Materials Research Express

PAPER

Enhanced drug efficiency of doped ZnO–GO (graphene oxide) nanocomposites, a new gateway in drug delivery systems (DDSs)

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Keywords: zinc oxide, graphene, chemical deposition, drug delivery

Abstract

Graphene oxide (GO) nanosheets were doped to zinc oxide (ZnO) nanoparticles using a facile chemical deposition method. X-ray diffraction (XRD) confirmed the presence of hexagonal structure and increased peaks broadening upon doping. Undoped and ZnO doped GO sheets showed morphology like nanoparticles, nanorods and flakes were observed under transmission and field emission electron microscopies respectively. An increase in absorption was observed in absorption spectra upon doping recorded via UV-visible spectroscopy. The hydrogen bonding between functional groups of GO and ZnO is responsible for limiting maximum drug loading efficiency. GO doped ZnO has higher drug loading efficiency of about 89% compared to ZnO (82%) and this trend reverse in drug releasing process. This study will provide an efficient design of the drug delivery system for dissolution enhancement according to the required drug release.

1. Introduction

Cancer, a complicated disease with hundred different types involves abnormal cell growth. World health organization (WHO) described cancer deaths statistics comprised of 5-years analysis and reported 14.1 million newly diagnosed, 32.6 million existing patients and 8.2 million deaths by cancer worldwide in 2012 [1–4]. Cancer cure is a hard challenge for science and medical experts involving current methods as surgery, radiotherapy, chemotherapy, immunotherapy and photodynamic therapy [5–10]. Chemotherapy involves agents such as (doxorubicin, mitomycin, daunorubicin and capetabine) distribution inside body providing non-target tumor specificity resulting in adverse effects like alopecia, loss of digestive capacities, and immunosuppression [11–14].

Targeted cancer therapy and drug delivery systems (DDSs) having capability to differentiate between cancerous and healthy cells with minimum side effects are utilized to prevent non-target tumor specificity [15]. Drug delivery systems (DDSs) comprising of nanomedicine with nanocarriers permit drugs and medical therapies to play role in more convenient, safe and efficient way [11]. Noble metal (Ag and Au) and metal oxide nanoparticles (Fe3O4, NiO, CoO, ZnO and SnO2) are promising materials for drug delivery [16–18]. Among these, zinc oxide (ZnO) nanoparticles involvement in cancer therapy has received much attention. Recently, ZnO in various structures like nanorods, nanospheres and nanodiscs found non-toxic exhibiting antibacterial, antitumor and photocatalytic activity has been used in DDSs. These structures are also biocompatible, cost
effective and richness in structure can be considered as a potential candidate for drug delivery application [19, 20].

Graphene based nanocomposites having novel physical applications in various field, used as transparent conductive electrodes and is explored widely in biomedical [21–26] for drug loading and release with low toxicity and high biocompatibility in DDS [27–29]. Among carbon nanostructures, Graphene oxide (GO) is modern posing two-dimensional (2D) structure with one-atom thickness having four times large surface area relative to other nanomaterials used. Aromatic drugs (doxorubicin, camptothecin and paclitaxel) can be loaded to GO nanosheets with high capacity via π-π stacking and hydrophobic interactions. Furthermore, GO can be prepared economical in large amounts compared to inorganic nanomaterials. GO functionality imparts specific biological properties upon introduction with covalent and non-covalent surface coatings [30–34]. Both ZnO and GO are biocompatible and can demonstrate success in diverse drug delivery applications. Our work relies on analysis of GO/ZnO formation and its comparison with ZnO in anticancer drug loading and release at different pH values.

In present study, GO was prepared using modified Hummers procedure and utilized to synthesis GO/ZnO nanocomposite. These undoped and doped GO were applied on anti-cancer drug DOX (figure 1) to check its drug loading and release capacity.

2. Experimental details

2.1. Materials

Graphite flakes, hydrogen peroxide-H2O2 (30%) and potassium permanganate-KMnO4 (99.5%) were purchased from BDH (England). Hydrochloric acid (HCl) (37%) from Merk (USA) and sodium nitrate-NaNO3 (99.0%) from DUKSAN (South Korea) were used. Sulfuric acid-H2SO4 (99.99%) and dialysis tubing (2000 MWCO) were procured from Sigma–Aldrich (USA). Sodium hydroxide (NaOH), ethanol (C2H5OH) and zinc chloride (ZnCl2) were obtained from Riedel-de Haën (Germany), AnalRe (USA) and Panreac Química (Spain), respectively. Doxorubicin HCl (10 mg 5 ml \(^{-1}\)) (DOX) was obtained from Fresenius Kabi (USA). All reagents were of analytical standard and utilized as received.

2.2. Preparation of graphene oxide sheets

Modified Hummers method was used to obtain GO from refined graphite powder [35]. First of all, H2SO4 (24 ml) and NaNO3 (0.51 g) were mixed with graphite powder (0.5 g) under vigorous stirring for 30 min at 0 °C then, KMnO4 (3 g) was added in solution at 10 °C and stirred for 3 h till 35 °C. Deionized water (DIW) was gradually added and temperature maintained at 100 °C for 2 h. H2O2 (10 ml) was introduced into water containing mixture. Subsequently, fabricated material was washed several times with HCl (0.1 M) aqueous solution to remove metal ions. Finally, obtained product dried at 70 °C for 24 h using oven to achieve GO sheets.

