Regeneration of Bombyx Mori Silk Nanofibers and Nanocomposite Fibrils by
the Electrospinning Process

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DEDICATIONS

To my parents for all of their support and guidance throughout my life and for putting up with me all these years. You’ve sacrificed a lot to see me get to where I am today and for that I’ll be forever grateful. I’d also like to thank the rest of my family for the continuous support shown over the years.
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ABSTRACT

Regeneration of *Bombyx mori* Silk Nanofibers and Nanocomposite Fibrils by the Electrospinning Process

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Frank K. Ko. Ph.D.

In recent years, there has been significant interest in the utilization of natural materials for novel nanoproducts such as tissue engineered scaffolds. Silkworm silk fibers represent one of the strongest natural fibers known. Silkworm silk, a protein-based natural biopolymer, has received renewed interest in recent years due to its unique properties (strength, toughness) and potential applications such as smart textiles, protective clothing and tissue engineering. The traditional 10-20 µm diameter, triangular-shaped *Bombyx mori* fibers have remained unchanged over the years. However, in our study, we examine the scientific implication and potential applications of reducing the diameter to the nanoscale, changing the triangular shape of the fiber and adding nanofillers in the form of single wall carbon nanotubes (SWNT) by the electrospinning process. The electrospinning process preserves the natural conformation of the silk (random and β-sheet). The feasibility of changing the properties of the electrospun nanofibers by post processing treatments (annealing and chemical treatment) was investigated. *B. mori* silk fibroin solution (formic acid) was successfully electrospun to produce uniform nanofibers (as small as 12 nm). Response Surface Methodology (RSM) was applied for the first time to experimental results of electrospinning, to develop a processing window that can reproduce regenerated silk nanofibers of a predictable size (d < 100nm). SWNT-silk multifunctional nanocomposite fibers were fabricated for the first time with
anticipated properties (mechanical, thermal and electrically conductive) that may have scientific applications (nerve regeneration, stimulation of cell-scaffold interaction). In order to realize these applications, the following areas need to be addressed: a systematic investigation of the dispersion of the nanotubes in the silk matrix, a determination of new methodologies for characterizing the nanofiber properties and establishing the nature of the silk-SWNT interactions. A new visualization system was developed to characterize the transport properties of the nanofibrous assemblies. The morphological, chemical, structural and mechanical properties of the nanofibers were determined by field emission environmental scanning microscopy, Fourier transform infrared and Raman spectroscopy, wide angle x-ray diffraction and microtensile tester respectively.
CHAPTER 1: INTRODUCTION

Nanoscale polymeric fibrous materials are the fundamental building blocks of living systems. From the 1.5-nm double helix DNA molecules, 30-nm diameter cytoskeleton filaments, to sensory cells such as hair cells and rod cells of the eyes, nanoscale fibers form the extracellular matrices of tissues and organs. Organic polymers can be electrospun to produce nanofibers of with diameters as small as 3 nm. The 3-nm diameter fibers have only 6 or 7 molecules across the fiber. These fibers have very large surface areas, which contain an abundance of potential reaction sites (sensing and bonding) which may have applications for the development of new nanoscale consumer products such as reinforcing fibers in textile composites, nonwettable fabrics, high performance membrane filters, cell-growth scaffolds, vascular grafts, wound dressings and drug delivery systems. The large surface area to volume ratio may enhance cellular migration and proliferation in tissue-engineered scaffolds. Of particular interest are natural and synthetic polymers such as *Bombyx mori* (silkworm silk), collagen, chitosan, polylactic, polyglycolic acid and related polymers. Silk is an environmentally stable and biocompatible material with unique physico-chemical properties which can be modified by controlling the genetic sequence. Therefore, it would be of interest to determine the properties of the regenerated electrospun silk nanofibers.

This thesis systematically studied the electrospinning parameters utilized to produce regenerated silk nanofibers (less than 100 nm in diameter). RSM was applied for the first time to the electrospinning data, to predict fiber diameter. The silk fibroin was characterized through the electrospinning processing steps of degumming,
dissolution in aqueous calcium chloride, dialysis, water removal (lyophilization), dissolution in formic acid and electrospinning (fiber formation). While the bioactive properties of silk nanofibers are desirable, the utility of the nanofibers is limited by poor mechanical properties. As previous research has shown that electrospun nanofibers do not possess satisfactory mechanical properties, nanofillers such as carbon nanotubes (CNT) were incorporated into the nanofibers in order to improve not only the mechanical properties of the nanofibers, but also to provide new tailorable properties such thermal and electrical conductivity. The availability of CNT provides attractive material design options to tailor the mechanical properties of the nanofibers for various applications. With a tensile strength and modulus of 30 GPa and 1 TPa respectively for the CNT, one can anticipate an enormous reinforcement effect with the use of only a small portion of CNT’s. Accordingly, by co-electrospinning of CNT with the Bombyx mori silk fibers, a nanocomposite combining the bioactivity and structural reinforcement can be fabricated to yield multifunctional strong and tough fibers. Post processing treatments such as methanol immersion and annealing were also carried out to impart new properties on the electrospun nanofibers.

A major goal of this thesis was the establishment of characterization techniques for the nanofibers. There is a need for determining new methodologies of characterizing the transport properties of the nanofibrous assemblies. Nanostructured materials interact with liquids in unique ways requiring the development of new modeling and characterization methods before their potential applications can be
realized. A new visualization system was developed to characterize the transport properties of the nanofibrous assemblies.
CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

People have been using polymers for thousands of years. Silk, cellulose, collagen, rubber and many more natural materials are examples of polymers. Life depends on polymers since the DNA, RNA and many other proteins in cells which comprise the body are all made up of polymers. Polymers have been the subject of vibrant scientific interest and in addition to natural materials; man-made or synthetic polymers have also been fabricated with equal or better properties in comparison to the ones synthesized by nature. This has resulted in a broad range of theoretical, numerical and experimental techniques to investigate the properties of these materials. In the world of natural fibers, silk has long been recognized as the wonder fiber for its unique combination of high strength and rupture elongation.

2.1 Production of silk
First discovered in China more than 4500 years ago and smuggled out to the West 2000 years later, silk is now produced across Asia and Europe, although the main sources are Japan, China and India. Manufacture of silk (sericulture) is still an industry requiring a great deal of manual labor, and the present cost of silk reflects this.

Silks are produced solely by arthropods and only by animals in the classes *Insecta*, *Arachnida*, and *Myriapoda*. Silk is defined as fibrous protein polymers containing highly repetitive sequences of amino acids and are stored in the animal as a liquid and organize into fibers when sheared or “spun” at secretion [1]. Silk is spun into fibers
by Lepidoptera larvae such as silkworms, spiders, scorpions, mites and flies. The silk is utilized for different functions which range from providing protective shelter, structural support, foraging and dispersal to reproductive uses. Generally, insects produce different types of silk proteins although this may be limited to only one within the same species. Spiders on the other hand, may produce as many as eight different types of silk fibers. Of particular interest are the silk fibers from silkworm Bombyx Mori and spider Nephila clavipes due to their intrinsic properties utilisable in high quality textiles, biotechnological and biomedical fields. Draglines of N. clavipes and A. aurentia spider silks are among the strongest spider silks that are known. The strength of the dragline of N. clavipes and dragline of A. aurentia obtained by forcible silking, are reported to be about 8 g/denier (~ 900 MPa) and 12 g/denier (~ 1300 MPa) respectively [2]. Considering the remarkable mechano-chemical properties of silk and fueled by the recent progress in biotechnology and nanotechnology, there is a revival of interest in using silk in different applications. Due to the diverse types of silk spun by arthropods, this study will concentrate only on silk spun by Bombyx mori as it is widely available and has great commercially importance.

Silk protein is synthesized in the silk glands of the silkworm where its secreted and stored in the lumen, thereafter its transformed into fibers by the stretching of the liquid silk through the head movement of the silkworm [3-6]. The mechanism of fiber formation is highly complex and the exact spinning mechanism is still an open question.
2.2 Physical, chemical and structural properties of silk fibers

2.2.1 Physical properties of silk

*B. mori* silk fibers are generally 10-20 µm in thickness (size) and each fiber is actually a duplet of two individual fibers, each with its own silk coating (sericin) and an inner core (fibroin). The fibroin consist of thousands of parallel fibrils (100-400 nm), which after ion-etching can be viewed by a scanning electron microscope. The fibrils give the microfilament its grainy structure. The fibers also contain small quantities of carbohydrate, wax and inorganic components which also play significant roles as structural elements during fiber formation. *B. mori* fibers are not circular in cross section but appear triangular as shown in Figure 1.

The fine structure of silk fibers gives them dynamic qualities of excellent luster, color, exquisite texture and superb temperature retainability.

2.2.2 Chemical properties of silk

The unique properties of the silk protein originate from its unique amino acid composition translated into an unusual primary structure and hierarchical structural organization. The molecular backbone of silk proteins consists of a chain of amino acids, each of which is built of four groups. Three of the groups, an amine group (−NH₂), a carboxyl group (−COOH), and a hydrogen group (−H), are common to all amino acids and are bound to a carbon molecule designated as the α-carbon. The fourth group of each amino acid, the “R” group or side chain, varies and the diversity
of silk proteins derives from the different size, shape, charge, hydrogen-bonding capacity, and chemical reactivity of these distinctive side chains.

![Micrographs of B. mori silk at different magnifications showing the triangular cross section of the fibers.](image)

Figure 1. ESEM micrographs of *B. mori* silk at different magnifications showing the triangular cross section of the fibers.

In addition, the interactions among the R-groups are affected by the rotation around the $\alpha$-carbon of the amino acid and this in turn allows the silk protein to fold in a variety of ways. Figure 2 shows the structure of 4 of the most abundant amino acid groups in *B. mori* silk.
B. mori silk is composed of a light (~ 25 kDa) and heavy fibroin chains (~ 350 kDa). The heavy chain protein consists of 12 repetitive regions called crystalline regions and 11 non repetitive interspaced regions called amorphous regions. The composition of the 5263 amino acid residues in the fibroin (in mol %) is 45.9% glycine (G), 30.3% alanine (A), 12.1% serine (S), 5.3% tyrosine (Y), 1.8% valine(V) and 4.7% other remaining amino acids [7].

The composition of the B. mori fibroin is shown in Table 1. The exact sequence of B. mori silk has been the subject of much research. Several authors have conducted experiments and derived models to describe the sequence. Mita et al [8] and Zhou and his group [9] utilizing cDNA sequencing method, employed shotgun sequencing strategy combined with traditional physical map-directed sequencing of the fibroin.
gene of the heavy chain, predicted the presence of unusual repeat sequences in the silk fibroin.

Table 1. Amino acid composition of *B. mori* silk [10]

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Symbol</th>
<th>Charge</th>
<th>Hydrophobic/hydrophilic</th>
<th>Amount (g/100g fibroin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>32.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>neutral</td>
<td>hydrophilic</td>
<td>42.8</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>neutral</td>
<td>hydrophilic</td>
<td>11.8</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>neutral</td>
<td>hydrophilic</td>
<td>14.7</td>
</tr>
<tr>
<td>Aspartate</td>
<td>Asp</td>
<td>-</td>
<td>hydrophilic</td>
<td>1.73</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>+</td>
<td>hydrophilic</td>
<td>0.90</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>+</td>
<td>hydrophilic</td>
<td>0.32</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Glu</td>
<td>-</td>
<td>hydrophilic</td>
<td>1.74</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>+</td>
<td>hydrophilic</td>
<td>0.45</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>3.03</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>0.68</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>0.87</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>1.15</td>
</tr>
<tr>
<td>Proline</td>
<td>Pro</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>0.63</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
<td>neutral</td>
<td>hydrophilic</td>
<td>1.51</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>0.10</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>neutral</td>
<td>hydrophobic</td>
<td>0.03</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>neutral</td>
<td>hydrophilic</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Their analysis demonstrates that the primary structure of *B. mori* silk fibroin may be approximately divided into four regions: a repetitive region R subdivided into three smaller regions (1, 2, and 3) and an amorphous region A (4), which are arranged alternatively along the molecular chain. Region (1) is the highly repetitive GAGAGS sequence which constitutes the crystalline part of the fibroin (94% of total chain), region (2) is the relatively less repetitive GAGAGY and/or GAGAGVGY sequences consisting of the semi-crystalline parts which contain hydrophobic moieties, region
(3) is similar to (1) plus an additional AAS chain, while region (4) is the amorphous part containing negatively charged, polar, bulky hydrophobic, and aromatic residues. Figure 3 depicts the schematic representation of the primary structure. The crystalline repetitive region is responsible for the secondary structure (anti-parallel $\beta$-pleated sheets) of the protein [11]. The crystalline domains are responsible for the strength of the material, whereas the amorphous domains allow the crystalline domains to orient under strain thereby introducing flexibility and further increasing the strength of the material.

Figure 3. Schematic representation of the primary structure of *B. mori* silk.[Asakura, T; Seguno, R; Yao, J; Takashima, H; Kishore, R. Biochemistry 2002, 41, 4415-4424]

2.2.3 Structural composition of silk
The molecular and crystal structure of silk fibroin has been the subject of great interest since the turn of the century. Ishikawa and Ono carried out X-ray diffraction studies of silk as early as 1913. Thereafter, the structure was examined by several other researchers [12, 13]. Marsh et al in 1955 determined that the crystal structure of silk fibroin was a regular arrangement of anti-parallel sheets which was the generally accepted structure for decades. However, since their crystal structure model was based on certain estimations, several authors found their model and analysis unacceptable and have tried to elucidate the structure [14-21]. The cell dimensions are based on models of polypeptides such as poly(L-Ala-Gly) and Gly-Ala-Gly-Ala-Gly-Ser. Three silk fibroin conformations have been previously identified by x-ray and electron diffraction, nuclear magnetic resonance (NMR) and infrared spectroscopy; random structure (low concentrations of fibroin), α-structure (silk I, type II β-turn, high concentrations formed in the absence of physical shear), and β-structure (silk II, antiparallel β-pleated sheet, formed with physical shear or exposure to solvents such as methanol) [5]. These conformations are easily inter-converted from one form to another depending on the processing conditions (temperature, application of stress or shear, pH, exposure to solvents etc).

