Video Article

Molecular Entanglement and Electrospinnability of Biopolymers

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Abstract

Electrospinning is a fascinating technique to fabricate micro- to nano-scale fibers from a wide variety of materials. For biopolymers, molecular entanglement of the constituent polymers in the spinning dope was found to be an essential prerequisite for successful electrospinning. Rheology is a powerful tool to probe the molecular conformation and interaction of biopolymers. In this report, we demonstrate the protocol for utilizing rheology to evaluate the electrospinnability of two biopolymers, starch and pullulan, from their dimethyl sulfoxide (DMSO)/water dispersions. Well-formed starch and pullulan fibers with average diameters in the submicron to micron range were obtained. Electrospinnability was evaluated by visual and microscopic observation of the fibers formed. By correlating the rheological properties of the dispersions to their electrospinnability, we demonstrate that molecular conformation, molecular entanglement, and shear viscosity all affect electrospinning. Rheology is not only useful in solvent system selection and process optimization, but also in understanding the mechanism of fiber formation on a molecular level.

Video Link

The video component of this article can be found at https://www.jove.com/video/51933/

Introduction

Electrospinning is a technique that is capable of producing continuous micro- to nano-scale fibers from a wide variety of materials. It has gained increasing academic and industrial interest[1]. Though the setup and practice of electrospinning seem straightforward, the ability to predict electrospinnability and control fiber properties remains a challenge. The reason may lie in the fact that there are many factors influencing the electrospinning process[2] and the process, especially the path travelled by the fiber, is chaotic[1]. Often an empirical “cook-and-look” approach is used for screening potential electrospinnable materials. However, to gain better control over the electrospinning process and resultant fiber properties, a more complete understanding of the mechanisms that govern electrospinnability is required. Several researchers have found that molecular entanglement of polymers in the spinning dope is an essential prerequisite for successful electrospinning[3-5].

Rheology is a powerful tool to probe molecular conformation and interaction in polymer dispersions. For instance, McKee *et al*. investigated the molecular conformation of linear and branched poly(ethylene terephthalate-co-ethylene isophthalate) copolymers in a solvent containing chloroform/dimethyl terephthalate (7/3, v/v), and determined that the polymer concentration had to be 2-2.5x the entanglement concentration for successful electrospinning[4].

There is currently renewed interest in fibers from biopolymers because of their advantages in biodegradability, biocompatibility, and renewability vis-à-vis their synthetic counterparts. Yet practitioners confront many challenges arising generally from their structural complexity, difficulty in thermal processing and inferior mechanical properties. Starch, found in plant tissues, is among the most abundant and inexpensive biopolymers on earth. Pure starch fibers fabricated using an electro-wet-spinning apparatus were recently described[6]. Pullulan is a linear polysaccharide produced extracellularly by certain bacteria. The regular alternation of (1→4) and (1→6) glucosidic bonds are believed to be responsible for several distinctive properties of pullulan, including excellent fiber/film forming capability[7,8]. Electrospinning of pullulan fibers from aqueous dispersion has been reported by a number of researchers[9,10]. In our previous publications, the electrospinnability of two biopolymers, starch[11] and pullulan[12], has been discussed. This report focuses on demonstrating the protocol for utilizing rheological principles in the investigation of the electrospinnability of these two biopolymers.

Protocol

1. Spinning Dope Preparation

1. Prepare a range of biopolymer concentrations to be investigated (0.1% to 30%, w/v) and be sure to consider moisture content of the biopolymer powder in these calculations. For each concentration, weigh the biopolymer (starch or pullulan) powder into a 50 ml test tube. Add aqueous dimethyl sulfoxide (DMSO) solution and a stir bar.
2. Place the tube into boiling water with constant stirring on a magnetic stirrer hotplate.
3. After about 1 hr, turn off heat and allow the dispersion to cool to room temperature. The dispersion is then ready for rheological testing and electrospinning.

