Carbohydrate Polymers 158 (2017) 51–57

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Guar gum oleate-*graft*-poly(methacrylic acid) hydrogel as a colon-specific controlled drug delivery carrier

D. Sathya Seeli, M. Prabaharan*

Department of Chemistry, Hindustan Institute of Technology and Science, Padur, Chennai 603 103, India

ARTICLE INFO

Article history:
Received 12 August 2016
Received in revised form 9 November 2016
Accepted 30 November 2016
Available online 2 December 2016

Keywords:
Guar gum
Hydrogel
Colon
Controlled release
Cytotoxicity

ABSTRACT

A novel type of ethylene glycol dimethacrylate (EGDMA) cross-linked guar gum oleate-*graft*-poly(methacrylic acid) (GGO-*g*-PMAc) hydrogel was prepared as a pH-responsive controlled release carrier for colon-specific drug delivery. The structure of GGO-*g*-PMAc hydrogel was characterized by FT-IR, ^{1}H NMR and X-ray diffraction (XRD) analysis. The swelling degree of the GGO-*g*-PMAc hydrogel at pH 7.4 was found to be higher than that at pH 1.2. The drug release studies performed in pH 7.4 and 1.2 buffer solutions at 37 °C revealed that the rate and amount of drug released from the GGO-*g*-PMAc hydrogel at pH 7.4 were higher than that at pH 1.2. The MTT assay revealed that there is no noticeable cytotoxicity of GGO-*g*-PMAc hydrogel at the concentration range of 0–100 μg/ml against the mouse mesenchymal stem cell line (C3H10T1/2). These results suggested that GGO-*g*-PMAc hydrogel can be a prospective pH-sensitive carrier for colon-targeted drug delivery.

1. Introduction

Guar gum is a natural polymer which is obtained from the seeds of *Cyamopsis tetragonolobus* (Deepak, Sheweta, & Bhupendar, 2014). It is a non-ionic galactomannan comprising of ~80% galactomannan, 12% water, 5% protein, 2% acidic insoluble ash, 0.7% ash and 0.7% fat. The backbone of guar gum is a linear β-D-(1–4) linked mannose units having α-D-galactopyranose units connected by (1–6) linkages (Panariello, Favaloro, Forbicioni, Caputo, & Barbucci, 2008). In recent years, guar gum and its derivatives are widely considered as potential materials for biomedical, pharmaceutical and environmental applications due to their bioavailability, biocompatibility, biodegradability, hydrophilicity and non-toxic nature (Mishra, Yadav, Mishra, & Behari, 2011; Prabaharan, 2011; Subhraseema & Usharani, 2015; Yadav, Sand, Mishra, & Behari, 2010; Yadav, Srivastav, Verma, & Behari, 2013). Due to the vulnerability to microbial degradation in the colon environment and stability over a wide pH range, guar gum based materials have capability to be used for colon-targeted drug delivery through the oral administration (Elias, Anil, Ahmad, & Daud, 2010; Madan et al., 2014; Sathya Seeli & Prabaharan, 2016).

The difficulties involved in the oral colon drug delivery are absorption and the degradation of drug molecules in the upper gastrointestinal (GI) tract that lead to systemic side effects and low bioavailability of the drug in the colon site. To overcome these drawbacks, various types of colon-specific drug delivery carriers such as time-dependent delivery carriers, pH-dependent delivery carriers and bacteria-dependent delivery carriers have been developed (George & Abraham, 2007; Kuntal, Tejraj, & Anandrao, 2011; Susan, Ellen, Jennifer, & Simon, 2015; Wakerly, Fell, Attwood, & Parkins, 1997). These carriers can deliver the drug more specifically to the colon site due to their physicochemical properties and thereby improve the bioavailability and absorption of drugs for the potential therapeutic effects. However, among the drug delivery carriers, in recent years, pH-dependent delivery carriers have attracted increasing attention because of their desirable properties and practical applications (Nazar & Umbreen, 2014; Sathya Seeli, Dhivya, Selvamurugan, & Prabaharan, 2016). Due to the presence of weakly acidic groups, the pH-dependent delivery carriers can present the limited swelling behavior in the acidic stomach fluid, which limits the drug release from the carriers. However, because of the increased pH in the large intestine and colon environment, the extent of swelling and thereby the amount of drug release from the hydrogel will be improved (Yihong, Huiqun, & Chaobo, 2007).

In this work, EGDMA cross-linked GGO-*g*-PMAc hydrogel was prepared as a colon-targeted drug delivery carrier by grafting PMAc onto GGO in the presence of EGDMA as a cross-linking agent and potassium persulfate as a free radical initiator. The GGO-*g*-PMAc hydrogel is expected to provide a pH-responsive character and sustained release of the loaded hydrophobic drug due to the presence

* Corresponding author.
E-mail address: mprabaharan@yahoo.com (M. Prabaharan).

http://dx.doi.org/10.1016/j.carbpol.2016.11.092
0144-8617/

of PMAc and amphiphilic GGO, respectively. In addition, due to the cross-linked polymer networks, this hydrogel would have the improved stability and swelling behavior in the aqueous media, which control the drug release mechanisms from the hydrogel (Todd & Daniel, 2008). Therefore, the GGO-*g*-PMAc hydrogel could be usefull to release the drugs more specifically to the colon in a controlled manner by avoiding the premature release of drugs in the stomach when travelling through the GI tract. In this study, the prepared GGO-*g*-PMAc hydrogel was analyzed with FT-IR, ^{1}H NMR and XRD techniques. The swelling character of the hydrogel was investigated in pH 1.2 and 7.4 buffer solutions at 37 °C. To assess the suitability of the hydrogel as a carrier for colon-targeted drug delivery, the hydrophobic model drug, ibuprofen, was encapsulated in the hydrogel and the amount of drug released was determined in pH 1.2 and 7.4 buffer media at 37 °C. In addition, the cytotoxicity of the GGO-*g*-PMAc hydrogel against the C3H10T1/2 cell line was evaluated using 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

