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Electrospun xanthan gum-chitosan nanofibers as delivery carrier of hydrophobic bioactives

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ABSTRACT

Viscoelastic gels of xanthan gum-chitosan(X-Ch) in formic acid were electrospun to produce nanofibers, stable in aqueous media, for the encapsulation and release of curcumin (Cu). After 120 h, the nanofibers released lower amount of curcumin (~20%) at pH 2.2 comparatively to the release in neutral media (~50%), suggesting that X-Ch nanofibers could be used as a carrier for the encapsulation of hydrophobic bioactive compounds with long-term pH-stimulated release properties.

1. Introduction

The efficient delivery of hydrophobic bioactives, requires proper encapsulation to overcome concerns related with their low solubility and instability in aqueous body fluids, and limited bioavailability. Electrohydrodynamic (electrospinning and electrospray) methods have been widely studied due to their high encapsulation efficiency, low process temperature using a range broad food bioactive and shell ingredient [1]. Moreover, electrospun fibers comes along with high surface area, tunable diameter and surface functionality, which makes them very attractive for encapsulation and controlled bioactive release [1,2].

Curcumin is a phenolic compound recognized by its pharmaceutical properties as an antioxidant, antimicrobial, anti-inflammatory agent and inhibitor of tumorigenesis and metastasis [3,4]. Due to its hydrophobicity and subsequent poor bioavailability, new delivery carriers have been investigated using electrospinning technology [5–7].

Chitosan (Ch) is a cationic polysaccharide consisting of N-acetyl glucosamine and glucosamine known for its biocompatibility, biodegradability, and mucoadhesivity [8] and ability to enhance gastrointestinal drug absorption [9]. Xanthan (X) gum is an anionic polysaccharide known for its peculiar physico-chemical properties [10] and has been used as encapsulating matrix [11]. A recent study from our group found that X-Ch-Cu nanofibers incubated with Caco-2 cells, resulted in enhancement of the *in vitro* absorption of Cu across cell monolayers, with a 3-fold increase of Cu permeability, compared to free-curcumin. This work aims to investigate the X-Ch nanofiber development, morphological and encapsulation properties and evaluate its potential as a Cu release carrier in various pH media.

2. Experimental

2.1. Materials

All chemicals including xanthan gum (Mw about 2000 kDa [12], chitosan (Mw 28 kD, degree of deacetylation (DD) of 89% and degree of polymerization (DP) of 175), curcumin and formic acid, were obtained from Sigma-Aldrich (Denmark).

2.2. Preparation and characterization of electrospun solutions and fibers

Xanthan (0.75% w/v) and chitosan (3% w/v) were dissolved in formic acid under vigorous stirring overnight at room temperature. Curcumin (2% w/v) was added to X-Ch solution, and stirred for 30 min. X, Ch and X-Ch (with and without curcumin) rheological properties were determined as described at [13]. X-Ch

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and X-Ch-Cu solutions were electrospun at 25 kV (ES50P-10 W, Gamma High Voltage Research, Inc., USA), feed rate of 0.01 mL/min (syringe pump, New Era Pump Systems, USA) using a 21G needle (Proto Advantage, Canada). Fibers were collected on a stainless steel plate, placed 10 cm from the needle tip. Scanning electron microscopy (SEM) and fiber diameter distribution analyses (100 data points) followed the protocol described in [5]. Atomic force microscopy (AFM) was performed on Multimode 8 in PeakForce QNM mode. TAP150A probes with normial spring constant of 5 N/m were used. To measure the adhesion force, deflection sensitivity calibration was performed on sapphire and spring constant (determined by thermal tuning). Adhesion map was formed by plotting the adhesion forces at each point, obtained from the retraction part of each force-distance curve. The encapsulation efficiency (EE) of the Cu within X-Ch nanofibers was determined by Cu fiber extraction using methanol in a sonication bath [5].

2.3. In vitro release studies

X-Ch-Cu nanofibers (3.0 mg) were suspended in 2 mL of Tris buffered saline solution (pH 2.2, 6.5 and 7.6) at 37 °C in a thermoshaker water bath. Supernatant aliquots (100 μL) were withdrawn and replaced with the same volume of fresh media. The amount of Cu released was determined using a NanoDrop One UV–Vis Spectrophotometer (Thermo Fisher Scientific, Denmark) at the optical wavelength of 420 nm. Triplicates were conducted for each sample.

3. Results and discussions

Formation of a viscoelastic network with elastic modulus values (G′) higher than the viscous modulus (G″) was observed for the X-Ch mixture in formic acid after 12.5 h (Fig. 1a). Individual xanthan and chitosan solutions exhibited G" higher than G'. The tan δ value (tan δ = G″/G′) for individual chitosan and xanthan solutions was 2,533 and 1,17, respectively, denoting a liquid-like behaviour, whereas the tan δ of X-Ch mixture was 0.144, confirming a gel-like behaviour as reported for other biopolymers gels [13].

