International Journal of Pharmaceutics 513 (2016) 528–537

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Quaternized chitosan/κ-carrageenan/caffeic acid–coated poly(3-hydroxybutyrate) fibrous materials: Preparation, antibacterial and antioxidant activity

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ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 17 September 2016
Accepted 21 September 2016
Available online 23 September 2016

Keywords:
Quaternized chitosan
κ-Carrageenan
Caffeic acid
Electrospinning
Antibacterial fibers
Antioxidant activity

ABSTRACT

Novel fibrous materials with antioxidant and antibacterial properties from poly(3-hydroxybutyrate) (PHB), quaternized chitosan (QCh), κ-carrageenan (Car) and caffeic acid (CA) were obtained. These materials were prepared by applying electrospinning or electrospinning in conjunction with dip-coating and polyelectrolyte complex (PEC) formation. It was found that the CA release depended on the fiber composition. X-ray diffraction analysis (XRD) and differential scanning calorimetry (DSC) revealed that CA incorporated in the fibers was in the amorphous state, whereas CA included in the coating was in the crystalline state. In contrast to the neat PHB mats, the CA-containing mats and the PEC QCh/Car-coated mats were found to kill the Gram-positive bacteria *S. aureus* and the Gram-negative bacteria *E. coli* and were effective in suppressing the adhesion of pathogenic bacteria *S. aureus*. Enhancement of the antioxidant activity of the fibrous materials containing both CA and QCh/Car coating was observed.

1. Introduction

In recent years the use of electrospinning for the preparation of nanofibrous materials with antibacterial and antioxidant properties designed for biomedical applications has attracted considerable attention. Various approaches have been reported for the preparation of such materials, including immobilization of drugs or agents with antibacterial and antioxidant activity in nanofibers by electrospinning (Ignatova et al., 2013b; Suganya et al., 2011; Toncheva et al., 2014), electrospinning of polymers with intrinsic antimicrobial and antioxidant properties and preparation of coatings from polymers with inherent antimicrobial and antioxidant activity or polyelectrolyte complexes based on them on the surface of electrospun micro- and nanofibers (Ignatova et al., 2013a; Kalinov et al., 2014b; Li and Hsieh, 2006). Among the natural polymers with intrinsic activity chitosan and its derivatives are particularly attractive for use in nanofibrous materials. This interest is mainly due to the valuable biological properties of chitosan including lack of toxicity, biodegradability, biocompatibility, wound healing capacity, intrinsic antibacterial properties and antioxidant activity (Hirano, 1999; Wan et al., 2013). The quaternized derivatives of chitosan (QCh) are known to be highly effective against bacteria and fungi (Jia et al., 2001; Kim et al., 1997). These chitosan derivatives exhibit good antioxidant activity, as well (Guo et al., 2007 and Wan et al., 2013). In aqueous solutions chitosan and its quarternized derivatives can form polyelectrolyte complexes with polyanions-natural and synthetic polyacids (Juntaprama et al., 2012; Kalinov et al., 2014a; Verheul et al., 2011). QCh-based nanofibrous materials have been prepared by one-pot electrospinning of mixed solutions of QCh/poly(vinyl alcohol), QCh/poly(vinyl pyrrolidone) or QCh/polylactide (Alipour et al., 2009; Ignatova et al., 2007). These electrospun fibers manifest good antibacterial activity. The combination of electrospinning and PEC formation is an effective approach for targeted modification of the properties and behaviour of the surface of fibrous polymer materials. It has been demonstrated that the formation of a PEC coating from *N*-carboxyethylchitosan or QCh on the surface of fibrous materials from polyesters leads to obtaining of novel antibacterial materials (Jiang et al., 2015; Kalinov et al., 2014b; Yancheva et al., 2010). One-pot electrospinning has been used to produce nanofibrous materials from QCh-based polyelectrolyte complexes, which display high stability in saline, as well as good antibacterial activity towards Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* (Kalinov et al., 2015). The incorporation of carrageenan in electrospun materials designed for medical and pharmaceutical applications is of great

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http://dx.doi.org/10.1016/j.ijpharm.2016.09.062
0378-5173/

interest because these anionic sulphated polysaccharides which are isolated from various types of red algae are biodegradable, biocompatible and exhibit antioxidant, antiviral, antiinflammatory, immunomodulating and anticoagulant activity (Cunha and Grenha, 2016; Necas and Bartosikova, 2013; Prajapati et al., 2014). Strong antioxidant activity and antiinflammatory, antimicrobial, antiviral, antimutagenic, and antitumor properties are exhibited by certain natural phenolic compounds, such as caffeic acid (CA) (Chang et al., 2010; Gülçin, 2006; Guzman, 2014). When included in nanofibrous materials, such compounds may impart them beneficial properties.

Up to now, studies have been focused on the incorporation of CA in fibrous materials based on aliphatic polyesters. CA has been incorporated in polycaprolactone (Kim et al., 2012) and polylactide (Llorens et al., 2013 and Llorens et al., 2014) fibers by electrospinning. CA has been chemically immobilized onto the surface of electrospun poly(L-lactic acid) fibers (Chuysinuan et al., 2012). Among the aliphatic polyesters representing suitable carriers of the natural phenolic compound CA polyhydroxyalkanoates, and poly(3-trihydroxybutyrate) (PHB), in particular, have attracted significant attention. The latter is a promising biomaterial because it degrades completely without releasing any toxic side products (Lenz and Marchessault, 2005). The micro- and nanofibrous materials based on PHB are characterized by good mechanical properties (Wang et al., 2008). Recently we have demonstrated the preparation of PHB/poly(ethylene glycol)-based nanofibrous materials containing CA and coated with a PEC of trimethylchitosan and alginate, which display good *in vitro* antitumor activity towards HeLa cells, good antibacterial activity and stimulate the proliferation of murine lymphocytes and murine peritoneal macrophages (Ignatova et al., 2016).

