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Rheological Aspects in Fabricating Pullulan Fibers by Electro-Wet-Spinning

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Electro-wet-spinning was used to fabricate continuous nonwoven microscale and nanoscale fibers from pullulan. We investigated the rheological properties of pullulan dispersions using DMSO:water mixtures as solvents with varying compositions. The relationship between electrospinnability and rheological properties were studied. In order to obtain well-formed pullulan fibers, the concentration of pullulan dispersion had to be 1.88 – 2.25 times the entanglement concentration, depending on DMSO:water ratio in the solvent. Shear viscosity was another important factor. The shear viscosities at 100 s\(^{-1}\) of electrospinnable pullulan dispersions fell into a range between 0.06 and 2.2 Pa\(\cdot\)s, regardless of solvent composition. Yet, there may still be other factors governing the fiber size as DMSO concentration changed. Pullulan fibers in the order of hundreds of nanometers to tens of microns were obtained. Increase in DMSO concentration in the solvent generally increased the fiber size and pore size in the electrospun pullulan fiber mat.

KEYWORDS: pullulan, fiber, nanofiber, rheology, electrospinning, electro-wet-spinning
1. Introduction

Electrospinning is a cost-efficient technique to produce continuous nonwoven microscale and nanoscale fibers from a wide variety of materials. Among the materials of interest, polysaccharide biopolymers have been identified as potential substitutes for synthetic polymers (Kong, Ziegler, & Bhosale, 2010). In addition to a sustainable and renewable supply of their constituent biopolymers, biofibers have advantages pertaining to their inherent biodegradability and biocompatibility. Therefore a variety of biopolymers, including polysaccharides, proteins, and DNA, have been successfully spun into fibers, especially by electrospinning (Kong et al., 2010; Kong & Ziegler, 2012, 2013).

Pullulan is a linear polysaccharide produced extracellularly by strains of Aureobasidium pullulans. The basic repeating unit of pullulan is α-(1→6) linked maltotriose, where three glucopyranose units are linked by α-(1→4) glycosidic bonds (Fig. 1). Other structures may exist as maltotetraose connected by α-(1→6) linkage. The regular alternation of (1→4) and (1→6) bonds are believed to be responsible for several distinctive properties of pullulan, for instance, structural flexibility, high solubility in water, adhesiveness, oxygen impermeability and excellent fiber/film forming capacities (Leathers, 2003; Singh, Saini, & Kennedy, 2008). Hence pullulan has many potential biomedical, food, paper and electronic applications (Cheng, Demirci, & Catchmark, 2011). Compared with films and gels, fibers possess porous 3-dimensional structure and high surface area that would benefit air permeation, moisture absorption, and action of active agents. Fiber mats made of pullulan are thus potentially useful in certain food applications, e.g. food packaging materials, and biomedical applications, e.g. wound dressing and drug delivery.

Production of pullulan fibers has been disclosed in a number of patents (Domoto & Tsuji, 1978; Fujii, Mori, & Tabuchi, 1991; Nomura, 1976; Ozaki, Nomura, & Miyake, 1996). The
utilization of electrospinning for fabricating pullulan fibers appeared only in a few recent reports (Sun, Jia, Kang, Cheng, & Li, 2013). Pullulan/montmorillonite (MMT) blend nanofibers with diameters in the range of 50 to 500 nm were prepared by electropsinning (Karim et al., 2009). The introduction of MMT improved the tensile strength and thermal stability of the pullulan matrix. Stijnman et al. studied the rheological properties and electrospinnability of a series of polysaccharides including pullulan (Stijnman, Bodnar, & Hans Tromp, 2011). For successful electrospinning, the polysaccharide dispersion had to fall into a narrow range defined by shear viscosity at 1000 s$^{-1}$ and the ratio of concentration to overlap concentration. Nanoscale pullulan fibers were obtained by using aqueous dispersions in these studies, and we have found it difficult to electrospin microscale pullulan fibers from aqueous dispersions. Though nanofibers are of interest because of their high surface area and small pore size, preference can be given to microscale fibers in some cases, for instance, combined nanoscale and microscale fibers may be the most appropriate architecture for mimicking extracellular matrix in tissue engineering application (Santos et al., 2008; Tuzlakoglu et al., 2005). The productivity of manufacturing nanofibers by electrospinning is usually much lower than that of microscale fibers. In addition, there are some safety concerns with the use of nanostructures in food, medical, and engineering applications (Meng, Xia, George, & Nel, 2009).

