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Graphene oxide based magnetic nanocomposites for efficient treatment of breast cancer

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ABSTRACT

The present work reports a simple one step synthesis of nanoscale graphene oxide magnetic composites (GO–IO) using ferrofluid (GO–IOF). The obtained GO–IO were compared with GO–IO obtained from *in situ* (GO–IOI) methods. Anastrozole (ANS) was loaded on the GO–IOI and GO–IOF *via* simple stirring method to form GO–IOA and GO–IOFA respectively. These GO–IO prepared by two techniques were characterized using spectroscopic techniques and vibrating sample magnetometer (VSM) analysis. Particle size and potential were measured using Malvern Zetasizer. Scanning electron microscopy (SEM) was used for studying the surface morphology of GO–IO, and in addition to this elemental analysis was also performed for confirming the presence of iron. The cell viability assay was carried out using the MCF-7 cell line. It revealed that GO–IOFA had reasonably high cytotoxicity (49.7%) compared to GO (13.1%), ANS (16.6), GO–IOI (13%), GO–IOF (13.6) and GO–IOIA (18.34%). Both, GO–IOIA and GO–IOFA showed improved cytotoxicity when compared with pure ANS. GO–IOF were found to exhibit superior magnetic activity due to higher iron content along with smaller particle size and higher loading efficiency compared to GO–IOI. The overall effect suggests that GO–IO can be utilized as efficient carriers for the loading of chemotherapeutic agents.

1. Introduction

Development of new drug delivery system has always been an area of interest for formulation developers. Recently, nanomaterials-based drug carriers have made a huge amount of interest owing to their unique properties and significant research is being conducted for interfacing these nanomaterials with a drug delivery system to explore nanotechnology in medicine. The unique properties of these nanomaterials include high and efficient drug loading, targeted delivery and controlled release of drugs and increased stability to name a few [1–5].

Graphene based nanomaterials have recently attracted attention for its use in drug delivery. 'Graphene' is the name assigned to a two-dimensional sheet of sp^2-hybridized carbon. It consists of a single-atom-thick planar sheet and possesses an unusual crystal and electronic tones. The aspect of graphene that is of greatest interest at the present time is its oxidized counterpart, graphene oxide (GO). GO has large p conjugated structure, which can form p–p stacking interaction with aromatic drug. Apart from p–p stacking interaction, presence of abundant functional groups such as carboxyl, hydroxyl, and epoxide functional groups on the edge, top, and bottom surfaces of GO further enhances its interaction with aromatic groups of drug. The interaction of these functional groups to aromatic groups of drug results in the formation of strong hydrogen bond which forms the basis of drug loading on GO and related structures. On the basis of such interaction, researchers reported the loading of various drugs including anticancer drugs on GO for the treatment of tumors, such as doxorubicin, camptothecin, ibuprofen and 5-fluorouracil through non-covalent physisorption (p–p stacking, van der Waals interaction or hydrogen bond) [6–14].

The targeting efficiency of GO based nanocarriers can be enhanced by using GO magnetic composites using iron oxide nanoparticles. Magnetic iron oxide (magnetite Fe_3O_4 or maghemite gamma-Fe_2O_3) nanoparticles exhibit super-paramagnetic nature, but suffer the problem of aggregation which limits their magnetic properties and also alters its stability. This problem may be solved by using GO based magnetic composites which will minimize the clumping/aggregation of magnetic nanoparticles thereby increasing the stability as well as preserving their unique magnetic property [15]. Various chemistry based processing methods have been reported for fabrication of graphene/magnetite nanocomposites (GO–IO) which includes *in situ* formation [16], solvothermal method [17], hydrothermal method [18], covalent bonding method [19], and electrochemical method [20]. These GO–IO possess unique properties including generation of heat in alternating magnetic fields or an ability to be pointed to a specific tissue or organ

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under influence of external magnetic field. The power generation of heat under alternating magnetic field can be used in cancer therapy.

The present investigation explores the different advantages of GO and magnetic nanoparticles for fabrication of an efficient platform for the delivery of anastrozole (ANS). This study demonstrates that GO based GO–IO can be used as efficient nanocarriers for the loading and delivery of water insoluble anticancer drug. In the present study, a novel one step method for the synthesis of GO–IO has been developed. The study involves the comparative study of GO–IO fabricated using *in situ* (GO–IOI) method and ferrofluid (GO–IOF) conjugation method. Use of ferrofluid for fabricating GO–IO is simple, efficient and less time consuming. To the best of our knowledge, we are the first who utilized ferrofluid for fabricating GO–IO and successfully loaded ANS on prepared GO–IO for treatment of breast cancer.

2. Experimental

2.1. Materials

Anastrozole (ANS) was gifted by Natco Pharmaceuticals Pvt. Ltd., Mumbai, Maharashtra. Ferrofluid was generously gifted by Ferro-Tech Pvt. Ltd., Bedford, USA. Graphite was kindly provided by Asbury Carbons, New Jersey. Sulfuric acid and hydrochloric acid were purchased from Merck Specialities Pvt. Ltd., Mumbai, and potassium permanganate and trisodium citrate were purchased from Loba Chemie Chemicals Pvt. Ltd, Mumbai. Hydrogen peroxide was purchased from RFCL Limited, Mumbai. Ferric chloride, ferrous sulfate and ammonia hydroxide were purchased from Himedia Lab Pvt. Ltd, Mumbai. All other chemicals and reagents were of analytical grade.