The present contribution aims at studying the possibility for preparation of antibacterial and antioxidant fibrous materials based on PHB, QCh, Car and the natural phenolic compound CA of various designs using electrospinning or a combination of electrospinning, dip-coating and PEC formation. The morphology of the fibrous materials was assessed by scanning electron microscopy (SEM). The surface composition and the thermal characteristics of the novel materials were determined by X-ray photoelectron spectroscopy (XPS) and differential scanning calorimetry (DSC). The *in vitro* release profile of CA from fibrous materials of various compositions was monitored. The antibacterial activity of the obtained fibrous materials towards Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* was assessed. The adhesion of the pathogenic bacteria *S. aureus* onto the mats was studied, as well. The impact of the fiber composition on the antioxidant properties of the materials was also monitored.

2. Materials and methods

2.1. Materials

PHB (330 000 g mol^{-1}, Biomer), formaldehyde solution (36% in water) (Fluka), CH_3I (Fluka), $NaBH_4$ (Fluka), NaI (Fluka), κ-carrageenan (Car) (Sigma-Aldrich) and CA (Acros Organics) were of analytical grade of purity. Dimethylformamide (DMF) (Merck) and chloroform (Merck) were used as received. Prior to use, *N*-methyl-2-pyrrolidone (NMP) (Fluka) was distilled under reduced pressure. Chitosan (Aldrich) with an average viscometric molar mass of 380 000 g/mol and a deacetylation degree of 80% was used. The quaternized chitosan derivative (QCh) *N,N,N*-trimethylchitosan iodide was prepared from chitosan according to known procedure (Kim et al., 1997). The quaternization degree of QCh was determined by ^{1}H NMR. The quaternization degree was calculated from the intensity ratio of the signal at 3.39 ppm for $-$R-N$^+$($C\underline{H}_3$)$_2$I$^-$ (where R = CH_2 or CH_3) to the signals at δ 3.64-4.54 ppm for H-2, H-3, H-4, H-5, H-6, H-6′ (6H). The degree of quaternization was 56%. The degree of methylation of the —OH functions was determined from the intensity ratios of the signal of $C\underline{H}_3$—O at 3.50 and 3.42 ppm for OH at C-3 and C-6 positions, respectively, and the H-2, H-3, H-4, H-5, H-6, H-6′ (6H) signals at δ 3.64-4.54. The degree of methylation of H-3 and H-6 was 97%.

S. aureus 749 and *E. coli* 3588 were purchased from the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria.

2.2. Preparation of the fibrous materials

Three types of CA-loaded fibrous materials were prepared. The first one consists of PHB fibers with incorporated CA; they are further denoted as CA/PHB. For their preparation solutions of PHB and CA in $CHCl_3$/DMF = 4/1 (v/v) were mixed at polymer concentration of 10 wt%. The PHB solution was obtained upon heating (60 °C). CA content was 5, 10 and 20 wt% with respect to the polymer weight. The CA/PHB mixed solution was placed in a syringe and was delivered by using an infusion pump (NE-300 Just InfusionTM Syringe Pump, New Era Pump Systems Inc., USA) at a constant rate of 2.0 mL/h. Electrospinning was performed at a voltage of 25 kV and a distance of 25 cm from the needle tip to the rotating collector (1200 rpm). The obtained mats were placed under reduced pressure at 30 °C for 8 h to remove any solvent residues.

The second type of materials consisted of CA/PHB fibers coated with QCh/Car polyelectrolyte complex; they are further designated as QCh/Car-(CA/PHB). To obtain these materials, CA/PHB mats were first immersed for 15 min in 0.1 wt% aqueous solution of Car (prepared with stirring for 2 h at 60 °C), and subsequently dried to constant weight. Then Car-coated mats were immersed into 0.1 wt % QCh solution (in water/ethanol = 3/1, v/v) at room temperature for 15 min followed by drying the mats to constant weight.

The third type of materials (denoted as CA/QCh/Car-PHB) was composed of PHB fibers coated with QCh/Car complex containing CA. First, 10 wt% PHB solution in $CHCl_3$/DMF = 4/1 (v/v) was electrospun; then, the obtained PHB mat was coated with Car by immersion for 15 min in 0.1 wt% aqueous solution of Car. Then the CA/QCh/Car-PHB mat was prepared by immersing the Car-coated PHB mat in solution of QCh and CA (water/ethanol = 3/1, v/v) at room temperature for 1 h and drying the mat to constant weight. The QCh was dissolved in water, and CA was dissolved in ethanol. Solution was then prepared by mixing the two solutions at total concentration of 0.4 wt%, and QCh/CA weight ratio of 1/1. The amount of the incorporated CA was 20 wt% with respect to the PHB weight and was determined spectrophotometrically (λ_{max} 292 nm, $CHCl_3$/DMF = 4/1 (v/v)).

The dynamic viscosity of the spinning solutions was measured using a Brookfield DV-II+ Pro programmable viscometer for cone/plate option equipped with a sample thermostated cup and a cone spindle, at 25 ± 0.1 °C. The electrical resistance of the spinning solutions was measured in an electrolytic cell equipped with two rectangular sheet platinum electrodes as previously described (Ignatova et al., 2007).

2.3. Fibrous materials characterization

The morphology of the fibrous materials was evaluated by scanning electron microscopy (SEM). The samples were vacuum-coated with gold prior to observation by SEM (Jeol JSM-5510). The average fiber diameter was estimated by Image J software (Rasband, 2006).

The surface chemical composition of the fibrous materials was determined by XPS. The XPS measurements were carried out in the ultrahigh-vacuum (UHV) chamber of an ESCALAB-MkII (VG