Therefore, it was the interest of this study to investigate the effect of electrospinning parameters on fabrication of both microscale and sub-microscale pullulan fibers. First, we investigated the effect of solvent composition and concentration on the electrospinnability of pullulan fibers with an emphasis on the rheological properties of pullulan dispersions (Kong & Ziegler, 2012). Then, we studied the effect of dimethyl sulfoxide (DMSO):water ratio in the solvent on fiber diameters and pore sizes.
2. Materials and Methods

2.1. Materials

Pullulan was kindly provided by Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). Dimethyl sulfoxide (DMSO) and ethanol were obtained from VWR International (Radnor, PA). Deionized water was used in the study.

2.2. Electrospinning

The preparation of spinning dope involved dissolving the appropriate amount of pullulan in an aqueous DMSO solution. The pullulan dispersion was heated in a boiling water bath with continuous stirring on a magnetic stirrer hotplate for about two hours. The pullulan dispersion was then allowed to cool to room temperature and deaerated if necessary. A 10 mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) with a 20 gauge blunt needle was used as the spinneret.

The electrospinning setup comprised a high voltage generator (ES40P, Gamma High Voltage Research, Inc., Ormond Beach, FL), a syringe pump (81620, Hamilton Company, Reno, NV), and a grounded metal mesh immersed in pure ethanol (Fig. 2). This electrospinning configuration can also be referred to as “electro-wet-spinning”. The fibrous mat deposited in the ethanol coagulation bath was then washed using pure ethanol and dried in a desiccator containing Drierite under vacuum. Electrospinning was conducted at room temperature.

The electrospinnability was not evaluated under constant process parameters. Instead, the electrospinnability of each pullulan dispersion was evaluated while varying three spinning parameters (feed rate, voltage, and spinning distance) within predetermined ranges: feed rates from 0.01 to 0.4 mL/h, and spinning distances from 5 to 10 cm. At each feed rate and spinning distance
combination, the voltage was gradually increased from 0 to 15 kV. The onset and ending voltages of continuous jet formation were recorded. The electrospinnability for pullulan dispersions was determined by visual and microscopic observation of the fibers formed.

2.3. Rheology

Pullulan dispersions in aqueous DMSO solutions (0%, 20%, 40%, 60%, 80%, and 100% v/v) were prepared for rheological characterization. For each DMSO concentration, pullulan concentrations of 0.1 to 20% (w/v) were prepared. Flow curves, i.e. shear viscosity versus shear rate, were generated using a cone and plate geometry on an ARES strain-controlled rheometer (TA Instrument, New Castle, DE). The cone and plate diameters were 50 mm and the gap was set at 0.043 mm. The cone angle was 0.04 radians. Viscosity data were collected in the shear rate range from 0.1 s⁻¹ to 100 s⁻¹ at 20 ºC.

2.4. Characterization

Fiber morphology was examined using a Phenom G2 Pro SEM (Eindhoven, The Netherlands) at an accelerating voltage of 5 keV. Five random fiber images were analyzed for fiber size and pore size using the Fibermetric application in the Phenom Pro Suite package. 500 different fiber segments were randomly measured by the software to obtain a diameter histogram.

3. Results and Discussion

3.1. Rheological properties

Flow curves of pullulan in DMSO dispersions with varying pullulan concentrations are given in Fig. 3. Unreliable data, i.e. out of the detection limit of the rheometer, were not plotted. In all DMSO concentrations, dispersions with 10% (w/v) or lower pullulan concentrations showed Newtonian behavior, i.e. shear viscosity was independent of shear rate, in the shear rate range 0.1-
As pullulan concentration increased beyond 10 % (w/v) shear thinning became noticeable. However, the shear thinning effect was weak, since the viscosity decreased much less than 1 order of magnitude over three decades of shear rate. The 20% (w/v) pullulan in pure and 80% DMSO dispersions did not fully enter the power law region even at the highest shear rate (100 s$^{-1}$). By fitting the power law model, $\eta=K\gamma^{n-1}$, to the data, the power law indices, $n$, were calculated to be 0.83 and 0.82 for 20 % (w/v) pullulan in 60% and 40% DMSO, respectively, indicating the presence of a weak shear thinning effect. Shear thinning became even more insignificant in 20% and 0% DMSO, with the power law indices of 0.93 and 0.95, respectively. Compared to high amylose starch in DMSO aqueous dispersions (Kong & Ziegler, 2012), the flow curves of pullulan suggested little asymmetry of pullulan molecules, i.e. rather spherical conformation, and thus the Newtonian behavior in the flow.

