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In situ sonochemical reduction and direct functionalization of graphene oxide: A robust approach with thermal and biomedical applications

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ABSTRACT

The rapid, robust, scalable and non-hazardous sonochemical approach for *in situ* reduction and direct functionalization of graphene oxide has been developed for non-toxic biomedical applications. The graphene oxide (GrO) was directly functionalized with tryptamine (TA) without using any hazardous acylating and coupling reagents. The reaction was completed within 20 min. An impact of ultrasound was inferred for a direct functionalization with other conventional methods. The evolved electronic states were confirmed with near edge X-ray absorption fine structure (NEXAFS). The direct covalent functionalization and formation of f-(TA) GrO was proven with FTIR, ^{13}C solid state NMR, XPS, XRD, Raman, HRTEM, AFM and TGA. The total percentage weight loss in TGA confirms an enhanced thermal stability of f-(TA) GrO. The f-(TA) GrO was further explored for an investigation of *in vitro* antimicrobial activity to ensure the health and environmental safety. An outstanding antibacterial activity of f-(TA) GrO was found against gram positive *Staphylococcus aureus* at MIC 128 mg mL^{-1}. It confirms a suitability of f-(TA) GrO for thermally stable antibacterial coating. The f-(TA) GrO showed 39.14–48.9% antioxidant activities, evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The inherent cytotoxicity of f-(TA) GrO was evaluated with SRB assay to living cells, MCF-7 and Vero. The estimated cell viabilities were >80% upon addition of f-(TA) GrO over a wide concentration range of 10–80 μg mL^{-1}. The high cytocompatibility of f-(TA) GrO confirms the low toxicity and an excellent biocompatibility. The morphological effect on Vero cell line, evidently confirmed the biocompatibility of f-(TA) GrO. Therefore, f-(TA) GrO was emerged as an advanced functional biomaterial for thermal and biomedical applications.

1. Introduction

Currently there is a much emphasis on functionalization of GrO not only to broaden its application abilities but also to induce the structural abilities for better SAR (structure activity relationship). Further, it becomes an advantageous and modern route when the GrO is functionalized for nontoxic applications, which have been adequately focused in this research work. In spirit of above, the several experimental routes are available for the functionalization but unfortunately, many of them involved the use of hazardous chemicals and non-greener methods. Thus, we have developed the greener sonochemical approach to way out for f-(TA) GrO preparation. Hence, the main emphasis of our work has been to develop the new material with novel routes of direct functionalization for the biomedical applications. Since an inception, the carbon allotropes have been the vital chemical materials for an improvement of human civilization through their extraordinary properties and applications mainly in the field of electronics, telecommunication for conversion of sound waves to an electrical audio signal, medical sciences, mechanical, thermal, supercapacitor and large surface area applications [1–9]. Similarly, the flat, hexagonal, two dimensional graphene has astonishing properties due to their higher surface area, lower weight, strength and stability. Thus, it could be utilized in several biomedical applications like scaffolds in tissue engineering, targeted dug delivery, antimicrobial and biocompatible coating and others. However, an exploitation of graphene for several biomedical applications is a challenge due to its stability and insolubility in organic solvents. Also easily available graphite (Gt) to be used as the starting material for chemical modification of graphene has the same problem. Therefore, due to the presence of surplus oxygen containing functionalities, graphene oxide (GrO), provides an alternative for chemical modification and acts as a precursor for wet chemical functionalization [10–12]. Hence, it is necessary to understand the properties and applications of GrO for the development of advanced functional materials. The wet chemical methods are employed to understand

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the chemistry of graphene for newer applications [13–24]. In addition, the various approaches of functionalization and surface modification of GrO attempted for new applications of chemically modified graphene in almost all the fields [25–34]. The sonochemical approach for direct functionalization of GrO was initiated by S. S. Maktedar and S. S. Mehetre et al. [35,36]. Thus, the science and mechanism of functionalization of GrO attracts global attention but limited research work is available on the robust sonochemical method for direct functionalization of GrO without using any hazardous reagents [35,36]. The direct functionalization of GrO was supposed to be more explored for the robust, scalable synthesis of functionalized GrO for biomedical applications. Due to the synergistic impact of various functionalities present over the surface of GrO, the functionalized GrO has been suitable for thermal, antimicrobial, antioxidant and biomedical applications [37–45]. The present studies report the functionalization of GrO with TA. A formation of f-(TA) GrO was achieved with sonochemical method within 20 min, without using any hazardous acylating and coupling reagent. The direct functionalization of GrO is possible only due to an effect of ultrasound irradiation where both the physical and chemical effects of an ultrasound arise out of an acoustic cavitation. The localized spots, generated during the bubble collapse within the liquid, have temperature of ~5000 K and pressure of hundreds of bars, which was responsible to induce the direct covalent functionalization of GrO with TA. The as prepared, f-(TA) GrO was thermally stable and found to be suitable for biomedical applications. The outstanding antibacterial activity of f-(TA) GrO against gram positive *Staphylococcus aureus,* confirms the suitability of f-(TA) GrO for thermally stable antibacterial coating. Additionally, the cytocompatibility on MCF-7 and Vero cell lines confirms the suitability of f-(TA) GrO, as a biomaterial for thermally stable biocompatible coating.