2.3. Preparation of ZnO and GO/ZnO nanomaterial

Facile chemical deposition route was adopted to synthesize ZnO and GO/ZnO nanomaterials [36]. To prepare GO/ZnO, GO sheets (0.1 g) added in DIW (40 ml) were kept under ultra-sonication for 30 min to disperse GO sheets and sodium hydroxide (NaOH, 0.2 g) and zinc chloride (ZnCl2, 0.1364 g) were mixed in above GO sheets solution. The solution was stirred at 90 °C for 6 h and then was cooled to room temperature. The cooled solution was washed several times with ethanol and DIW to remove impurities. Refined product was obtained after
heating at 90 °C for 24 h to achieve GO/ZnO nanomaterial powder (figure 2). Same procedure was employed without addition of GO to fabricate ZnO nanoparticles.

2.4. Drug loading
Conjugation of nanomaterial to DOX was attained by forming dilution of DOX with DIW (100 μg ml⁻¹). Subsequently, ZnO (10 mg) were dispersed in DIW (2 ml) separately and then mixed in 1 ml of DOX dilution (100 μg ml⁻¹). Samples placed in ultra-sonication for 20 min for proper conjugation of medicine with GO nanomaterials. All samples were placed in dark spot at orbital shaker for 24 h and ultra-centrifugation was carried out at 15,000 rpm afterward. Finally, supernatant from all samples was characterized using UV–vis spectrophotometer at 480 nm absorbance to measure loading efficiency. Loading efficiency and capacity were calculated by equations [17] using UV–vis spectrophotometer at 480 nm absorbance to measure loading efficiency (figure 3).

\[
\text{% Loading Efficiency} = \frac{[\text{DOX}_i - \text{DOX}_f]}{\text{DOX}_i} \times 100
\]

\[
\text{Loading Capacity (μg mg}^{-1} = \frac{[\text{DOX}_i - \text{DOX}_f]}{\text{Drug}_{\text{carrier}}} \]

Whereas, DOX_i and DOX_f are the initial amount (μg) of desired drug and unbound drug in supernatant, respectively and Drug_{carrier} is total quantity (mg) of nanoparticles.

2.5. Drug release
For drug release, a standard curve was used to predict the concentration of DOX (figure 4(b)). The obtained pallet after centrifugation containing DOX loaded on GO/ZnO nanomaterials was placed in dialysis tubing after mixing with 3 ml buffer solution. Drug on loaded (GO/ZnO) nanomaterials start to release as dialysis tubing suspended in 100 ml of buffer solution beaker. Drug release was checked for 24 h in which, 3 ml aliquots were withdrawn each hour and analyzed through UV–vis spectrophotometer. Meanwhile, 3 ml fresh buffer was added again in beaker to maintain sink condition (figure 4(a)). Two different pH values (3 and 11) were used to analyze pH variation on drug-releasing properties. Drug Release percentage was calculated by the equation below [37]:

\[
\text{Drug Release %} = \frac{D_t}{D_o} \times 100
\]

Whereas, Dt is the drug released by nanomaterials at given interval while Do is the total amount of drug loaded on nanoparticles.
2.6. Characterization
Nanomaterials were characterized via x-ray diffraction (XRD), UV–vis spectroscopy (UV–vis) and field emission scanning electron microscopy (FE-SEM). For XRD, PANalytical Xpert Highscore PRO was utilized for crystallinity and phase detection for \(2\theta\) variation from 5°–70° with radiation source Cu K\(\alpha\) (\(\lambda \sim 0.154\) nm). Drug loading and release properties of nanohybrids (ZnO, GO/ZnO) DOX along with optical characteristics were examined through UV–vis-Genesys 10S spectrophotometer. Information regarding surface morphology and elemental composition were examined by JSM-6460LV FE-SEM using Cu-grid coupled with EDX spectrometer.

3. Results and discussion
Representation of XRD patterns of GO, ZnO and GO/ZnO are indicated in figure 5. The Braggs diffraction peaks from ZnO and GO doped ZnO samples, the observed planes are identical (100), (002), (101), (102), (110), (103) and (112) at different diffraction angles 31.5°, 34.3°, 36.1°, 47.3°, 56.3°, 62.5° and 67.7° respectively [38]. The observed peaks indicates crystalline behavior with hexagonal structure (JCPDS 01-079-0208). It was...
observed that crystallinity of ZnO decreased upon GO with slight shift towards lower angles. The calculated average crystallite sizes through Debye–Scherrer’ formula [39] are 33.56 nm (ZnO) and 18.41 nm (GO/ZnO). A significant decrease in crystallite size was observed due to incorporation of GO in ZnO sample compared to ZnO [38].

