Electrospinning of Octenylsuccinylated Starch-Pullulan Nanofibers from Aqueous Dispersions

Songnan Li – University of Alabama
Lingyan Kong – University of Alabama
Gregory R. Ziegler – Pennsylvania State University

Deposited 08/31/2020

Citation of published version:
Electrospinning of Octenylsuccinylated Starch-Pullulan Nanofibers from Aqueous Dispersions

Songnan Li a,b, Lingyan Kong b,*, Gregory R. Ziegler a,*

a Department of Food Science, Pennsylvania State University, 341 Food Science Building,
University Park, PA, 16802, USA
b Department of Human Nutrition and Hospitality Management, The University of Alabama,
Tuscaloosa, AL, 35487, USA

* Corresponding authors.

Address:
341 Rodney A. Erickson Food Science Building, University Park, PA 16802, USA (G. Ziegler)
482 Russell Hall, 504 University Blvd, Tuscaloosa, AL 35487, USA (L. Kong)

E-mail address:
lkong@ches.ua.edu (L. Kong)
grz1@psu.edu (G. Ziegler)
Abstract

We aimed to develop a greener process for dry-electrospinning food-grade modified starch through the elimination of organic solvents. The rheological properties and electrospinnability of aqueous dispersions of commercial octenylsuccinylated (OS) starches with various molecular weight (Mw) were investigated, yet only nanofibers with beads or defects could be obtained from OS starch with the highest Mw, i.e., Purity Gum® Ultra (PGU). Further improvement in the fiber morphology was achieved by adding pullulan (PUL) as a minor component in the spinning dope. Smooth, continuous, and bead-free nanofibers (147-250 nm) were obtained from the PGU-PUL dispersions. Shown on an electrospinnability map, the successful electrospinning of 12%, 15%, and 20% (w/v) aqueous PGU dispersions required a minimum addition of 6%, 5%, and 3% (w/v) of PUL, respectively. The addition of PUL contributed to establishing sufficient molecular entanglement for electrospinning. This study provides a promising green process to produce starch-based nanofibers for use in various applications, e.g., drug delivery, wound dressing, and tissue engineering.

Keywords

Modified starch; octenylsuccinylated starch; pullulan; rheology; electrospinning; nanofiber
1. Introduction

Electrospinning, an electrohydrodynamic processing technique, employs a high electrostatic force to stretch a viscoelastic jet derived from a polymeric solution or melt into fine fibers. Both synthetic polymers and natural biopolymers can be fabricated into micro- and nano-scale fibers via electrospinning. Because it is cost-effective, applicable to a large variety of materials, capable of controlling fiber size and morphology, and easily scaled, electrospinning has garnered much attention (Xue, Wu, Dai, & Xia, 2019). A variety of natural biopolymers, such as chitosan, hyaluronic acid, collagen, cellulose, starch, and soy proteins, have been electrospun into micro- and nanofibers with potential uses in food technology, packaging, filtration, controlled drug delivery, tissue engineering, wound dressing, and other biomedical applications (Ameer, Pr, & Kasoju, 2019; Mendes, Stephansen, & Chronakis, 2017).

Starch is the second most abundant renewable biopolymer on earth after cellulose and widely used in paper products, bioplastics, engineered products (e.g., composite fibers and films), agricultural products (e.g., water release material and seed carrier), and biomedical products (e.g., in drug delivery and tissue scaffold compositions) (Masina et al., 2017; Sweedman, Tizzotti, Schafer, & Gilbert, 2013). The electrospinning of micro- to nano-scale starch fiber mats with random and aligned orientation has been demonstrated by a number of researchers (Kong & Ziegler, 2012, 2014a; Vasilyev, Vilensky, & Zussman, 2019; Wang, Kong, & Ziegler, 2019a). Kong
and Ziegler (2012) developed a wet-electrospinning technique using an ethanol coagulation bath to fabricate pure starch fibers from a dimethyl sulfoxide (DMSO)/water solvent medium. There are drawbacks to this technique: the drying step after wet electrospinning is time-consuming (>6 h), the fiber mat was fragile, and the fiber diameter was in the micron range (Kong & Ziegler, 2013, 2014a). The plasticizing effect of water during storage (Wang, Kong, & Ziegler, 2018), incorporating nanocellulose-cationic starch (Wang, Kong, & Ziegler, 2019b), and achieving fiber alignment with a rotating drum collector (Wang et al., 2019a) were all found to improve the tensile strength of starch fiber mats by a modest amount. Starch-based nanofibers (~146 nm) were electrospun from aqueous solvent with the addition of sodium palmitate and pullulan (PUL) (Wang & Ziegler, 2019). However, this was still a wet-spinning process.

