Chapter 20

Solution Blowing of Soy Protein Fibers

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Solution blowing of soy protein (sp)/polymer blends was used to form monolithic nanofibers. The monolithic fibers were blown from blends of soy protein and nylon-6 in Formic Acid. The sp/nylon-6 ratio achieved in dry monolithic nanofibers formed using solution blowing of the blend was equal to 40/60. In addition, solution blowing of core–shell nanofibers was realized with soy protein being in the core and the supporting polymer in the shell. The shells were formed from either polyacrylonitrile (PAN), or nylon-6. The sp/nylon-6 ratio achieved in dry core–shell fibers was 32/68. For sp/PAN the ratio achieved in dry core–shell fibers was 29/71. The nanofibers developed in the present work contain significant amounts of soy protein and hold great potential in various applications of nonwovens.

Introduction

Modern technology is experiencing a significant shift towards biobased polymers caused by their biodegradability, renewable sources, or just “green” agricultural biomolecular origin. The shift is driven by a necessity to reduce dependence on synthetic materials, namely on petroleum-derived polymers.
As a result, significant efforts are directed toward production of biodegradable materials from cotton, corn, potato (1–5) and soy protein and development of biodegradable composites (6–8). In particular, there is a growing emphasis on making soy-based “green materials”. The reason behind the drive towards soy-based materials can be attributed to the fact that soy protein is one of the cheapest and most widely grown vegetable products with a wide range of uses. Booming SoyDiesel production (9, 10) facilitates increasing production of soy, while using only soy oil, and leaving behind abundant residual soy protein.

It is emphasized that soy nanofibers were unavailable at present until our recent work (11), as to our knowledge. In the present work we employ a novel method - solution blowing (11, 12) - to produce monolithic and core–shell nanofibers containing soy protein. We denote as monolithic the fibers made of sp/polymer blends and distinguish them from core–shell fibers where sp is located in the core, and a polymer is predominantly in the shell. It is emphasized that in this work it was impossible to form pure sp fibers. Indeed, pure soy protein is very brittle and does not lend itself to fine fiber formation. In Ref. (13) electrospinning with pure sp was attempted and resulted in spraying only.

**Experimental Section**

**Materials**

Polyacrylonitrile (PAN) and nylon-6, two common industrial fiber-forming polymers were used. In particular, nylon-6 was chosen since it is soluble in formic acid which is also a solvent for soy protein isolate, enabling formation of homogeneous solution blends. On the other hand, PAN is soluble in N,N-Dimethyl Formamide (DMF), which is a non-solvent for soy protein isolate. The soy protein isolates PRO-FAM 955 (sp 955) and PRO-FAM 974 (sp 974) were generously donated by ADM Specialty Food Ingredients. PRO-FAM 955 and PRO-FAM 974 have the same composition but differ in the way they were processed, even though it might be expected that their denaturation in solution can make them practically indistinguishable. Both PRO-FAM 955 and PRO-FAM 974 were recommended by the United Soybean Board (USB) and furnished by ADM.

**Solution Preparation**

Solution preparation for blowing of sp/nylon-6 monolithic and core–shell fibers and their rheological characterization are described in detail in Ref. (11) and are not reproduced here. For the solution blowing of core–shell fibers with PAN in the shell of the fiber, a 10 wt% PAN solution dissolved in DMF (no soy protein added) was used. The core was formed from the following blend: 0.75 g of sp 955 was mixed in 10 g of DMF, and then 0.5 g of 8 wt% PAN solution in DMF was added to enhance core spinnability (viscoelasticity).

**Setup and Experimental Procedure**

A detailed description can be found in Refs. (11) and (12).
Results and Discussion

Blends of Soy Protein and Nylon-6: Monolithic Fibers

The fibers obtained from the blend of soy protein and nylon-6 are shown in Figure 1. They have no visible grains of protein, as it was completely dissolved in formic acid. Note that the fibers in the SEM images in Figure 1 a,b (PRO-FAM 955) look rather curly, and some knobs are also visible in Figure 1c where another type of soy protein (PRO-FAM 974) was used for comparison. A macroscale web image corresponding to Figure 1c is shown in Figure 1d.

