Polyethylene Glycol-Graphene Oxide Modified with Mesalazine; Synthesis, Characterization, and In-Vitro Drug Releasing Investigation

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ABSTRACT: *Regarding the importance of targeted delivery of pharmacies, a novel drug delivery system was designed by mesalazine (Mes) anchoring on Graphene Oxide (GO) modified with polyethylene glycol (PEG) to obtain 2.6 mmol Mes on 1 g of GO-PEG. The new compound was produced through esterification reactions of GO with PEG and subsequent esterification with Mes. The nano drug was characterized with common analysis methods including Raman, FT-IR, UV-Vis, Energy Dispersive X-ray, X-ray Diffraction, and Transmission Electron Microscopy. The in vitro Mes releasing from the composite was evaluated at two pHs of 3.5 and 7.4 for simulation of the gastric and intestine conditions. It was found that the compound is more stable in acidic media and slowly releases Mes at pH=4. The reason for this phenomenon is considered the ester bonds are produced in acidic media and is not been hydrolyzed in these conditions. The initial results for in vitro experiments indicated that the new drug would be a promising candidate for use in vivo.*

KEYWORDS: *Drug research; Mesalazine; Graphene oxide; Nanocarrier.*

INTRODUCTION

Concern about the damage of pharmacies to the normal cells encourages the researchers to design supported drugs for direct release in the target cells. Three kinds of drug delivery systems have been developed oral, systemic, and local, in which oral releasing as the traditional strategy is controlled by pH and enzymes [1]. With increasing the number of employed supports for drug delivery systems, the performances of various compounds have been evaluated precisely to select the best one. In this regard, Graphene Oxide (GO) is the center of attention due to its advantages such as easy drug loading, high surface area, sustainability, and inexpensive nature [2]. Moreover, drug release from GO could be controlled by various conditions like temperature, pH, or infrared/laser irradiation.

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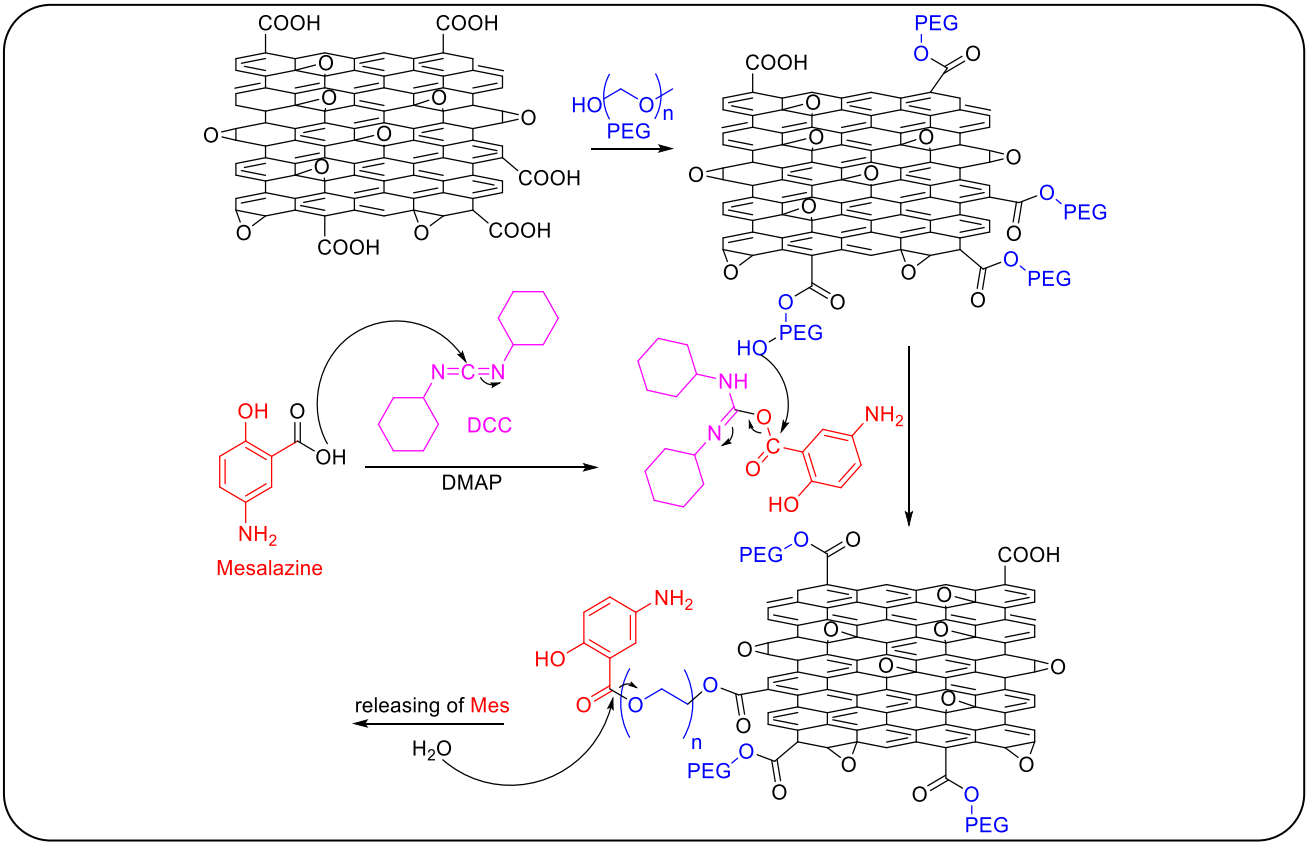