Lancuski, Vasilyev, Putaux, and Zussman (2015) used aqueous formic acid to dissolve starch and obtained starch-formate fibers with diameters ranging from 80 to 300 nm using a dry-electrospinning process. Though formic acid can chemically gelatinize starch granules, it may also decrease the molecular weight and cause the formylation of starch (Lancuski et al., 2015). Furthermore, there were kinetic constraints, as the solutions were spinnable for a short window in time only (Lancuski et al., 2015). Current techniques relying on organic solvents may limit the commercialization and utilization of starch fibers, especially for food and biomedical applications. Dry-electrospinning of starch nanofibers using green solvents, e.g., aqueous solvents, would lower the cost of chemicals and waste treatment, minimize chemical residues, and ensure non-irritability.
Native starches possess poor solubility in aqueous solvents resulting from strong hydrogen bonding between starch chains (Zhang et al., 2017). Various modification techniques (i.e., physical, chemical, enzymatic, genetic, and blended treatments) can alter the gelatinization, swelling, solubility, pasting and retrogradation characteristics of starch (Masina et al., 2017). Among modified starches, octenylsuccinate (OS) starch, i.e., starch after chemical modification with octenyl succinic anhydride, has been used as a high-performance, cost-effective replacement for gum Arabic in food applications. Most commercial OS starches are derived from waxy maize (e.g., Purity Gum™, CAPSUL™, and Hi-CAP™) or normal maize (e.g., DRYFLO™) starches through octenylsuccinate substitution and enzymatic hydrolysis (Sweedman, Tizzotti, Schafer, & Gilbert, 2013). OS starches have better water solubility or dispersibility making them suitable for formulations such as liquid beverage emulsions. Here we used OS starch, a GRAS (Generally Recognized as Safe) additive in food and cosmetics, to obtain a relatively stable dispersion of starch in water at concentrations suitable for electrospinning.

PUL was added to aid the electrospinning of OS starch. PUL is an extracellular and water-soluble microbial polysaccharide produced by Aureobasidium pullulans, and currently exploited in food and pharmaceutical industries due to its unique characteristics (non-ionic, non-toxic, non-immunogenic and non-carcinogenic) (Prajapati, Jani, & Khanda, 2013). The unique glycoside linkages of α-1,6 and α-1,4 in PUL endow this polymer with distinctive physical traits, including
adhesive properties and the ability to form films and fibers. Electrospun PUL fiber mats with and without other polymers or additives have been reported (Karim et al., 2009; Kong & Ziegler, 2014b; Li et al., 2017). PUL addition made the wet-electrospinning of nanofibers from aqueous dispersions of high-amylose starch (HAS) sodium palmitate inclusion complexes feasible (Wang & Ziegler, 2019).

Based on our previous work electrospinning starch-sodium palmitate inclusion complexes, we hypothesized that OS starch would be electrospinnable but that this would depend on the degree of substitution and molecular weight. In this study, we attempted to dry-electrospin commercial OS starches of various molecular weight (Mw) from their aqueous dispersions, and further improve the nanofiber morphology by blending with PUL. Both sufficient molecular entanglement and shear viscosity were found to be determining factors dictating the electrospinnability. An electrospinnability map as a function of OS starch and PUL concentration was obtained. Nano-sized OS starch-PUL fibers were successfully fabricated by dry-electrospinning and could be a promising nanofiber material for various applications in the food, pharmaceutical, cosmetic, and biomedical fields.

2. Materials and methods

2.1. Materials

Three commercial OS starches, including Purity Gum® Ultra (PGU), Purity Gum® 2000
(PG2000), and Hi-Cap® 100 (HC100), were kindly supplied by Ingredion (Bridgewater, NJ). Food
grade pullulan (PUL) was purchased from Hayashibara Biochemical Laboratories Inc. (Okayama,
Japan). Deionized water (DI water) was used to prepare all aqueous solutions.

