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A bio-injectable algin-aminocaproic acid thixogel with tri-stimuli responsiveness

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ABSTRACT

In this article a novel bio-injectable algin-aminocaproic acid (Alg-ACA) tri-stimuli responsive thixogel system is reported. The designed soft thixotrophic hydrogel (thixogel) was characterized using various analytical techniques such as FT-IR, NMR, SEM, AFM and DSC. The soft thixogel system was further investigated for stress responsiveness using different rheological studies which confirmed the thixotropic nature of the gel [Thixotropic area (A_r) of Alg-ACA (1:0.5), Alg-ACA (1:1) and Alg-ACA (1:2), were 23.5%, 43.1%, and 27.59%, respectively, which were higher than that of Na-Alg (2.08%)]. The thixogel also demonstrated temperature and ultrasonication responsiveness. This tri-stimuli responsive soft thixogel system was rendered flowable (fluid) on applying the described physical stimuli and recovered its "rigid" gel structure upon removal of the applied stimuli. This approach of synthesizing a thixogels may be applicable to a broad variety of other natural polymers and has the potential for use in biomedical applications.

1. Introduction

In recent years, multi-stimuli responsive gels have emerged as an attractive candidate and new functional material in material chemistry with wide applications such as drug and gene delivery devices (Chatterjee, Haik, & Chen, 2003; Mano, 2008; Taylor, Tanna, Taylor, & Adams, 1995), several sensors and actuators (Matsumoto, Yoshida, & Kataoka, 2004; Suzuki, 2006). Stimuli responsive gels considered to have this property due to the weak non-covalent bonding interactions i.e. hydrogen bonding, π–π stacking, electrostatic and van der Waals interactions (An et al., 2004; Loos, Feringa, & Esch, 2005; Wang, Zhang, & Zhu, 2005). Currently, natural polymer based materials are demonstrated as key formulation ingredients for the fabrication of drug delivery systems (García-González et al., 2010; Jagur-Grodzinski, 2010; Joshi & Müller, 2009; Pose-Vilarnovo et al., 2004). Natural polysaccharides are especially attractive because they are renewable, cheap, biodegradable and abundantly available natural polymers in the biosphere, and are tuneable to for the development of advanced materials with novel properties (Huang, Yuan, & Chen, 2006; Malafaya, Silva, & Reis, 2007). With this in view, preparations of polysaccharide based hydrogels have attracted considerable attention as a stimuli responsive material for the utility in various fields. Several investigations exist wherein polysaccharide based multi-stimuli responsive gels have been reported (Dumitriu, Mitchell, & Vasile, 2011; Mocanua, Souguirb, Picton, & Cerf, 2012; Mocanua, Mihai, Dulong, Picton, & Cerf, 2012; Prabaharan & Mano, 2006).

Sodium alginates, one of the oldest natural polymers have become an extremely important polysaccharide in the field of biomedical applications because of their potential applications (Boateng, Matthews, Stevens, & Eccleston, 2008; Ghidoni et al., 2008; Lee & Mooney, 2012; Ramsey & Wozniak, 2005; Soonshiong et al., 1993; Zimmermann, Shirley, & Zimmermann, 2007). Sodium alginate has been widely used for many applications in the field of drug delivery and tissue engineering due to its biocompatibility, non-immunogenicity, relatively low cost, and easy gelling ability with divalent cations such as Ca^{2+} (George & Abraham, 2006). The thickening and gel-forming ability of sodium alginate are the key factors for use of this polymer as a conventional drug carrier in the field of drug delivery (Tønnesen & Karlsen, 2002). The present research work demonstrated fictionalization of sodium alginate by aminocaproic acid via amide formation using carbodiimide chemistry. Aminocaproic acid (ACA) is a one of the well-known anti-fibrinolytic agents used for the treatment of excessive

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bleeding, especially bleeding resulting from systemic hyperfibrinolysis, urinary fibrinolysis or several surgeries such as cardiac, spine and liver transplantation (Cheriyan et al., 2013; Gardner & Helmer, 1980; Kang et al., 1987). Aminocaproic acid (ACA) is a synthetically derived from lysine, an amino acid used to inhibit the conversion of plasminogen to plasmin and also directly inhibit the proteolytic activity of plasmin (Kang et al., 1987). Further it has been observed that the gelling properties of sodium alginate can be induced via amide formation by adding different amine substrates on to it (Chhatbar, Prasad, Chejara, & Siddhanta, 2012; Chejara, Kondaveeti, Prasad, & Siddhanta, 2013). With this in view, this study reported the synthesis of amide conjugates of native biopolymer sodium alginate using aminocaproic acid, i.e. amide bond formation between the amine group of ACA and carboxyl group of sodium alginate which was observed to have ultrasound-induced gel forming ability, injectable property and tri-stimuli responsiveness to various stimuli. To our knowledge, this is the first report on synthesis of alginate-aminocaproic acid based amide conjugate. The developed materials may have potential use as a drug delivery system in diverse biomedical applications due to their tri-stimuli responsiveness as well as injectable ability.

2. Experimental

2.1. Materials and methods

Sodium alginate (Na-Alg, M_w = 75 kDa), aminocaproic acid (ACA) and N,N′-dicyclohexylcarbodiimide were of AR grade and purchased from Sigma–Aldrich® Inc. (St. Louis, MO, USA). Other general chemicals and solvents were of analytical grade and were used as received.

