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Synthesis of a novel acrylated abietic acid-*g*-bacterial cellulose hydrogel by gamma irradiation

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ABSTRACT

Acrylated abietic acid (acrylated AbA) and acrylated abietic acid-grafted bacterial cellulose pH sensitive hydrogel (acrylated AbA-*g*-BC) were prepared by a one-pot synthesis. The successful dimerization of acrylic acid (AA) and abietic acid (AbA) and grafting of the dimer onto bacterial cellulose (BC) was confirmed by ^{13}C solid state NMR as well as FT-IR. X-ray diffraction analysis showed characteristic peaks for AbA and BC; further, there was no effect of increasing amorphous AA content on the overall crystallinity of the hydrogel. Differential scanning calorimetry revealed a glass transition temperature of 80 °C. Gel fraction and swelling studies gave insight into the features of the hydrogel, suggesting that it was suitable for future applications such as drug delivery. Scanning electron microscopy observations showed an interesting interpenetrating network within the walls of hydrogel samples with the lowest levels of AA and gamma radiation doses. Cell viability test revealed that the synthesized hydrogel is safe for future use in biomedical applications.

1. Introduction

Cellulose-based hydrogels have been synthesized and studied for diverse applications. Water-soluble derivatives of cellulose have frequently been used to form hydrogels, by using only the cellulose derivative or by using its polymer blend with other natural or synthetic polymers (Chang & Zhang, 2011; Edgar, 2007). Hydrogels of water-soluble cellulose derivatives such as carboxymethyl cellulose and hydroxypropylmethyl cellulose are synthesized by exposing a paste (10%, w/v or above) of the cellulose derivatives to high-energy ionizing radiation (Pekel, Yoshii, Kume, & Güven, 2004; Yoshii et al., 2003). These hydrogels, among others, have been used in a variety of biomedical applications such as tissue engineering scaffolds and drug delivery systems, and are obtained from guar gum, pectin, chitosan, and dextran (Shukla & Tiwari, 2012). Hydrogels based on native cellulose can be prepared by dissolving cellulose in a solvent with or without water-soluble vinyl monomers such as acrylic acid (AA) and acrylamide. Hydrogels composed solely of hydrophilic vinyl monomers are considered as first-generation/conventional super-porous hydrogels. However, because of their poor mechanical properties, these hydrogels do not form products that are mechanically sustainable under stress, and are not suited for skin or oral applications (Mastropeitro, Omidian, & Park, 2012). In order to overcome this issue, these hydrogels have been combined with native bacterial cellulose (BC) for obtaining superior mechanical, swelling, and stimuli-responsive properties. Recently, syntheses using micronized BC with electron beam irradiation and BC flakes with microwave irradiation have followed the aforementioned theme (Amin, Ahmad, Halib, & Ahmad, 2012; Halib, Amin, & Ahmad, 2009; Pandey & Amin, 2013). Cellulose has also been combined with organic compounds, and this is in contrast to its combination to synthesize hydrogels with water-soluble vinyl monomers mentioned earlier. An exemplary study reported the combination of the ester of dehydroabietic acid with cellulose. In the study, the O-acylation reaction was conducted in an ionic liquid to yield cellulose dehydroabietate, which had better solubility and better resistance to acids and bases as compared to native cellulose (Xu, Duan, Huang, & Li, 2011). Abietic acid (AbA) is another organic resin acid that has been combined with hydroxypropylcellulose to form a macromolecular pro-drug through formation of ester linkage (Hussain, 2007). AbA constitutes more than 90% of the rosin along with dehydroabietic acid and isomeric L-pimaric acid (Wang, Yao, Wang, Tang, & Jiang, 2013). It is a monocarboxylic acid

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substituted phenantherene (a bulky hydrophobic moiety), which has various applications including synthesis of various bioactive molecules, preparation of pellets of drugs with retarded release and block copolymers for the synthesis of micellar nanoparticles for drug delivery (Abdulla, 2008; Nande, Barbade, Morkhade, Patil, & Joshi, 2006; Wang et al., 2013).

L-Pimaric acid (LPA), an isomer of AbA that differs only in the site of saturation within the phenantherene nucleus, has been dimerized with AA to form acrylated L-pimaric acid (Bicu & Mustaţa, 2007). This bi-functional compound furthered the esterification of isomeric levopimaric acid, a reaction otherwise reported as difficult to carry out. The esterification of acrylated LPA was carried out with ethylene carbonate (1,3-dioxolan-2-one) (Mustata & Bicu, 2010). These studies show that chemical modification of AbA and its isomers precedes polymer synthesis in the generation of novel materials with different applications. Modified AbA can also be used to synthesize hydrogels (swollen cross-links) i.e. in contrast to the aforementioned non-swelling polymers. Chiral hydrogels have been obtained using AbA derivatives. They retain the configuration of the parent L-abietic acid, whereby AbA acts as the source of chirality for *N*-propargyl abietamide, the copolymer, and its hydrogel with achiral moieties such as *N*-isopropyl acrylamide (NIPAam). The hydrogel showed considerable enantioselective recognition toward molecules with the L-configuration (Yao, Zhang, Zhang, Yang, & Dang, 2013). Such hydrogels have been reported and prepared by combining native BC with AA. The combination of LPA and AA has also been reported in the synthesis of the subsequent polymer. However, the synthesis of hydrogels with AbA or modified AbA, AA, and cellulose has not been reported.