The random structure or less ordered chains are the polymeric secretions that cannot be spun or easily stretched [1]. However, they can easily be transformed into α and β-sheet structures by mechanical shearing and treatment in organic solvents under various reaction conditions.

In the β-sheet structure, hydrogen bonds are formed between adjacent segments of polypeptide chains [22]. The polypeptide chains are aligned side by side in a parallel
or antiparallel direction in four possible configurations: 1) polar-antiparallel sheet proposed by Marsh *et al* [13]; 2) polar-parallel sheet (methyl groups of alanine residues grouped on just one side); 3) antipolar-antiparallel sheet (methyl groups pointed alternately to opposite sides of the sheet), and 4) antipolar-parallel model. Figure 4 shows the schematic of two possible β-sheet configurations (parallel and anti-parallel).

![β-sheet configurations of Gly-Ala-Gly-Ala-Gly-Ser amino acid sequences](image)

Figure 4. β-sheet configurations of Gly-Ala-Gly-Ala-Gly-Ser amino acid sequences a) parallel b) antiparallel.

Both the direction of adjacent polypeptide chains and the polarity of the molecules composing them, determine how the protein sheets stack into a three-dimensional crystalline matrix. The β-sheet sheets are closely packed with intersheet distances of 3.5 Å for glycine-glycine interactions and 5.7 Å for alanine-alanine interactions [23]. If the β-sheet sheets are parallel to each other and assume the same direction, the hydrogen bonds between the sheets become distorted with 5.27 Å spacing between
sheets. As a result of the great internal strains generated by parallel packing of β-sheets, they are easily destabilized and hence parallel β-sheets of less than five polypeptide chains are rare.

The β-sheet structure was originally characterized as having a polar-antiparallel structure. On this basis Warwicker [23] classified the β-sheet structured silks into 5 groups with regards to the length of the c-axis of the unit cell matrix. The dimensions of the unit cells of B. mori fibroin are: a (9.3 Å), b (9.44 Å) and c (6.95 Å). However, current research has shown that the β-sheet structures are comprised of two antipolar-antiparallel sheet structures with different orientations which occupy the crystal sites [24].

The α-structure (silk I, type II β-turn), despite its long history of study, remains poorly understood because it easily transforms into silk II which make determination of its orientation by X-ray and electron diffraction a challenge [25-27]. Therefore, most research conducted on silk I structures are based on models of peptide chains such as (AG)_n, resulting in conflicts in the determination of the structure.

B. mori silk fibroin can exist in two distinct structures in the solid state, namely silk I (pre spinning) and silk II (post spinning). A third structure, silk III, with a threefold helical crystal structure has recently been observed by Valluzzi et al (1999) in films prepared from aqueous fibroin solutions using the Langmuir Blodgett (LB) technique [28]. The films prepared had either a uniaxially oriented crystalline texture, with the helical axis oriented perpendicular to the plane of the film or the helical axes lying roughly in the plane of the film at the air-water interface.
The crystallinity and degree of orientation of the silk crystals in fiber, film and solution forms have also been widely studied over the years as they are factors that influence the mechanical properties of the material [29-33].

2.3 Electrospinning process

2.3.1 Fabrication of microfibers

Synthetic fibers are produced typically by three easily distinguishable methods: dry spinning [34, 35], wet spinning [36-39] and melt spinning [40-42]. Melt spinning processes use heat to melt the fiber polymer to a viscosity suitable for extrusion through a spinnerette. Solvent spinning processes utilize organic solvents, which usually are recovered for economic reasons, to dissolve the fiber polymer into a fluid polymer solution suitable for extrusion through a spinnerette. Melt spun polymeric fibers include high density polypropylene (HDPE), polyesters, nylon and polyolefins. Examples of dry solvent spun polymers are cellulose acetate, cellulose triacetate, acrylic, vinyon and spandex; while wet spun fibers include acrylic and modacrylic.

Almost all these processes rely on a pressure driven extrusion of a viscous polymer fluid and the fibers produced range from 2-500 microns in diameter.

With the trend in science moving towards smaller materials (nanotechnology), it was only a matter of time before researchers started thinking of reducing the size of materials and examining the properties of these materials at much smaller scales (nanoscale). Reduction in sizes of materials to nanoscale levels will enable the control of basic material properties without changing their chemical compositions. Utilization
of novel technologies to fabricate nanofibers presents the opportunity to develop new families of products with various applications. Nanofibers are solid state linear nanomaterials characterized by flexibility and an aspect ratio greater than 1000: 1. According to the National Science Foundation (NSF), nanomaterials are matters that have at least one dimension equal to or less than 100 nanometers [43]. Therefore, nanofibers are fibers that have diameter equal to or less than 100 nm. Materials in fiber form are of great practical and fundamental importance. The combination of high specific surface area, flexibility, thermal and electrical conductivity; and superior directional strength makes nanofibers a preferred material form for many applications ranging from clothing to reinforcements for aerospace structures. Other potential market applications include filtration, structural composites, healthcare, energy storage and cosmetics.

2.3.2 Methods of fabricating nanofibers

Several technologies are available to produce nanofibers including the template method [44-46], self assembly [47, 48], vapor grown [49], phase separation [50, 51], drawing [52], melt blowing [53, 54], multi-component fiber splitting and spinning; and electrospinning.

The drawing method is a process similar to dry spinning in fiber industry, which can produce individual long single nanofibers. However, the drawback of this method is that only viscoelastic materials can be drawn into nanofibers.

The template method uses a nanoporous membrane as a template to make nanofibers of solid (a fibril) or hollow (a tubule) shape. This method allows the fabrication of
nanometer tubules and fibrils of various raw materials such as electrically conducting polymers, metals, semiconductors and carbons. The disadvantage of this method is that single continuous nanofibers can’t be produced.

The self-assembly process utilizes the ability of individual, pre-existing components to organize themselves into desired patterns and functions. However, the process is time-consuming in processing continuous polymer nanofibers.

The phase separation method is a multi process involving dissolution, gelation and extraction using different solvents, freezing, and drying which results in a nanoscale porous material. The process however, takes relatively long period of time to transfer the solid polymer into the nano-porous foam.

Melt blown fibers are created by melt blowing a fiber with a modular die. The fibers produced are a mixture of both micron and submicron sizes. This technique lends itself to the use of thermoplastic polymers in a relatively inexpensive spinning process. However, melt blown fibers typically do not have good mechanical properties (strength), primarily because less orientation is imparted to the polymer during processing and low molecular weight polymers are employed.

Nanofibers can also be prepared using a multi component fiber comprised of a desired polymer and a soluble polymer. The soluble polymer is then dissolved out of the composite fiber, leaving microfilaments of the other remaining insoluble polymer. However, utilization of this process leads to low manufacturing yields due to the fact that significant portion of the multicomponent fiber must be destroyed to produce the microfilaments. Pike modified this process of fabricating nanofibers by splitting the fibers in a melt spinning process [55]. He was able to produce fibers with diameters
as low as 300 nm with a small fiber diameter distribution. Multicomponent fibers having two or more polymeric components may also be mechanically split into finer fibers comprised of the respective components. The single composite filament thus becomes a bundle of individual component microfilaments.

Thus, the electrospinning process seems to be the only feasible process which can be further developed for mass production of continuous nanofibers from various polymers.

2.3.3 Electrospinning of nanofibers

The most publicized method of fabricating nanofibers is the electrostatic spinning or electrospinning process. The electrospinning process, which was patented by Formhals in 1934 [56] enables polymeric fibers with diameters in the range of a few nanometers to several microns to be fabricated, depending on the type of polymer and the processing conditions. The electrospinning technique involves the generation of a strong electric field between a polymer solution or melt contained in a reservoir such as a glass syringe with a capillary tip or needle, and a metallic collection plate as shown in Figure 5.
When the voltage reaches a critical value, the charge overcomes the surface tension of the deformed drop (Taylor cone) of suspended polymer solution formed on the capillary tip or needle, and a jet is produced. The diameter of electrically charged jet decreases under electro-hydrodynamic forces, and under certain operating conditions this jet undergoes a series of electrically induced bending instabilities during passage to the collection plate, which results in extensive stretching. The stretching process is accompanied by a rapid evaporation of the solvent, which leads to a reduction in the diameter of the jet. The dried fibers are deposited randomly or in aligned manner on the surface of the collection plate. The fiber diameter can be controlled by varying the processing parameters such as polymer solution concentration, viscosity, applied charge and electric field; type of solvent employed, distance from tip of capillary to the collection plate, flow rate, diameter and angle of spin of the spinneret.
Over the years, a lot of researchers conducted work on modeling the behavior and flow of monomeric fluid jets. In 1969, Taylor [57] derived the condition for the critical electric potential needed to transform the droplet of liquid into a cone (commonly referred to as the Taylor cone) and to exist in equilibrium under the presence of both electric and surface tension forces as:

\[ V_c^2 = 4 \frac{H^2}{L^2} \left( \ln \frac{2L}{R} - \frac{3}{2} \right) (0.117 \pi \gamma R) \]  

(1)

where \( V_c \) is the critical voltage, \( H \) is the distance between the capillary tip and the ground, \( L \) is capillary length, \( R \) is capillary radius and \( \gamma \) is surface tension of the liquid. He began with the observation of a droplet in equilibrium at the end of the capillary and observed its deflection under applied fields. Taylor discovered that cones with a half angle of 49.3° were the only ones that met this criterion. A similar equation was found by Hendricks et al in 1964 [58].

\[ V = 300 \sqrt{20 \pi \gamma r} \]  

(2)

where \( r \) is the radius of the pendant drop. However, this equation is only valid for slightly conductive, monomeric fluids displaying the cone jet mode. The models proposed by Taylor and Hendriks do not take into account the dependency of viscosity and conductivity which play important roles in the process. This can greatly influence the equilibrium angle (49.3°) that balances the surface tension and
electrostatic forces as derived by Taylor. Despite the fact that viscosity and conductivity terms were not included in these models, the relationship between surface tension and applied voltage serves as a useful guide for electrospinning of slightly conducting, medium-to-low viscosity solutions. In 1971, Baumgarten [59] employed the process to polymer solutions. His spun acrylic resin-dimethyl formamide (DMF) systems at various concentrations and viscosities, thereby producing fibers in the range of 0.05-1.1 µm in diameter. He also highlighted the effects of solution viscosity, surrounding gas, flow rate, etc. on the fiber diameter and jet length. The results of his experiments showed that as the solution viscosity increased the fiber diameter increased (approximately proportionally) to the jet length. He established a relationship between fiber diameter and solution viscosity expressed by the follow equation:

\[ d = \eta^{0.5} \]  

where \( d \) is fiber diameter and \( \eta \) is solution viscosity in poise. He also stated that the fiber diameter is highly dependent on the applied electric field. An increase in the applied voltage leads to increases in the electrostatic stresses, which, in turn, produces smaller diameter fibers. Baumgarten also conducted research on the effects of humidity on the spinning of fibers. He concluded that in dry air with relative humidity less than 5%, spinning could only run for a few minutes due to the drying out of the droplets; and in humid air (> 60% R.H), the formed fibers did not dry properly and became fused together.
Berry also conducted studies which showed that the diameter of fibers produced is influenced not only by the concentration of the polymer but also by its molecular conformation [60-63]. He stated that the degree of entanglement of polymer chains in solution could be described by a dimensionless number called the Berry number (\(Be\)). If a polymer is dissolved in a solvent and the concentration is very dilute, the polymer molecules are so far apart in the solvent that individual molecules rarely touch each other and \(Be\) is less than unity. When the polymer concentration is increased, at some overlap concentration, the individual molecules interact and therefore become entangled; in this instance, \(Be\) is then greater than unity. The \(Be\) can be used as a processing index for controlling the diameter of electrospun fibers. Ko et al [64] studied the influence of polymer molecular conformation in solution, described by \(Be\), on a electrospun poly(L-lactic acid)/chloroform system and confirmed the following relationship between \(Be\), solution concentration and intrinsic viscosity:

\[
Be = [\eta] c
\]

\(\text{where } [\eta] \text{ is the intrinsic viscosity of the polymer i.e. the ratio of specific viscosity to concentration at infinite dilution and } c \text{ is the concentration of the solution.}
\]

In the 1980’s several other authors [65-67] researched the process experimentally and determined that the physical properties such as onset potential, capillary radius, and liquid conductivity all affect the process, yet much remains to be understood. Hayati et al [68, 69] studied the effect of electric field and environment of pendent drops on the ability to form stable jets. They concluded that the conductivity of the liquid was
a major factor in determining the onset of the jet disruption. Highly conductive fluids were found to drip from the capillary and with increases in voltage, form very erratic jets that broke into many droplets. On the other hand, insulating materials were unable to hold a surface charge and therefore no electrostatic forces built up at the interface. In the case of semi-conducting fluids (conductivity in the range of $10^{-6} - 10^{-8} \, \Omega^{-1} \, \text{m}^{-1}$), it was possible to form stable jets erupting from a conical base. In more recent years, scientists such as Cloupeau et al [70] and Grace et al [71] studied the effects of flow rate, applied potential, capillary size, and fluid properties such as surface tension, fluid conductivity, and viscosity on the electrospinning process.

Since then, the process has been attempted to produce various products such as synthetic vascular grafts [72], tubular products [73], acrylic fibers [59, 74, 75]. However, most industries deemed the process not to be cost effective and the only commercial product to date are filters.