2.2. Synthesis of the Alginate-aminocaproic acid (Alg-ACA) conjugates

Synthesis of alginate based amide conjugate was carried out using a method adapted from the literature (Abulateefeh, Khanfar, Al Bakain, & Taha, 2013). Sodium alginate (1.04 g, 5 mmol carboxylate) in 90 mL of distilled water was stirred for one hour, the pH was then adjusted to 3–4 using hydrochloric acid (0.5 M). Thereafter, dicyclohexylcarbodiimide (DCC, 5 mmol, 1.03 g) was added. After 2 h, aminocaproic acid (0.33–1.31 g i.e. 2.5–10 mmol according to various ratios) was added to the reaction mixture. Subsequently, the pH of the reaction mixture was raised to 9 using sodium hydroxide solution (2.0 M). After 24 h, the reaction was terminated by precipitation with hydrochloric acid (10 mL, 1.5 M) and acetone (100 mL). The precipitate was separated by filtration on a Whatman filter paper no. 41. The generated precipitate was washed thoroughly thrice with 25 mL each of acetone, ethanol and diethyl ether, followed by drying the product under vacuum at 45 °C.

2.3. Degree of substitutions

The degree of substitution, which represents the quantity of aminocaproic acid chains per hexuronic acid residues, was calculated by comparing the ratio of methylene protons correlated with carbons C1′–C5′ of the ACA chain in the Alg-ACA graft to carbons C2–C5 of alginate protons using Eq. (1) (Elomaa et al., 2004).

$$DS = 5A_{\text{Alg-ACA}}/4A_{\text{Na-Alg}} \quad (1)$$

where $A_{\text{Alg-ACA}}$ is the area of methyl protons of the Alg-ACA graft and $A_{\text{Na-Alg}}$ is the area of methyl proton signals of the sodium alginate unit.

2.4. Characterization of Alg-ACA conjugates

The amide conjugates of alginate coupled with ACA (Alg-ACA) were characterized by Fourier Transform Infrared (FT-IR) spectroscopic analysis in which spectra were recorded on a Perkin Elmer Spectrum 2000 FT-IR spectrometer, employing a single-reflection diamond MIRTGS detector (PerkinElmer Spectrum 100, Llantrisant, Wales, UK). ^{13}C and ^{1}H NMR spectra for derivatives were recorded on a Bruker Advance-II 500 (Ultra shield) spectrometer, Switzerland, at 500 MHz. Samples were dissolved in D_2O (30–40 mg mL^{-1}), and spectrum for Alg-ACA was recorded at 70 °C while for sodium alginate and ACA spectra were recorded at 30 °C using d_6-DMSO (ca. 39.4 ppm) as internal standard. ACA was dissolved in d_6-DMSO (15–20 mg mL^{-1}).

2.5. Microstructure and morphology characterizations of Alg-ACA conjugate

Surface morphology and microstructure of the synthesized derivatives were studied using scanning electron microscopy (SEM) and atomic force microscopy (AFM). SEM analysis was performed using a Phenom™ SEM (FEI Company, OR, USA). Vacuum oven dried samples were mounted on a sample holder and coated with gold via sputter coating (SPI Module™ Sputter Coater, SPI Supplies, PA) before analysis. The surface topography changes were observed by AFM. Samples were measured by AFM with tapping mode in air using a Dimension 3100 Veeco AFM. All scans were performed using commercial High-Resolution Tapping Mode silicon probes with the nominal tip radius of 8 nm. The amplitude set point was typically 75% of the free air oscillation amplitude. The resonance frequency of the cantilever was 300 kHz.

2.6. Thermal analysis of Alg-ACA conjugates

The thermal behaviour of the prepared conjugates and individual polymer were determined by differential scanning calorimetry (DSC) on Mettler Toledo, DSC1 (STARe System, Schwerzenback, Switzerland). Samples (10 mg) were placed into 40 μL aluminium pans and were heated from 20 to 350 °C at a rate of 10 °C/min.

2.7. Ultrasound-induced gelation of Alg-ACA conjugates

For the preparation of gel, the conjugate Alg-ACA was homogenously dispersed in distilled water (conc. of 3% w/v) via ultrasonication (pulse: Amp 1; 80% for 30 s) using SONICS Vibra cell (Model CV18; 130 W; 20 kHz) at 60 °C followed by cooling of the mixture to 20 °C.

2.8. Rheological transitions of the Alg-ACA conjugate thixogels

Rheological measurements were performed on a Haake Mars (II) Modular Advanced Rheometer system using cone plate geometry (Rotor C35/1, D = mm, 1° Titan) at a gap of 0.050 mm. The hydrogels prepared of Alg-ACA conjugates (3% w/v) as well as of alginate (3% w/v) were homogenized prior to assessment. Following different types of experiments were performed: (a) apparent viscosities with different shear rates, (b) hysteresis loop test, (c) time-dependent thixotropic property determination, (d) yield stress, and (e) creep recovery. Dynamic viscosities of all samples were determined with varying shear rates at 25 °C. Oscillatory frequency sweeps for all samples were performed from 50 to 0.10 rad s^{-1} at a constant shear stress of 1 Pa. For evaluation of thixotropy, varying shear rates in the range of 0.1–500 s^{-1} for 600 s were applied to the samples, where an upward curve was immediately followed up by a downward curve. Areas under the upward