Physical characteristics of the starches and PUL, including viscosity average molecular weight
(Mw), degree of substitution (DS), and moisture content (MC), are summarized in Table 1. The
viscosity average molecular weight of the OS starch samples was determined by viscometric
measurements and the Mark’s variation of Staudinger’s formula (Dokić, Dokić, Dapčević, &
Krstonošić, 2008; Kerr, Cleveland, & Katzbeck, 1951). The DS of OS starches was determined
using excess alkali saponification and back titration with HCl, according to Chiu et al. (2017). The
MC of OS starch and PUL samples were determined using an air-oven method (AACC method
44-15.02) (AACC International, 1999). Measurements were made in triplicate and data are
reported as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Samples</th>
<th>PGU</th>
<th>PG2000</th>
<th>HC100</th>
<th>PUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mw (×10^4 g/mol)</td>
<td>1.24</td>
<td>3.8</td>
<td>2.3</td>
<td>10~20</td>
</tr>
<tr>
<td>DS (×10^{-2})</td>
<td>0.16 ± 0.04</td>
<td>4.72 ± 0.10</td>
<td>4.77 ± 0.02</td>
<td>n.a.</td>
</tr>
<tr>
<td>MC (%)</td>
<td>5.45 ± 0.13</td>
<td>5.58 ± 0.16</td>
<td>4.61 ± 0.10</td>
<td>6.15 ± 0.21</td>
</tr>
</tbody>
</table>

a Mw, molecular weight; Mw of PUL was obtained from manufacturer’s datasheets.
b DS, degree of substitution.
c MC, moisture content.
2.2. Dope preparation

To prepare the spinning dopes, an appropriate amount (0.1-30%, w/v) of OS starch, PUL or their mixtures were dispersed into DI water. These dispersions were heated in a boiling water bath with continuous stirring on a magnetic stirrer hotplate for 1 h, and then allowed to cool to room temperature (20 °C). The surface tension of the dispersions was measured via an automated tensiometer (Rame-hart model 260, Succasunna, NJ, USA) using pendant drop mode. The conductivity of the dispersions was measured using a calibrated EXTECH conductivity meter (EC100, FLIR Systems, Inc., Waltham, MA, USA). Measurements were made in triplicate and data are reported as mean ± standard deviation.

2.3. Rheology

The apparent shear viscosity of spinning dopes differing in polymer concentration was measured using a strain-controlled rheometer (ARES, TA Instrument, New Castle, DE, USA) with cone and plate geometry (50 mm), a gap of 0.043 mm, and cone angle of 0.04 radians. Flow curves, i.e., apparent shear viscosity versus shear rate increasing from 0.1 to 100 s⁻¹, were collected at 20 °C.

In this study, the relative viscosity at 100 s⁻¹, close to the shear rate of the electrospinning process, was plotted against polymer concentration to illustrate the scaling relationship. The log-log plot of relative viscosity, \( \eta_r \) (Eq. (1)) of each aqueous dispersion at 100 s⁻¹, against polymer
concentration was fitted by linear equations to calculate the overlap and entanglement concentrations.

\[ \eta_r = \frac{\eta}{\eta_s} \]  

(1)

Where \( \eta_s \) is the measured viscosity of DI water and \( \eta \) is the shear viscosity of polymer dispersions at 100 s\(^{-1}\), which is the approximate shear rate at the tip of spinneret (Greiner & Wendorff, 2008). The overlap concentration (\( C^* \)) and entanglement concentration (\( C_e \)) were taken as those concentrations where abrupt changes in the power law exponent for the concentration dependence of viscosity were observed.

2.4. Electrospinning

The above spinning dopes were loaded into 12 mL syringes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with 22-gauge blunt needles (Hamilton Company, Reno, NV, USA) as the spinneret. The electrospinning apparatus comprised a high voltage power supply (ES40P, Gamma High Voltage Research, Inc., Ormond Beach, FL), a syringe pump (81,620, Hamilton Company, Reno, NV), and a piece of grounded aluminum foil as the collector. Electrospinning of each OS starch dispersion was conducted by varying three spinning parameters (flow rate, voltage, and spinning distance) until the onset of a Taylor cone was observed at the tip of the spinneret. The electrospinning process was performed at room temperature (20 °C) in an enclosed Plexiglas box (Kong & Ziegler, 2014a; Wang & Ziegler, 2019). The electrospinnability was determined by visual
inspection, and the resulting mat was collected for further analysis.