After a period of low research activities, interest in the process has exploded since the mid-1990’s mainly due to the invigorating work by Reneker and coworkers [76], [77]. Doshi and Reneker electrospun fibers from water soluble poly(ethylene oxide) with diameters ranging from 50-5000 nm. In their work, they described the electrospinning process, the processing conditions, fiber morphology, and potential applications of the fibers. Reneker and Chun [77] electrospun more than 20 polymers, including polyethylene oxide, nylon, polyimide, DNA, polyaramid, and polyaniline. They were able to produce polymer fibers with diameters ranging from 40 nm-20 µm. Since then, an exponential growth of research in electrospinning has occurred leading to new materials and new potential applications. Most of the studies focused on the
effects of electrospinning parameters on the morphology and structure of spun fibers. Jaeger et al [78] studied electrospun PEO fibers and observed chain packing utilizing atomic force microscopy (AFM). They concluded that at the molecular level, electrospun PEO fibers possess a highly ordered surface layer, which could be the result of the electrospinning process. Not only the morphology and molecular structure of the fibers were studied but also transport properties such as in the work conducted by Gibson and Rivin [79]. They concluded that the electrospun nonwoven fiber layers present minimal impedance to moisture vapor diffusion required for evaporative cooling. Table 2 shows some polymers that have been electrospun since the resurgence of the technology in the 1990’s.

Nanofibers have been electrospun from many kinds of synthetic polymers, DNA and other naturally occurring biopolymers, and polymer precursors of carbon and ceramic fibers. In addition to circular in cross section nanofibers, a variety of fibers with different shapes can be produced utilizing the electrospinning process such as, branched fibers, flat ribbons, and longitudinally split fibers [80].

2.4 Synthesis, properties and applications of carbon nanotubes

One of the ways nanotechnology has advanced the state-of-the art has been to enhance and improve the properties of existing conventional classes of materials. Polymer composites, for example, have been a mainstay of high-performance materials for nearly three decades, offering a whole host of tailorable properties, such as high strength and stiffness, dimensional and thermal stability. With the advent and
application of nanofibers, polymer nanofiber composites could become even more attractive.

Table 2. Examples of polymers that have been electrospun since its resurgence.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent</th>
<th>Fiber diameter</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyyacronitrile (PAN)</td>
<td>Dimethyly formamide (DMF)</td>
<td>0.05-1.1 μm</td>
<td>1971</td>
</tr>
<tr>
<td>PEO (polyethylene oxide)</td>
<td></td>
<td>0.05- 2 μm</td>
<td>1981</td>
</tr>
<tr>
<td>PEO</td>
<td>Water</td>
<td>0.05- 5 μm</td>
<td>1995</td>
</tr>
<tr>
<td>Poly(ethylene teraphthalate) PET</td>
<td>Trifluoroacetic acid + Dichloromethane</td>
<td>0.3 - 2 μm</td>
<td>1996</td>
</tr>
<tr>
<td>PLLA (poly-L-lactide acid)</td>
<td>Dichloromethane</td>
<td>100 nm- 5 μm</td>
<td>2001</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>Dichloromethane</td>
<td>101 nm- 5 μm</td>
<td>2001</td>
</tr>
<tr>
<td>Polynlycarbazole</td>
<td>Dichloromethane</td>
<td>102 nm- 5 μm</td>
<td>2001</td>
</tr>
<tr>
<td>PLGA (poly(D,L-lactide-co-glycolide); 85:15</td>
<td>Tetrahydrofuran (THF) + DMF; 1:1</td>
<td>500-800 nm</td>
<td>2002</td>
</tr>
<tr>
<td>Fibrinogen (human and bovine)</td>
<td>Hexaflouro-2-propanol (HFIP)</td>
<td>80-700 nm</td>
<td>2002</td>
</tr>
<tr>
<td>PAN</td>
<td>DMF</td>
<td>~ 100 nm</td>
<td>2002</td>
</tr>
<tr>
<td>Polyamide-6</td>
<td>Formic acid</td>
<td>&gt; 50 nm</td>
<td>2003</td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td>Dichloromethane</td>
<td>&gt; 50 nm</td>
<td>2003</td>
</tr>
<tr>
<td>Poly(ethylene-co-vinyl acetate) PEVA + Bovi</td>
<td>Dichloromethane</td>
<td>5 - 20 μm</td>
<td>2003</td>
</tr>
<tr>
<td>Polycaprolactone (PCL)</td>
<td>Chloroform + Methanol (3:1)</td>
<td>0.3 - 5 μm</td>
<td>2003</td>
</tr>
<tr>
<td>PEO</td>
<td>Methanol</td>
<td>0.08 - 1 μm</td>
<td>2003</td>
</tr>
<tr>
<td>Vanadium sol + Poly(vinylacetate)</td>
<td>Ethanol</td>
<td>0.5 - 1.5 μm</td>
<td>2003</td>
</tr>
<tr>
<td>Poly(vinyl alcohol) PVA + casein</td>
<td>Water + triethanolamine</td>
<td>100 - 500 nm</td>
<td>2003</td>
</tr>
<tr>
<td>Poly(vinyl chloride) PVC + Polyurethane</td>
<td>Dimethly acetamide (DMAc)</td>
<td>0.285- 2 μm</td>
<td>2003</td>
</tr>
<tr>
<td>Virus + Poly(vinyl pyrolidone) PVP</td>
<td>HFIP</td>
<td>100-200 nm</td>
<td>2004</td>
</tr>
</tbody>
</table>

Polymer nanocomposites have the potential to usher in a new era in materials development, just as polymer composites altered the face of industry thirty years ago.
The reinforcement of nanofibers with fillers such as carbon nanotubes is receiving substantial attention today, mainly due to the fact that carbon nanotubes offer opportunities not only to enhance mechanical and physical responses but also to impart unique electrical and thermal properties to the nanofibers. Furthermore, carbon nanotubes are the world’s strongest material in terms of tensile strength and are lightweight and flexible. The following sections will provide a synopsis of the synthesis, properties and applications of carbon nanotubes.

Since the discovery of carbon nanotubes (CNT) by Ijima in 1991 [81], they have become one of the most intensively studied materials in the last decade. The nanotubes were made up of carbon atoms arranged in a graphene layer folded into tubes (a few nanometers in diameter and up to hundreds of micrometers in length), which were nested inside one another, with ends closed by conical caps. These carbon nanotubes were called multiwall carbon nanotubes (MWNT). In 1993, Ijima [82] also discovered single wall carbon nanotubes (SWNT) which were synthesized similarly to the MWNT. Figure 6 shows schematics and images of SWNT and MWNT.
2.4.1 Synthesis of carbon nanotubes

Currently, there are several methods employed to make carbon nanotubes such as laser ablation [85], electric arc discharge [86] and chemical vapor deposition (CVD) [87] of hydrocarbon gases such as acetylene, ethylene, ethanol, etc over catalytic metal particles (usually cobalt or nickel, iron). In general, the CVD method has shown the most promise in being able to produce larger quantities of nanotube (compared to the other methods) at lower cost. In addition the CVD method leads to less mixtures of carbon materials.

2.4.2 Structure and properties of carbon nanotubes

Simply speaking, carbon nanotubes can be thought of as graphene sheets with a hexagonal lattice that have been wrapped into a tube and capped at each end with half
of a fullerene sphere. There are two main types of nanotubes: SWNT and MWNT. SWNT’s consist of a single graphene sheet seamlessly wrapped into a cylindrical tube while MWNT’s are made up of layers of such nanotubes that are concentrically arranged within one another. Depending on the way in which the sheets are rolled up, several different kinds of tubes can be distinguished by the so-called chirality indices (n, m) (Figure 6a), the two integer numbers which define the vector on the graphene sheet which connects two points that will become equivalent after having rolled up the sheet. A nanotube is considered to be an armchair type when the integers are equal i.e. \(n = m\); zigzag when either \(n = 0\) or \(m = 0\); and chiral when \(n\) and \(m\) are other values than those mentioned previously. Due to the symmetry and unique electronic structure of graphene, the structure of a nanotube strongly affects its electrical properties. All armchair SWNT’s are metals; those with \(n - m = 3k\), where \(k\) is a nonzero integer, are semiconductors with a tiny band gap; and all others are semiconductors with a band gap that inversely depends on the nanotube diameter. Nanotubes are composed entirely of sp\(^2\) bonds, similar to graphite, which are stronger than the sp\(^3\) bonds found in diamond. This bonding structure provides them with their unique strength. Nanotubes have a tendency to align themselves into "ropes" held together by Van der Waals force. Nanotubes can merge together under high pressure, trading some sp\(^2\) bonds for sp\(^3\) bonds, thus giving great possibility for producing strong, unlimited-length wires through high-pressure nanotube linking. The diameter and length of the carbon nanotubes may vary depending on the method of production. SWNT’s have diameters ranging from 0.4 – 3 nm with average diameters of approximately 1.2 nm. MWNT’s on the other hand, can range from several
nanometers to tens of nanometers in diameter. The length of the CNT’s are typically in the microscopic range which normally result in aspect ratio of about 100 to as high as 10,000 depending on the method of synthesis. Due to their extreme small size, SWNT structures contain very low defect density or are almost defect free, a major contributing factor to their extraordinary properties. The measured specific tensile strength of CNT can be as high as 100 times that of steel. There have been a large number of studies, both theoretical and experimental of the mechanical properties of carbon nanotubes. The reported Young’s modulus of SWNT is in the order of 1 - 1.2 TPa [88 – 92] , tensile strength in the order of 37 GPa at a failure strain of 6% [93, 94] . However, there are discrepancies in the values of reported tensile modulus and strength for SWNT’s which range from 0.4 - 5 TPa and 37 - 200 GPa respectively. These discrepancies are a result of the different methods of calculation and assumption of the nanotubes wall thickness, which leads large variation in the cross sectional area and hence deviations in modulus and strength. Table 3 shows a comparison of Young’s modulus of carbon nanotubes estimated by various groups of researchers. The most impressive feature of SWNT’s is the density-normalized tensile modulus and strength, which is ~19 and ~56 times that of steel wire, and ~2.4 and ~1.7 times that of silicon carbide rods [95, 89]. These exceptional mechanical properties have stimulated further study of SWNT reinforced nanofiber polymers to attain lightweight and high strength materials.
Table 3. Young’s modulus of carbon nanotubes as estimated by various researchers [96].

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>E (TPa)</th>
<th>ν</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yakobson</td>
<td>1996</td>
<td>5.5</td>
<td>0.19</td>
<td>Molecular Dynamic</td>
</tr>
<tr>
<td>Zhou <em>et al</em></td>
<td>2001</td>
<td>0.77</td>
<td>0.032</td>
<td>Theoretical</td>
</tr>
<tr>
<td>Lu</td>
<td>1997</td>
<td>1.0</td>
<td>0.28</td>
<td>Molecular Dynamic</td>
</tr>
<tr>
<td>Tu</td>
<td>2002</td>
<td>4.7</td>
<td>0.34</td>
<td>Theoretical</td>
</tr>
<tr>
<td>Chang and Gao</td>
<td>2003</td>
<td>1.325</td>
<td>0.26</td>
<td>Molecular Dynamic</td>
</tr>
<tr>
<td>Krishnan <em>et al</em></td>
<td>1998</td>
<td>1.25</td>
<td>-</td>
<td>Theoretical</td>
</tr>
<tr>
<td>Li and Chou</td>
<td>2003</td>
<td>1.05</td>
<td>-</td>
<td>Finite Element Modeling</td>
</tr>
<tr>
<td>Yu <em>et al</em></td>
<td>2000</td>
<td>0.27-0.95</td>
<td>-</td>
<td>Experimental</td>
</tr>
<tr>
<td>Li <em>et al</em></td>
<td>2000</td>
<td>0.79</td>
<td>-</td>
<td>Experimental</td>
</tr>
<tr>
<td>Demczyk <em>et al</em></td>
<td>2002</td>
<td>0.9</td>
<td>-</td>
<td>Experimental</td>
</tr>
</tbody>
</table>

In addition to the unique mechanical properties, carbon nanotubes are also excellent conductors of heat [97]. However, unlike bulk materials where the thermal conductivity is the same in all directions, SWNT’s conduct better along the tube axis. The major benefit of SWNT’s is in their electrical conductivity. The electronic structure of SWNT’s has been extensively studied and is known to reflect confinement of electron waves to the one dimensional (1D) molecular cylinder. SWNT’s can possess current densities of up to $10^{11}$ A m$^{-2}$ [98].

The measured room temperature thermal conductivity of SWNT is greater than that of natural diamond and the basal plan of graphite [99]. It has been indicated that electronic properties of the SWNT are highly dependent on the chirality and diameter of the nanotubes [100, 89].
2.4.3 Potential applications of carbon nanotubes

The mechanical (strength, stiffness and toughness), thermal and electrical properties of CNT’s especially SWNT’s, allow for many applications, for example, batteries, fuel cells, fibers and cables, as well as biomedical and pharmaceutical materials. The CNT’s can be mixed with other materials to improve on existing properties or provide new ones such as in uses as additives in thermoplastics and thermosets. Small amounts of SWNT’s added to epoxy resins have resulted in increases in hardness, electrical and thermal conductivities. The thermal deformation temperature and modulus can also be increased by adding small quantities of SWNT’s to the material.

2.5 Characterization techniques for nanofibers/nanocomposites

Scientists have worked with nanoscale materials for centuries, but until recently lacked the analysis capability to fully realize its application potentials. The last few decades have dramatically changed that, first with ground-breaking tools and equipments for investigating the microscale, then even more astounding tools that enabled investigation of individual atoms. The structure, morphology, and geometry of nanofiber assemblies and the porosity and tensile properties of nanofiber mats can be investigated through conventional techniques and instruments. But new techniques are needed for the mechanical testing of single nanofibers. Although measurement of mechanical properties such as tensile modulus, strength, and elongation is difficult due to the small size of the fibers, these properties are crucial for the proper use of nanofiber mats. Continuous modifications of current tools and techniques are required
in order to provide a better understanding of the nanoscale materials that have been fabricated. The characterization techniques to determine the nanofiber and nanocomposite assembly properties can be organized into 3 major groups: 1) physical 2) compositional or chemical and 3) mechanical.