Zero shear viscosities, $\eta_0$, were approximated from the flow curves by using the actual or extrapolated values for apparent viscosity at 0.1 s$^{-1}$, and used to calculate specific viscosity, $\eta_{sp}=（\eta_0-\eta_s）/\eta_s$. In order to determine the entanglement concentration in each DMSO solution, $c_e$, specific viscosity data were plotted against pullulan concentration (Fig. 4). The $c_e$ was thus determined from the intercept of the fitted lines in the semidilute unentangled and the semidilute entangled regimes. Entanglement concentrations were determined to be from 3.99% to 4.41% (w/v) in 20% and higher DMSO concentrations, without an apparent trend. It suggested that the DMSO concentration had little impact on the conformation of pullulan molecules in the solvent. In aqueous dispersion, pullulan had an observed entanglement concentration of 5.33% (w/v).

In the semidilute unentangled regime, the specific viscosity, $\eta_{sp}$, was proportional to $c^{1.39}$ in pure DMSO. This concentration dependence is close to reported values for starch in pure DMSO ($\eta_{sp} \sim c^{1.40}$) (Kong & Ziegler, 2012), chitosan in 80% aqueous acetic acid solution ($\eta_{sp} \sim c^{1.3}$).
(Klossner, Queen, Coughlin, & Krause, 2008), and for linear and branched poly(ethylene terephthalate-co-ethylene isophthalate) (PET-co-PEI) in mixture solvent chloroform/dimethylformamide ($\eta_{sp} \sim c^{1.41}$) and ($\eta_{sp} \sim c^{1.39}$) (McKee, Wilkes, Colby, & Long, 2004), respectively. This scaling dependence is also close to the theoretically predicted value ($\eta_{sp} \sim c^{1.25}$) for neutral, linear polymers in semidilute unentangled regime in a good solvent (de Gennes, 1979). In 80% or lower DMSO concentrations, the concentration dependences of specific viscosity became lower, i.e. from 0.88 to 1.16. This suggested a less extended or a rather spherical conformation of pullulan polymer in these solvents at low concentrations.

In the semidilute entangled regime, we observed that the concentration dependences of specific viscosity ranged from 3.54 to 4.12, lower than theoretical prediction for linear polymers in a good solvent ($\eta_{sp} \sim c^{4.8}$) (de Gennes, 1979) and some reported values ($\eta_{sp} \sim c^{6.0}$) for linear PET-co-PEI (Klossner et al., 2008) and chitosan (Klossner et al., 2008). This concentration dependence is very close to that of the other random coil polysaccharides, including dextran, carboxymethylamylose, alginate, lambda-carrageenan, and hyaluronate, e.g. 3.3 (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981), and suggests that the pullulan molecules were entangled but did not interact strongly.

3.2. Correlation with electrospinnability

A series of pullulan dispersions in each DMSO concentration were subject to electrospinning on the apparatus shown in Fig. 2. The fiber forming ability (electrospinnability) was examined in the predetermined process parameter ranges. A spinnability map illustrating regions of spinnability at varying concentrations of DMSO and pullulan was constructed (Fig. 5). Pullulan dispersions with good fiber forming ability are designated by the shaded area. Some fibers from boundary conditions were shown in Fig. 6. When the dispersions had good electrospinnability, a continuous
and stable jet could be induced and pullulan fibers were deposited on the surface of the coagulation bath without accompanying sprayed particles. Scanning electron microscopy was also employed to evaluate the fiber morphology. Good fibers are continuous, uniform, smooth, and defect-free (Fig. 6B-F). At lower concentrations outside of the shaded area, fibers did not spin smoothly and were interrupted by electrospraying, which resulted in particle-embedded fibers or beaded fibers (Fig. 6A). Poor fibers with a high content of particles are fragile and are difficult to collect from the coagulation bath. At concentrations higher than the shaded area, the jet did not develop whipping instability, and the process appeared like simple wet-spinning. However, it is important to keep in mind that the electrospinnable region was determined within the parameter ranges defined in this study, i.e. feed rate from 0.01 to 0.4 mL/h, spinning distances from 5 to 10 cm, and voltage from 0 to 15 kV. By using greater parameter ranges, the shaded electrospinnable area could be expanded.