Fig. 1: Synthesis and mechanism of Mes-PEG-GO and Mes releasing mechanism.

Therefore, some drugs were deposited on GO by covalent and noncovalent interactions [2]. Regarding the covalent binding to GO, the existence of active carboxylic groups on this carrier makes it easy to support a wide variety of drugs containing alcohols through an esterification reaction [3]. Polyethylene glycol (PEG) is the most demanding compound to bond to GO as it offers superior solubility, stability, and biocompatibility [4]. Furthermore, functionalization of GO with PEG provides an opportunity for the attachment of some drugs with active moiety susceptible to react with alcohols [5-17]. Folic acid, paclitaxel, 3,3′-Diindolylmethane, Ginsenoside Rh2, and doxorubicin are some active pharmacies which successfully delivered by GO [2]. In addition, valuable drug delivery systems were reported using PEG-GO to carry doxorubicin [18], Au-Histidine@ZnO nanoparticles [19], curcumin [20], and Camptothecin [21].

Mesalazine (Mes) or mesalamine is the 5-amino salicylic acid, which has three active functional groups of alcohol, amine, and carboxylic acid [22]. Mes as a drug is used in the treatment of ulcerative colitis and Crohn's disease, which are two inflammatory bowel diseases [22]. Carboxylic acid group on Mes makes it susceptible for the reaction with alcohols like PEG. Therefore, herein we employed PEG-GO to support Mes through esterification reaction (Fig. 1). PEG gives some fascinating properties to GO as a support for the drug delivery agenda [7,8,23,24] as well as provides desired functionality to react with Mes.

EXPERIMENTAL SECTION

Material and Methods

All reagents were purchased from Sigma-Aldrich and used without further purification. PEG-6000 Molecular Biology was provided by Sigma-Aldrich. GO was prepared by the modified Hummer method [22]. Transition Electron Microscopy (TEM) micrographs were obtained with a Philips CM100 BioTWIN transmission electron microscope. Fourier Transform InfraRed (FT-IR) spectroscopy of the Jasco 6300 FTIR instrument was used to characterize the prepared samples in the range of 400-4000 1/cm. Energy Dispersive X-ray (EDX) Spectroscopy was carried out by SEM FEI Quanta 200. The powder

X-Ray Diffraction (XRD) pattern was prepared by Bruker, D8 ADVANCE X-ray diffractometer with a Cu-K_α radiation source (λ = 1.5406 Å). UV–visible spectrophotometer (Biowave II, Biochrom WPA Ltd., UK) was employed to provide spectra from solutions of dispersed samples in H_2O under ultrasonication.