2.5.1 Physical characterization

Physical characterization includes determining the surface texture, morphology and structure of the materials. The surface characterization involves standard surface techniques such as x-ray photoelectron spectroscopy (XPS), secondary-ion mass spectrometry (SIMS), scanning tunneling microscopy (STM), scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HRTEM) and low energy electron diffraction (LEED). The morphology of the nanofibers/nanocomposites produced can be determined by using techniques such as SEM, HRTEM and atomic force microscopy (AFM).

The structure of the nanofibers/nanocomposites can be determined by techniques such as wide x-ray diffraction and scattering (WAXD), small angle x-ray scattering (SAXS). This also allows the determination of the crystallinity of the materials. Other physical properties such as glass transition, melt and crystallization temperatures can be obtained from tools such as differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA).
2.5.2 Chemical or compositional characterization

The chemical or compositional properties of the nanofibers/nanocomposites can be determined by techniques such as Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR).

2.5.3 Mechanical characterization

The mechanical properties of the nanofiber/nanocomposite can be elucidated by tools such as AFM, Kawabata micro-tensile testers (KES) and dynamic mechanical analyzer (DMA). It’s worth noting that apart from the AFM method where individual nanofiber/nanocomposites could be isolated and manipulated for the mechanical tests, all other test methods require a collection of these nanofibers either in random mat or aligned yarn forms.

The techniques utilized in this study for the characterization of the nanofibers/nanocomposite fabricated through the electrospinning process will be discussed in detail in the corresponding chapters and sections.
CHAPTER 3: OBJECTIVES

One of the most important applications of traditional (micro-sized) fibers, such as carbon, glass, and Kevlar is for structural or reinforcement purposes. Proteins in particular, have been identified as a promising basis for a diverse class of novel high performance structural polymer materials. Silk has long been recognized as the ultimate model of strong and tough material. For example, dragline silk from *Nephila clavipes* is three times tougher than aramid fibers and five times stronger than steel [101]. The prerequisite for such a widespread use is the cost effective production in sufficient quantities for commercial fiber manufacturing. However, efforts to produce spider silk on a commercial scale have not been fruitful as spiders are territorial animals i.e. cannibalistic in nature and can not be farmed. Current researches in biotechnology have shown some potential for low-cost, large-scale production but to date, none of these methods are able to produce commercial amounts of silk. On the other hand, silkworm silk is widely available, produced on a large commercial scale (sericulture) and also possesses unique properties which make it a material of great interest to be studied for its nanoscale properties.

Based on the evidence of reduction in fiber size leading to improvement in mechanical properties of the material as shown by the research of Jelinski *et al* [104], it was hypothesized that reducing *B. mori* fibers to the nanoscale level through the electrospinning process, may lead to improvement in its mechanical properties.

Since *B. mori* nanofibers also have potential uses in the biomedical and composite industries, it is envisioned that incorporating single wall carbon nanotubes into the
nanofibers will lead to further improvements in the properties of the nanofibers. New tailorable properties such as reduction in static electricity, thermal and electrical conductivity, higher tensile modulus and strength can be expected. The effects of post processing treatments on the electrospun silk fibers such as annealing and methanol immersion were also studied.

The objectives of this thesis are:

3.1 Fabrication of nanofibers

This section presents an investigation into the feasibility of producing truly nanoscale (d < 100 nm) regenerated *B. mori* silk fibers using the electrospinning process.

3.2 Prediction of fiber diameter and optimization of electrospinning process

This section presents an analysis based on Response Surface Methodology (RSM) to predict and determine the optimum electrospinning parameters in order to obtain *B. mori* nanofibers. RSM was carried out to provide a systematic means for the optimization of the process parameters. An empirically determined response function was obtained by a linear regression analysis using observed responses (e.g. fiber diameter) and coded variables (e.g. electric field strength and polymer concentrations). The relationship between the response and variables is visualized by a response surface or contour plot. From the graphical representations as contour plots, the operating conditions (electric field, concentration) necessary to generate nanofibers with the desired diameter and other properties of the electrospun fibers can be predicted.
3.3 Characterization of electrospun nanofibers

This section provides a general understanding of the structural changes that occur during the various stages of the electrospinning process of regenerated silkworm silk and establishes standard characterization methodologies for the characterization of the compositional (e.g. FTIR, Raman), structural (e.g. SEM, TEM, AFM, Raman) and physical properties (AFM, microtensile test etc) of the electrospun nanofibers.

3.4 Evaluation of mechanical properties of nanofibers

This section presents methods of changing the mechanical properties of the electrospun nanofibers. One method is achieved by co-electrospinning regenerated silkworm silk with SWNT. Other methods employed are post-processing treatments, namely annealing and immersion in methanol.

3.4.1 Single wall carbon nanotubes reinforcement

An investigation was carried out on the reinforcement effects of SWNT’s on the morphological, chemical and mechanical properties of electrospun silk nanofibers.

3.4.2 Chemical treatment of electrospun nanofibers

The effect of chemical treatment (immersion of electrospun nanofibers in methanol) on the morphology, secondary structure, crystallinity and mechanical properties of the fibers was evaluated.
3.4.3 Annealing of electrospun nanofibers

The effect of annealing the electrospun nanofibers (heating above the glass transition temperature $T_g$) on the morphology, structure, and mechanical properties of the fibers was evaluated.

3.5 Determination of transport properties of nanofibrous silk membranes

This section of the thesis presents a method of gaining a better understanding of the wetting and fluid flow properties of the nanofibrous membranes, developing theoretical and experimental tools to characterize the transport properties including the mechanism(s) involved in the process.
CHAPTER 4: MATERIALS AND METHODS

4.1 Introduction

The experimental parts of this thesis can be subdivided according to the objectives into the following sections: 1) fabrication of nanofibers through the electrospinning process 2) prediction of fiber diameters and optimization of the electrospinning process 3) characterization of electrospun fibers 4) improvement of electrospun fiber properties by post processing treatments such as addition of carbon nanotubes, methanol treatment and annealing; 5) determination of transport properties of nanofibrous silk membranes.

The following sections are descriptions of the materials and methods employed in attaining the afore mentioned objectives.

4.1.1 Spinning dope preparation

Silk fibers were purchased from Taiwan Textile Research Institute (TTRI). The fibers were heated (100 °C for 30 min) in aqueous Na₂CO₃ (0.02 M) and rinsed with water to extract sericin. The extracted fibers were readily dissolved in 50% aqueous CaCl₂ (100 °C, then cooled) to obtain silk concentration of 6% solution. All concentration measurements were done in weight by weight (w/w). The solution was poured into regenerated cellulose dialysis tubing (Fisher Scientific: T3 membrane, pore size 25 Å) to carry out dialysis against 1000 ml of deionized water (for 48 h at 23 °C). The regenerated silk fibroin sponge was obtained by lyophilization (Labconco 2.5 Plus Freezone lyophilizer). The silk sponge solution (5–20%) was electrospun in formic acid (98–100%).
4.1.2 Electrospinning of nanofibers

The silk–formic acid solution was placed in a 3-ml syringe (18-G needle (0.84 mm) and spinning angle 45°). The tip-to-collection plate (covered with aluminum foil) distance varied from 5 to 10 cm vertically under the needle tip (Figure 7). The electric field expressed in terms of voltage/distance between the collection plate (cathode) and the needle tip (anode) ranged from 2 to 5 kV/cm.

Figure 7. Schematic diagram of electrospinning technique. The silk solution is filled in a reservoir like syringe and high voltage is passed through it. The fibers are collected at the collection plate, which is grounded.
4.1.3 Process optimization and empirical modeling

In order to obtain a more systematic understanding of these process conditions and to establish a quantitative basis for the relationships between electrospinning parameters and fiber diameter, response surface methodology was employed. A factorial experiment was designed to investigate and identify the relative significance of the processing parameters on fiber diameter (Table 4).

Table 4. Factorial design of experiment

<table>
<thead>
<tr>
<th>Factor</th>
<th>Factor Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (%)</td>
<td>5, 8, 10, 12, 15, 19.5</td>
</tr>
<tr>
<td>Electric field (kV/cm)</td>
<td>2, 3, 4, 5</td>
</tr>
<tr>
<td>Spinning distance (cm)</td>
<td>5, 7, 10</td>
</tr>
</tbody>
</table>

4.1.4 Characterization methods

4.1.4.1 Electron scanning environmental microscopy

The morphology and structure of the electrospun nanofibers can directly be observed by field emission environmental scanning electron microscope (Phillips XL-30 ESEM). ESEM also allows for 3-D observation of the sample fibers. The observations are made from the secondary electron line from the sample material. Since *B. mori* silk is extremely poor in electrical conductivity, it is necessary to coat
the samples with a conducting material such as gold or platinum. A Denton vacuum Desk II sputtering machine was employed for this purpose as shown in Figure 8. The operating conditions were 40 mA, 26.66 kPa and the gold sputtering time was 60 seconds. The nanofibers were first fixed with the aid of carbon tape to the sample holder and then placed in the sputtering machine for gold coating.

![Figure 8. Picture of a Denton Vacuum Desk II sputtering machine.](image)

The gold sputtered electrospun nanofibers were examined by ESEM under high vacuum as shown in Figure 9 and the average fiber diameter and its distribution were determined from 100 random fibers obtained from each spinning conditions. The operating parameters for the ESEM were electron beam ranging from 10-15 kV and spot size ranging from 3-5.
4.1.4.2 Raman spectroscopy

Raman spectroscopy is a very useful tool for determining the conformation of proteins not only in the solid state but also in solutions. It enables us to follow the conformational changes, which occur during the processing stages of electrospun nanofibers such as chemical modification and crystallization. It can also reveal information about the proteins secondary structure and anisotropy. In Raman spectroscopy, the sample is radiated with monochromatic visible or near infrared light from a laser. This brings the vibrational and rotational energy levels in the molecule to a short-lived, high-energy collision state, which returns to a lower energy state by emission of a photon. Normally, the photon has a lower frequency than the laser light,
and the difference in frequency between the frequency of the laser and that of the scattered photon is called the Raman shift [147]. Raman spectra usually contain many sharp bands that are characteristic of the specific molecules in the sample. Figure 10 shows the Renishaw 1000 spectrometer with a 780 nm diode laser utilized for the characterization of electrospun nanofibers.

Figure 10. Photograph of Renishaw 1000 Raman spectrometer.

Deconvolution of the Raman spectra was done by peak fitting application provided by GRAMS software which uses an iterative fitting of Gaussian functions with the data minimized by the chi-squared criterion.
4.1.4.3 Fourier transform infra red spectroscopy

FTIR spectroscopy was used to follow the conformation changes (from random coil/helical to β-sheet) that occur in the silk fibroin/formic acid solution during the dope preparation stage and the electrospinning process that produces solid nanofibers. FTIR spectroscopy is a characterization technique widely used in physics, chemistry, and biology. It has the advantages of high spectral resolution, good signal-to-noise ratios, and the ability to measure a broad region of the spectrum in a short amount of time. The infrared spectrum is formed because of the absorption of electromagnetic radiation at frequencies that correlate to the vibration of specific sets of chemical bonds from within a molecule. The vibrational spectrum of a molecule is considered to be a unique physical property and is characteristic of the molecule.

Figure 11 shows a photograph of the Excalibur Series Digilab-UMA 600 FTIR spectrometer used in the study. Each spectrum was acquired in reflectance mode with accumulations of 200-400 scans, resolution of 4 cm$^{-1}$ and a spectral range of 700-7000 cm$^{-1}$. 
4.1.4.4 Wide angle x-ray diffraction

WAXD is a powerful technique for determining the three dimensional structure of molecules, including complex biological macromolecules such as proteins and nucleic acids, that form crystals or regular fibers. It can be used to determine not only the type and structure of the crystals in the nanofibers but also the crystallinity and degree of orientation of the crystals/crystallites.

Figure 12 shows the photograph of the Siemens D500 WAXD used in the study.
4.1.4.5 Mechanical properties

Conducting mechanical tests on nanofibers is a challenging task due to the small size of the fibers. The nanofibers tested in this study were an aggregation of fibers either in random mat or aligned yarn forms. The mechanical properties of the electrospun nonwoven mat were measured on a Kawabata KES-G1 microtensile tester (Figure 13). The tests were carried out at room temperature (23°C) and humidity. The microtensile tester is able to provide information on the properties of the fiber constructs such as yield stress, Young’s modulus and elongation at break.
4.1.4.6 Thermal properties

To measure the thermal properties of the electrospun silk fibers, a differential scanning calorimeter (Perkin Elmer DSC 7 ) and a thermogravimetric analyzer (Perkin Elmer TGA 7 ) attached to a thermal analysis controller TAC7/DX were utilized.

4.1.4.7 Transport properties of nanofibrous membranes

The Micro Absorb Meter, which has been designed and constructed at Textile Research Institute, Princeton (TRI) is illustrated in Figure 14. This device measures
the volume of a micro sized droplet deposited on a porous substrate, as a function of
time during the spontaneous absorption with a millisecond resolution. Based on the
theory of fast absorption, the method allows us to extract the dynamic wetting
properties of fluids invading micro/nanometer pores.

Figure 14. The Micro Absorb Meter: During the spontaneous absorption of droplets
by nanofibrous substrates, high strain rates and stresses are achieved. The optical
device monitors the process with a millisecond resolution.

A visualization system was also employed which is schematically described in Figure
15. It is suitable for characterization of wettability and absorbency of porous
fibers/fiber bundles/yarns. It is based on an analysis of the dynamics of droplet
absorption. The technique assumes the use of a particular model for fluid transport in
pores thus relating the microscopic transport properties with the shape of the visible droplet.

![Schematic diagram of the PC-based imaging system.](image)

Figure 15. Schematic diagram of the PC-based imaging system.

