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Functionalized graphene oxides for drug loading, release and delivery of poorly water soluble anticancer drug: A comparative study

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ABSTRACT

In this work, the modification of graphene oxides (GOs) have been done with hydrophilic and biodegradable polymer, polyvinylpyrrolidone (PVP) and other excipient β −cyclodextrin (β-CD) through covalent functionalization for efficient loading and compatible release of sparingly water soluble aromatic anticancer drug SN-38 (7-ethyl-10-hydroxy camptothecin). The drug was loaded onto both GO-PVP and GO-β-CD through the π-π interactions.The release of drug from both the nanocarriers were analyzed in different pH medium of pH 7 (water, neutral medium), pH 5 (acidic buffer) and pH 12 (basic buffer). The loading capacity and the cell killing activity of SN-38 loaded on functionalized GO were investigated comprehensively in human breast cancer cells MCF-7.Our findings shown that the cytotoxicity of SN-38 loaded to the polymer modified GO was comparatively higher than free SN-38. In particular, SN-38 loaded GO-PVP nanocarrier has more cytotoxic effect than GO-β-CD nanocarrier against MCF-7 cells, indicating that SN-38 loaded GO-PVP nanocarrier can be used as promising material for drug delivery and biological applications.

1. Introduction

The exponentially emergent call for advances in the efficient diagnosis and treatment of various malignant diseases has stimulated a wide range of interdisciplinary science community to innovate an efficient and undisruptive drug delivery system. Graphene, with a sp^2-hybrirdized 2D framework has produced pioneering results and attracted a great research interest across the globe owing to its remarkable mechanical strength, electrical as well as thermal conductivity and large specific surface area [1–4]. Because of these extraordinary properties, graphene provides essentially infinite prospective for various applications such as green energy, biomedical, electronics, and nanocomposites etc. Graphene oxides (GOs) develop during oxidation of graphene, which have oxygenated hydrophilic functionalities at the surface of GO such as hydroxyl (-OH), epoxy (> O), and at the edges such as carboxylic (-COOH) groups. These functional groups promote the intercalation of water molecules into the covered passage and they can be easily detached by ultra-sonication that helps to produce highly dispersible GO sheets in aqueous medium [5–8]. The exfoliated GO can be further functionalized for drug delivery applications. Owing to these potent hydrophilic groups present on the surface of GO, the assistance of being high dispersion in water and physiological environments is attained by GO. Due to these oxygen containing groups, GO can be further functionalized covalently or noncovalently for its required applications accordingly [9]. Importantly, GO has specific and large surface area which is exclusively accessible from top and bottom sides of GO sheets and offer effective immobilization/loading of various chemicals as well as biomolecules (drugs, genes, proteins, etc.). Graphene has enormous potential to penetrate through the plasma membrane resulting into the enhanced cellular uptake of desired micro [10,11] and macromolecules [12,13] with excellent biocompatibility made GO a magical vehicle for drug delivery applications. The targeted drug delivery concept predominantly shows potential application in the biomedical field, where the developments of different therapeutic systems are significant to get better efficacy, reduces dose amount and eliminate adverse effects.

As we know, the majority of anticancer drugs are either insoluble or sparingly soluble in water as well as in biological medium due to their

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hydrophobic nature. But, they can acquire the hydrophilic nature by combining with highly polarized groups. This is one of the reasons why there is exponentially growing interest in exploring the potential of GO in drug delivery systems (DDSs).

The capability of polymer grafted GO sheets to stack and deliver anticancer drugs to the targeted cells has been confirmed previously [10–14]. Several research groups have demonstrated the potential of carbon nanomaterials for loading and delivery of anticancer drugs [15–19]. For example, Pan et al. [20] functionalized GO with adipic acid and sodium alginate and loaded the anticancer drug doxorubicin hydrochloride (DOX·HCl) on it. They reported that their developed nanocarrier exhibited better cytotoxicity to Hela cells compared to pure drug.

Bao et al. [21] synthesized chitosan-functionalized graphene oxide and showed that this nanocarrier was effective vehicle for anticancer drug delivery of camptothecin (CPT). Sahoo et al. [22] developed the two nanocarrier systems such as polyvinyl alcohol (PVA) functionalized multi walled carbon nanotubes (MWCNT) and GO in order to achieve efficient loading and targeted delivery of anticancer drug and investigated the cytotoxic effect of both systems on various cancer cells. The cytotoxicity of CPT was better in MWCNT-PVA compared to GO-PVA.

However, in drug delivery applications, the role of functionalized GO with hydrophilic and biocompatible polymers and other excipients are in core interest as they have suitable attaching sites to combine with drugs through noncovalent interaction.