2.5. Characterization

Observation of as-spun fiber mats was performed using a Phenom G2 Pro scanning electron microscope (SEM, Phenom-World, Eindhoven, the Netherlands) at an accelerating voltage of 5 keV. The samples were not coated with metal, the support was a standard SEM aluminum stub and the sample was adhered to the stub using double-sided carbon tape. Open software, Image J (National Institutes of Health, Bethesda, MD), was used for analyzing fiber diameters from the SEM images (Angel, Guo, Yan, Wang, & Kong, 2020). Five images were used for each sample and at least 150 different segments were randomly measured.

3. Results and discussion

3.1. Rheological properties and electrospinnability of OS starches

Previous studies have revealed that rheological properties of a polymer dispersion are crucial for determining its electrospinnability. For a polymer dispersion to form a coherent viscoelastic jet and be elongated by the electrostatic force, sufficient molecular entanglements need to be established to resist the breakup of the jet. Meanwhile, the resistance to deformation, i.e., shear viscosity, should be within an appropriate range for sufficient stretching of the jet that leads to a reduction in fiber diameter. To explore the electrospinnability of OS starches, the rheological
properties of their aqueous dispersions were characterized.

Flow curves of OS starch (HC100, PG2000 and PGU) aqueous dispersions varying in concentration (0.1-30%, w/v) are shown in Figure S1. Unreliable data, i.e., data lower than the detection limit of the rheometer, were not plotted.

Both relative viscosity ($\eta_r$) and specific viscosity have been used to distinguish unentangled from entangled regimes (Kong & Ziegler, 2014b; Wang & Ziegler, 2019). Here log $\eta_r$ at 100 s$^{-1}$ was plotted against log starch concentration (Figure 1A-C). Three regions of concentration dependence can be clearly distinguished: the dilute regime, semidilute unentangled regime, and semidilute entangled regime, respectively. In the dilute regime, the concentration dependences of the three OS starches were all very low, i.e., 0.05, 0.11, and 0.17, for HC100, PG2000 and PGU, respectively, indicating that no polymer chain overlap occurred. Beyond the overlap concentration ($C^*$), i.e., in the semidilute unentangled regime, the overlap is insufficient to cause any significant degree of entanglement (Colby, 2009). The concentration dependencies in the semidilute unentangled regime were 0.64, 1.03, and 1.06 for HC100, PG2000, and PGU, respectively. These values were lower than theoretically predicted (~$c^{1.25}$) (McKee, Wilkes, Colby, & Long, 2004) or observed for neutral linear polymers (de Gennes, 1979), including HAS in pure DMSO ($\eta_{sp} \sim c^{1.40}$) (Kong & Ziegler, 2012), chitosan in 80% aqueous acetic acid solution ($\eta_{sp} \sim c^{1.30}$) (Klossner, Queen, Coughlin, & Krause, 2008), and for branched polymers, e.g., poly (ethylene terephthalateco-ethylene isophthalate) in chloroform/dimethylformamide mixture solvent ($\eta_{sp} \sim c^{1.39}$) (McKee et
This suggests that the OS starches, which were modified from waxy maize starch (i.e., highly branched amylopectin), had a less extended or a rather spherical conformation in the aqueous dispersions at low concentrations (Sweedman et al., 2013).

In the semidilute entangled regime, the concentration dependencies were 1.70, 2.25, and 2.65 for HC100, PG2000, and PGU, respectively. These values were higher than the reported value ($\eta_r \sim c^{1.60}$) for HAS-palmitate-PUL in DI water (Wang & Ziegler, 2019), comparable to that of starches with various amylose content in 95% (v/v) DMSO aqueous solution ($\eta_r \sim c^{2.14-2.87}$) observed by Kong and Ziegler (2012), and lower than the theoretical predictions for linear polymers in a good solvent ($\eta_{sp} \sim c^{4.80}$) (de Gennes, 1979). This implies that although the OS starches begin to interact above the entanglement concentration, they could not entangle as sufficiently as linear polymers.

In both the semidilute unentangled and semidilute entangled regimes, the concentration dependence increased with the relative molecular weight.

