Carbohydrate Polymers 88 (2012) 465–473

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Synthesis and characterization of thermo- and pH-responsive bacterial cellulose/acrylic acid hydrogels for drug delivery

Mohd Cairul Iqbal Mohd Amin[a,*], Naveed Ahmad[a], Nadia Halib[b], Ishak Ahmad[c]

[a] Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[b] Medical Technology Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia
[c] Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 20 August 2011
Received in revised form 8 December 2011
Accepted 13 December 2011
Available online 30 December 2011

Keywords:
Acrylic acid
Bacterial cellulose
Drug-delivery system
Electron-beam irradiation
Hydrogel
Swelling behavior

ABSTRACT

To assist in identifying the utility of novel materials in drug-delivery applications, this study investigated the use of bacterial cellulose (BC), a natural biopolymer, in the synthesis of hydrogels for drug-delivery systems. BC was combined with different proportions of acrylic acid (AA) to fabricate hydrogels by exposure to accelerated electron-beam irradiation at different doses. Fourier transform infrared analysis revealed that the AA had been successfully grafted onto the cellulose fibers and allowed for prediction of the reaction mechanism in the synthesis of hydrogels. Thermal and morphological characterization indicated the formation of thermally stable hydrogels with pore size determined by AA content and irradiation dose. The results of swelling and in vitro drug-release studies revealed the hydrogels to be both thermo- and pH-responsive. Such thermo- and pH-responsiveness, in addition to their morphological characteristics, suggests that these BC/AA hydrogels are promising candidates as controlled drug-delivery systems.

1. Introduction

The conventionally passive role of pharmaceutical excipients in pharmaceutical products as providers of weight, volume, flowability, and consistency is rapidly evolving to the more active roles of drug-performance enhancers that target drug delivery at the site of action and protect the drug from degradation inside the body (Beneke, Viljoen, & Hamman, 2009; Guenther, Smirnova, & Neubert, 2008). This evolution has prompted researchers to discover and explore new materials for drug-delivery applications. From the application point of view, many natural polymers, such as starch, cellulose, chitosan, carrageenan, and alginate, have been explored due their stability, availability, sustainability and low level of toxicity (Malafaya, Silva, & Reis, 2007; Säkkinen, Seppälä, Heinänen, & Marvola, 2002). These biopolymers also offer the advantages of biodegradability, biocompatibility, and capability of chemical modification which confers them principle properties for their application to drug-delivery systems (Domb & Kost, 1997). While many successful drug-delivery systems have been developed using conventional biopolymers, the rapid evolution of the pharmaceutical industry demands continued exploration to identify novel biomaterials that can be used in devising intelligent drug-delivery systems with efficient in vivo performance.

One such biopolymer of great interest is bacterial cellulose (BC) synthesized by *Acetobacter xylinum*. BC possesses many unique structural and biochemical properties, including an ultrafine nanofibrous network structure (Patel & Suresh, 2008), bioadaptibility (Hong & Qiu, 2008), chemical stability, and non-toxicity (Grzegorczyn & Slezak, 2007; Moreira et al., 2009). Due to its uniform and ultrafine fibrous network structure, BC has excellent water-absorbance capacity and mechanical properties, including high tensile strength and elastic modulus (Czaja, Young, Kawecki, & Brown, 2008; Hua et al., 2009; Tatsuya, Sachiko, Kaoru, & Yoshinari, 2011). These advantageous properties make BC an attractive material for the fabrication of intelligent adsorptive materials for drug-delivery applications, such as hydrogels.

Hydrogels are adsorptive materials composed of hydrophilic polymers linked through chemical or physical crosslinking in such a manner that they can absorb and retain large volumes of water within their three-dimensional network without dissolution. In addition to being thermo responsive, they can be fabricated to become pH responsive by the addition of acrylic acid (AA). Such pH- and thermo-responsiveness, in addition to their swelling capacity and biocompatibility, allows hydrogels to serve as versatile materials in many biomedical and pharmaceutical applications. Such applications include the delivery of antibodies, antibiotics, enzymes, hormones, and contraceptives that act through a variety of routes, including oral, subcutaneous, and intramuscular routes. These characteristics allow hydrogels to play important roles in sustained-release, controlled-release, targeted-release, and

* Corresponding author. Tel.: +60 3 9289 7690; fax: +60 3 2698 3271.
E-mail address: mciamin@pharmacy.ukm.my (M.C.I. Mohd Amin).

0144-8617/$ – see front matter.
doi:10.1016/j.carbpol.2011.12.022

protective drug-delivery systems (Hamidi, Azadi, & Rafiei, 2008; Hennink, De Jong, Bos, Veldhuis, & Van Nostrum, 2004; Peppas, Bures, Leobandung, & Ichikawa, 2000). Nevertheless, their great potential has been hampered by one major challenge: the difficulty of fabricating sufficiently mechanically strong hydrogels using conventional chemical crosslinking methods without compromising their intelligent-swelling characteristics (Haraguchi, Takehisa, & Fan, 2002; Karaaslana, Tshabalalab, Yelleb, & Buschle-Diller, 2011).

