International Journal of Pharmaceutics 484 (2015) 276–282

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Graphene-oxide stabilization in electrolyte solutions using hydroxyethyl cellulose for drug delivery application

Hanieh Mianehrow [a], Mohamad Hasan Mohamadzadeh Moghadam [a], Farhad Sharif [a], Saeedeh Mazinani [b,*]

[a] Department of Polymer Engineering and Colour Technology, Amirkabir University of Technology, 15875-4413 Tehran, Iran
[b] Amirkabir Nanotechnology Research Institute (ANTRI), Amirkabir University of Technology, 15875-4413 Tehran, Iran

ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form 25 February 2015
Accepted 27 February 2015
Available online 28 February 2015

Keywords:
GO–HEC nanohybrid
GO stabilization
Electrolyte solutions
Drug delivery applications
Folate-targeted drug delivery

ABSTRACT

Stabilization of graphene oxide (GO) in physiological solution is performed using hydroxyethyl cellulose (HEC) to make the resultant nanohybrid suitable for targeted drug delivery purposes. Short and long term stability of GO suspensions with different ionic strengths were assessed using ultraviolet–visible spectroscopy (UV–vis), atomic force microscopy (AFM) and zeta potential measurements. Results depicted that HEC effectively stabilized GO in electrolyte solutions and the mechanism of stabilization appeares to be depended on HEC content. Drug loading and release behavior of folic acid (FA) as a model drug, from GO–HEC nanohybrid were studied to assess its application in drug delivery systems. Results showed the nanohybrid could be highly loaded by folic acid. Moreover, HEC content in the nanohybrid played an important role in final application to make it applicable either as a carrier for controllable drug release or as a folate-targeted drug carrier. In addition, according to cytotoxicity results, the nanohybrid showed good biocompatibility which indeed confirms its potential application as a drug carrier.

1. Introduction

Graphene oxide (GO), a 2D carbonaceous nanomaterial, has been the subject of many studies in the field of targeted drug delivery for cancer therapy in recent years (Akhavan et al., 2012; An et al., 2013; Depan et al., 2011; Hu et al., 2012; Huang et al., 2011; Justin and Chen, 2014; Liu et al., 2008; Maity et al., 2014; Miao et al., 2013; Shi et al., 2013; Sun et al., 2008; Wang et al., 2013, 2014; Weaver et al., 2014; Wen et al., 2012; Yang et al., 2008, 2010a; Yue et al., 2013; Zhang et al., 2010). Despite of pronounced properties of GO, a tendency to form agglomerate in biologic medias, in which either ionic strength or pH is sufficiently high or low respectively, is inevitable (Bao et al., 2011; Chowdhury et al., 2013b; Hong et al., 2011; Hu et al., 2012; Wang and Hu, 2013). This phenomenon highly reduces GO effectiveness as a drug carrier. The amphiphilic nature of GO as well as its planar structure make it possible to incorporate hydrophilic and/or hydrophobic biomolecules which gives the opportunity to prevent its instability in such a medium (Hu et al., 2012; Maity et al., 2014; Rana et al., 2011; Yang et al., 2008; Zhang et al., 2011). Many efforts have been conducted to utilize biomolecules as a stabilizing agent; however, developing new biomaterials in order to manage GO bioactivity and colloidal stability in ionic medium has remained an open area of research.

GO nanosheets can be dispersed in aqueous media due to the ionization of hydroxyl and carboxyl groups (Chowdhury et al., 2013a; Overbeek, 1999; Wang and Hu, 2013). According to DLVO theory, repulsive interaction between two neighboring GO sheets overcomes van der Waals (vdW) attractive forces between them; however, an increase in ionic strength (or a decrease of pH) of media results in the shrinkage of electric double layers around GO sheets which makes it aggregate (Hong et al., 2011; Li et al., 2008; Novoselov et al., 2004). When electrostatic force is lowered, a possible path to stabilize GO sheets might be applying a protective layer which acts as steric hindrance around them.