In dry monolithic nanofibers formed using solution blowing of soy protein (PRO-FAM 955) blends with nylon-6 the protein/polymer ratio of sp/nylon=40/60 was achieved, as determined by the initial solution composition after the solvent had evaporated.

![Figure 1](http://pubs.acs.org/doi/abs/10.1021/bk-2012-1105.ch020)

**Figure 1.** Panels (a) and (b) show SEM images of the monolithic fibers formed by blowing a blend of nylon-6 and soy protein PRO-FAM 955 in formic acid. The zoomed-in view of a section of (a) is shown in panel (b). The fibers are rather curly. Panel (c) shows for comparison fibers formed by blowing a blend of soy protein PRO-FAM 974 and nylon-6 in a 17/83 ratio in formic acid. The macroscale web shown in panel (d) corresponds to the nanofibers of panel (c). (Courtesy of the American Chemical Society).

PRO-FAM 974 product data shows nominal amino acid and other mineral contents, 0.62 wt% sulfur from cystine and methionine, and the elements Na (1.2-
1.6 wt%), K (0.024 - 0.3 wt%), Ca (0.05 – 0.15 wt%), P (0.7 – 1.0 wt%), Fe (0.008 – 0.013 wt%) and Mg (0.025 – 0.10 wt%). EDS spectra from SEM image collection was employed to detect the unique atomic markers from soy protein isolate to differentiate from nylon-6 in a monolithic blend of nanofibers such as shown in Figure 1c. Figure 2a shows the EDS spectra for a formic acid solution cast film of soy protein isolate PRO-FAM 974. The unique markers, P and S are clearly seen. An EDS spectra of 40/60 sp/nylon-6 monolithic blend nanofibers containing some beads is shown in Figure 2b. It is emphasized that the blend of sp/nylon-6 blown to form the fibers used in Figure 2 was absolutely clear, which is an indication that it was homogeneous and no undissolved protein grains were present. When such a blend is strongly stretched during the solution blowing process, solid nanofibers are formed. Sometimes some droplets are disconnected and emitted by high-speed gas stream, or beads are formed, which can be attributed to capillary instability. When the cast films of soy protein was examined using EDS unique markers, (P, S) can be clearly seen, which can also be seen clearly in the nanofiber. The quantities of S from the soy protein amino acids are comparable to theoretical (0.6 wt% in pure sp and 0.2% in the 40/60 sp/nylon-6 blend). It should be mentioned that Al was omitted in the analysis since it was due to the aluminum foil was used as nanofiber collection substrate. An EDS spectra was collected with SEM zoomed into a single bead as shown in Figure 2c. The P and S peaks are clearly visible indicating the presence of soy protein. Finally, the EDS spectra were collected with SEM zoomed to a nanofiber-only region as shown in the box labeled S3 of Figure 2d (image) and e (spectra). P and S are again clearly visible at levels consistent with a monolithic blend. These EDS spectral data suggest compositional uniformity of a monolithic sp/nylon-6 solution resulting in nanofibers and some beads with the same composition. The EDS analysis of the beads showed both S and P, which proves that soy protein precipitated in the beads. The EDS analysis of a visually fiber-only region (Figure 2e box labeled S3) clearly revealed P and S. In particular, such a region in box S3 in Figure 2d shows bundles of physically separated nanofibers with no matrix. This proves that the soy protein and nylon-6 co-precipitated to form monolithic fibers. In other words, the soy protein was mixed with nylon-6 in the nanofibers and not lying in between the fibers.