Preparation of Mes-PEG-GO

The first stage of the preparation was enriching the carboxylic acids on GO to facilitate the modification reactions which totally depends on the existence of these functional groups. The number of carboxylic acids on GO was increased by the addition of NaOH (40 mL, 120 mg/mL) to GO suspension (40 mL, 2 mg/mL) and sonication for 4 h [20]. Next, HCl solution (6 mL, 12 M) was added to the mixture and then, the solid was separated through centrifuging and washed with deionized water (DI-H_2O) (100 mL). The obtained GO (2 g) was activated by ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (1 g) and *N*-hydroxysuccinimide (NHS) (0.6 g) under stirring for 24 h. PEG-6000 (0.5 g) was poured into the mixture, and stirring continued for 24 h. Finally, the mixture was centrifuged (8000 rpm, 25 ºC) for 15 min and the residue was washed with DI-H_2O (3 × 20 mL) to yield 2.2 g of PEG-GO.

A vessel containing obtained PEG-GO (2 g), *N*,*N'*-dicyclohexylcarbodiimide (DCC) (1 g), 4,4'-dimethylaminopyridine (DMAP) (0.04 g), H_2O (10 mL) was stirred at 90 ºC for 1 h and then, Mes (0.5 g, 3 mmol) was added to the mixture. After 24 h stirring at 90 ºC, the suspension was filtered off, and the residue was washed with DI-H_2O (3 × 20 mL) to give Mes-PEG-GO.

In vitro release of Mes from Mes-PEG-GO

Releasing of Mes was investigated in citric acid/phosphate-buffered saline at pH 3.5 and 7.4. For providing 100 mL buffer with pH 7.4, 9.15 mL of citric acid (0.1 M) and 90.85 mL of Na_2HPO_4 (0.2 M) were mixed. To adjust pH of the buffer at 3.6, 67.80 mL of citric acid (0.1 M) and 32.20 mL of Na_2HPO_4 (0.2 M) were mixed. Both of solutions were treated with 0.8 g NaCl, 0.02 g KCl, and 2 mL tween (2%). Mes-PEG-GO (20 mL, 2 mg/mL) was poured in a dialysis tube with 12 kDa molecular weight cut off and incubated with a shaker incubator (37 ºC, 100 rpm). The Mes molecular diffusing out of the tube are sampled for analysis by UV-Vis spectroscopy. For UV-Vis analysis, the calibration curves were prepared by various concentrations of Mes in two pHs adjusted in 3.5 and 7.4 since the carboxylic group of Mes impressed the absorption of Mes with the pH change. The absorption intensity for each sample was adopted with the calibration curves and the concentration of Mes was extracted from the curves.

RESULTS AND DISCUSSION

GO enriched with carboxylic acid groups is susceptible to the reaction with alcohols. PEG as a reactive alcohol can participate in the esterification reaction, especially with GO [23-26]. The obtained compound, PEG-GO, was employed in the second esterification reaction with Mes in the presence of DCC/DMAP as the reaction catalyst (Fig. 1). The produced Mes-PEG-GO was characterized with FT-IR spectroscopy and for following the changes, that spectrum was compared with spectra of PEG-GO and Mes (Fig. 2). Spectrum of PEG-GO showed absorption peaks at 3450, 2930, 1710, 1600, and 1040 related to OH of PEG/GO, CH of PEG, C=O of GO, C=C of GO, and C-O of PEG, respectively. FT-IR spectrum of Mes-PEG-GO revealed all the peaks related to PEG-GO as well as an absorption band at 1650 belonging to NH bending vibrational mode of Mes.

Raman spectroscopy is one of the important techniques to characterize GO composites with characteristic peaks related to D-bands and G-bands. Raman spectrum of Mes-PEG-GO also showed D-band at 1356 1/cm and G-band at 1582 1/cm which attributed to the out-of-plane vibrations arising from the structural defects and in-plane vibrations of C=C, respectively (Fig. 3). These peaks were also revealed in the spectrum of GO. The I_D/I_G for Mes-PEG-GO was calculated to be 0.84 which is some lower than the ratio determined for GO as 1.09. This change offers that the functional groups between the GO layers were decreased and maybe some aggregations occurred during the modification reaction. This could be elucidated by the TEM images before and after modifications.