Stabilization of GO in ionic media with various biocompatible molecules such as DNA (Patil et al., 2009), heparin (Lee et al., 2011), polyethylene glycol (Liu et al., 2008; Sun et al., 2008; Wen et al., 2012; Wojtoniszak et al., 2012), chitosan (Bao et al., 2011; Rana et al., 2011) and pluronic F127 (Hu et al., 2012) has been explored to make it suitable for biomedical applications. Among all materials, polysaccharides with their hydrophilic, biocompatible, non-toxic and biodegradable nature appeared to be more attractive

* Corresponding author at: Amirkabir Nanotechnology Research Institute (ANTRI), Amirkabir University of Technology, 424 Hafez Avenue, Amirkabir University of Technology (Polytechnic of Tehran), 15875-4413 Tehran, Iran. Tel.: +98 21 66402441; fax: +98 21 66402441.
E-mail addresses: haniye.mianehro@gmail.com (H. Mianehrow), mohamad_m_m_s@yahoo.com (M.H.M. Moghadam), sharif@aut.ac.ir (F. Sharif), s.mazinani@aut.ac.ir (S. Mazinani).

http://dx.doi.org/10.1016/j.ijpharm.2015.02.069
0378-5173/

candidates along with GO for biological applications (Rodri'guez-Gonza'lez et al., 2012; Yang et al., 2011, 2010b).

In this work, stabilization of GO with hydroxyethyl cellulose (HEC), a water soluble, non-ionic derivative of cellulose, is examined to enhance the stability of GO in saline solutions as a typical physiological media with similar ionic strength. Then, loading and release behavior of folic acid (FA), as a model drug, from GO–HEC nanohybrid is assessed to examine the suitability of the resultant nanohybrid as a nanocarrier for controllable drug release or folate-targeted drug delivery.

2. Experimental

2.1. Materials

Graphite flake with average lateral size of 20–100 micron, potassium permanganate, nitric acid 67%, sulfuric acid 98%, hydrochloridric acid 37%, hydrogen peroxide 30%, NaCl and folic acid were purchased from Merck Chemicals Co., hydroxyethyl cellulose with molecular weight of 300,000 g/mol and 1.5 (MS) was purchased from Kelong Chemical Co. (China), and all materials were used as received.

2.2. Synthesis of GO

GO was synthesized from natural graphite via preoxidation/thermal expansion of graphite followed by modified Hummer's method (Hummers and Offeman, 1958; McAllister et al., 2007). At first step, 10 g of natural graphite flake were mixed with 3:1 volume ratio of H_2SO_4:HNO_3 for 24 h. Then 400 ml of water was added to the resultant solution to quench the reaction. The resultant product was then filtered and washed out with water and dried in vacuum. In the second step, 2 g of the product was mixed with 200 ml of H_2SO_4 and 6 g potassium permanganate for 24 h resulting in a thick paste. Then 600 ml of distilled water was added to the paste, and the reaction was terminated by addition of aqueous solution of H_2O_2 (30 wt.%), resulting in a yellow brown mixture. Then the mixture was centrifuged and washed out three times with 10% HCl solution and then three times with water. The procedure was followed by sonication for 30 min to produce GO stable aqueous dispersion. At the end of this process, the mixture was centrifuged for 15 min at 4500 rpm in order to remove un-exfoliated graphite oxide particles. Synthesized GO full characterization could be found in the Supplementary data.

2.3. Preparation of GO–HEC nanohybrid and investigation of its dispersability in saline solution

As-prepared GO dispersion (5.4 mg/ml) was re-dispersed in 20 ml of distilled water to reach 0.2 mg/ml concentration and it was mixed with different amount of HEC powder to produce different mass ratios of HEC:GO (0.5:1, 0.7:1, 1:1, 1.5:1, 2:1, 5:1, 10:1, 20:1). For simplicity, the nanohybrid containing the HEC:GO mass ratio of *x* was named GO–HEC *x*. It was followed by 15 min severe stirring to dissolve HEC in water. The resultant mixture was then sonicated for 15 min for complete dissolution of HEC resulting in a crystal clear dispersion of GO–HEC nanohybrid. At the end, the dispersion of nanohybrid was dialyzed against distilled water for 3 days to remove unabsorbed HEC chains.