In this study, we aimed to synthesize and characterize acrylated AbA and graft it onto BC to form a hydrogel. We then evaluated the cytocompatibility of the hydrogel. The proposed synthesis of the hydrogel is a one-pot process, as the modification of AbA and the grafting of acrylated AbA onto BC can be effected in the same reaction.

2. Experimental

2.1. Materials

Nata de coco was obtained from the local food market. AbA (85%) was obtained from Acros Chemicals, Belgium. AA was procured from Sigma Aldrich (St. Louis, MO, USA). All reagents were of analytical grade and used without further purification (except *nata de coco*).

2.2. Preparation of microfibrillated BC (MFBC)

BC was prepared according to a previously reported method (Amin, Abadi, Ahmad, Katas, & Jamal, 2012). Briefly, food-grade *nata de coco* was subjected to acid removal by soaking in water until a neutral pH was obtained. Next, it was treated with 0.1 N NaOH solution in a hot water bath for 1 h at 90 °C. After the pH became neutral, the mixture was lyophilized and finally ground to microfibrillated BC using a *Pulverisette 14* (Fritsch, Idar-Oberstein, Germany) rotor mill.

2.3. Synthesis of acrylated AbA and acrylated AbA-g-bacterial cellulose hydrogel

An aqueous dispersion (1%, w/v) of the microfibrillated BC (300–600 μm) was obtained by mechanical homogenization at 11,000 rpm with a mechanical homogenizer (IKA Labortechink Ultra Turrax T25, Germany). To produce three different aqueous dispersions, 3 g of AbA (0.01 mol) and three different volumes of AA (20.58 ml (0.3 mol), 27.45 ml (0.4 mol), and 34.03 ml (0.5 mol)) were added separately to make up the volume of the BC aqueous dispersions to 100 mL. Homogenization was continued until no phase separation was observed. The three formulations were irradiated in an oxygen-rich atmosphere at radiation doses of 30 kGy, 40 kGy, and 50 kGy with a *Gammacell 220* ^{60}Co radiation source (Maximum activity; 410Tbq, Dose rate; 2.076 kGy/h). Hydrogels were named according to the different volumes of AA and radiation doses used (Table 1).

2.4. ^{13}C solid state NMR

The selected hydrogel sample was purified in distilled water. It was freeze-dried and finely ground to make it suitable for ^{13}C NMR analysis in solid state. Solid-state cross-polarization/magic angle spinning (CPMAS) ^{13}C NMR spectra of the hydrogel was recorded using an NMR spectrometer (Avance 400; Bruker, Germany). A 100 mg of the sample was packed into the 4-mm inner diameter cylindrical zirconium oxide MAS rotor with an O-ring seal and end cap. Chemical shifts were recorded in ppm with reference of 29.50 ± 0.10 ppm (CH) and 38.56 ± 0.10 ppm (CH_2) using adamantine as the external standard.

2.5. FT-IR analysis

All the freshly prepared hydrogels were placed in distilled water, such that the distilled water was replaced twice a day for two weeks to ensure removal of any possible un-reacted monomers present and only purified hydrogel would be available for analysis. Afterwards the hydrogels were freeze-dried and powdered. The FT-IR samples were prepared by mixing the powder in KBr and fusing the mixture into a transparent disc using a hydraulic press. The FT-IR analysis was performed by using an FT-IR spectra 2000 spectrophotometer (Perkin-Elmer, Waltham, USA) under the following conditions: range: 4000–500 cm^{-1}, resolution: 4 cm^{-1}, number of accumulation scans: 16.

2.6. Differential scanning calorimetry (DSC)

The hydrogel samples were studied using a Diamond differential scanning calorimeter (Perkin-Elmer, Waltham, USA). For analysis, 5 mg of each sample was sealed in an aluminum pan specifically meant for solid samples. The samples were analyzed over the temperature range 0–200 °C at a rate of 20 °C/min and a nitrogen purge of 25 mL/min.

2.7. Scanning electron microscopy (SEM)

The hydrogels were cut into circular discs and freeze-dried prior to the porosity analysis. The samples were coated by using a gold sputter coater under an argon atmosphere and mounted onto the aluminum stub of a LEO 1450 VP scanning electron microscope. Images were recorded at different magnifications.

2.8. Gel fraction

The freshly prepared hydrogels were cut into discs, which were then dried in an oven at 60 °C until they reached a constant weight. The samples were purified by soaking in distilled water for a week. The soaking water was replaced with fresh distilled water at every 12 h in order to remove any un-reacted monomers. Subsequently, the hydrogels were removed and dried overnight after a week. Then, the final weight was measured and the gel fraction was obtained using the following equation:

$$\frac{W_f}{W_i} \times 100$$