EDX analysis was performed on Mes-PEG-GO, which the obtained spectra showed the presence of C, O, and N atoms with 53.4, 42.99, and 3.61 w%, respectively (Fig. 4). As can be seen from Scheme 1, the existence of N atoms is attributed to Mes molecules deposited on Mes-PEG-GO. As a result, the presence of N atoms in the Mes-PEG-GO structure obviously revealed successful loading of Mes

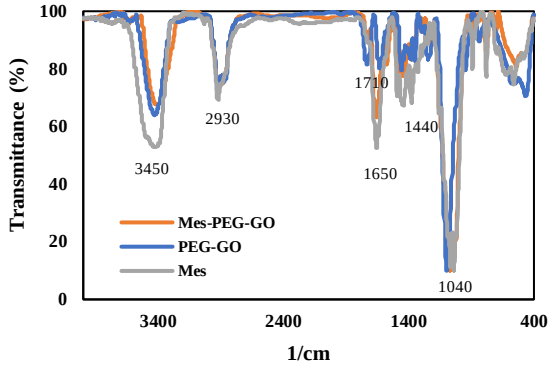

Fig. 2: FT-IR spectra of Mes, PEG-GO, and Mes-PEG-GO.

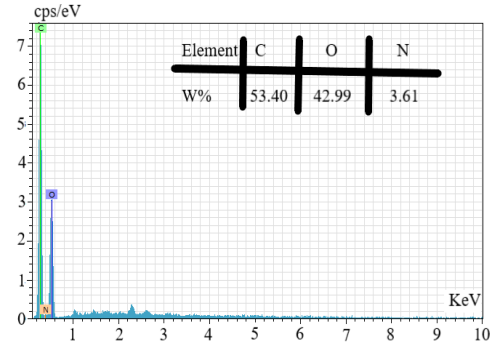

Fig. 4: EDX analysis of Mes-PEG-GO.

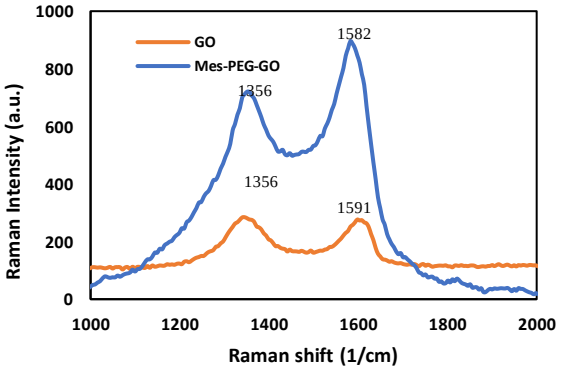

Fig. 3: Raman spectrum of Mes-PEG-GO.

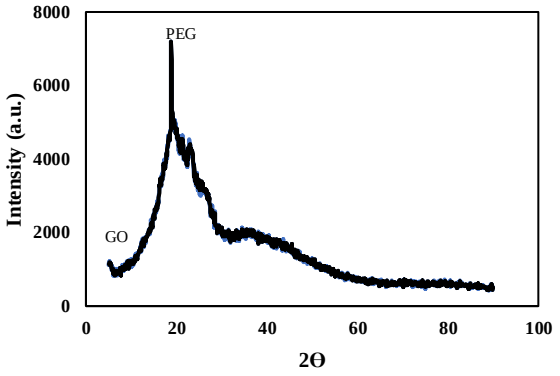

Fig. 5: XRD pattern of Mes-PEG-GO.

on the new compound. Since the molecular weight of N is 14, mol% of N is 2.6 mmol per 1 g of Mes-PEG-GO. With respect to each of Mes has one N, 2.6 mmol Mes was loaded on Mes-PEG-GO. The related equations for this calculation are as the following:

m_N per 1 g sample = (w% of nitrogen) × (1 g sample/100 g sample)

mol of Mes = mol of nitrogen = m_N per 1 g sample / M_N

Where m_N is the mass of nitrogen in the analyzed sample, w% is the weight percent of nitrogen obtained from the analysis, and M_N is the molecular weight of nitrogen.