2. Steady Shear Rheology

1. Warm up the rheometer and set the stage temperature at 20 °C. Calibrate the gap between the probe (25 mm cone used) and the stage (plate).
2. Load 0.41 ml of the biopolymer dispersion onto the center of the stage and lower the probe to set the position (0.053 mm gap for 25 mm cone). Make sure that the dispersion evenly spreads within the gap.
3. Perform the rheological test with the following experimental parameters: sweep mode: log, initial rate: 100 sec^{-1}, final rate: 0.1 sec^{-1}, points per decade: 10, delay before measure: 5 sec, measure time: 10 sec, and directions per measure: two (both clockwise and counterclockwise).
4. Analyze rheological data to estimate appropriate dispersion concentrations for electrospinning trials.
    1. Plot apparent shear viscosities against shear rates as a function of polymer concentrations. For each flow curve, approximate zero shear viscosities, η_0, by the actual or extrapolated values (*e.g.*, at low concentrations where the low shear rate data are unreliable) for apparent viscosity at 0.1 sec^{-1}.
    2. Calculate specific viscosity: $\eta_{sp} = (\eta_0 - \eta_s)/\eta_s$, where η_s is the viscosity of the solvent.
    3. Plot specific viscosities as a function of concentration. Identify semidilute unentangled and entangled regimes. The semidilute unentangled regime begins from the low concentration end with a small slope, and the semidilute entangled regime has a greater slope following the unentangled regime. Fit power-law regression models in both regimes. The power values are the slopes (concentration dependence) in semidilute unentangled and entangled regimes on a log-log plot. The intersection of the two fitted lines is the entanglement concentration, c_e.

3. Electrospinning Parameter Variation

1. Assemble the electrospinning setup as shown in **Figure 1**. Load the syringe with dispersion of appropriate composition, *e.g.*, 15% (w/v) starch or pullulan in 100% DMSO, onto the syringe pump. Clip the high voltage wire (positive) to the needle. Connect the coagulation bath containing pure ethanol to ground by immersing the ground wire (negative) into the bath. Use a lab jack to adjust the distance between the syringe needle and coagulation bath. Immerse a metal mesh in the bath to collect the fiber mat after electrospinning.
2. Spin the biopolymer in the following parameter ranges: feed rate from 0.1 to 0.4 ml/hr, spinning distance from 5 to 10 cm, and voltage from 0 to 15 kV.
    1. Start with a spinning distance of 5 cm. For the first feed rate (0.1 ml/hr), ramp the voltage up slowly from 0 V. Pay attention to the shape of the dispersion extruded at the needle tip and note when the dripping dispersion is accelerated and then elongated.
    2. Note the voltage at which a tiny jet initiated from the drop surface, indicating electrospinnability of the solution. Record the voltage at which a continuous jet initiates, if any.
    3. Examine the complete range for each of the three parameters and note successful spinning conditions. Collect the fibers only when there is a continuous jet from the tip.
3. After a few minutes of collecting, rinse the fiber mat with pure ethanol. Place the fiber mat into a desiccator containing desiccant under vacuum.
4. Repeat for each biopolymer concentration for complete characterization.

