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

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## ABSTRACT

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

28 KEYWORDS: pullulan, fiber, nanofiber, rheology, electrospinning, electro-wet-spinning

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## 30           **1. Introduction**

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

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

51           Production of pullulan fibers has been disclosed in a number of patents (Domoto & Tsuji,  
52 1978; Fujii, Mori, & Tabuchi, 1991; Nomura, 1976; Ozaki, Nomura, & Miyake, 1996). The

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

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

76        **2. Materials and Methods**

77        *2.1. Materials*

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

81        *2.2. Electrospinning*

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

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

94        The electrospinnability was not evaluated under constant process parameters. Instead, the  
95 electrospinnability of each pullulan dispersion was evaluated while varying three spinning  
96 parameters (feed rate, voltage, and spinning distance) within predetermined ranges: feed rates from  
97 0.01 to 0.4 mL/h, and spinning distances from 5 to 10 cm. At each feed rate and spinning distance

98 combination, the voltage was gradually increased from 0 to 15 kV. The onset and ending voltages  
99 of continuous jet formation were recorded. The electrospinnability for pullulan dispersions was  
100 determined by visual and microscopic observation of the fibers formed.

### 101 *2.3. Rheology*

102 Pullulan dispersions in aqueous DMSO solutions (0%, 20%, 40%, 60%, 80%, and 100 %  
103 v/v) were prepared for rheological characterization. . For each DMSO concentration, pullulan  
104 concentrations of 0.1 to 20% (w/v) were prepared. Flow curves, i.e. shear viscosity versus shear  
105 rate, were generated using a cone and plate geometry on an ARES strain-controlled rheometer (TA  
106 Instrument, New Castle, DE). The cone and plate diameters were 50 mm and the gap was set at  
107 0.043 mm. The cone angle was 0.04 radians. Viscosity data were collect in the shear rate range  
108 from 0.1 s<sup>-1</sup> to 100 s<sup>-1</sup> at 20 °C.

### 109 *2.4. Characterization*

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

## 114 **3. Results and Discussion**

### 115 *3.1. Rheological properties*

116 Flow curves of pullulan in DMSO dispersions with varying pullulan concentrations are given  
117 in Fig. 3. Unreliable data, i.e. out of the detection limit of the rheometer, were not plotted. In all  
118 DMSO concentrations, dispersions with 10% (w/v) or lower pullulan concentrations showed  
119 Newtonian behavior, i.e. shear viscosity was independent of shear rate, in the shear rate range 0.1-

120  $100 \text{ s}^{-1}$ . As pullulan concentration increased beyond 10 % (w/v) shear thinning became noticeable.  
121 However, the shear thinning effect was weak, since the viscosity decreased much less than 1 order  
122 of magnitude over three decades of shear rate. The 20% (w/v) pullulan in pure and 80% DMSO  
123 dispersions did not fully enter the power law region even at the highest shear rate ( $100 \text{ s}^{-1}$ ). By  
124 fitting the power law model,  $\eta = K\dot{\gamma}^{n-1}$ , to the data, the power law indices,  $n$ , were calculated to be  
125 0.83 and 0.82 for 20 % (w/v) pullulan in 60% and 40% DMSO, respectively, indicating the  
126 presence of a weak shear thinning effect. Shear thinning became even more insignificant in 20%  
127 and 0% DMSO, with the power law indices of 0.93 and 0.95, respectively. Compared to high  
128 amylose starch in DMSO aqueous dispersions (Kong & Ziegler, 2012), the flow curves of pullulan  
129 suggested little asymmetry of pullulan molecules, i.e. rather spherical conformation, and thus the  
130 Newtonian behavior in the flow.

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

140 In the semidilute unentangled regime, the specific viscosity,  $\eta_{sp}$ , was proportional to  $c^{1.39}$  in  
141 pure DMSO. This concentration dependence is close to reported values for starch in pure DMSO  
142 ( $\eta_{sp} \sim c^{1.40}$ ) (Kong & Ziegler, 2012), chitosan in 80% aqueous acetic acid solution ( $\eta_{sp} \sim c^{1.3}$ )



143 (Klossner, Queen, Coughlin, & Krause, 2008), and for linear and branched poly(ethylene  
144 terephthalate-*co*-ethylene isophthalate) (PET-*co*-PEI) in mixture solvent  
145 chloroform/dimethylformamide ( $\eta_{sp} \sim c^{1.41}$ ) and ( $\eta_{sp} \sim c^{1.39}$ ) (McKee, Wilkes, Colby, & Long,  
146 2004), respectively. This scaling dependence is also close to the theoretically predicted value ( $\eta_{sp}$   
147  $\sim c^{1.25}$ ) for neutral, linear polymers in semidilute unentangled regime in a good solvent (de Gennes,  
148 1979). In 80% or lower DMSO concentrations, the concentration dependences of specific viscosity  
149 became lower, i.e. from 0.88 to 1.16. This suggested a less extended or a rather spherical  
150 conformation of pullulan polymer in these solvents at low concentrations.