NaCl was then added to the samples to reach the final concentration of 75 and 150 mM (similar salt concentrations are often used in biological studies (Hong et al., 2011)). Thereafter, samples were centrifuged for 10 min at 4500 rpm to evaluate the stability of GO–HEC in saline solutions.

2.4. FA loaded GO–HEC nanohybrid and in-vitro release analysis

Loading of FA on the resultant nanohybrid was performed by mixing of 10 ml of FA (with 80 ppm concentration at pH 9) with 15 mg of as-prepared nanohybrid overnight in a dark room. The mixture was then centrifuged at 6000 rpm for 1 h to remove the unloaded drug from the mixture. The drug loading efficiency of the nanohybrid was measured by UV–vis spectrophotometer and was calculated as follows:

Drug loading efficiency (%) = 100 × [Total concentration of FA − Concentration of FA in the supernatant)/Total concentration of FA]

The release behavior of the loaded nanohybrid was monitored in phosphate buffer solution (PBS) with adjusted pH at 7.4 and 5.3. Briefly, 15 mg of GO–HEC–FA including 5 ml of the buffer was sealed in a dialysis tube with 12 kDa pore size. The dialysis tube was then immersed in 25 ml of the same saline buffer and placed in a shaking bed with a rotational speed of 150 rpm. 3 ml of release media was withdrawn every hour for the first 10 h and thereafter every 24 h until 72 h. Released folic acid in the samples was measured by UV–vis spectrometer.

2.5. Cytotoxicity of GO–HEC nanohybrid

In-vitro cytotoxicity of GO–HEC nanohybrid at different concentrations of 5, 10, 20, 50 μg/ml was evaluated by MTT

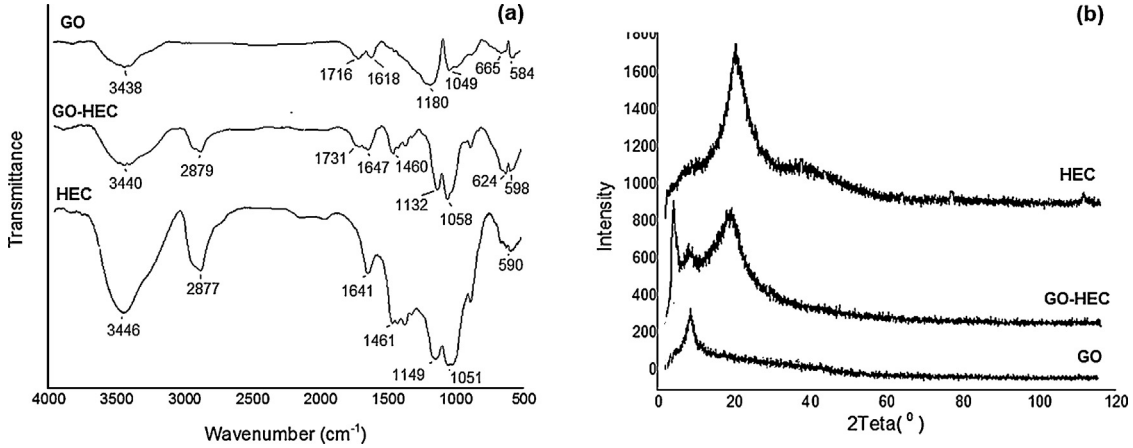

Fig. 1. FTIR spectra of GO, HEC, GO–HEC nanohybrid (a) and XRD pattern of GO, HEC, GO–HEC nanohybrid (b).