**Core–Shell Nanofibers of Soy Protein and Polymer**

**Core–Shell Nanofibers of Soy Protein and PAN**

The optical images of the core–shell PAN-based fibers formed at the core flow rate of 1.2 mL/h are shown in Figure 3 a,b, and at the core flow rate of 2.2 mL/h - in Figure 3 c,d. It can be seen that for the higher core flow rate the presence of the core is much more unambiguous compared to that at the lower core flow rate. However, it should be emphasized that an increase in the core flow rate makes the blowing process prone to interruptions and can result in dripping instead of core–shell fiber formation. It can be seen in Figure 3 d that in some cases the core was not present throughout the whole fiber (indicated by arrow), albeit such defects were relatively rare. Note also, that there are no visible soy protein grains.
Several PAN-based core–shell samples were prepared for heat treatment followed by SEM observation. The resulting fibers were heated at 350 °C for 3 hours in air followed by heating at 750 °C for 1 hour in N$_2$ atmosphere. For control, soy protein isolate was also subjected to the same treatment and it was found that it was never fully destroyed and left residual quite comparable in volume to the original soy protein. Therefore, it was established that after such treatment the PAN-based core–shell samples will not be hollow, as in the case of PMMA-PAN samples in Ref. (12), even though the soy protein was subjected to such high temperatures when there must be some thermal degradation of it. Note that PAN shell definitely does not fully disappear under the same thermal treatment but rather carbonizes (12). In addition, it was expected that both materials (soy protein and PAN) shrink differently under such thermal treatment, and thus some cross-sectional differences will be visible in SEM images. It was expected that DMF in the core of the core–shell fiber should evaporate through the shell within about 10 sec (14). However, in contrast to Ref. (14), this process did not result in a hollow structure as in Ref. (14) where the core material was a relatively dilute polymer solution, which resulted in its precipitation onto the shell.
Figure 3. Optical images of the PAN-based core−shell fibers in the cases of the core flow rate of 1.2 mL/h in (a) and (b), and 2.2 mL/h in (c) and (d). The shell flow rate was kept constant at 4.5 mL/h in all the cases. Cores and shells are typically easily distinguishable. In (d) the core interruption at a certain location is marked by an arrow.

The SEM images in Figure 4 show the fracture pattern of the PAN-based core−shell nanofibers before heat treatment. The difference in the fracture between the core and the shell (a pit-like pattern of the core versus a straight cut of the shell) manifests different physical properties of soy protein coagulant in the core and PAN precipitant in the shell. The SEM images in Figure 5 show that the structure of the fibers after heat treatment is rough and possesses some nano-texture (indicated by arrows). The shell walls of the heat-treated PAN-based fibers possess porous structure resulting from DMF evaporation.

Two examples of macroscopic samples of core−shell soy protein-PAN nonwovens collected on a wire ring as a film or in a bucket as a bulky fluffy mass are given in Figure 6.

Core−Shell Nanofibers of Soy Protein and Nylon-6

The SEM images in Figure 7 show the cross-sectional view (Figure 7a) and side view (Figure 7b) of the nylon-6 based core−shell nanofibers. Denaturation of soy protein by formic acid results in complete solubilization of the former, while
the evaporation of the latter after fibers were formed leaves a porous nylon-6 shell seen in Figure 7b. The mechanisms of pore formation in nanofibers were discussed before in the context of electrospinning in Refs. (14) and (15). They can be attributed to such factors as direct permeation of the shell by vapor escaping from the core, as well as to the breath figures, i.e. solvent evaporation cooling, which triggers water droplet condensation onto nanofibers from the surrounding humid air. The image in Figure 7a shows a significant grayness gradient between the core and the shell, which is attributed to a significant electron contrast of soy protein in the core and nylon-6 in the shell.

Figure 4. Panels (a) and (b): SEM images of core−shell fibers (soy protein and a small amount of PAN in the core and 10 wt% of PAN in the shell) before heat treatment. In panels (a) and (b) the arrows point at the darker pit-like fracture of the core. This type of fracture is more prominent in panel (b). It looks like the core and shell have different stiffness, which results in different fracture patterns.