Morphological analysis of GO, ZnO and GO:ZnO, FE-SEM and HR-TEM was deployed to samples, the observed structure and size are illustrated in figure 6. FESEM layered structure of GO in figure 6(a) can be seen but more visible in HR-TEM image as shown in figure 6(b). FESEM of ZnO is demonstrating an integrated structure consisting of nanorods and flakes figures 6(c), (d) [40]. The merging π–π stacking wrinkled GO sheets with ZnO resulted slight conversion of nanorods to particles along with agglomeration of flakes present in ZnO structure can be seen in figure 6(d).
The chemical composition of GO, ZnO and GO conjugated ZnO was investigated using EDX (figure 7). The presence of O, C and Zn involvement in the product was confirmed by EDX analysis and observed ratios are described in inset of figure (a)–(c). The peaks of O and Zn are manifested to ZnO while, C content ascribed to GO nanosheets. Rest of the detected elemental peaks (K, Mn, Na) in the GO samples could be due to KMnO₄, NaOH were utilized during GO synthesis process. Cu coating of samples for FESEM analysis was found responsible for Cu peak in desired material.

Absorption spectra of synthesized materials GO, ZnO and doped ZnO was collected between absorption and wavelength which played vital role to monitor band gap energy probed via UV–vis spectrophotometer (figure 8). GO showed absorption edge band around 230 nm representing (C=C) with shoulder peak in GO around ~300 nm (C=O bonds) [41] and ZnO at 375 nm exhibits a characteristic absorption peak of wurzite hexagonal phase of ZnO [42]. The absorption increased between 245–400 nm with GO doping and this increase in absorption suggests about the bandgap variation of fabricated nanostructured. To attain energy bandgap from Tauc’s plot: firstly, Beer–Lambert relation was used to calculate absorption coefficient ($\alpha$), $\alpha = (2.303A)/d$, here A presents absorbance and d is path length of glass cuvette (10 mm) having colloidal solution. In next step, Tauc’s equation ($\alpha hv = B(hv - E_g)^n$) is used to obtain energy bandgap. In this equation, B, h and v are constant, Plank’s
constant and frequency of photon respectively, $E_g$ is bandgap energy and $n$ illustrates type of optical transition. Hence $n = 1/2$ is put in present case attributed to direct bandgap of prepared material and Tauc's plot ($\alpha h\nu^2$ versus $h\nu$) is depicted in (figure 8(b)). Finally, bandgap of prepared nanomaterials were calculated by Tauc transformation spectra and extrapolation used on X-axis. Energy bandgap of pure ZnO found 3.28 eV and with GO bandgap energy increased around 3.32 eV.

Drug loading efficiency (LE) and loading capacity (LC) of ZnO and GO/ZnO were evaluated using equations (1) and (2) and UV–vis absorption spectra (DOX loading spectra) as displayed in figure 9(a). A significant increase in LE of GO:ZnO was observed which could be due to existence probability of hydrogen bonding between –OH and –COOH groups of GO and –OH and –NH$_2$ groups of DOX [44]. LE calculated for ZnO and GO/ZnO was found 82 and 89% respectively. On the other hand, LC of control sample was $\sim$6.30 $\mu$g mg$^{-1}$ and doped ZnO has 7.47 $\mu$g mg$^{-1}$ which is low in comparison to LE (figure 9(b)) as we used very small weight ratio of DOX compared to nanoparticles. Drug release profile analyzed in different media is demonstrated in figures 9(c), (d). Cumulative drug release % for ZnO-DOX was observed highest (100%) in basic (pH = 11) along with lowest (46%) in acidic solution (pH = 3) and same trend found for GO/ZnO-DOX due to partial dissociation of the hydrogen-bonding interaction under acid and basic environments. Mostly, ZnO exhibited enhanced drug releasing efficiency in both media but basic media was found more suitable.

4. Conclusion

In present work, GO was fabricated by modified Hummers method and successfully incorporated in synthesized ZnO nanoparticles ($\sim$18.41 nm average crystallite size) using facile chemical deposition route. XRD pattern revealed that ZnO hexagonal lattice and decrease in crystallinity was found upon doping. The merging $\pi-\pi$ stacking wrinkled GO sheets with ZnO lattice resulted conversion of nanorods to flakes examined by FE-SEM. The calculated bandgap of doped ZnO is found 3.32 eV relative to ZnO (2.28 eV) is attributed to increase in absorption upon doping. Drug LE increased with doped GO content (89%) and ZnO possessed (82%)
corresponding to hydrogen bonding between GO and ZnO. The release rate showed reverse behaviour from LE as ZnO attained high rate about 46% (∼pH = 3) and 100% (∼pH = 11) in comparative estimate to GO/ZnO having 35% (∼pH = 3) and 41% (∼pH = 11). This drug loading and releasing approach and design would be beneficial and provide a new gateway with dissolution enhancement in drug delivery systems.

Acknowledgments

Authors are grateful to higher education commission (HEC), Pakistan for financial support through start research group (SRGP) project number 21-1669.

Conflict of interest

Authors confirm that this manuscript has no conflict of interest.

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