151 In the semidilute entangled regime, we observed that the concentration dependences of  
152 specific viscosity ranged from 3.54 to 4.12, lower than theoretical prediction for linear polymers  
153 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  
154 PET-*co*-PEI (Klossner et al., 2008) and chitosan (Klossner et al., 2008). This concentration  
155 dependence is very close to that of the other random coil polysaccharides, including dextran,  
156 carboxymethylamylose, alginate, lambda-carrageenan, and hyaluronate, e.g. 3.3 (Morris, Cutler,  
157 Ross-Murphy, Rees, & Price, 1981), and suggests that the pullulan molecules were entangled but  
158 did not interact strongly.

### 159 3.2. Correlation with electrospinnability

160 A series of pullulan dispersions in each DMSO concentration were subject to electrospinning  
161 on the apparatus shown in Fig. 2. The fiber forming ability (electrospinnability) was examined in  
162 the predetermined process parameter ranges. A spinnability map illustrating regions of spinnability  
163 at varying concentrations of DMSO and pullulan was constructed (Fig. 5). Pullulan dispersions  
164 with good fiber forming ability are designated by the shaded area. Some fibers from boundary  
165 conditions were shown in Fig. 6. When the dispersions had good electrospinnability, a continuous

166 and stable jet could be induced and pullulan fibers were deposited on the surface of the coagulation  
167 bath without accompanying sprayed particles. Scanning electron microscopy was also employed  
168 to evaluate the fiber morphology. Good fibers are continuous, uniform, smooth, and defect-free  
169 (Fig. 6B-F). At lower concentrations outside of the shaded area, fibers did not spin smoothly and  
170 were interrupted by electrospinning, which resulted in particle-embedded fibers or beaded fibers  
171 (Fig. 6A). Poor fibers with a high content of particles are fragile and are difficult to collect from  
172 the coagulation bath. At concentrations higher than the shaded area, the jet did not develop  
173 whipping instability, and the process appeared like simple wet-spinning. However, it is important  
174 to keep in mind that the electrospinnable region was determined within the parameter ranges  
175 defined in this study, i.e. feed rate from 0.01 to 0.4 mL/h, spinning distances from 5 to 10 cm, and  
176 voltage from 0 to 15 kV. By using greater parameter ranges, the shaded electrospinnable area could  
177 be expanded.

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

185 In addition to molecular conformation, other factors may influence the electrospinnability of  
186 pullulan dispersions, e.g. shear viscosity, conductivity, and surface tension. The shear viscosities  
187 at  $100 \text{ s}^{-1}$  were used to represent the processing flow viscosity of pullulan dispersions (Fig. 7),  
188 although the shear rate involved in electrospinning is much higher than the highest shear rate in

189 the rheological tests. The viscosity decreased with decreasing DMSO concentration. The upper  
190 and lower viscosity limits of the electrospinnable region were observed to be 0.06 and 2.2 Pa•s,  
191 respectively. The upper limit coincided well with starch dispersions (Kong & Ziegler, 2012), while  
192 pullulan could have a lower viscosity and still be spinnable. Even though sufficient molecular  
193 entanglement had been fulfilled at high pullulan concentrations, the high viscosity of the dispersion  
194 limited electrospinnability and resulted in simple wet-spinning. At lower concentrations in the  
195 range from  $c_e$  to  $c^*$ , sufficient molecular entanglement was not present to prevent the stream from  
196 breaking and forming discrete particles. A conclusion could be drawn, from a rheological point of  
197 view, that molecular entanglement and shear viscosity together influenced the electrospinnability  
198 of pullulan dispersions, which is consistent with our previous findings for starch spinning.

### 199 *3.3. Effect of DMSO concentration*

200 Fiber diameter generally increased with DMSO concentration, and sub-microscale fibers  
201 only resulted from low DMSO concentrations. Pullulan in different DMSO dispersions at the same  
202 concentration of 12% (w/v) were subjected to electrospinning and compared in terms of the  
203 resulting diameter and pore sizes (Fig. 8). Continuous smooth fibers were obtained from all  
204 pullulan dispersions. As a general trend, with increasing DMSO concentration in the spinning dope  
205 a longer spinning distance, a smaller applied voltage, and a slower feed rate was required to  
206 produce good fibers. With constant electrospinning parameters, the fiber diameter decreased as  
207 DMSO concentration decreased. Pullulan fibers from 100% to 40% DMSO solvents were largely  
208 in the micron range, whereas fibers from 20% and 0% DMSO fell into the submicron range. The  
209 pore size also experienced a huge reduction as DMSO concentration decreased from 40% to 20%.  
210 The most prevalent pore size range decreased from 1.65 – 19.54  $\mu\text{m}^2$  to 0.01 – 0.18  $\mu\text{m}^2$ . The fiber  
211 and pore size of samples from 20% and 0% DMSO were about the same, which is also consistent

212 with other reports on electrospinning pullulan from aqueous solutions (Karim et al., 2009; Sun et  
213 al., 2013). The coagulation bath was actually not necessary for collecting fibers from pullulan in  
214 low DMSO concentrations. While the rheological properties could explain and predict  
215 electrospinnability of pullulan fibers, the fiber size seemed to be affected by other solution  
216 properties, e.g. surface tension and conductivity, which are important in electrospinning process  
217 (Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005). For instance, the intrinsic surface tension of the  
218 solvent is a function of DMSO:water ratio (Catalán, Díaz, & García-Blanco, 2001). These aspects  
219 would demand investigation in the future.

#### 220 **4. Conclusions**

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

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