In the present work, we synthesized GO by expanded graphite powder by using modified Hummer's method. The resulting GO is soluble in water due to highly polar hydroxyl, epoxy and carboxyl groups. Due to these polar and chemically reactive groups, GO further modified with hydrophilic and biocompatible polymer PVP covalently to improve the solubility of GO in biological mediums. We have also developed the other nanocarrier system based on GO and β-CD to compare the loading and cytotoxicity effect of drug. Later, the anticancer drug SN-38 (active metabolite of irrinotecan, an analog of camptothecin) was loaded onto GO functionalized with PVP and β-CD. SN-38 is a cytotoxic quinolone alkaloid, which is known to have anticancer activities. Being an aromatic drug SN-38 has a sparingly soluble nature in physiological solutions which is the main cause for its low bioavailability. The drug SN-38 was stacked noncovalently (via π-π stacking and intermolecular hydrogen bonding) onto PVP and β-CD grafted GO. Subsequently, in view of the biological importance of those SN-38 loaded nanocarriers, it was designed to check *in-vitro* cytotoxicity study against human breast cancer cells using MTT assay. This research work is dedicated to developing a rationale by comparing various parameters such as loading capacity, release profile and cell killing efficiency of SN-38 by using two different carriers GO-PVP and GO-β-CD.

2. Materials and methods

2.1. Materials

Expanded Graphite powder with particle size (~100 μm) was acquired from Sigma-Aldrich. Potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), sulphuric acid (H_2SO_4), hydrogen peroxide (H_2O_2, 30% aq.) were purchased from Himedia; polyvinylpyrrolidone (PVP, M_w ~ 40000), betacyclodextrin (β-CD, M_w ~ 1135) were purchased from Calbiochem; DCC (*N*,*N*',Dicyclohexyl Carbodiimide), DMAP (4-Dimethylaminopyridine) were purchased from SRL andSN-38 (7-ethyl-10-hydroxy camptothecin)were acquired from Sigma. Minimum Essential Medium Eagle (MEM) with NEAA, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), penicillin-streptomycin were purchased from Himedia (Assam, India). Unless otherwise indicated, all reagents were acquired from Sigma-Aldrich.

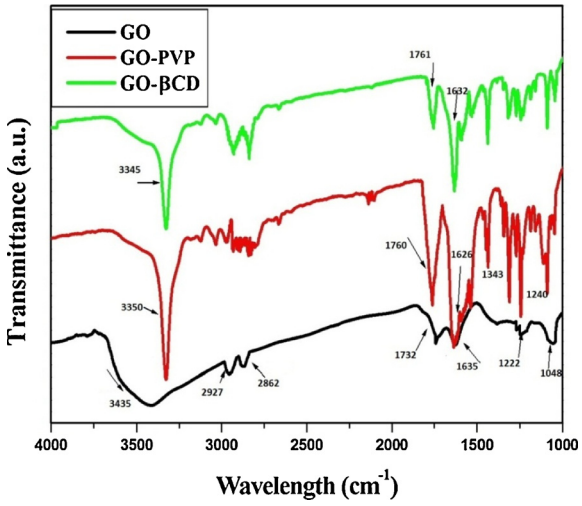

Fig. 1. Fourier Transform infrared (FT-IR) spectra of GO, GO-PVP and GO-β-CD.

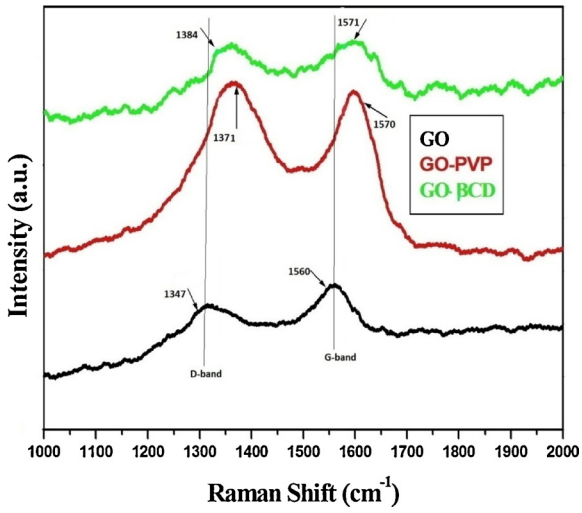

Fig. 2. Raman spectra for GO, GO-PVP, and GO-β-CD.

Table 1
Raman data of GO, GO-PVP and GO-β-CD.

System	D-band (cm^{-1})	G-band (cm^{-1})	I_D/I_G
GO	1347	1560	0.82
GO-PVP	1371	1570	1.12
GO-β-CD	1384	1571	0.98

2.2. Synthesis of GO

Expanded graphite powder has been utilized to synthesize the GO sheets by following the modified Hummer's method [23]. Briefly, mixture of calculated amount of the expanded graphite powder and 1 g of $NaNO_3$ were placed into a round bottom flask. Later 46 mL of H_2SO_4 was slowly added drop by drop and stirred continuously in an ice water bath. After stirring continuously for 4 h in an ice-water bath, 6 g of $KMnO_4$ was added to the slurry mixture of graphite and $NaNO_3$ at a heating temperature of 32 °C over about 2 h. After vigorously stirring the solution for 2 h, 92 mL DD water was added to the solution and maintain the temperature 95 °C. Then after over 2 h of constant stirring 200 mL of DD water was added and constantly stirred for 1 h. Finally, 20 mL of H_2O_2 (30%) was added and stirred continuously for 1 h at the room temperature. The final oxidized product has been purified by a