2. Experimental section

2.1. Materials

Graphite flakes, concentrated sulphuric acid (95–98%), phosphoric acid (>85 wt.% in H_2O), concentrated hydrogen chloride, Tryptamine (98%), were procured from Sigma-Aldrich Co. 30% hydrogen peroxide was provided by S.D Fine chemicals. Rankem supplied analytical grade potassium permanganate. Absolute alcohol was obtained from Scvuksmandli Ltd. India. All the chemicals were used without further purification.

2.2. Synthesis of graphene oxide

Initially, graphite oxide (GtO) was prepared with an efficient oxidation process [10] where the graphite flakes were oxidized with mixture of conc. H_2SO_4/H_3PO_4 in 9:1 ratio (180:20 mL). The mixture was poured in 500 mL RB flask containing a mixture of graphite flakes (1.5 g, 1 wt equiv) and $KMnO_4$ (9.0 g, 6 wt equiv), the reaction produced 35–40 °C temperature. The exotherm produced was cooled at RT then, oil bath was heated over magnetic stirrer with hot plate to maintain 50 °C. The RB flask holding reaction mixture was kept inside oil bath and heated to 50 °C with constant stirring for 12 h. For workup, the reaction was cooled to RT and poured onto ice cold water (~200 mL) with 30% H_2O_2 (1.5 mL). The reaction mixture was centrifuged at 6000 rpm for 0.5 h, to separate larger aggregates. The exhaustive washing of remaining solid was done for two times, for each washing in succession with 200 mL of water, 150 mL of 30% HCl, and 100 mL of ethanol. For each wash the filtrate is centrifuged at 6000 rpm for 0.5 h and the supernatant decanted away. After multiple-wash process the remaining material was coagulated with 100 mL of ether. The brownish coloured solid cake obtained as sediment and it was vacuum-dried overnight at 50 °C, and reported as oxidized GtO [11,12]. The GtO was further exfoliated in ethanol with an ultrasound for 3 h, for graphene oxide (GrO) preparation.

2.3. Direct functionalization of graphene oxide

The 1 mg/mL concentration of GrO in ethanol was used for exfoliation, to prepare the homogeneous dispersion. The 0.05 g GtO in 50 mL ethanol was subjected to an intense ultrasonic treatment for 3 h. After the exfoliation, the raised temperature was measured to be 45 °C. A formation of homogeneous light brown coloured dispersion confirmed a GtO exfoliation and formation of GrO. The ultrasound enhanced a chemical reactivity of GrO and a kinetically active homogeneous GrO dispersion was subjected to 0.25 g TA, and within 20 min, the reaction is completed after TA addition to GrO. It indicates a formation of blackish dark brown coloured agglomerate. The f-(TA) GrO was washed batch wise thrice with 20 mL distilled water and ethanol respectively and centrifuged at 6000 rpm for 0.5 h, for each washing. After washing a formation blackish coloured compound was observed, it suggested an *in situ* reduction of GrO and also noted as a direct functionalization. The functional material was dried at 50 °C under vacuum for 24 h. The structural characterization with high ends analytical techniques depicted f-(TA) GrO formation mechanism of direct amidation through sonochemical reaction (Scheme 1).

2.4. Ultrasonic superiority over conventional methods

The superiority of ultrasound process was inferred by performing the same reaction of TA with GrO under conventional reflux condition in EtOH and H_2O at 95 and 85 °C respectively. The 1 mg mL^{-1} dispersion of GrO was prepared by adding 0.05 g GtO into 50 mL EtOH that was subjected to ultrasonic treatment for 3 h, for preparation GrO dispersion. To this dispersion, the 0.25 g TA was added and refluxed in EtOH at 95 °C with constant stirring for 12 h. After this, the reaction is allowed to cool at R.T. and the formation of black coloured agglomerates was observed. The obtained product was washed batch wise thrice with 20 mL EtOH and centrifuged at 6000 rpm for 0.5 h, for each washing. The product was dried at 50 °C under vacuum for 24 h, which did not show the signature of f-(TA) GrO due to an absence of amide peak in FTIR (†ESI supplementary information Fig. S1). It inferred that the TA is not covalently attached with GrO on lines of direct functionalization except the reduction. It inferred that the conventional reflux in EtOH at 95 °C was not suitable for direct amidation. However, it was confirmed that the direct GrO functionalization is attained only due to an effect of ultrasound irradiation. Both the physical and chemical effects of ultrasound arise out of acoustic cavitation. Mechanistically, the localized spots, generated during the bubble collapse within the liquid, raised the temperature to ~5000 K and pressure of hundreds of bars. Such changes are responsible to induce the direct covalent functionalization of GrO with TA which is not possible with conventional method. Additionally, an impact of direct functionalization was observed in different solvent. The same reaction was performed in water. However, no signature of amide peak was obtained in conventional water reflux (†ESI Fig. S2). It inferred a suitability of EtOH as a solvent for direct GrO functionalization. Hence, our proposed mechanism for direct GrO functionalization seems to be feasible (Scheme 1).

2.5. Analysis and characterization

The synthesized compounds were prepared using REMI 1MLH magnetic stirrer with hot plate. The Oscar ultrasonic Microclean-103 model operated at 30 kHz frequency was used for the all the