XRD for Mes-PEG-GO showed a similar pattern to the previous report of PEG-GO with peaks of GO and PEG [24,27]. The pattern indicated 002 diffractions of GO at 2θ=5.11 and a broad band at about 25 for PEG (Fig. 5) [23]. It is the stacked form of products that is the reason for broadening of peaks which can be observed in TEM image (Fig. 6).

TEM micrographs of Mes-PEG-GO showed aggregated sheets of GO compared to GO (Fig. 6). It is the hydrogen binding of PEG's alcohols functionalities on both chain sides that is responsible for the gathering of sheets. Totally, the images obviously indicate the formation of graphene sheets which we expected from TEM.

UV-Vis spectra were prepared for PEG-GO and Mes-PEG-GO to evaluate the absorption bands and changes (Fig. 7). The spectrum for PEG-GO was demonstrated peaks at 229 and 297 nm [20]. Modification of PEG-GO with Mes led to the appearance of a new absorption at 337 nm which is in accordance with the spectrum reported for Mes [28].

Since GO previously indicated strong bonding with various molecules, it was necessary to evaluate Mes releasing from GO [29-31]. Mes extrication from Mes-PEG-GO was studied in two buffers at pH = 3.5 and 7.4 as the pHs of gastric and bowel. It was found that 38 w% of Mes of Mes-PEG-GO was released at pH = 3.5 after 72 h, in which a peak at 285 nm was observed for the Mes. As shown in Fig. 8, the emancipation rate was increased at pH = 7.4 to 63 w%. The low stability of Mes-PEG-GO in basic conditions is attributed to the low stability of ester bonds

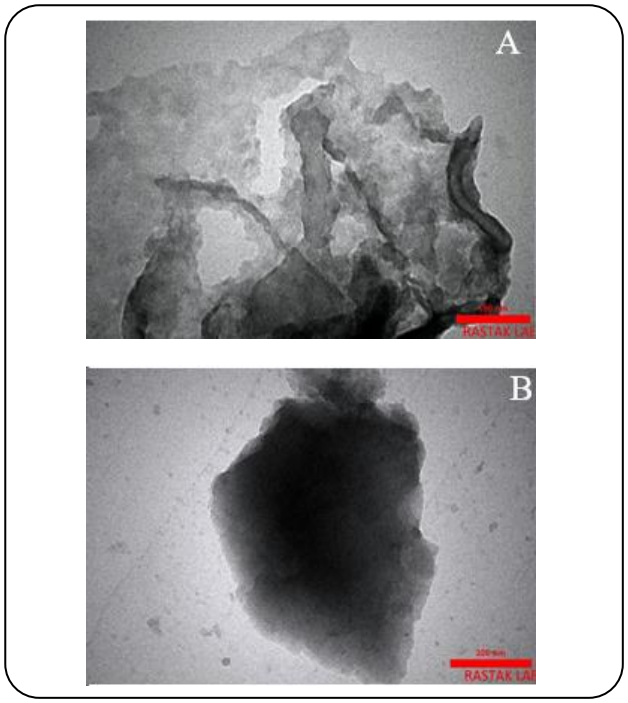

Fig. 6: TEM images of GO (A) and Mes-PEG-GO (B).