The wettability and rate of absorption of liquid on the silk membrane were measured using the visualization device shown above. The visualization system gives the ability to obtain the dynamics of the complete droplet shape for slow and fast absorption processes.
CHAPTER 5: REGENERATION OF B. MORI SILK BY ELECTROSPINNING

5.1 Background and Significance

Polymer fibers of various diameters (µm to nm) have applications including use as reinforcing fibers in composite materials [105-107], biocatalysts [108], non wetting surface layer in ordinary textiles [109], high performance membranes (filters) [110] and tissue engineering applications like fabrication of cell-growth scaffolds [111, 112], vascular grafts, wound dressings [113, 114] and drug delivery [115-119]. These applications benefit from the small fiber diameters and porous structure of the fabrics. The large surface area to volume ratio also allows cellular migration and proliferation in tissue engineered scaffolds.

Several methods have been developed to fabricate highly porous biodegradable scaffolds including fiber bonding, solvent casting, particle leaching, phase separation, emulsion freeze drying, gas foaming and 3D-printing technique [120]. However, the simplicity of electrospinning is an attractive advantage for scaffolds construction. The small diameter structures produced by electrospinning process have a high surface area to volume ratio and morphology similar to natural tissues. In addition, the static charge of electrospun fibers incorporated into membrane filters enhances filtration efficiency [121].

Presently, biocompatible and biodegradable polymeric biomaterials are used in fabrication of scaffolds. These materials include collagen, poly-(lactide-co-glycolide), poly-(lactic acid), poly-(glycolic acid) and poly-(caprolactone). However, many of these scaffolding materials have insufficient mechanical integrity and often
induce an inflammatory response [122]. The good mechanical properties of silk fibers (because of its secondary structure, β-sheets) make it a unique candidate for scaffolds [123-129].

Renewed interest in silk fibers for biomedical applications is because of their favorable mechanical and biocompatibility properties. Although many organisms are capable of producing silk, silkworms and spiders are the two main producers of silk. Silks have remarkable mechanical properties that make their applications attractive for use in biomedical, military and industrial fields. As mentioned earlier, spider silk production has its limitations. Therefore, silkworm silk is a much better alternative for use in commercial applications.

Among silkworms, the domesticated *Bombyx mori* is well known and its silk has been of interest for over 5000 years not only for its textile properties of texture, tenacity and dyeing [109] but also its use in cosmetics creams, lotions, makeup, powders, bath preparations and pharmaceuticals [132].

The use of electrospinning process as a technique for achieving highly porous silk scaffolds having nanoscale fiber diameter is currently of great interest. The electrospinning of *B. mori* cocoon silk was first reported by Zarkoob *et al.* [133-135]. They used solution of 0.23–1.2 wt% silk in hexafluoro-2-propanol. Silk fibers with diameters in the range of 6.5–200 nm were produced with crystallographic order equivalent to that of the original natural fibers.

Buchko *et al* [136] electrospun recombinant hybrid silk-like polymers with fibronectin functionality in 96% formic acid solution. They were able to produce nanofibers with diameters ranging from ~ 60-200 nm. Nanofibers were also
fabricated by Jin and coworkers [137] and by Ogho et al [109] through the electrospinning process. Weixan [138] was able to produce nanofibers by electrospinning a mixture of silk fibroin and chitin.

Initial research on electrospinning of silk by Cappello et al [39] encountered problems with choosing a solvent and controlling the conformational transitions of the fibroin during electrospinning process. The solvent for dissolving silk should not interfere with the biocompatibility of the processed material when exposed to cells in vitro or in vivo. Maintaining the secondary structure of silk (β-sheets) in the electrospun fibers is necessary to attain optimal mechanical properties. Jin and coworkers electrospun different blends of poly-(ethylene oxide) (PEO) and silk using aqueous hexaflouro-2-propanol (HFIP). Uniform fibers (800 ± 100 nm) were obtained but this technique involves the use of PEO which might alter the mechanical properties of the fibers. Natural B. mori fibers have tensile strength of 500-700 MPa, Young’s modulus of 5-15 GPa and elongation of 4-19 %. Ohgo et al. used hexafluoroacetone-hydrate (HFA) for preparation of the recombinant silk. The average diameter of the fibers obtained using this solvent was 300 nm. The use of HFA-hydrate might compromise the biocompatibility of the fibers and so methanol was used to remove HFA.

The first objective of this thesis is to study the processing parameters in electrospinning and to produce B. mori silk nanofibers. The goal is to obtain uniform nano scale silk fibers by the electrospinning process and to carry out detailed statistical analysis of the effects of the electrospinning parameters on fiber diameter.
5.2 Materials and methods

Silk fibers were heated (100 ºC for 30 min) in aqueous Na₂CO₃ (0.02 M) and rinsed with water to extract the sericin covering. The extracted fibers were dissolved in 50% aqueous CaCl₂ (100 ºC, then cooled) to obtain silk concentration of 6 wt % solution. The solution was dialyzed against deionized water for 48 h at room temperature. The dialyzed fibroin was lyophilized to obtain regenerated silk fibroin sponge. The silk sponge solution (5–20%) was dissolved in formic acid (98–100%) and electrospun as described earlier.

5.3 Results

5.3.1 Effect of silk polymer concentration on fiber diameter

It was observed that silk concentration plays a major role in fiber diameter. No fibers were formed at less than 5% silk concentration for any electric field and spinning distances. This was because at this concentration, there obviously were not sufficient molecular chain entanglements in the polymer solution to prevent the breakup of the electrically driven jet and hence droplets were formed. Table 5 shows a summary of the fiber formation dependency on fibroin concentration. Figure 16 and 17 show the morphology of fibers obtained at the electric fields of 3 and 4 kV/cm, respectively, at silk/formic acid concentrations of 5, 8, 10, 12, 15, and 19.5% with a constant tip-to-collection plate distance of 7 cm. At 8% concentration less than 30 nm diameter fibers were formed with beads (drops of polymer over the woven mesh) and they were not uniform and were branched off (Figure 17). At 10% concentration with 5 cm
spinning distance and 2, 3 and 4 kV/cm electric fields, drops were formed instead of fibers. Continuous fibers were obtained above 12% regardless of electric field and distance. This was due to the fact there were sufficient molecular chain entanglements in the polymer solution to prevent the breakup of the electrically driven jet and to allow the electrostatic stresses to further elongate the jet to form fibers. At 19.5%, the average fiber diameter was much larger than that of fibers spun at lower concentrations.

The distribution of fiber diameters at 12, 15 and 19.5% concentration is shown in Figure 18. Fiber diameters less than 100 nm were successfully electrospun at each concentration and the distribution of the fiber diameter was plotted. The fiber diameter distribution is skewed to the left but the values of skewness were under acceptable limits and thus the distribution was assumed as normal to carry out further statistical analysis on the data.
Figure 16. The morphology of fibers at electric field of 3kV/cm at concentrations from 5 to 19.5% with a constant spinning distance of 7 cm. The figure also shows the average standard deviation, maximum and minimum values of the fiber diameter.

Table 5. Fiber and bead formation dependence on fibroin concentration

<table>
<thead>
<tr>
<th>Concentration (w/w)</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>No fiber</td>
</tr>
<tr>
<td>8</td>
<td>Fibers with lots of beads</td>
</tr>
<tr>
<td>10</td>
<td>Fibers with beads</td>
</tr>
<tr>
<td>12</td>
<td>Uniform continuous fibers</td>
</tr>
<tr>
<td>15</td>
<td>Uniform continuous fibers</td>
</tr>
<tr>
<td>19.5</td>
<td>Continuous but not uniform fibers</td>
</tr>
</tbody>
</table>
Figure 17. The morphology of fibers at electric field of 4kV/cm at concentrations from 5 to 19.5% with a constant spinning distance of 7 cm.
Figure 18. The distribution of fiber diameter at concentrations of 12, 15 and 19.5% with a constant spinning distance of 7 cm. The Gaussian distribution of the fiber diameter can also be seen from the values of the skewness.

5.3.2 Effect of voltage and spinning distance on morphology and diameter

The process parameters were optimized by studying the influence of electric field and distance on fiber diameter. Figure 19 shows the relationship between mean fiber diameter and electric field with concentration of 15% at spinning distances of 5, 7 and 10 cm.
Figure 19. The relationship between mean fiber diameter and electric field with concentration of 15% (w/w) at spinning distances of 5, 7 and 10 cm.

The mean fiber diameter obtained at 2 kV/cm is larger than other electric fields. The relationship between the fiber diameter and concentration at 2, 3 and 4 kV/cm is shown in Figure 20 along with the regression line. The results suggest an exponential relationship between fiber diameter and silk concentration. The effect of two factors, concentration and electric field on fiber diameter was investigated by two-way analysis of variance. The interaction effect between two factors is also obtained from this analysis.
Figure 20 depicts that the concentration apparently has more effect on the fiber diameter than electric field. Multiple regression analysis was carried out to evaluate the contribution of concentration and electric field on the fiber diameter. The fiber diameters were transferred to the natural logarithmic values and then linear model was applied to the data as seen in Table 6. It depicts the analysis of variance and coefficients for this model.
Table 6. Analysis of variance for the two factors (electric field and concentration) and coefficients of the model.

<table>
<thead>
<tr>
<th>Model(^a)</th>
<th>F</th>
<th>Sig.</th>
<th>R</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>778.171</td>
<td>0.000</td>
<td>0.862</td>
<td>0.742</td>
</tr>
<tr>
<td>Variable</td>
<td>Unstandardized Coefficients</td>
<td>Standardized Coefficients: (\beta)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(_0)</td>
<td>7.653</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric field: (x_1)</td>
<td>C(_1)</td>
<td>-0.611</td>
<td>-0.668</td>
<td></td>
</tr>
<tr>
<td>Concentration: (x_2)</td>
<td>C(_2)</td>
<td>-0.497</td>
<td>-2.027</td>
<td></td>
</tr>
<tr>
<td>(x_1^2)</td>
<td>C(_{11})</td>
<td>0.04556</td>
<td>0.346</td>
<td></td>
</tr>
<tr>
<td>(x_2^2)</td>
<td>C(_{22})</td>
<td>0.02189</td>
<td>2.668</td>
<td></td>
</tr>
<tr>
<td>(x_1 x_2)</td>
<td>C(_{12})</td>
<td>0.01373</td>
<td>0.325</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Model: \(\ln y = C_0 + C_1 x_1 + C_2 x_2 + C_{11} x_1^2 + C_{22} x_2^2 + C_{12} x_1 x_2\) where \(y\) is the fiber diameter.

The standardized coefficients explain the contribution of each parameter to the dependent variable (fiber diameter). The coefficient of determination (\(R^2\)) was found to be 0.742. However, difference between the observed and predicted values was within one standard deviation. The standardized coefficient of the concentration was much larger than that of the electric field.

5.4 Discussion

In this section of study, solution concentration was found to be the most significant factor controlling the fiber diameter in the electrospinning process.
5.4.1 Effect of polymer concentration on fiber diameter

It was not possible to obtain fibers below the silk concentration of 5% because a stable drop at the end of spinneret was not maintained. In the short distance as well as low concentration (10%), the solution reaches the collection plate before the solvent fully evaporates. This explains the formation of droplets and beads at the low concentration and distance. Fewer beads were observed in electrospun fibers at higher concentration. Increase in the regenerated silk concentration in the formic acid increases the solution viscosity. Fong et al. [140] showed that the viscosity plays a major role in the formation of beads. They indicated that higher viscosity tends to facilitate the formation of fibers without beads. The results obtained in this study are consistent with theirs. Dietzel et al. [139] demonstrated that solution surface tension and viscosity play important roles in determining the range of concentrations from which continuous fibers can be obtained in electrospinning. At low concentrations beads are form instead of fibers and at high concentrations the formation of continuous fibers are prohibited because of inability to maintain the flow of the solution at the tip of the needle resulting in the formation of larger fibers. Continuous nanofibers were obtained above 12% regardless of electric field and distance and at higher concentration of 19.5% the average fiber diameter was larger than at lower concentrations.

5.4.2 Effect of electric field and spinning distance on fiber diameter

Two-way analysis of variance was carried out at the significance level of 0.05 to study the influence of electric field and distance on fiber diameter. For 12 samples
(four levels of electric field and three levels of distance), multiple comparison analysis was carried out to find out the significant differences in mean fiber diameter among samples. The results suggest that there is no significant difference (at 0.05 level) in fiber diameter between 7- and 10-cm spinning distance at the same electric field and concentration of 15%. This study indicated that the fiber diameter increases exponentially with increasing concentration. Similar relationship between fiber diameter and solution concentration was observed for PEO/water solution [139]. Jin et al [137] observed that electric field of more than 2 kV/cm was necessary for fiber formation in silk/HFA and silk/PEO solutions. Our results were consistent with Jin et al. as we obtained the fibers of less than 100 nm at 3 and 4 kV/cm electric fields.

Summary:

The electrospinning of B. mori silk fibroin in formic acid was processed and fiber diameters ranging from 12 to 1500 nm were obtained depending on the electrospinning conditions. Morphology of fibers and distribution of fiber diameter were investigated by varying the electric field, concentration of regenerated silk solution and distance between tip and collection plate. The concentration of regenerated silk solution was the most dominant parameter to produce uniform and continuous fibers. Uniform fibers with diameters less than 100 nm were produced at the spinning conditions of 12–15% concentration and electric fields of 3 to 4 kV/cm.
6.1 Background and Significance

In the preceding chapter, the important electrospinning processing parameters that affect fiber morphology and diameter of regenerated silk nonwoven mat were identified [141]. It was also empirically determined that the silk solution concentration was the dominant parameter in producing uniform and continuous fibers. Uniform fibers (less than 100 nm diameter) were obtained from 12 to 15 \% (w/w) silk in formic acid concentrations and electric fields of 3 kV/cm and 4 kV/cm [141].