Entanglement concentrations ($C_e$) of OS starches in aqueous dispersions were obtained as the intercept of the fitted lines of the semidilute unentangled and semidilute entangled regimes (Figure 1A-C). The $C_e$ values were determined to be 12.68% (w/v), 12.36% (w/v), and 6.43% (w/v) for HC100, PG2000, and PGU, respectively. As would be expected, these values decreased with the increasing relative molecular weight of the OS starches (HC100 < PG2000 < PGU). Higher molecular weight polymers may occupy greater hydrodynamic volume and thus require a lower concentration to entangle (Gupta, Elkins, Long, & Wilkes, 2005). Moreover, these results were far
higher than those of HAS palmitate-PUL in DI water (3.63%, w/v) (Wang & Ziegler, 2019) and HAS in aqueous DMSO (3.61%, w/v) (Kong & Ziegler, 2012). It appears that in DI water these OS starches need to be more concentrated to establish significant molecular entanglement as might be expected for a branched conformation.

In addition to the fulfillment of molecular entanglement, the apparent viscosity of the polymer dispersion at a high shear rate must fall into an appropriate range for electrospinnability. According to Kong and Ziegler (2012), the electrospinnable viscosity range (at 100 s\(^{-1}\)) for pure HAS in aqueous DMSO dispersions was 0.2-2.2 Pa·s. Figure 1D presents the shear viscosities of OS starch aqueous dispersions at 100 s\(^{-1}\) as a function of starch concentration (0.1-30%, w/v). The shear viscosities at the same concentration followed the order of PGU > PG 2000 > HC100, which would be expected from their relative molecular weights. Polymers of higher molecular weight would possess a larger surface area, facilitating the entanglement of polymer chains, and thus greater intermolecular friction and resistance to flow (Colby, Fetters, & Graessley, 1987). PGU of 8-20% (w/v) and PG2000 of 20-30% (w/v) demonstrated shear viscosities at 100 s\(^{-1}\) in the range from 0.2-2.2 Pa·s, suggesting possible working conditions.
Figure 1. Plots of relative viscosity (A-C: HC100, PG2000 and PGU) and shear viscosity at 100 s⁻¹ (D) of OS starch aqueous dispersions as a function of starch concentration (0.1-30%). The overlap concentration (C*) and entanglement concentration (Cₑ) and slopes of fitted lines in three regimes are illustrated.

Electrospinning of PGU dispersions (15-30%, w/v) was attempted using predetermined parameters (fixed spinning distance of 5-10 cm, feed rate of 0.2-0.6 mL/h, and voltage of 15-25 kV), and the products are shown in Figure S2. OS starch nanofibers with beads start to form from 30% (w/v) PGU, that is the onset concentration for successful electrospinning of PGU aqueous dispersions was at least 4.7 times of its Cₑ (6.43%, w/v, as shown in Figure 1C). This value is higher than the ranges observed for HAS in DMSO/water solutions (1.2-2.7 times), PUL (1.9-2.3
times), and HAS-palmitate-PUL (2.8 times) aqueous solutions (Kong & Ziegler, 2012, 2014b; Wang & Ziegler, 2019). This phenomenon could be explained by the insufficient chain entanglement between molecular chains of the OS starch derived from waxy maize starch (Sweedman et al., 2013). In contrast, HC100 and PG2000 dispersions could not be spun into fibers even at 30% (w/v), but could only produce nano- and micro-size particles (Figure S3). This could be attributed to their low shear viscosity or much higher $C_e$. During the electrospinning process, with insufficient molecular entanglement and low viscosity, the jets of polymer dispersion are unstable and tend to break up and form droplets, resulting in electrosprayed particles (Niu, Shao, Luo, & Sun, 2020). Gupta et al. (2005) investigated the electrospinnability of poly (methyl methacrylate) with Mw ranging from 12,470 to 365,700 g/mol, and found that in the semidilute unentangled regime, polymer droplets were formed at lower Mw (12,470 and 17,710 g/mol), some limited fiber formation connected by polymer droplets for increased Mw (125,900 g/mol), beaded fibers for higher Mw (205,800 and 365,700 g/mol), while uniform and bead-free fibers were observed for all the samples in the semi-dilute highly entangled regime. Tao and Shivkumar (2007) reported that at constant concentration the structure of electrospun poly (vinyl alcohol) changed from beads, to beaded fibers, to complete fibers and to flat ribbons as the Mw increased from 9,500 g/mol to 155,000 g/mol.