Figure 5. Panels (a) and (b): SEM images of the core−shell (soy protein/ PAN) fibers after heat treatment. The arrows indicate the presumably soy protein core surrounded by the porous carbon shell.
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Figure 6. Macroscopic samples of core–shell soy protein-PAN nanofibers collected as a film on a wire ring (panel a), and as a bulky fluffy deposit in a bucket (panel b). Both samples were moved to Petri dishes also seen in the image.

Figure 7. Panels (a) and (b) show SEM images of core–shell fibers made with fully denaturated soy protein and nylon-6. The core is clearly visible in the cross-sectional view in panel (a) because of a significant electron contrast between soy protein and nylon-6. The core boundary in panel (a) is also traced by a white contour to make it more visible in printed reproduction and marked by the arrow. The side view (b) shows that the nylon-6 shell of this core–shell fiber is porous. (Courtesy of the American Chemical Society).

In dry core–shell nanofibers the sp/nylon-6 ratio was 32/68, as determined by the initial solution compositions and the corresponding flow rates, after the solvent had evaporated.

Fluorescence Microscope Imaging

Fluorescence imaging was used to map soy protein inside fibers formed by solution blowing of blends of nylon-6 with sp in formic acid, and core–shell fibers with sp in the core and nylon-6 in the shell. The fiber mats were washed twice with phosphate-buffered saline (PBS) and then blocked with PBS containing 3%
BSA (500 µl per well) at room temperature for 30 min. Then the fiber mat was washed twice by PBS. After that, a rabbit anti-soy antibody at a dilution 1:2,000 in PBS was added overnight at 4 °C. Thereafter, the fiber mats were washed twice again with phosphate-buffered saline (PBS), and then a goat anti-rabbit IgG FITC antibody (Cat. No.10004302, Cayman, USA) was added at a dilution 1:100 in PBS for one hour at room temperature. Finally, the fiber mats were washed twice with phosphate-buffered saline (PBS). Fluorescent images were recorded using a Nikon microscope (Eclipse E800). Illuminating light was focused on the sample through a 20 ×/0.75 NA objective or a 10 ×/0.25 NA objective, and images were recorded using a CCD camera (CoolSnap fx, Roper Scientific, Tucson, AZ).

Figure 8 demonstrates that soy protein is uniformly distributed in the fibers with the stained soy protein visible everywhere as green color. It is emphasized that soy protein can show some fluorescence under the illuminating light. However, typically staining is required for more reliable observation. On the other hand, nylon-6 does not possess any fluorescence of its own, and cannot be stained using the aforementioned procedure.

![Image](https://example.com/image.png)

**Figure 8.** Optical image (panel a: black and white) versus fluorescent images (panel b: in color) of the fibers formed by solution blowing a blend of nylon-6 and soy protein in formic acid. Images (a) and (b) were taken exactly at the same place of a fiber mat. Both images were taken with a 100× microscope objective. The green color indicates the presence of soy protein. (Courtesy of the American Chemical Society).

The core–shell fibers are depicted in Figure 9. The images in Figure 9 reveal that the diameter of the green fluorescence fibers in panels (b) and (d) is smaller than the corresponding diameter of the same fibers in the black-and-white optical images in panels (a) and (c). In particular, in panel (a) the optical image of a fiber has a diameter of 1 µm, whereas the fluorescent image of the same fiber is only 0.4 µm in diameter. This proves that soy protein is, indeed, located in the core of the core–shell fibers as intended. It is emphasized that the fluorescent markers could easily reach the soy protein core via the nylon-6 shell through multiple pores clearly visible in Figure 7b and also reported previously for the other types of core–shell fibers (14, 15).
Figure 9. Optical images (panels a and c: black and white) and fluorescent images (panels b and d: in color) of core–shell fibers with soy protein and a small amount of nylon-6 in the core and 20 wt% solution of nylon-6 in formic acid used to form the shell. Panels (a) and (b) corresponding to exactly the same place in the fiber mat were taken with a 40× microscope objective. Panels (c) and (d) corresponding to exactly the same place in the fiber mat were taken with a 100× microscope objective. Green fluorescence indicates the presence of soy protein. (Courtesy of the American Chemical Society).