The purpose of this section is to identify the optimum combination of electric field and polymer concentration in order to produce silk fibers with diameters less than 100 nm. To investigate the interaction of these variables and tip-to-collection plate distance (referred to as the spinning distance), the experiments were regulated at two spinning distances.

In order to obtain a more systematic understanding of these process conditions and to establish a quantitative basis for the relationships between electrospinning parameters and fiber diameter, Response Surface Methodology (RSM) [142] was employed in this study. The objective is to develop an empirical model to guide future experiments and to spin uniform nanoscale fibers for future applications.

RSM has been used successfully for material and process optimization [142] in numerous studies including thermoplastic elastomer [143], diamond-like carbon films [144] and poly (vinyl alcohol) hydrogels [145]. This approach has the advantage of
taking into account the combined effects of several parameters and it uses statistical methods to fit an empirical model to the experimental data. The use of a model to describe the effects of electrospinning parameters permits the representation of the influencing parameter in a simple and systematic way and prediction of the results of the experiments with different parameter combinations. Thus RSM gives an overview of the processing parameters and their influence on each other. Furthermore, it helps to obtain the surface contours of these parameters using experimental and predicted values. These contour plots outline the processing window and point out the direction to attain the optimum condition in the form of an Eigen value. For more detailed explanation on RSM, references [142, 146] can be made use of. A brief introduction is provided herein.

6.1.1 Response surface methodology (RSM)

RSM is used in situations where several variables influence a feature (called the response) of the system. The steps in the procedure are described briefly as follows.

I. Identification of variables $\zeta_1, \zeta_2, \zeta_3 \ldots \ldots \text{ for response } \eta$

II. Calculation of corresponding coded variables $(x_1, x_2, x_3 \ldots \ldots)$ by using the following equation.

$$
 x_i = \frac{\zeta_i - [\zeta_{.0} + \zeta_{.1}]/2}{[\zeta_{.0} - \zeta_{.1}]/2}
 $$

(5)
where, $\zeta_{A_i}$ and $\zeta_{B_i}$ refer to the high and low levels of the variables $\zeta_i$, respectively.

III. Determination of the empirical model by multiple regression analysis to generate theoretical responses ($\hat{Y}$). The second-order model is widely used in RSM. The general equation for response $\eta$ of the second-order model is given by:

$$
\eta = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j=2}^{k} \beta_{ij} x_i x_j
$$

where, $k$ is the number of factors, $x_i$ are the coded variables and $\beta$ are coefficients

IV. Calculation of the coefficients $\beta$ to fit the experimental data as closely as possible.

The relationship between the response and the variables is visualized by a response surface or contour plot to see the relative influence of the parameters, to find an optimum parameter combination, and to predict experimental results for other parameter combinations.

The electric fields and concentrations are the two variables identified in our study.

When $k=2$, the Empirical model from the general equation (6) becomes

$$
y = \beta_0 + \beta_{11} x_1 + \beta_{22} x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \epsilon
$$

(7)
where, $y$ is the natural logarithm of the fiber diameter, $x_1$ is the coded electric field variable, $x_2$ is the coded concentration variable, and $\epsilon$ is the random error term.

RSM procedure to optimize the process parameters for the electrospinning silk is shown in Figure 21.

Figure 21. RSM procedure to optimize the electrospinning condition of regenerated silk.
6.2 Materials and methods

6.2.1 Materials and electrospinning

Details of spinning dope preparation and electrospinning conditions were reported earlier in chapter four (sections 4.1.1 and 4.1.2 respectively). The silk-formic acid solution was electrospun at a 45° spinning angle, a voltage of 10 to 35 kV and a constant spinning distance of either 5 or 7 cm.

The fiber morphology was examined by ESEM. For each experiment, fiber diameter and distribution were determined from 100 measurements of the random fibers.

6.2.2 Experimental design

A factorial experiment was designed using two factors (electric field, concentration) under constant electric field. For the application of RSM to develop the process, sequential experiments were carried out. Initially, the experiment was designed with spinning distance of 7 cm then other spinning distances were considered. Previous study showed that the effect of spinning distance on fiber diameter between 7 cm and 10 cm (15 % silk concentration) was insignificant. Thus a lower spinning distance of 5 cm was used for subsequent experiments. These experiments were planned according to the modified central composite design [142]. This type of design defines the minimum number of experimental combinations in the experimental domain to be explored in order to obtain the maximum information for adjusting the proposed model. For a quadratic model, experiments must be performed for at least three levels
of each factor. These levels are best chosen equally spaced. The two factors (silk concentration and electric field) and three levels resulted in nine possible combinations of factor settings. A schematic of the experimental design is shown in Figures 22A and 22B. The values in the bracket are coded variables ($x_1$: electric field, $x_2$: concentration) and numbered values at coordinate point represents mean fiber diameter obtained by experiment.

Figure 22. Experimental design A: spinning distance 7cm, B: spinning distance 5cm. The values at the coordinate points show the average fiber diameter (nm) of 100 measurements and coded values are shown in the brackets (electric field, concentration). NF: no fiber formation
The coded values were calculated using equation (5) of the electric field ($\zeta_1$) and concentration ($\zeta_2$). In this case, the high value of the electric field ($\zeta_{A1}$) is 4 kV/cm and the low value is 2 kV/cm ($\zeta_{B1}$). The high value of the concentration is 20 % ($\zeta_{A2}$) and the low value is 10 % ($\zeta_{B2}$). In the case of 5 cm spinning distance, the critical concentration for fiber formation was above 12 % and there were no fibers formed at concentrations of 10 % or less. This resulted in an uneven spacing of the levels as can be seen in Figure 22B. Coded and natural variables are listed in Table 7.

Table 7. Design of experiments (variables and levels).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Coded variables</th>
<th>Natural variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$x_1$</td>
<td>$x_2$</td>
</tr>
<tr>
<td>Spinning distance: 5cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-1</td>
<td>-0.6</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>-0.6</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-0.6</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spinning distance: 7cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
6.3 Results

6.3.1 Response function

By linear regression analysis of equation (7) the numerical values for coefficients ($\beta_0$, $\beta_1$, $\beta_2$, $\beta_{11}$, $\beta_{22}$, $\beta_{12}$) were obtained. The fitted second-order equation for the natural logarithmic fiber diameter is given by

$$\hat{y} = 4.484 - 0.115x_1 + 0.907x_2 + 0.178x_1^2 + 0.159x_2^2 + 0.05616x_1x_2$$  \hspace{1cm} (8)

for 5cm spinning distance and

$$\hat{y} = 4.362 - 0.148x_1 + 0.931x_2 + 0.03334x_1^2 + 0.523x_2^2 + 0.131x_1x_2$$  \hspace{1cm} (9)

for 7cm spinning distance.

P-values (a measure of the statistical significance) and $R^2$ (a measure of the percent of the response being represented by the variables) for regression models (equations (8) and (9)) are shown in Table 8. P-values for both regressions are less than the significance level of 0.05, validating adequacy of these models. Values of $R^2$ are 0.947 for 5 cm spinning distance and 0.958 for 7 cm spinning distance. The models predict a variability of 95% in new data for 5 cm spinning distance and 96% for 7 cm spinning distance.
Table 8. Significance probability (P-value) and correlation coefficient of linear regression for response surface equations.

<table>
<thead>
<tr>
<th>Spinning distance</th>
<th>P-value</th>
<th>R</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>5cm</td>
<td>0.006</td>
<td>0.984</td>
<td>0.947</td>
</tr>
<tr>
<td>7cm</td>
<td>0.007</td>
<td>0.992</td>
<td>0.958</td>
</tr>
</tbody>
</table>

where \( y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \epsilon \)

6.3.2 Response surfaces of fiber diameter as a function of concentration and electric field

6.3.2.1 Effects of concentration on fiber diameter

Figure 23 shows contour plots in the case of 7 cm spinning distance. The response indicates that changes in fiber diameter are more responsive to electric field at the low solution concentration. Low concentration of fibroin gives low fiber diameters. 10% concentration gives fiber diameters ranging from 35-80 nm while a 20% concentration of fibroin gives fiber diameters above 300 nm. The interaction effect of the electric field can also be observed. The corresponding experimental mean fiber diameters used to build this function are shown in Figure 23 (symbol +). The residual (difference between the experimental fiber diameters and the predicted fiber diameter) is less than the standard deviation of the predicted fiber diameters. In Figure 24, five points (symbol o) show the corresponding experimental mean fiber diameters from new experiments. The residuals at these conditions are also less than the standard deviation of the experimental fiber diameters except one combination
(4 kV/cm, 8 %). However, this result follows the direction obtained from contour plots suggesting lower concentration gives lower fiber diameter.

Figure 23. Contour plots of fiber diameters (nm) as a function of electric field and solution concentration for 7 cm spinning distance. The corresponding experimental mean fiber diameters (nm) are placed in the contour plot (symbol + at experimental design and symbol o at new experiments). Fibers are not formed in the shaded area.

The responses however are iso-level curve response (Figure 23) indicating that nanofibers can be produced over a range of process conditions. The figure also shows that nanofibers can be theoretically produced at low concentrations (less than 8 %) and low electric fields (less than 2 kV/cm) but this was not possible experimentally indicating that one should not extrapolate from empirical models.
6.3.2.2 Effects of electric field on fiber diameter

The contour plot for the 7 cm spinning distance shows that the fiber diameter decreases as the electric field is increased at a given or fixed silk concentration. For a 10 % concentration, increasing the electric field from 1 to 5 kV/cm leads to a decrease in the fiber diameter from 100 to 35 nm. Keeping the electric field constant, an increase in the concentration increases the fiber diameter in the spinnable region. At a higher concentration the effect of the electric field becomes negligible. This is apparent from the slopes of the curves which become smaller as the concentration increases. For example, at 20 % the fiber diameter is ~500 nm irrespective of the electric field. Thus electric field has more effect on the fiber diameter at low concentrations of silk.

6.3.2.3 Optimum processing window for nanofibers

The center of the response surface system called the stationary point is a point representing minimum values of the response or the smallest fiber diameter. The stationary point is mathematically described [142] by the following equations (10 and 11).

The fitted regression model, equation (8), in matrix notation,

\[
\hat{y} = b_0 + x'b + x'Bx
\]  

(10)

Where \( b_0 \), \( b \), \( \hat{B} \) are estimates of the intercept, the linear, and the second-order coefficients respectively. \( x'=[x_1, x_2] \) and \( \hat{B}=2 \times 2 \) symmetric matrix.
The stationary point $x_s$ is obtained by

$$x_s = \hat{\mathbf{B}}^{-1}\mathbf{b}/2$$

(11)

The stationary points were calculated using equation (11). For spinning distance of 7 cm the coded values are $x_s = [5.263, -1.549]$ and $\hat{y} = 3.25$. The corresponding physical values are 8.3 kV/cm (electric field), 7.3 % (concentration) and 25.8 nm (fiber diameter). Although this condition is outside of the experimental design region, it indicates the direction chosen for process conditions to obtain the smaller fiber diameter. In the case of 7 cm spinning distance, lower concentration with higher electric field produces smaller fiber diameter.

6.3.2.4 Effect of spinning distance on fiber diameter

The experiments for 5 cm spinning distance was designed to confirm the region producing nanofibers found in the case of the 7 cm spinning distance. As shown earlier (Figure 21), a 7 cm spinning distance exhibits larger fiber producing region than a 5 cm spinning distance from experiments.

Figure 24 shows the contour plots of fiber diameter calculated in the case of 5 cm spinning distance. High concentrations of fibroin lead to large fiber a diameter that is consistent with the trend observed in the case of 7 cm spinning distance. 20 % concentration forms fibers with diameters in the 300-500 nm range. A lower concentration of 12 % produces fibers with diameters in the 150-60 nm range. At a given or fixed concentration of fibroin, the combined effect of the electric field is
seen to cause reduction in fiber diameter, as was observed in the case of the 7 cm spinning distance. For a 12% concentration, an increase in electric field from 1 to 5 kV/cm results in reduction in fiber diameter from 150 to 60 nm. The values of mean fiber diameter from 100 measurements are also shown at corresponding conditions (symbol +, Figure 24). The residual is less than the standard deviation of the experimentally obtained fiber diameters.

Figure 24. Contour plots of fiber diameters (nm) as a function of electric field and solution concentration for 5 cm spinning distance. Corresponding experimental values of mean fiber diameters (nm) are placed in the contour plot with symbol +. Fibers are not formed in the shaded area.

Comparing fiber diameters at 5 cm and 7 cm spinning distances at the same electric field and concentration (see Figure 23 and Figure 24) shows that fiber diameters
obtained at 7cm spinning distance are lower than those at 5 cm spinning distance in the concentration range of 10 to 18 %. For 12% silk concentration, an increase in electric field from 2 to 4 kV/cm leads to a decrease in fiber diameter from 70 to 45 nm and from 76 to 56 nm for 7cm and 5 cm spinning distances respectively. The difference is greater at higher electric field (5 kV/cm). One might suggest that this result is due to the longer spinning distance which enables the polymer to evaporate solvent more efficiently even at same electric field strength thus leading to smaller fiber diameter in comparison with the diameter obtained at 5cm spinning distance. However, higher concentration such as 20 % and at an electric field less than 4 kV/cm, the fiber diameters obtained for 7 cm spinning distance were larger than those for a 5 cm spinning distance. This result might suggest that a critical point has been reached at this concentration whereby interaction effects of smaller spinning distance and electric field lead to a decrease in fiber diameter size.

6.4 Discussion

The use of RSM to study the optimum combinations of electrospinning parameters to produce nanofibers from regenerated silk was introduced. Contour plots relating fiber diameter to electric field and solution concentration were generated for spinning distances of 5 and 7 cm. These plots will assist in future studies and production of electrospun nanoscale silk fibers.