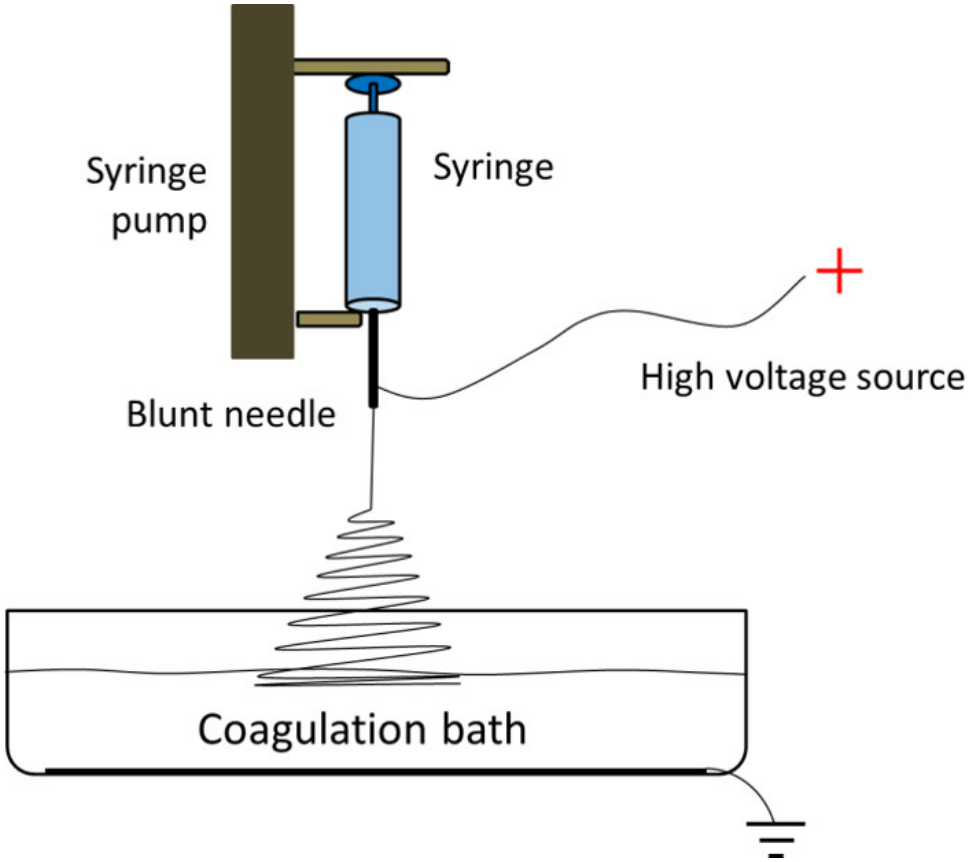

Figure 1. Schematic drawing of the electro-wet-spinning setup. The biopolymer dispersion is extruded from a syringe pump. A high voltage DC power supply provides high voltage to the blunt needle and grounds the coagulation bath. The polymer jet from the needle tip travels through a straight path and then develops a rapid whipping path (aka whipping instability).

4. Morphological Characterization

1. Cut a piece of dried fiber mat and immobilize it onto a SEM stub using carbon tape.
2. Load the sample stub into the SEM instrument and obtain images for analysis.

Representative Results

Flow curves of the biopolymer dispersions as a function of biopolymer concentration and DMSO concentration in solvent were obtained. Two representative figures show the flow curves of starch (**Figure 2A**) and pullulan (**Figure 2B**) as a function of their concentration in pure DMSO solvent. The specific viscosities were plotted against biopolymer concentration (**Figure 3A** for starch and **Figure 3B** for pullulan). From these plots, entanglement concentrations were obtained as the intercept of the fitted lines in the semidilute unentangled and the semidilute entangled regimes.