In addition to the dope solution, other electrospinning parameters, including voltage, flow rate and distance, are also key factors that have significant influence on the as-spun fiber morphology.
Further trials with 30% of PGU were carried out by varying the spinning distance (5-11 cm), voltage (21-26 kV), and flow rate (0.2-1 mL/h), and their results are shown in Figure S4. Even the optimized electrospinning parameters (distance of 9 cm, voltage of 24 kV and flow rate of 0.2 mL/h) could not produce bead-free fibers (Figure 2). The result suggested that sufficient chain entanglement in the polymer solution is necessary for electrospinning fibers, otherwise electrospraying will take place, and instead of fibers, particles, beads, or droplets are formed on the collector. Fong, Chun, and Reneker (1999) reported that the formation of beaded nanofibers results from capillary breakup of the electrospinning jets by surface tension, altered by the presence of electrical forces. In addition, starches containing high amylopectin content (≥75%) were not electrospinnable at any concentration in 95% DMSO, despite their high Mw, due to the molecular conformation of amylopectin, a dense spheroid, resulting from a high degree of branching (Kong & Ziegler, 2012). These results implied that sufficient chain entanglement in the polymer dispersion plays a more important role than Mw per se in the electrospinning process. Approaches for electrospinning OS starch-based fibers by increasing chain entanglement might include further increasing polymer concentration, decreasing the surface tension by adding salts (Li et al., 2017), or blending with other water-soluble polymers, such as PUL (Wang & Ziegler, 2019) and hyaluronic acid (Sun, Perry, & Schiffman, 2019).
Figure 2. SEM image of electrospun OS starch nanofibers produced from 30% of PGU aqueous dispersion with optimized electrospinning parameters (spinning distance of 9 cm, voltage of 24 kV, and flow rate of 0.2 mL/h).

3.2. Electrospinnability of OS starch with PUL

PUL is an excellent fiber-forming polymer and can be added to enhance molecular entanglement and thus aid in electrospinning of a non-fiber-forming polymer (Aceituno-Medina, Mendoza, Lagaron, & López-Rubio, 2013; Blanco-Padilla, López-Rubio, Loarca-Pina, Gómez-Mascaraque, & Mendoza, 2015; Drosou, Krokida, & Biliaderis, 2018; Wang & Ziegler, 2019; Yang, Xie, Liu, Kong, & Wang, 2020). Flow curves of aqueous PUL dispersions as a function of concentration (0.1-30%, w/v) and its entanglement concentration determination are shown in Figure S5. The $C_e$ of PUL was determined to be 6.08% (w/v), lower than that of PGU (6.43%, w/v). Successful electrospinning of PUL in water requires a minimum concentration of 14% (w/v) (Figure S6), where fair fibers with slight beading were formed. The transition of beaded-fiber to bead-free fiber for electrospinning of PUL was observed during the increase of PUL concentration
from 8% to 15% in aqueous dispersions (Li et al., 2017). The minimum electrospinnable concentration of PUL is 2.3 times $C_e$, lower than that of PGU (4.7 times), despite its lower molecular weight ($1-2 \times 10^5$ g/mol). The more linear and flexible chain structure of PUL facilitated molecular entanglement.

Aqueous dispersions of PUL (1-13%, w/v) and OS starch (12%, 15%, 20%, w/v) were blended, tested for shear viscosity, and electrospinning attempted. Figure 3 shows the SEM images of electrospun PGU-PUL mats at varying polymer concentrations. The addition of PUL enabled the formation of smooth, continuous, randomly oriented, and bead-free nanofibers. The lowest PUL addition levels for achieving good nanofibers from 12%, 15%, and 20% (w/v) PGU were 6%, 5%, and 3%, respectively. This result indicated that a relatively small addition of PUL played a vital role in establishing sufficient molecular entanglement for electrospinning. A similar effect was seen in our previous study on the electrospinning of aqueous HAS-palmitate-PUL dispersions (Wang & Ziegler, 2019). PUL could hinder starch association, modify dispersion properties (e.g., reduced conductivity and decreased shear viscosity), as well as promote molecular entanglement, which were suggested to be responsible for the successful electrospinning.
Figure 3. SEM images of electrospun PGU-PUL nanofibers from varying polymer concentrations.
Table 2. Properties of OS starch and spinnable PGU-PUL dispersions at room temperature (≈ 20 °C).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Shear viscosity at 100 s⁻¹ (Pa·s)</th>
<th>Conductivity (uS/cm)</th>
<th>Surface tension (nN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% PGU</td>
<td>2.07</td>
<td>1712 ± 29</td>
<td>57.81 ± 0.62</td>
</tr>
<tr>
<td>20% PG2000</td>
<td>0.20</td>
<td>2519 ± 26</td>
<td>34.56 ± 0.14</td>
</tr>
<tr>
<td>20% HC100</td>
<td>0.06</td>
<td>2498 ± 28</td>
<td>36.04 ± 0.62</td>
</tr>
<tr>
<td>12%PGU+6%PUL</td>
<td>1.52</td>
<td>1103 ± 22</td>
<td>59.99 ± 0.38</td>
</tr>
<tr>
<td>15%PGU+5%PUL</td>
<td>1.77</td>
<td>1307 ± 25</td>
<td>55.36 ± 0.54</td>
</tr>
<tr>
<td>20%PGU+3%PUL</td>
<td>3.02</td>
<td>1581 ± 22</td>
<td>55.86 ± 1.20</td>
</tr>
</tbody>
</table>