The response surfaces indicated that silk nanofibers (less than 100 nm) could be produced from solutions in the range of 10 to 16.5 % concentration with electric fields over 2 kV/cm and for 7 cm spinning distance. For a spinning distance of 7 cm
the contour plot predicted the fiber diameter less than 40 nm. Figure 25 shows that at experimental parameters of 10%, 3kV/cm and 7cm spinning distance it's possible to produce fibers with diameters less than 30 nm. However, experimental results indicated that the electrospun nonwoven mat contain beads at a concentration of 10% or less (see Figure 26A). In order to produce fibers without beads concentrations above 12% are required as seen in Figure 26B.

Figure 25. The morphology of fibers at a spinning concentration of 10%, an electric field of 3kV/cm and a spinning distance of 7cm.
Figure 26. The morphology of fibers at two different conditions: A: Concentration 10%, electric field 4 kV/cm and spinning distance 7cm. B: Concentration 15%, electric field 5 kV/cm and spinning distance 7cm.
From the fact that different contour shapes were obtained for 5 cm and 7 cm spinning distances, these results might suggest a coupling effect or interaction between spinning distance and electric field strength, which should also be considered when a simple normalization to electric field (voltage/spinning distance) is used.

The use of the stationary point to determine the optimum combinations of the electric field and concentration to generate nanofibers gives a usual indication of a trend, but must be used with caution as often some values fall beyond the experimental region. The stationary point for a spinning distance of 5 cm is $x_s = [0.79, -2.9]$, physical values were 3.8 kV/cm and 0.04 %. This stationary point at 5 cm spinning distance represents the condition under which fibers cannot be formed because silk concentration is too low. In such a case, further experiments with broader range may be designed to improve the process.

It must be noted that variations may exist in the properties of *B. mori* silk obtained from different sources or processed differently. These differences for the natural polymer may cause variations in experimental results.

Summary:

RSM analysis was applied to the experimental results to develop a processing window that will produce nanoscale regenerated silk fibers by electrospinning process. Contour plots relating fiber diameter to electric field and solution concentration were generated for spinning distances of 5 cm and 7 cm. From the RSM models, a concentration of 8 to 10 % and electric field of 4 to 5 kV/cm were found to be the sufficient condition for achieving nanofibers of diameters less than 40
nm. The difference between the experimental fiber diameters and the predicted fiber diameters is less than the standard deviation of the experimental fiber diameters. The model will serve as a useful guide to obtain uniform nanoscale silk fibers in future at various combinations of process variables and to construct the response surfaces for mechanical, chemical and physical properties.

The characterization of the properties of an electrospun nonwoven silk mat will be reported in the subsequent chapter.
7.1 Background and Significance

In this chapter, the effect of electrospinning process on the structure, morphology and properties of the silk fiber assemblies were studied. The silk fibroin is characterized through the processing steps of degumming, dissolution in aqueous calcium chloride, dialysis, water removal, dissolution in formic acid and fiber formation. The compositional, structural and mechanical properties of the pristine and electrospun silk fiber assemblies were compared. The electrospinning process of silk involves conformational changes in the silk fibroin from random coil to $\beta$–sheet and vice versa. An understanding of the structural changes that occur during the various stages of the electrospinning process is essential for the proper use of the regenerated silk for various applications.

7.2 Materials and method

The spinning dope and electrospinning process were carried out as previously described in Chapter 4. The critical electrospinning parameters (electric field and fibroin concentration) identified in Chapter 6 of the thesis were optimized by a modified RSM method. Schematics of these experimental designs are shown in Figures 27A-27C. The electric field ranged from of 2 to 4 kV/cm and the concentration from 9 to 15%. Voltages of 10 to 50 kV and spinning distances of 5, 7 and 10 cm were utilized.
Figure 27. Experimental design A: spinning distance 7 cm, B: spinning distance 5 cm and C: spinning distance 10 cm. The values at coordinate point show mean fiber diameter of 100 measurements and coded values are shown in the brackets (electric field, concentration).

7.3 Results

7.3.1 Fiber morphology and diameter distribution

As discussed earlier in Chapter 5, concentration was found to be the most important parameter influencing the fiber diameter produced in the electrospinning process. For spinning distances of 5, 7 and 10 cm at low electric fields of 2–3 kV/cm, the fiber diameter was found to increase with an increase in fibroin concentration. A 9%
fibroin concentration produced fibers with diameters ranging from 8 to 223 nm. A 15% fibroin concentration yielded fibers with diameters from 12 to 397 nm.

Figure 28 shows the ESEM micrographs of electrospun fibers at spinning distance of 10 cm, electric field strength of 3 kV/cm and polymer concentrations of 9, 12 and 15 wt%. The fibers had smooth surfaces and appeared circular in cross-section.

<table>
<thead>
<tr>
<th>10cm, 3kV</th>
<th>Magnification</th>
<th>Fiber diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000x</td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td>5000x</td>
<td>35000x</td>
</tr>
<tr>
<td></td>
<td>AV: 55.2</td>
<td>STDV: 28.5</td>
</tr>
<tr>
<td></td>
<td>Max: 117.0</td>
<td>Min: 7.8</td>
</tr>
<tr>
<td>12%</td>
<td>AV: 62.6</td>
<td>STDV: 32.9</td>
</tr>
<tr>
<td></td>
<td>Max: 175.8</td>
<td>Min: 14.9</td>
</tr>
<tr>
<td>15%</td>
<td>AV: 98.6</td>
<td>STDV: 58.4</td>
</tr>
<tr>
<td></td>
<td>Max: 212.7</td>
<td>Min: 15.6</td>
</tr>
</tbody>
</table>

Figure 28. ESEM micrographs of electrospun silkworm silk fibers and their corresponding processing parameters of electric field of 3 kV/cm, spinning distance of 10 cm and concentrations of 9, 12 and 15% respectively.

Fiber diameter distributions at a fibroin concentration of 9%, an electric field of 3 kV/cm and the three spinning distances are shown in Figure 29 (A)–(C). The average
fiber diameters were $70 \pm 23$, $61 \pm 27$ and $55 \pm 26$ nm at spinning distances of 5, 7 and 10 cm, respectively.

Figure 29. Fiber Diameter Distribution: A: for 9% silk, 5cm spinning distance and electric field of 3 kV/cm, B: for 9% silk, 7 cm spinning distance and electric field of 3 kV/cm and C: for 9% silk, 10 cm spinning distance and electric field of 3 kV/cm.

7.3.2 Raman spectroscopy

The conformational changes of the secondary structure of silk fibroin, which occur during the electrospinning process, were analyzed by Raman spectroscopy. The
spectra for pristine, degummed and electrospun fibers are shown in Figure 30A. Pristine and degummed silk fibroins display characteristic conformational bands in the range 1650–1667 and 1241–1279 cm$^{-1}$ which correspond to amide I and complex amide III, respectively [148, 149].

Figure 30. (A) Raman spectroscopy of pristine, degummed and electrospun nonwoven silk mat. (B) Secondary structural compositions of silk fibroin showing the fraction of Amide I to Amide III conformations.
In this study, the amide I (random coil) pristine band was observed at 1665 cm\(^{-1}\) and the amide III (\(\beta\)-sheet) pristine band at 1231 cm\(^{-1}\). These well-defined bands were chosen because they give a clear indication of changes in the secondary structures from random to \(\beta\)-sheet. The degummed silk also shows absorption bands at these wavelengths. No significant spectral changes were observed indicating that the fibroin conformation is unchanged during the degumming process. The Raman spectra of the electrospun fiber are essentially the same as that of the pristine and degummed fibers although minor bands and some differences in peak intensities appear. This shows that the electrospinning process preserves the natural conformation of the fibroin. Figure 30B shows the amide I (1665 cm\(^{-1}\), random) to amide III (1228 cm\(^{-1}\), \(\beta\)-sheet) ratio of the electrospun fiber is less than that of the pristine fiber. This means that the electrospun fiber has higher \(\beta\)-sheet content than the pristine fiber.

7.3.3 FTIR spectroscopy

The secondary structure of \(B.\ mori\) silk fibroin consists of the major conformations including- random coils (silk I) and \(\beta\)-sheet (silk II) [150]. No differentiation is made between random coils and silk I structures because of their similar conformational structures [25]. FTIR spectroscopy was used to follow the conformational changes that occur during the electrospinning process. Random coils show strong absorption bands at 1665 (amide I), 1540 (amide II), and 1235 cm\(^{-1}\) (amide III) and the \(\beta\)-sheets show absorption bands at 1628 (amide I), 1533 (amide II) and 1265 cm\(^{-1}\) (amide III) [151]. Figure 31A shows the FTIR spectra of degummed silk, silk fibroin (6%
aqueous calcium chloride), silk fibroin (aqueous post dialysis) and silk fibroin (12% aqueous formic acid). There are no significant differences in the major peaks. This indicates that there are no significant conformational differences between the degummed fiber and the fibroin solutions.

Figure 31B shows the FTIR spectra of pristine, degummed, dried fibroin sponge and electrospun silk fibers. There are no significant differences in the major bands. However, there are significant differences in peak intensities, which indicate changes in the amount of the secondary structure (concentration). The percent changes in the intensity of the 1663 cm$^{-1}$ band (amide I, random) relative to the pristine silk are 0, 10 and 6 for the degummed fiber, dried fibroin sponge and electrospun silk mat respectively.

The amide I crystallinity index, calculated as the intensity ratio of 1624 and 1663 cm$^{-1}$ bands, is shown in Table 9 for each step of the electrospinning process. The amide I represents the most abundant secondary structure of fibroin and shows strong absorption bands at 1663 cm$^{-1}$ (random) and 1624 cm$^{-1}$ (β-sheet).
Figure 31. (A) FTIR spectra of - (1) dialyzed silk fibroin in water, (2) 6% silk fibroin in calcium chloride solution, (3) degummed silk fiber, (4) 12% silk fibroin in formic acid. (B) FTIR spectra of -(1) electrospun silk mat, (2) dried silk sponge, (3) pristine and (4) degummed silk.
Table 9. Crystallinity index of silk fibroin determined for each step of the electrospinning process. The index is calculated from the amide I peak intensities at 1624 and 1663 cm\(^{-1}\)

<table>
<thead>
<tr>
<th>Type of silk</th>
<th>Peak Ratio (1624/1663), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pristine</td>
<td>48.8</td>
</tr>
<tr>
<td>Degummed</td>
<td>48.8</td>
</tr>
<tr>
<td>Fibroin in CaCl(_2)</td>
<td>49.5</td>
</tr>
<tr>
<td>Fibroin after dialysis</td>
<td>48.2</td>
</tr>
<tr>
<td>Dried sponge</td>
<td>48.7</td>
</tr>
<tr>
<td>Fibroin in formic acid</td>
<td>49.0</td>
</tr>
<tr>
<td>Electrospun silk mat</td>
<td>50.2</td>
</tr>
</tbody>
</table>

Figure 32 shows the qualitative values of the crystallinity index indicative of secondary structure changes. These results indicate an increase in the \(\beta\)–sheet of the electrospun nanofiber in comparison with the pristine and degummed fibers.
Figure 32. Crystallinity index of silk fibroin at each step of the electrospinning process.

7.3.4 Structural analysis by wide angle x-ray diffraction

WAXD was used to determine the crystalline structure of the fibroin. Figure 33 shows the WAXD patterns of pristine and electrospun fibers. The similarities between the patterns of the two systems strongly suggest structural similarity between the pristine and electrospun fibers. The pristine fiber was characterized by the presence of five diffraction peaks at 13.7, 16.5, 18.2, 25.3 and 28.1°, corresponding to silk (I) and silk (II) crystalline d-spacings of 6.5, 5.4 (II), 4.9 (II), 3.5 (I), 3.2 (I) Å respectively. The electrospun fiber was characterized by diffraction peaks at 13.7, 16.6, 18.3, 25.4 and 28.6°, corresponding to silk (I) and silk (II) crystalline d-spacings of 6.5, 5.3 (II), 4.8 (II), 3.5 (I) and 3.1 (I) Å respectively.
Within experimental error, these results are in agreement with the findings of Asakura [25], Hayakawa [152] and Jin [5]. The diffraction patterns indicate preferred orientation of the molecular axis of the crystals along the fiber axis. The results show that both types of fibers are comprised of mixtures of random (silk (I)) and $\beta$–sheet (silk (II)) crystals i.e. none of the fibers consist of only one type of crystal. The unknown d-spacing value of 6.5 may be due to other types of conformations such as helical and $\beta$–turns or distortions by the presence of conformers and/or segments in which torsion angles deviate from those of random or $\beta$–sheet. The crystallinity of the fibers was calculated by the method of Herman [153]. The crystallinity of the pristine (natural) and electrospun fibers was 48 and 39% respectively.
Due to the nanoscale size of the electrospun fibers, it is not possible to determine their orientation by conventional methods such as infrared dichroism. Birefringence is another method that is used to determine the molecular orientation of electrospun fibers. Lyons [154] was able to show that there is indeed attenuation of the chains on a molecular level of melt-electrospun isotactic polypropylene. However, due to the nanoscale size of the electrospun silk, this method could not be employed.