From the electrospinnability map, there appeared a correlation between the entanglement concentration, \( c_e \), and the concentration at which good fibers start to form, i.e. the critical concentration for electrospinnability, \( c^* \). The observed values of \( c^*/c_e \) fell in the narrow range from 1.88 to 2.25. These \( c^*/c_e \) values of pullulan agree well with reported \( c^*/c_e \) values for electrospinning defect-free fibers from polyesters (McKee et al., 2004) and polymerized ionic liquids (Chen & Elabd, 2009), but are narrower in range than previously observed for amylose (Kong & Ziegler, 2012).

In addition to molecular conformation, other factors may influence the electrospinnability of pullulan dispersions, e.g. shear viscosity, conductivity, and surface tension. The shear viscosities at 100 s\(^{-1}\) were used to represent the processing flow viscosity of pullulan dispersions (Fig. 7), although the shear rate involved in electrospinning is much higher than the highest shear rate in...
the rheological tests. The viscosity decreased with decreasing DMSO concentration. The upper and lower viscosity limits of the electrospinnable region were observed to be 0.06 and 2.2 Pa*s, respectively. The upper limit coincided well with starch dispersions (Kong & Ziegler, 2012), while pullulan could have a lower viscosity and still be spinnable. Even though sufficient molecular entanglement had been fulfilled at high pullulan concentrations, the high viscosity of the dispersion limited electrospinnability and resulted in simple wet-spinning. At lower concentrations in the range from $c_e$ to $c^*$, sufficient molecular entanglement was not present to prevent the stream from breaking and forming discrete particles. A conclusion could be drawn, from a rheological point of view, that molecular entanglement and shear viscosity together influenced the electrospinnability of pullulan dispersions, which is consistent with our previous findings for starch spinning.

### 3.3. Effect of DMSO concentration

Fiber diameter generally increased with DMSO concentration, and sub-microscale fibers only resulted from low DMSO concentrations. Pullulan in different DMSO dispersions at the same concentration of 12% (w/v) were subjected to electrospinning and compared in terms of the resulting diameter and pore sizes (Fig. 8). Continuous smooth fibers were obtained from all pullulan dispersions. As a general trend, with increasing DMSO concentration in the spinning dope a longer spinning distance, a smaller applied voltage, and a slower feed rate was required to produce good fibers. With constant electrospinning parameters, the fiber diameter decreased as DMSO concentration decreased. Pullulan fibers from 100% to 40% DMSO solvents were largely in the micron range, whereas fibers from 20% and 0% DMSO fell into the submicron range. The pore size also experienced a huge reduction as DMSO concentration decreased from 40% to 20%. The most prevalent pore size range decreased from $1.65 – 19.54 \ \mu m^2$ to $0.01 – 0.18 \ \mu m^2$. The fiber and pore size of samples from 20% and 0% DMSO were about the same, which is also consistent
with other reports on electrospinning pullulan from aqueous solutions (Karim et al., 2009; Sun et al., 2013). The coagulation bath was actually not necessary for collecting fibers from pullulan in low DMSO concentrations. While the rheological properties could explain and predict electrospinnability of pullulan fibers, the fiber size seemed to be affected by other solution properties, e.g. surface tension and conductivity, which are important in electrospinning process (Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005). For instance, the intrinsic surface tension of the solvent is a function of DMSO:water ratio (Catalán, Díaz, & García-Blanco, 2001). These aspects would demand investigation in the future.

4. Conclusions

In conclusion, electrospinnability of pullulan and its correlation with rheological properties in different DMSO dispersions were studied. The formation of good fibers required that the pullulan concentration had to be 1.88 to 2.25 times the entanglement concentration $c_e$ depending on the DMSO concentration. In addition, shear viscosity is also of importance. All electrospinnable dispersions showed a shear viscosity at $100^{-1}$ in the range of 0.06 to 2.2 Pa$\cdot$s. As DMSO concentration increased in the solvent, sub-microscale to microscale pullulan fibers could be obtained.
References