From these electrospinning attempts, a map illustrating regions of electrospinnability at varying concentrations of PGU and PUL was constructed (Figure 4A). PGU-PUL dispersions with good fiber forming ability are marked above the blue dotted line. When reading this map, it is important to note that the electrospinnable region was determined using the parameter ranges defined in this study, i.e., feed rate from 0.1 to 0.4 mL/h, spinning distance from 5 to 10 cm, and voltage from 5 to 15 kV. Expanding the parameter ranges, if possible, could alter the electrospinnable area. It is also worth noting that the voltage applied for PGU-PUL aqueous dispersions (5-15 kV) was lower as compared to that of PGU (21-26 kV), implying a more stable jet was formed at a lower voltage. The shear viscosities at 100 s⁻¹ of these PGU-PUL dispersions were plotted in Figure 4B as a function of polymer concentration. The viscosity of PGU dispersions increased with the increasing PUL addition. Electrospinnable dispersions were above the blue dotted line. When PGU concentration increased from 12% to 20%, the lowest viscosity required for the PGU-PUL dispersions increased from 1.52 to 3.02 Pa·s (Table 2). The shear viscosity of all electrospinnable dispersions was below 7.50 Pa·s. A conclusion could be drawn, from a rheological point...
of view, that molecular entanglement and shear viscosity together influenced the
electrospinnability of PGU-PUL dispersions, which is consistent with our previous
findings (Wang & Ziegler, 2019).

Figure 4. Evaluation of electrospinnability (A) and apparent viscosity at 100 s\(^{-1}\) (B) of PGU-PUL dispersions as a function of polymer concentration

3.3. Effect of polymer concentration on nanofiber diameter

Figure 5 shows SEM images with diameter histograms of fibers electrospun from
12-20% (w/v) PGU blended with a fixed PUL concentration of 12% (w/v), i.e. below
that where PUL alone is spinnable. Continuous smooth fibers were obtained from all
PGU-PUL dispersions. As PGU concentration increased from 12% to 20%, the mean
diameter of electrospun PGU-PUL fibers increased from 150±34 nm to 250±41 nm.
Figure 6 presents SEM images with diameter histograms of fibers electrospun from 15% (w/v) PGU with varying PUL concentrations (8-13%, w/v). There was also an increasing trend in mean fiber diameter from 147±26 nm to 209±57 nm with increasing PUL concentration which is expected since more total polymer would lead to thicker fibers. Previously reported diameters of HAS fibers were around a few micrometers by wet-electrospinning from aqueous DMSO solutions (Kong & Ziegler, 2014a) and 80 to 300 nm by dry-electrospinning from formic acid solutions (Lancuski et al., 2015). The diameters of PUL fibers from wet-electrospinning and dry-electrospinning were 300 nm-15 μm (Kong & Ziegler, 2014b) and 100-700 nm (Li et al., 2017; Sun, Jia, Kang, Cheng, & Li, 2013), respectively. Starch-based fibers at nanoscale (~146 nm) were obtained from aqueous HAS-palmitate-PUL dispersions by wet-electrospinning (Wang & Ziegler, 2019).
Figure 5. SEM images and diameter histograms of fibers electrospun from 12-20% (w/v) PGU blended with a fixed PUL concentration of 12% (w/v).
Figure 6. SEM images and diameter histograms of fibers electrospun from 15% (w/v) PGU with varying PUL concentrations (8-13%, w/v).

Conclusion

In this work, three types of commercial OS starches were studied for the relationship between rheological properties in aqueous dispersions and their electrospinnability. The OS starches were all modified from waxy maize starch and their relative Mw followed the order of PGU > PG2000 > HC100. Their concentration dependences follow the same order in the semidilute unentangled and entangled
regimes, while their entanglement concentration was inversely related to apparent Mw (Table 3).