Electron-beam radiation (ionizing radiation) can initiate crosslinking that produces pure, sterile, and residue-free hydrogels. Unlike conventional chemical crosslinking methods, this method does not require the addition of catalysts or any other additives to modify the material in a manner that protects the inherent biocompatible and biodegradable properties of natural polymers (Hennink & van Nostrum, 2002). Moreover, electron-beam radiation requires no involvement of a radioactive source, eliminating the risk of hazardous toxicity due to the formation of radioactive substances. Using this method, the degree of crosslinking and the pore size of hydrogels, which strongly determine the extent of swelling, can be easily controlled by varying the irradiation dose (Anjali, Manohar, Sabharwalb, Bhardwajb, & Majalib, 2000; Said, Abd Alla, El-Naggar, 2004). As such, this method has been found to be very useful in preparing hydrogels for pharmaceutical applications in which even a low level of contamination is undesirable.

The aim of this work was to investigate the use of novel materials in drug-delivery applications by synthesis and characterization of thermo- and pH-responsive hydrogels for use in controlled drug-delivery systems. BC was combined with AA at several ratios to fabricate hydrogels by exposure to accelerated electron-beam irradiation at different doses. After fabrication, FT-IR, thermal and morphological characterization of the hydrogels was performed to investigate the structure, stability and the porosity of the hydrogels. This characterization was followed by both swelling and in vitro drug-release studies to evaluate the thermal and pH responsiveness of the hydrogels and their overall potential as drug-delivery systems.

2. Experimental

2.1. Materials

Bacterial cellulose (BC) was prepared from *nata de coco* that had been purified, lyophilized, ground, characterized, and then identified as described in British Pharmacopeia (2010). Acrylic acid (AA), bovine serum albumin (BSA), and phosphate buffer saline (PBS) were supplied by Sigma–Aldrich, USA. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) without enzymes were prepared according to the procedure described by the United States Pharmacopeia (2010). Distilled water was used to prepare aqueous solutions and dispersions, and 12 cm × 12 cm × 0.5 cm plastic trays were used as molds for the preparation of the hydrogels.

2.2. Synthesis of BC/AA hydrogels

Lyophilized BC was ground to a powder of particle size ranging from 20 to 200 μm. AA was added to a 1% (w/v) dispersion of BC in distilled water to make 20:80, 30:70, and 40:60 AA:BC mixtures. To ensure thorough mixing, each mixture was stirred by a mechanical homogenizer (IKA Labortechink Ultra Turrax T25, Germany) for 30 min, then poured into a mold for subjection to electron-beam radiation of 35 or 50 kGy in air at the accelerator facility (EPS-3000, Japan) of the Malaysian Nuclear Agency. The resulting hydrogels were named as 208035, 208050, 307035, 307050, 406035, and 406050 hydrogels, on the basis of AA:BC ratio and electron-beam irradiation dose.

2.3. Gel fractions of hydrogels

Freshly prepared hydrogels were cut into disks 1 cm in diameter and 2 mm in thickness and dried in an oven at 60 °C to a constant weight. The dried hydrogels were then subjected to extraction in distilled water at ambient temperature for 7 days to remove free BC and AA, after which the extracted hydrogels were dried in an oven at 60 °C to a constant weight. The percent gel fraction was calculated using the following equation:

$$\frac{G_d}{G_i} \times 100 \quad (1)$$

where G_d is the initial dried weight before extraction and G_i is the constant dried weight of the hydrogels after extraction.

2.4. FT-IR analysis

FT-IR analysis was conducted to identify the structure of the hydrogels using the Perkin Elmer FT-IR Spectra 2000. Hydrogels were cut into 1 cm × 1 cm × 2 mm thin films, directly placed on the top plate of a diamond attenuated total reflection (ATR) and scanned over a range of 4000–500 cm^{-1}.

2.5. Thermogravimetric analysis

A Perkin Elmer STA 6000 was used to perform thermogravimetric analysis (TGA) of the BC, AA, and hydrogels. In preparation for TGA, the hydrogels were ground to powder form. Approximately 10 mg of BC, AA, and hydrogel powder was placed in the sample pan for analysis over a temperature range of 50–900 °C at a heating rate of 10 °C/min under a nitrogen purge (25 mL/min). The differential thermogravimetric (DTG) curve was derived from the TGA results using the Pyris 1 software program.

2.6. Differential scanning calorimetry

The glass-transition temperatures (T_g) of the purified hydrogels were determined using a Perkin Elmer Diamond differential scanning calorimeter to perform differential scanning calorimetry (DSC). Approximately 10 mg of all samples was sealed in a standard aluminum pan. The analysis was conducted over a temperature range of 0–150 °C at a 20 °C/min heating rate under a nitrogen purge (20 mL/min).

2.7. Morphological analysis

The morphologies of the porous structure of the BC/AA hydrogels were examined by scanning-electron microscopy (SEM). To prepare the hydrogel samples for examination under a Leo1450 VP (Germany) SEM, they were mounted on an aluminum stub with double-sided carbon tape and coated with gold in sputter coater (SC500; BioRad, UK) under argon atmosphere.

2.8. Swelling studies

To measure the swelling ratio (SR), dried and weighed hydrogels disks were immersed into 50 mL solutions of PBS at different pH values (2–10) and temperatures (25–50 °C). The swollen samples were weighed during time intervals at which excess of buffer was removed by blotting with filter paper. The SR percent was calculated using the following equation:

$$SR\% = \frac{G_s - G_d}{G_d} \times 100 \quad (2)$$