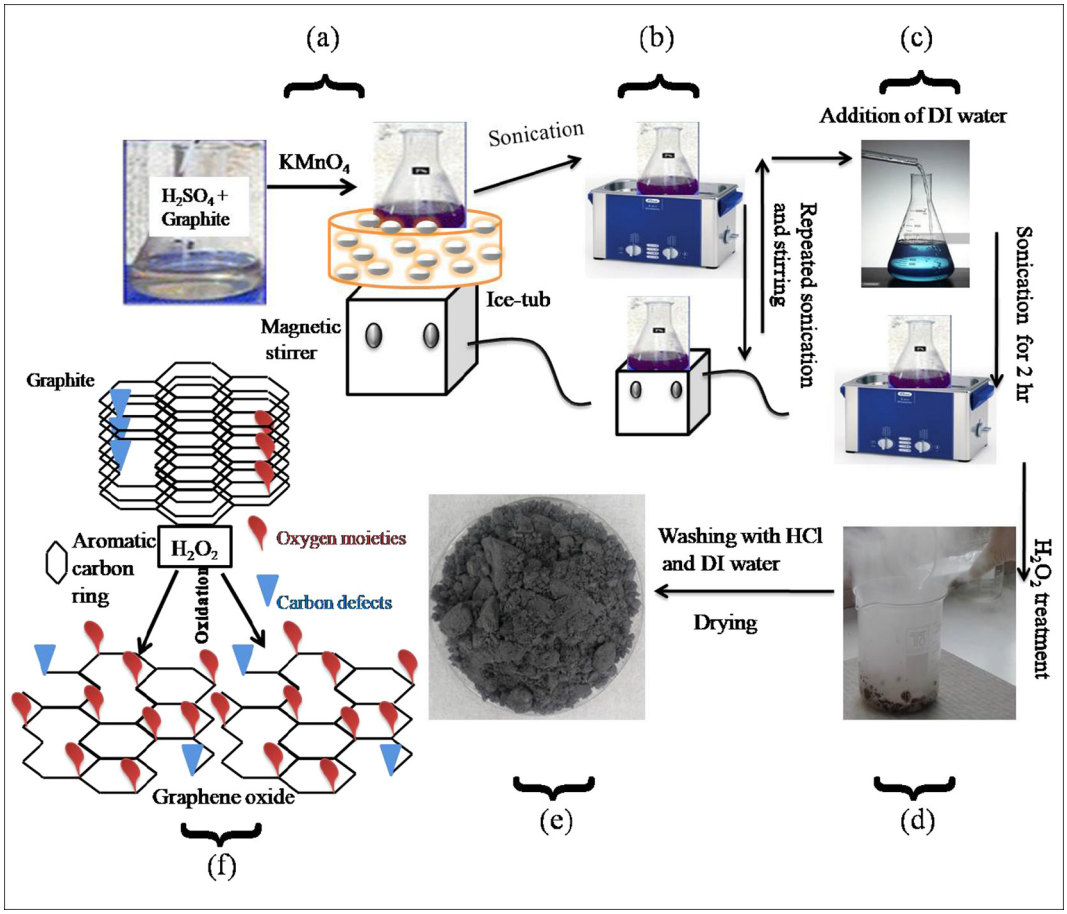

Fig. 1. (a–d) show the addition of constituents chemicals (i.e., graphite powders, H_2SO_4 and $KMnO_4$), ice-bath, magnetic stirring, sonication, quenching of reaction via addition of DI water and oxidation steps in the synthesis of GO. (e) Shows the dried GO powder. (f) Shows the schematic of carbon defects, broken bonds in the aromatic ring and attachment of functional oxygen groups in the GO sheets.