Table 3. Rheological characteristics of biopolymer samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Exponent n in relationship $\eta_r \propto C^n$</th>
<th>$C^*_r$</th>
<th>$C_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C^* &lt; C_r$</td>
<td>$C^* &lt; C &lt; C_e$</td>
<td>$C_e &lt; C$</td>
</tr>
<tr>
<td>HC100</td>
<td>0.05</td>
<td>0.64</td>
<td>1.70</td>
</tr>
<tr>
<td>PG2000</td>
<td>0.11</td>
<td>1.03</td>
<td>2.25</td>
</tr>
<tr>
<td>PGU</td>
<td>0.17</td>
<td>1.06</td>
<td>2.65</td>
</tr>
<tr>
<td>PUL</td>
<td>0.11</td>
<td>1.07</td>
<td>3.40</td>
</tr>
</tbody>
</table>

$C^*$: overlap concentration; $C_e$: entanglement concentration.

The shear viscosity at 100 s$^{-1}$ was also positively related with apparent Mw. Electrospinning of OS starch dispersions at varying concentrations up to 30% (w/v) was attempted, yet only the PGU dispersions of the highest concentration showed a promising hint of nanofiber formation. The HC100 and PG2000 dispersions were not electrospinnable, instead, micro to nano-scale particles were obtained. Further optimizing electrospinning parameters (distance, voltage, and flow rate) for the 30% (w/v) PGU dispersion could not produce bead-free and bead-free nanofibers, and thus PUL was added to aid in molecular entanglement. Additions of a relatively small amount of PUL into PGU dispersions were able to create continuous, smooth, and bead-free nanofibers. Electrospinnable PGU-PUL dispersions were demonstrated on an electrospinnability map, and their shear viscosity at 100 s$^{-1}$ fell into a range from 1.52 to 7.50 Pa·s. The mean diameter of PGU-PUL nanofibers obtained ranged from 147 to 250 nm in this study and was affected by total polymer concentration. This study successfully fabricated nanofibers from food-grade starch and PUL with a greener electrospinning process than using DMSO solvent or formic acid solution. Further studies are suggested to focus on the physicochemical properties of the starch-based
nanofibers and the utilization of the nanofibers. The nanofibers obtained showed bead-
free morphology and may find potential uses in food, pharmaceutical, cosmetic, and
biomedical applications.

Acknowledgments

This work was supported by the USDA National Institute of Food and Agriculture
Federal Appropriations under Project PEN04708. China Scholarship Council is
acknowledged for sponsoring Songnan Li as a visiting student (No. 201906150085) at
the Pennsylvania State University and the University of Alabama.

Declarations of interest

None

Supplementary material

Figure S1. Flow curves of OS starches (HC100, PG2000, and PGU) as a function of
starch concentration (0.1-30%, w/v).

Figure S2. SEM images of electrospun OS starch nanofibers produced from 15-30%
(w/v) of PGU aqueous dispersions with fixed spinning distance of 5~10 cm, feed rate
of 0.2~0.6 mL/h, and voltage of 15~25 kV.

Figure S3. SEM images of electrospun OS starch particles produced from 30% (w/v)
of HC100 and PG2000 aqueous dispersions with fixed spinning distance of 5~10 cm,
feed rate of 0.2~0.6 mL/h, and voltage of 15~25 kV.

Figure S4. SEM images of electrospun OS starch nanofibers produced from 30% (w/v)
of PGU aqueous dispersion with various electrospinning parameters (A1-4: distance of
5, 7, 9, and 11 cm with fixed feed rate of 0.2 mL/h and voltage of 21 kV; B1-4: voltage
of 21, 22, 24, and 26 kV with fixed feed rate of 0.2 mL/h and distance of 9 cm; C1-4:
feed rate of 0.2, 0.4, 0.75, and 1 mL/h with fixed distance of 24 kV).

Figure S5. Flow curve and plot of specific viscosity of PUL aqueous dispersions as a
function of concentration (0.1-30%, w/v).

Figure S6. SEM images of electrospun nanofibers produced from 10-15% (w/v) of PUL
aqueous dispersions with fixed spinning distance of 5~10 cm, feed rate of 0.2~0.6 mL/h,
and voltage of 5~15 kV.