Statistical Image Analysis

Nonwovens produced by solution blowing are comprised of the entangled individual fibers. They practically never end until the process is stopped. In this sense, they are similar to electrospun nanofiber mats. Therefore, measurements of fiber lengths and aspect ratios are meaningless. On the other hand, cross-sectional diameter can vary even along the individual fibers due to the effect of the turbulent pulsations in gas and elastic wave propagation (16, 17). Therefore, measurements of fiber diameter distribution can be important for the structural characterization of the solution-blown nonwovens and their further applications. Figure 10 depicts the diameter distributions measured from SEM images of sp/nylon-6 fibers blown from blends, while Figure 11 contains the corresponding data for sp/nylon-6 core–shell fibers. It is emphasized that the blend-blown (monolithic) fibers all belong to the submicron range, whereas the core–shell fibers can involve infrequent outliers as large as 2 µm. Still the core–shell fibers are submicron in average.
Figure 10. Diameter distribution in sp/nylon-6 blend solution blown nanofibers. (Courtesy of the American Chemical Society).

Figure 11. Diameter distribution in sp/nylon-6 core–shell solution blown fibers. (Courtesy of the American Chemical Society).
Conclusion

Solution blowing of monolithic and core–shell soy protein-polymer fibers was demonstrated. The blend fibers were produced from soy protein and nylon-6. They had a protein/polymer ratio of 40/60 in the dry sp/nylon-6 fibers. The core–shell fibers were produced from either protein/PAN, or protein/nylon-6 in the core and, respectively, PAN or nylon-6 in the shell. The protein/nylon-6 had a protein/polymer ratio of 32/68 in the dry core–shell fibers. The individual fibers and their nonwovens were analyzed using optical and scanning electron microscopy (with EDS). Soy protein and nylon-6 provided a significant electron contrast to make the protein core clearly distinguishable from the polymer shell. In the present work the monolithic nanofibers made from sp/nylon-6 blends were basically non-porous in the scale of 10-100 nm, since the images in this scale (e.g. Figure 1c) do not resolve any visible porosity, which still might be present on the smaller scale. The core–shell sp/nylon-6 fibers had pores in the shell in the scale of 10 nm (Figure 7b). Porous shells probably allow low molecular weight fluids to communicate with the core when core–shell fibers are submerged into them, which make them attractive for many applications. In addition, using staining with primary rabbit anti-soy antibody and secondary FITC antibody, soy protein has been successfully marked in the fibers. It was shown that in the blend-blown fibers, soy protein was distributed everywhere in the fibers. These results were in agreement with EDS data from SEM which indicated homogeneous sp/nylon-6 blends. In the core–shell sp/nylon 6 fibers, soy protein was located only in the fiber cores, as designed. The monolithic (blend-blown) fibers all belong to the submicron range, whereas the core–shell fibers can involve infrequent outliers as large as 2 μm. Nevertheless, the core–shell fibers are submicron in average.

The monolithic and core–shell nano- and micro-fibers containing significant amounts of soy protein developed in this work hold great potential as an effective method of utilizing of the one of the most abundant natural materials. In particular, it is foreseen as an effective method of utilization and valorisation of residual material left by Soy Diesel production, which can be useful in various applications of nonwovens. Moreover, Soy Protein Isolates (SPI) used in the present work contain 90% protein, while Soy Protein Concentrates (SPC) contain 50-70% protein (18). The highest protein content soy product commercially available was used for this study. Therefore, the use of SPC was not investigated.

In addition, note that solution blown the sp/nylon-6 nanofiber mats produced in the present work look stronger than many electrospun nanofiber mats reported so far (19) and are not brittle. A detailed study of their tensile properties is underway and will be reported in future.

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References