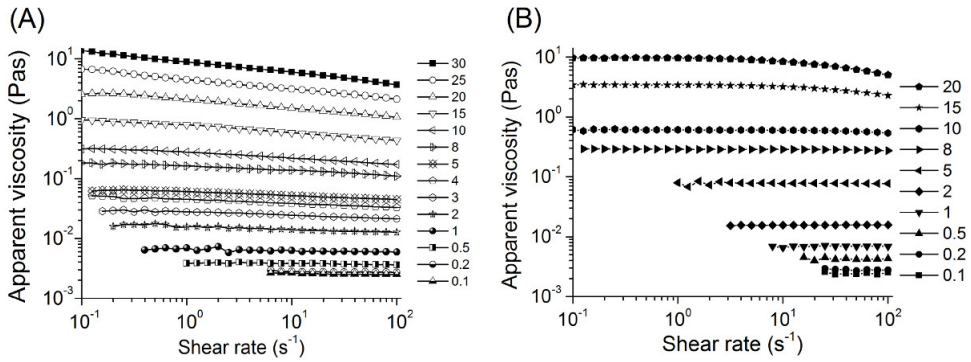

Figure 2. Flow curves of (**A**) Gelose 80 starch and (**B**) pullulan in pure DMSO as a function of concentration (%, w/v) at 20 °C. In both figures, starch and pullulan of low concentrations were less viscous to produce sufficient torque at low shear rates. These unreliable data were thus not plotted. In general, the two biopolymers showed Newtonian behavior at low concentrations, *i.e.* the apparent shear viscosity was independent of shear rate. Shear thinning became apparent as their concentration increases, especially beyond 10% (w/v). Yet the shear thinning behavior was weak. The 15% and 20% (w/v) pullulan dispersions only showed the early phase of the power law region upon high shear rates, while the starch dispersions did not show significant reduction in viscosity over the shear rate range from 0.1 to 100 sec^{-1}. Reprinted with permission from ref 11, Copyright (2012) American Chemical Society, and with permission from ref 12, Copyright (2014) Elsevier.

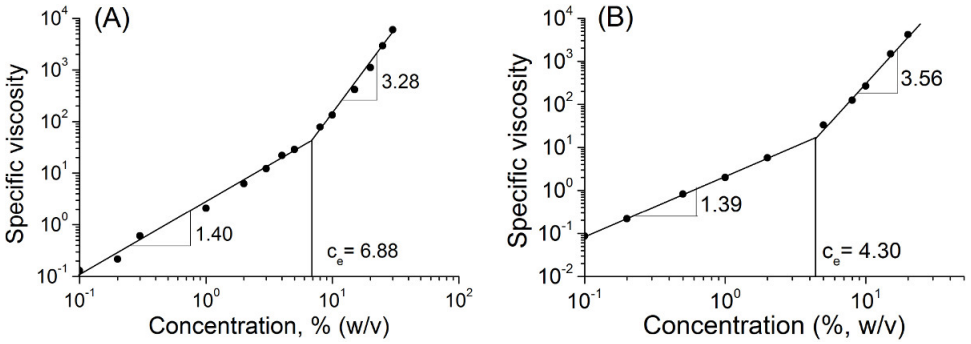

Figure 3. Plot of specific viscosity versus (**A**) Gelose 80 starch and (**B**) pullulan concentration in pure DMSO. The slopes of the fitted lines in semidilute unentangled (left side) and the semidilute entangled (right side) regimes indicate the concentration dependence of specific viscosity, aka scaling dependence[4]. Pullulan showed stronger concentration dependence than starch in the entangled regime. The intercept of the two fitted lines was termed as the entanglement concentration (c_e) at which biopolymers start to overlap in the dispersion. Starch required a higher concentration than pullulan to start entangling. Reprinted with permission from ref 11, Copyright (2012) American Chemical Society, and with permission from ref 12, Copyright (2014) Elsevier.

Electrospinning was attempted for all of the biopolymer dispersions, and results judged in terms of electrospinnability, *i.e.* jet forming ability during electrospinning, and morphology of the fibers formed. A dispersion of good electrospinnability formed a stable and continuous jet that resulted in continuous and smooth fibers without droplets. A dispersion that was not able to electrospin could not form a stable jet or develop whipping instability. Either tiny droplets or thick fibers were deposited into the coagulation bath. **Figure 4** shows representative good and poor fibers evaluated from their appearance. **Figure 5** summarizes evaluation of electrospinnability at varying concentrations of DMSO in solvent and the biopolymer in dispersion for starch and pullulan, respectively. In addition to entanglement concentrations, shear viscosities at 100 sec^{-1} were plotted against biopolymer concentration, where regions of electrospinnability were denoted (**Figure 6**).