2.2. Synthesis of GO

GO was synthesized from graphite flakes by an oxidation process using modified Hummers method [21,22]. Graphite flakes (2 g) were dissolved in 50 mL of conc. H_2SO_4 by maintaining the temperature at 0 °C with vigorously stirring for 3 h. To this solution, $KMnO_4$ (6 g) was added slowly while keeping the temperature below 15 °C. The $KMnO_4$ was added slowly for 2 h, after which the mixture was kept for stirring for further 1 h. The temperature was slowly raised to 35 °C, keeping the mixture under stirring until the mixture becomes pasty brownish; which was accompanied by an increase in viscosity of the mixture. The pasty brownish mixture was diluted with deionized water, followed by the slow addition of H_2O_2 (7 mL) to arrest the oxidation process which alters the color of mixture from dark brown to bright yellow, indicating the high level of oxidation of graphite. The obtained mixture was centrifuged at 25 000 rpm to collect the pellets of GO. Recovery of settled pellets was performed by simple decantation of supernatant. The obtained GO was washed three times with an HCl aqueous solution (1 M) followed by repeated rinsing with deionized water until the pH of GO dispersion reached 4–5 and dried at 35 °C.

2.3. Synthesis of GO–IOI (in situ synthesis)

The *in situ* synthesis of GO–IO (GO–IOI) was performed according to previously described method with slight modification [23]. Initially, GO (1.5 g) was dispersed in deionized water (100 mL) by stirring. The dispersion was exfoliated by sonication to obtain evenly distributed GO sheets. The exfoliation leads to thickening of GO dispersion which confirms the exfoliation of GO. The exfoliated GO dispersion was mixed in three necked flask followed by addition of a mixture of $FeCl_3$ (0.33 g) and $FeSO_4$ (0.38 g) in 100 mL deionized water. The resultant mixture was heated up to 80 °C with continuous stirring and finally the pH of the mixture was adjusted to 12 with the addition of 25% ammonia solution. After addition of ammonia solution, tri-sodium citrate (1 g) was added to the mixture and kept at 95 °C for 1 h. The resultant black color suspension was centrifuged at 15 000 rpm. The obtained GO–IOI were washed with deionized water three times, separated using magnet and finally dried at 60 °C.

2.4. Synthesis of GO–IOF (ferrofluid technique)

The GO–IO was prepared using ferrofluid (GO–IOF). Initially GO (100 mg) was dispersed in deionized water (10 mL) with stirring. The dispersion was exfoliated by sonicating the dispersion for 3 h, which led to the conversion of dispersion into viscous solution. The viscosity was decreased by the addition of 5 mL of water and dispersion was further sonicated for 1 h for complete exfoliation. After exfoliation of GO dispersion, 0.2 mL of ferrofluid was added to the GO dispersion. The mixture was sonicated for 30 min followed by stirring for 3 h. Finally, the dispersion was centrifuged at 15 000 rpm to obtain GO–IOF. It was washed with deionized water and poured in silica crucible for drying at room temperature.

2.5. Loading of ANS

The loading of ANS on synthesized GO–IO was performed using a passive loading method. ANS was dissolved in acetone at a concentration of 10 mg/mL. GO–IOI and GO–IOF were dispersed in deionized water and sonicated for 30 min, separately. After sonication, both GO–IO dispersions were mixed into drug solution with slow stirring at room temperature for 24 h. The drug solution was then centrifuged at 16 000 rpm for 30 min to obtain ANS loaded GO–IO *i.e.* GO–IOIA and GO–IOFA. Obtained GO–IO pellets were washed three times with deionized water. The concentration of ANS in the supernatant was measured using standard curve of ANS.

The loading efficiency of GO–IO was calculated using the following formula

$$\text{Loading efficiency } (\%\text{LE}) = \frac{(\text{Dtotal} - \text{Dsupernatant})}{\text{Dtotal}} * 100.$$

The drug loading ability of GO–IO was calculated using the following formula

$$\text{Drug loading ability } (\%\text{DL}) = \frac{\text{DGO–IO}}{\text{WGO–IO}} * 100.$$

Where, Dtotal = total amount of ANS taken; Dsupernatant = amount of ANS present in supernatant; DGO–IO = amount of ANS on loaded GO–IO; WGO–IO = total amount of GO–IO taken.

2.6. Characterization

2.6.1. Spectroscopic analysis

Ultraviolet–visible (UV–vis) absorption spectra of plain GO, IO, GO–IOI and GO–IOF were measured with a spectrophotometer (UV-1800 PC, Shimadzu, Japan), where the light path length was 1 cm. FTIR spectra of samples were recorded on IR spectrometer (Fourier Transform Infrared Spectroscopy–IR Affinity 1700, Shimadzu, Japan) at a resolution of 4 cm^{-1} with a maximum of 100 scans at frequencies ranging from 400 to 4000 cm^{-1}. For getting the IR spectra, the samples were thoroughly mixed with KBr in a ratio of 1:100 in the crucible. The mixture was then filled in powder sampling cells for spectral analysis.

2.6.2. Particle size and zeta potential

Particle size analysis of GO, GO–IOI and GO–IOF was carried out using a Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK) equipped with a 4.0 mW internal laser, which works on the principle of dynamic light scattering. The samples were diluted with double-distilled water in a disposable polystyrene cell prior to measurements to obtain dispersion with a concentration below 0.5 mg/mL (to avoid multiple scattering). All measurements were performed at 25 °C, at a