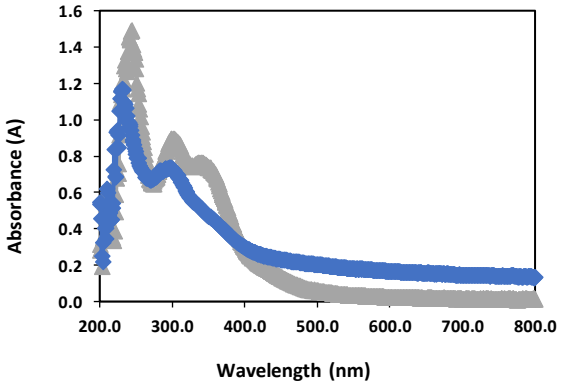

Fig. 7: UV-Vis spectra for PEG-GO (blue), and Mes-PEG-GO (grey).

in these conditions while esters are stable in acidic media. As a fact, acidic media is used for the construction of esters from carboxylic acids and alcohols, which confirms high stability of esters in low pHs. Therefore, regardless of other parameters such as the impact of enzymes, it can be concluded that Mes-PEG-GO would prefer to release Mes in a destination with basic pH compared to gastric with acidic pH. For Mes as a pharmacy to treat inflammatory bowel disease, safe passing from gastric and absorption in the intestine is very worthy. As a result, our compound has the potential to pass from the gastric to be released in the intestine.

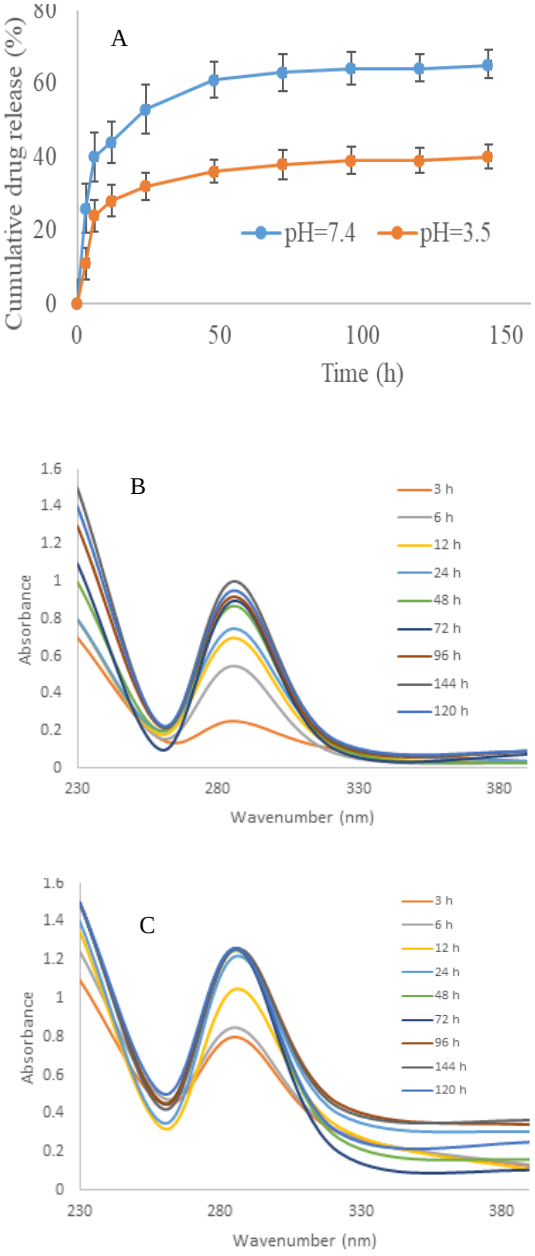

Fig. 8: Cumulative amounts of Mes released from Mes-PEG-GO at two different pHs (A), UV-Vis absorbance of the released drug at pH = 3.5 (B), and UV-Vis absorbance of the released rug at pH = 7.4 (C).

CONCLUSIONS

In conclusion, Mes was deposited on GO modified with PEG by esterification reaction. Loading of Mes on GO-PEG composite was approved by analyses such as FT-IR, EDAX, and UV-Vis spectroscopies. Also, the graphene sheets were confirmed with TEM images. Releasing of Mes (2.6 mmol) supported on PEG-GO (1 g)

was investigated in two pHs of 3.5 and 7.4 as the model of gastric and intestine media, in which the composite was more stable in acidic media and slowly destructed in basic conditions. The reason for this phenomenon is considered as the esters are produced in acidic pH. The initial results indicated that the new drug would be a promising candidate for the test in vivo studies.

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