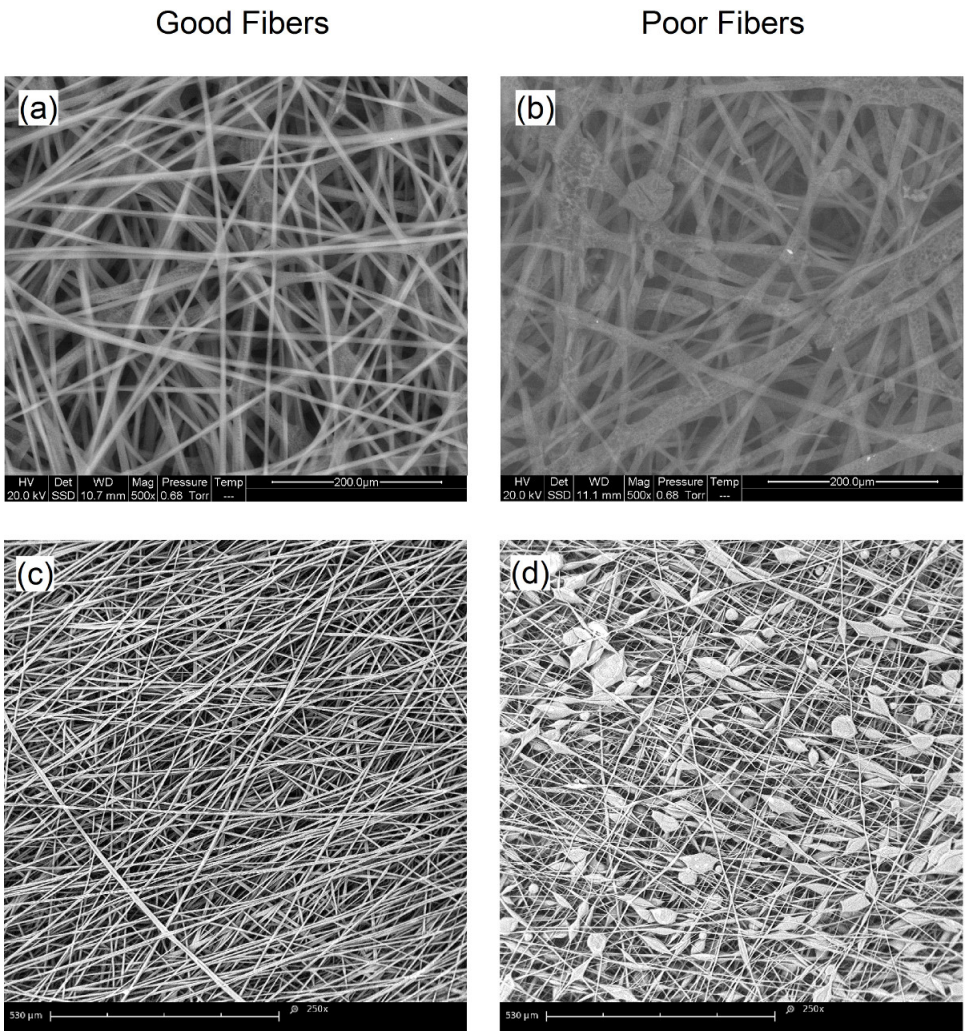

Figure 4: Scanning electron micrographs of good (left) and poor (right) starch and pullulan fibers. Good fibers are smooth, continuous, and randomly oriented, while poor fibers may have beads, breaks, and droplets as shown in the figure (red circles). (**a**) 10% (w/v) Gelose 80 starch in 95% (v/v) DMSO, (**b**) 8% (w/v) Gelose 80 starch in 80% (v/v) DMSO, (**c**) 17% (w/v) pullulan in 40% (v/v) DMSO, and (**d**) 9% (w/v) pullulan in 80% (v/v) DMSO. Reprinted with permission from ref 11, Copyright (2012) American Chemical Society, and with permission from ref 12, Copyright (2014) Elsevier.