2. Experimental

2.1. Materials

Guar gum was purchased from Sigma Chemical Company and cleaned by refluxing with diethyl ether prior to use. Oleic acid, 4-dimethylaminopyridine (DMAP), methacrylic acid, EGDMA, potassium persulfate and ibuprofen were purchased from Sigma–Aldrich. The mouse mesenchymal stem cells (C3H10T1/2 cell line) were obtained from National Centre for Cell Sciences (NCCS), Pune, India. All other chemicals used were of analytical reagent grade.

2.2. Synthesis of GGO

GGO with different degree of substitution (DS) was synthesized by reacting 100 ml of 1% (w/v) guar gum aqueous solution with the requisite amounts of oleic acid in the presence of DMAP as shown in Table 1. The reaction was performed at room temperature under constant stirring. After 24 h of reaction period, the reaction mixture was poured into ethanol solution (200 ml) under stirring in order to precipitate the product. Thereafter, the precipitate was filtered, washed many times with ethanol and vacuum dried at 55 °C for 8 h. The DS of the oleic acid group in GGO was determined by acid-base titration (Sarkar & Singhal, 2011).

2.3. Preparation of GGO-g-PMAc hydrogel

GGO-*g*-PMAc hydrogel was prepared by reacting 2.22 g (25.8 mmol) of methacrylic acid with 0.5 g (0.82 mmol) of GGO in presence of 0.02 g (0.1 mmol) of EGDMA using 0.05 g (0.185 mmol) of potassium persulfate as a free radical initiator in 100 ml of distilled water. The reaction was performed under constant stirring and nitrogen environment at 70 °C. After 3 h, the product formed was cooled to room temperature and washed repeatedly with 60/40% (v/v) ethanol-water mixture in order to eliminate the homopolymers and unreacted materials. Finally, the product was dried at 55 °C in the vacuum oven for 8 h and stored in the desiccators for further use. The grafting parameters such as grafting percentage (G%) and grafting efficiency percentage (GE%) of the hydrogel were calculated using the following formulas (Mishra, & Goutam, 2011).

$$G\% = [(\text{weight of pure graft copolymer} - \text{weight of GGS})/\text{weight of GGS}] \times 100$$

$$GE\% = [(\text{weight of pure graft copolymer} - \text{weight of GGS})/\text{weight of monomer}] \times 100$$

2.4. Characterization

The FT-IR spectra of the products in the range of 4000–400 cm^{-1} were recorded on a double-beam Perkin-Elmer 1600 FT-IR spectrometer. ^{1}H NMR spectra of the products were measured on a Bruker AV3 HD spectrometer at 25 °C. The XRD patterns of the samples were tested by a Brucker's D-8 advanced wide-angle X-ray diffractometer using Ni filter Cu Kα radiation source. Absorbance measurements were conducted on a Shimadzu UV–vis spectrophotometer (UV-2450) at 222 nm.

2.5. Swelling studies

The swelling characters of the GGO-*g*-PMAc hydrogel were determined in pH 1.2 and 7.4 buffer solutions at 37 °C. The dry hydrogel was precisely weighed (W_0) and submerged in buffer solutions. At prearranged time periods the swollen hydrogel was weighed (W_t) after the removal of excess water by the tissue paper. The degree of swelling of the hydrogel at time t was determined using the following formula.

$$\text{Degreeofswelling}, \% = [(W_t - W_0)/W_0] \times 100$$

where W_t and W_0 are the weights of the hydrogel at time t and in the dry state, respectively.

2.6. Drug loading and release studies

The model drug, ibuprofen, was encapsulated into the hydrogel by dipping accurately weighed amount of hydrogel in 10 ml of ethanol-drug solution (50 mg/ml, pH 7) in a small glass beaker at room temperature under stirring. After 48 h, the hydrogel-drug solution was filtered out using a Whatman filter paper. Then, the concentration of drug in the filtrate was determined by using a UV–vis spectrophotometer at 222 nm. The drug loading percent by the hydrogel (A) was calculated using the following formula.

$$A = [V \times (C_0 - C_1)]/W \times 100$$

where V is the volume of ibuprofen solution (ml), C_0 is the initial concentration of ibuprofen (mg/ml), C_1 is the concentration of ibuprofen solution after adsorption by the hydrogel (mg/ml), and W is the weight of the hydrogel (g).

The *in vitro* drug release studies were carried out in a glass beaker holding 100 ml of buffer solutions (pH 1.2, 6.8 and 7.4) at 37 °C (Prabaharan, Grailer, Pilla, Steeber, & Gong, 2009). A definite amount (50 mg) of drug loaded hydrogel was immersed in the buffer medium and kept in a laboratory shaking water bath retaining the constant temperature (37 °C) and stirring (100 rpm). Thereafter, 2 ml of drug solutions were removed at regular intervals and the volume of each sample was restored by the same volume of fresh buffer solution. The amount of released ibuprofen was evaluated on a spectrophotometer at 222 nm. All the release experiments were conducted in triplicate.

2.7. Cytotoxicity studies

The cytotoxicity of GGO-*g*-PMAc hydrogel against C3H10T1/2 cell line was assessed using MTT assay (Khaled et al., 2016). In brief, the C3H10T1/2 cells, which were maintained under standard conditions (5% CO_2, 37 °C, 10% FBS containing DMEM), were seeded