Flow rheological studies (Fig. 1b) reveal that xanthan in formic acid has a shear thinning behaviour [13]. The chitosan solution shows nearly Newtonian behaviour at the shear rates of 0.1–100 s^{-1}, and higher viscosity values than xanthan solution. However, a substantial viscosity increase was observed for X-Ch mixture. X and X-Ch mixture followed a power-law thinning behaviour of $\eta = m \cdot \gamma^{n-1}$ where η is the apparent viscosity, γ the shear rate, and m the flow index. The power law index values (n) were in the range of 0.551–0.437 for X-Ch and X-Ch-Cu, which are necessary to produce electrospun fibers [13]. The formation of a viscoelastic gel network, and an apparent viscosity increase of the X-Ch mixture is due to the oppositely charged X-Ch polyelectrolytes electrostatic interactions [14].

Fig. 2 shows individual, uniform and randomly oriented X-Ch nanofibers with average diameters of 750 nm. Note that both individual xanthan and chitosan solutions at the aforementioned concentrations could not be electrospun into fibers. The average diameter of electrospun xanthan-chitosan nanofibers slightly increased to 910 nm with the addition of 2% curcumin, due to the increase of the solution viscosity (Fig. 1b). The EE of Cu within X-Ch nanofibers was 69.4 ± 4.1%.

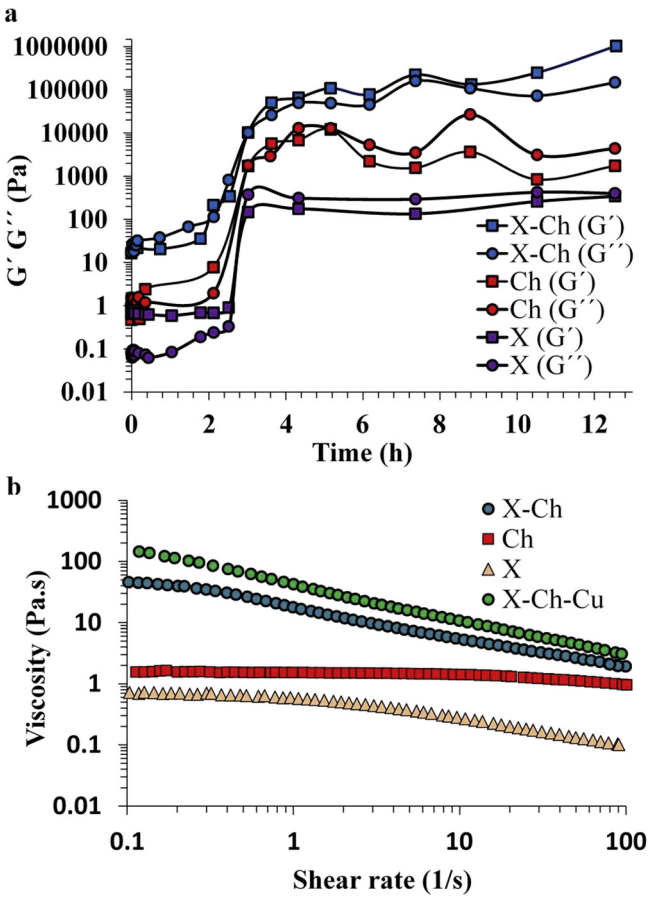

Fig. 1. Viscoelastic properties over time (a) and flow curves (b) of X, Ch, X-Ch and X-Xh-Cu solution.

The X-Ch and X-Ch-Cu fibers' adhesive properties were quantified by Peakforce QNM, which measures adhesion force between the AFM tip (silicon) and nanofibers at each pixel (Fig. 3). Adhesion forces from the top of the nanofibers are shown in the line profiles of X-Ch and X-Ch-Cu, with the average values of 10 nN, and 4 nN, respectively. X-Ch nanofibers displayed adhesive properties that decreased with the encapsulation of curcumin, due to the hydrophobic nature of this bioactive. The adhesive maps indicate that curcumin is incorporated homogeneously within X-Ch-Cu nanofibers.

Fig. 4 shows an 8–10% sustained release of curcumin from X-Ch nanofibers over 12 h for all pH studied (2.2, 6.5 and 7.4) with no burst effect [6]. Beyond that, the release of curcumin at pH 2.2 was much lower than the other media, with no significant increase for up to 120 h. On the other hand, the Cu release conducted in media at pH 7.4 and 6.5 was increased beyond 12 h up to 45 and 50% respectively [6]. It is to note that X-Ch and X-Ch-Cu nanofibers remained intact in all release media after 10–12 h at 37 °C (data not shown). However, after 120 h X-Ch-Cu nanofibers slightly swelled in buffer at pH 6.5 and 7.4 (about 3.5 times the initial fiber diameter), while at pH 2.2 the diameter increased about 1.5 times.

It is suggested, that the oppositely charged xanthan-chitosan mixture in formic acid is associated electrostatically [14]. When the electrospun nanofibers are immersed in the release media at different pH(s), the electrostatic equilibrium for both