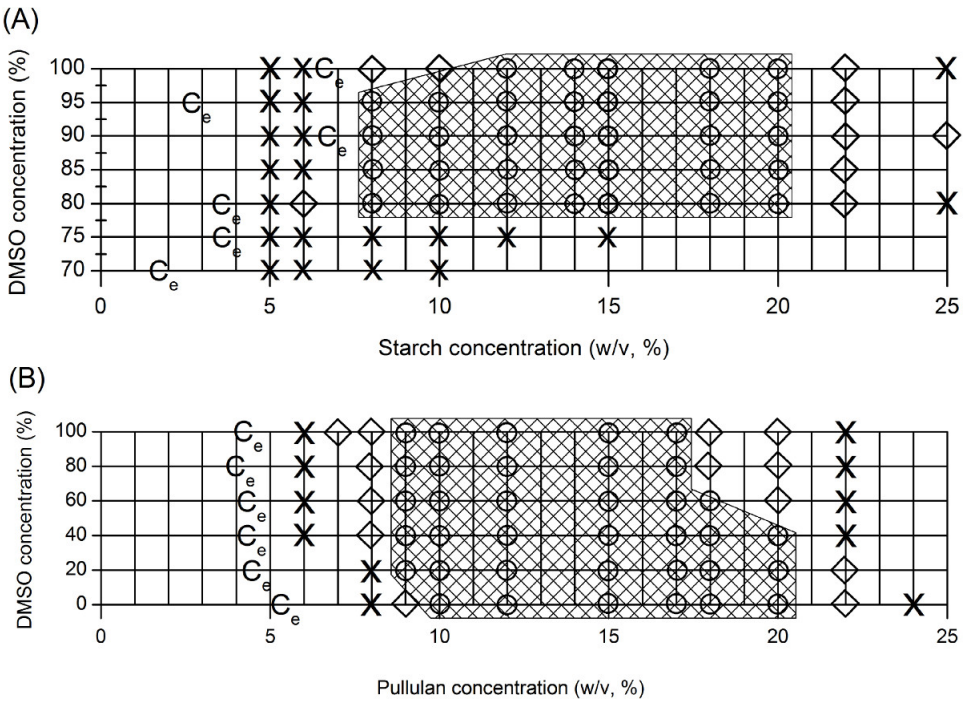

Figure 5. Evaluation of electrospinnability of (**A**) Gelose 80 starch and (**B**) pullulan dispersions as a function of DMSO concentration in solvent and biopolymer concentration in the dispersion: good electrospinnability (circles), poor electrospinnability (diamonds), and unable to electrospin (X's). Shaded areas roughly represent electrospinnable regions. Entanglement concentrations are also approximately labeled. Reprinted with permission from ref 11, Copyright (2012) American Chemical Society, and with permission from ref 12, Copyright (2014) Elsevier.

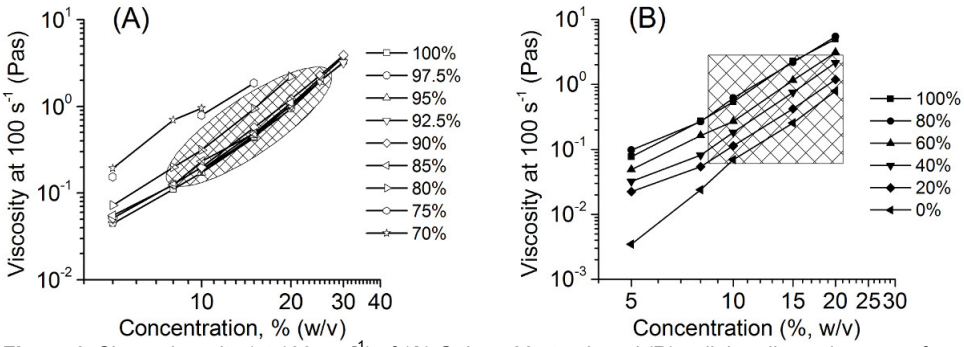

Figure 6. Shear viscosity (at 100 sec^{-1}) of (**A**) Gelose 80 starch and (**B**) pullulan dispersions as a function of biopolymer concentration in different DMSO/water solvents. Shaded areas roughly represent the electrospinnable region. Reprinted with permission from ref 11, Copyright (2012) American Chemical Society, and with permission from ref 12, Copyright (2014) Elsevier.

Discussion

Rheology is an essential tool to study the processing of polymers, including conventional fiber spinning and electrospinning[13]. From the steady shear rheological studies, polymer conformation and their interactions in different solvents can be resolved (**Figures 2** and **3**). At concentrations not high enough for biopolymer molecules to overlap with one another, their concentration dependence was around 1.4 (**Figure 3**), which was in good agreement with reported values of other polymers in good solvent[3,4]. After the biopolymer molecules start to entangle, the specific viscosity showed a much higher dependence on concentration. A greater n value indicates a stronger intermolecular interaction. Many random coil polysaccharides showed similar concentration dependence, with an n value of approximately 3.3 [14]. Pullulan showed stronger interaction than starch in solvents of high DMSO content, possibly due to the molecular nature of the two biopolymers. The starch used had some highly branched components (~20% amylopectin), while the pullulan should be linear. Of course, the molecular weights, which were unknown, would also have an influence.

The entanglement concentration would depend on the conformation of the biopolymer in the dispersion. For example, the entanglement concentration of starch in 92.5% (w/v) DMSO aqueous solution is much lower than that in pure DMSO[11]. It implies that starch molecules exist in a more extended conformation in 92.5% (w/v) DMSO aqueous solution so that they occupy a larger hydrodynamic volume and tend to overlap more easily. The entanglement concentrations of pullulan did not vary as drastically as those of starch with varying solvent quality, probably because both water and DMSO are good solvents for pullulan and have little effect on the molecular conformation. Water, which is not a good solvent for starch, made the scenario much complicated, since undissolved starch molecules would affect the rheological response.

To spin good fibers, the concentration had to be 1.2-2.7 and 1.9-2.3x the entanglement concentration for starch and pullulan, respectively (**Figures 4** & **5**). This range is narrower for pullulan, probably also due to less conformation difference in the solvents. It was interesting to note that a dispersion at entanglement concentration, when polymers start to entangle with one another, was not electrospinnable. Probably, high shear force involved in electrospinning impede chain overlap and long range polymer interaction that might have already been established at static and low shear conditions, and thus an enhanced and sufficient entanglement is required. In addition, shear viscosity also played an important role (**Figure 6**). The electrospinnable starch and pullulan dispersions fall into a similar range of shear viscosity at 100 sec^{-1}, with an upper boundary of 2.2 Pa·sec.

The procedure described herein can be modified in correspondence with the equipment and materials used in other studies. The dissolution of polymers is the first critical step in this protocol, because we found that partially dissolved starch dispersions (***e.g.***, in 85% (w/v) DMSO) produced unstable steady shear viscosity data that prevented an accurate determination of c_e. While conducting steady shear measurements, we prefer to start from the highest shear rate. By doing so, the dispersion is evenly distributed within the gap by the help of a high shear rate. The electrospinning step required much practice. Attention should be paid to the shape change of the droplet at the needle tip. Safety precautions during electrospinning should not be neglected. The main danger of electrospinning comes from the high voltage used in the process, although the current is relatively low. Electrospinning experiments should be performed in a fume hood in order to expel solvent vapor that may pose health hazards if one is exposed to it for a long time. Avoid close distance and even contact between the charged needle tip and the coagulation bath, because these will result in a short and fire hazard.

The rheological methods employed in the current study do have limitations. For instance, it should be noted that the actual shear rate involved in electrospinning is much higher than 100 sec^{-1} [1]. In addition to shear rheology studied, elongational rheology, which characterize the stretching of dispersion along the trajectory, may also play an important role[15]. The rheometer used in this study is not capable of characterizing elongational viscosity.

Rheological studies can provide valuable information on biopolymer conformation in dispersions and their processing properties. This protocol is potentially useful in electrospinning of many other biopolymers and their blends, in terms of solvent system selection, optimization of parameters, and fiber forming mechanism on a molecular level.

Disclosures

The authors declare that they have nothing to disclose.

Acknowledgements

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