7.3.5 Mechanical analysis by tensile testing

The mechanical properties of the electrospun random fiber mats were determined utilizing a Kawabata KES-G1 microtensile tester. Strips measuring 0.5 by 4 cm were mounted on paper sample holders (3-cm gauge length) as shown in Figures 34 and 35. The samples were mounted on thick paper frames using cyanoacrylate glue (QuickTite super glue gel, LocTite) to fix the ends of the sample to the paper end tabs as illustrated in Figure 35. The weight of each sample was recorded and used in the tensile properties calculations. Double-sided stick tape was used to secure the ends of the strips to the end tabs. A strain rate of 0.02 / sec, sensitivity setting of 5 and frequencies of 50 Hz were used in the tensile tests. The results of the experiment were computed in load (gram force) vs. displacement. The specific stress in gm/Tex was then calculated using the following equation assuming fully a dense structure:

\[
Stress (gm/Tex) = \frac{\text{Force (gm)} / \text{specimen width (mm)}}{\text{Areal density (gm/m}^2\text{)}}
\]  

(12)
The areal density is simply the weight (gms) of the nonwoven silk test strip divided by the area of the test specimen. The stress in gm/Tex was converted to N/Tex by multiplying it by 0.0098. This was converted into the stress in GPa by multiplying it by the density of silk, taken as 1.25 gm/cc. The conversion factor is shown in the equation below:

\[
Stress \ (MPa) = Stress \ (gm/\text{Tex}) \times 0.81633
\]  \hspace{1cm} (13)

Figure 34. Picture of mounted electrospun random strips after mechanical tests.
The strain was calculated by dividing the displacement by the gauge length. Figure 36A shows a typical stress-strain plot of the mechanical tests. The initial part of this curve shows a high resistance to deformation due to cohesive forces in the fiber assembly as a result of the large number of fiber-to-fiber contacts that is a consequence of the nanoscale fiber diameter. A pseudo-yield point is also observed after the initial linear portion of the stress-strain curve followed by a gradual reduction in modulus caused by the initiation of fiber slippage. The fiber slippage leads to slight fiber alignment along the tensile axis that results in a slow increase in stresses being generated. Further increases in strain (stretching) lead to decreases in the cross section of the test specimen and thus the number of fibers in the cross-section resulting in failure of the fiber assembly.
Figure 36. Stress-strain plots of random mat electrospun silk fibers: A) typical sample B) average.

The initial modulus of the electrospun random mat as calculated from the slope of the initial part of the stress-strain curve was 515 MPa. The breaking strain and ultimate tensile strength of the fiber mat were 0.032 and 7.25 MPa respectively. This compares quite favorably to the results obtained in an earlier study by Yang et al, wherein a
breaking strain of 0.014 and a modulus of 1.29 MPa was observed for spun-bonded natural silk nonwoven fabrics [155]. Figure 36 A shows the average stress-strain plot of the random mats.

An observation of the deformation mechanism of the nonwoven mat revealed nano-level drawing in some of the fibers as shown in Figure 37. This implies that the molecular structures of the fibers can be further oriented. The fact that not all the fibers experienced the same drawing effect suggests that mechanical properties of the silk have not been fully realized.

Figure 37. ESEM of electrospun nanofiber after mechanical tensile testing showing nano-drawing effect.
According to the mechanics of nonwoven fabrics [156], the theoretical Young’s modulus of a fiber ($E_f$) can be estimated as a first approximation to be 1.37 GPa from the modulus of a nonwoven fabric ($E_F$) as follows:

$$E_f = \frac{8}{3} E_F$$  \hspace{1cm} (14)

This value is an order smaller than that of natural $B. \text{mori}$ silk fibers shown in Figure 38 with average Young’s modulus, ultimate tensile strength and strain at break of $12.98 \pm 5.13$ GPa, $501.5 \pm 114.2$ MPa and $18.45 \pm 4.37$ % respectively. The average fiber diameter used in the calculations was $10.6785$ µm. The stress is calculated as in Equation 13 with the inclusion of the areal density as:

$$A_d = \frac{m}{A} (gm/m^2)$$  \hspace{1cm} (15)

where $A$ is the area of the silk fiber ($\pi d^2/4$), $d$ is the average fiber diameter and $m$ is the weight of the fiber.
From Figure 38, it is seen that there is variability in the modulus of the as received (natural) silk fibers. This is mainly due to the individual differences in the diameter of
the fibers, which can vary even if the fibers are obtained from the same bave of the cocoon. The variability of the components of the fiber which depend on the rearing period, conditions and diet of the silkworm silk also lead to the deviations observed.

Based on the results of the mechanical tests of the random fibers obtained, it’s clear that further alignment of the silk fibers is required and proper drawing of the aligned fiber assemblies will be necessary in order to attain the required mechanical properties of the nanofibers.

Mechanical tests of aligned electrospun silk fibers:
Electrospun aligned fibers were obtained by collecting the fibers between electrodes as designed by Lam [96]. A schematic of the method of collection is shown in Figure 39. A set of parallel electrodes were mounted parallel to the polymer jet to collect oriented fibers. This setup allows the simultaneous fabrication of both aligned and random fiber mat assemblies. The nanofibers were periodically collected until the desired tow size or denier was obtained. Fibers were collected and placed on a holder until sufficient amount of fibers were accumulated to form a filament-yarn assembly with as little twists as possible. The aligned nanofiber bundles were slightly twisted to gain a certain degree of integrity within the yarn. Approximately one twist per centimeter was applied. Mounting of the yarn was done by applying a small drop of the cyanoacrylate adhesive close to the outer edges of the end tabs. To prevent stress concentration imposed by the grips during testing, a small drop of silicon rubber was applied on the inner edges of the end tabs. At least five samples were tested
depending on the availability of the materials. The load and displacement data of each sample was converted to stress-strain plot.

![Schematic of method of obtaining aligned electrospun silk fibers and yarns](image)

**Figure 39.** Schematic of method of obtaining aligned electrospun silk fibers and yarns [96].

For the aligned fiber tow the specific stress of the yarn was estimated by first determining the specific yarn cross-sectional area ($A_{sp}$) based on the measured linear density ($denier = gm/9000m$):

$$
A_{sp} (cm^2) = \frac{denier (gm/9000m)}{9 \times 10^5 \times \rho (gm/cm^3)}
$$

(16)
where $\rho$ is the silk density (1.25 gm/cm\(^3\)).

The specific stress (MPa) of the random nanofibers strip was calculated from Equation 12. The elastic modulus was estimated from the slope of the linear portion of the stress-strain curve.

Figure 40A and 40B shows the typical stress-strain curves and average plot of the aligned fiber yarn assemblies respectively.

It’s evident that there’s an improvement in the mechanical properties of the silk as depicted by the stress-strain curves. Ultimate tensile strengths (UTS) and Young’s modulus of up to 23 MPa and 630 MPa were obtained respectively. The average UTS, Young’s modulus and strain at break were 19.1 ± 10.3 MPa, 312 ± 192 MPa and 5.8 ± 1.7 % respectively.

The standard deviations observed are due to the differences in the denier of the fiber assemblies tested. The mechanical properties of silk have a denier dependency as illustrated in Figure 41.

A variability in the denier of the tested samples leads to deviations in the Young’s modulus. It is also possible to increase the modulus by fabricating smaller denier samples.
Figure 40. Stress-strain curves of electrospun aligned silk fiber yarn assemblies: A) typical samples B) average plot.
7.4 Discussion

*Bombyx mori* fibroin is comprised of dimorphic structures (random coil and β–sheet), which can easily be interconverted during fibroin processing. The conformational changes i.e. transition between random coil and β–sheet were evaluated at each step of the electrospinning process.

The relative amounts of the random coil and β–sheet structures were measured by FTIR. The FTIR spectra show that there are no conformational changes in the relative amounts of random and β–sheet contents of the pristine and degummed fibers. Dissolution in calcium chloride leads to conversion of random structure into β–sheet
as shown by an increase in the crystallinity index of dissolved fibroin. The crystallinity indexes of degummed fibroin and fibroin dissolved in calcium chloride are 48.8 and 49.5 % respectively as shown in Figure 29. This agrees with studies that show cations such as Ca^{2+}, Na^+ and K^+ in the fibroin solution facilitate the transition of random coil to \(\beta\)-sheet [157]. Conversely, removal of Ca\(^{2+}\) cations reverses the conformational transition. This is consistent with our observation of the dialyzed fibroin that has a lower index of 48.2%. The aqueous environment also favors the transition of \(\beta\)-sheets into random coils. The index increases slightly during the drying process to produce a sponge due to an increase in intramolecular hydrogen bonding of the fibroin.

Dissolution of fibroin in formic acid increased the crystallinity index to a greater extent than dissolution in water, which is consistent with the observation of Um et al [32]. They reported that formic acid promotes the \(\beta\)-sheet crystallization, therefore reducing the hydrodynamic radius of the fibroin molecules and increasing intramolecular hydrogen bonding of the fibroin. Um also noted that removal of formic acid from the fibroin leads to a further increase in the \(\beta\)-sheet content. Elimination of formic acid during the electrospinning process produces nanofibers with a crystallinity index of 50.2 %, the highest observed. The electrospinning process also promotes fibroin molecular alignment [158], thereby enhancing crystallization.

The Raman results also indicate that there are no significant conformational differences between the pristine, degummed and electrospun fibers. A decrease in the relative amounts of amide I/amide III of the electrospun fiber in comparison with the
pristine fiber is an indication of an increase in β–sheet content. This is consistent with the FTIR result that also shows an increase in β–sheet content.

The XRD results compliment the findings by Raman and FTIR regarding the crystalline structure of the electrospun silk fiber in comparison with the pristine sample. The pristine and electrospun fibers are both comprised of mixtures of random (silk (I)) and β–sheet (silk (II)) crystals. The crystallinity of the pristine and electrospun fibers was 48 and 39% respectively.

The discrepancies in the value of the crystallinity (XRD) and crystallinity index (FTIR) are due to the difference in the detecting method employed. FTIR is sensitive to short-range ordered structures and XRD is sensitive to long-range ordered structures [32].

The natural silk fibers give a characteristic response for visco-elastic materials - asymptotic in shape, with strain hardening resulting from molecular orientation at high degrees of strain. Qualitatively, the electrospun mat is also visco-elastic in nature, but the shape of the stress-strain curve for the electrospun material is slightly different from the pristine fiber. When a strain is applied to the electrospun mat, fibers oriented in the direction of cross-head displacement are stretched uniaxially, while fibers oriented at some angle relative to the principal strain direction experience a rotation. In addition, the electrospun silk mat has a lower density than that of the natural fibers due to its porous structure and a lower degree of orientation of the crystallites along the fiber axis as depicted earlier in Table 9. These two factors explain the relatively low stress in the electrospun fibers in comparison with the natural fibers. As the strain in the electrospun mat increases and more fibers become
oriented in the direction of strain, the relative number of fibers stretched to fibers rotated increases, and the stress in the electrospun mat increases.

The electrospun samples (both random mat and aligned fiber constructs) seemingly have premature failure which is another difference between their stress-strain curves and that of the natural fiber.

The fundamental differences observed in the stress–strain behavior of the natural, random mat and aligned fiber constructs can further be interpreted by comparing their morphology and molecular orientation. The strain applied to the electrospun random mat and aligned yarn construct serves to orient both the fibers and the molecules within the fibers. Molecular orientation also occurs in the natural fiber when a strain is applied. Strain-induced orientation is illustrated in the stress-strain curve of the natural fibers at high degrees of strain, as shown in Figure 37. While the electrospun random mat is globally isotropic, the individual fibers comprising the mat have a certain degree of molecular orientation prior to stretching. Molecular orientation in the electrospun fibers can be shown with polarized light microscopy. Although the orientation of electrospun fibers is not perfect, it is sufficient to study the molecular orientation in the fibers. The results obtained by Pedicini et al [159] indicate that molecular orientation is, in fact, induced by the process of electrospinning, and is a contributing factor to the ‘stiffer’ stress–strain response, and premature failure, of the electrospun polyurethane when tested in uniaxial tension.

The stress–strain behavior of electrospun silk random mat and aligned yarn constructs have been observed and compared to the natural silk fiber. The three forms of the
silk fibers have characteristically different stress–strain responses resulting from the following phenomena:

- Differences in degree of molecular orientation within the fibers. Experimental results have proven that electrospinning process leads to higher molecular orientation in fibers. However due to relaxation processes which occur after fiber formation, the molecular orientation is reduced.
- Differences in crystallinity and crystal orientation along the fiber axis.
- Differences in fiber sizes (denier).

Electron microscopy, vibrational spectroscopy and polarized light microscopy can be used to illustrate the changes, which occur upon stretching of the natural, electrospun random mat and aligned fibers and their molecular orientations. The apparent molecular orientation in the electrospun fibers also leads to the pronounced reduction in elongation to failure of the electrospun fibers relative to the natural fibers.

The modulus of the electrospun random silk mat as determined by the mechanical tests was 515 MPa with a breaking strain of 0.032. These values are lower than that of natural silk fibers because of fiber slippage caused by frictional forces between fibers during testing.

The mechanical properties of the aligned silk yarns were surprisingly lower than the random mat tested. The results show that the initial modulus of the random mat (515 MPa) is higher than that of the aligned fiber (312 MPa). This can be explained by the fact that the higher stresses recorded for the random mat may be due to bonded fibers (glued together by the solvent) and/or physical entanglements.
between the fibers. The apparent modulus of the aligned fiber (342 MPa) is higher than that of the random mat (176 MPa), which is the expected result.

However, the mechanical properties of the electrospun random and aligned fiber constructs are still lower than those of the natural fibers. This may be as a result of the lower crystallinity of the electrospun silk fibers (~39%) in comparison with the natural fibers (~48%). Considering the fact that the crystalline part (ordered region) of the fibroin contribute to the strength and toughness of the fibers while the amorphous part (random region) contribute to its elasticity, its not too surprising that mechanical properties of the electrospun fibers differ from the natural ones. Other factors that play roles in the mechanical property differences are the orientation effect of the crystals and molecular chains alignment along the axial direction of the fibers. If there are differences in the orientation of the crystals comprising the crystalline part of the fibers, there will be differences in the mechanical properties of the fibers. The more the crystals are aligned and oriented along the fiber axis, the higher will be the strength and modulus of the fibers. On the other hand, when the crystals are not aligned and oriented along the fiber axis, the result is lower mechanical properties.

The molecular orientation of the chains comprising the fibers also play a large role in the stress-strain curve of the fibers and hence in determining the mechanical properties. It is widely believed that the electrospinning process leads to a higher degree of orientation of the molecular chains that form the individual nanofibers. A higher degree of molecular chain alignment along the fiber axis will lead to higher mechanical properties. However, since the mechanical properties of the electrospun fibers are not comparable with the natural fibers, the only plausible reason for the
difference may be due to the afore mentioned factors i.e. differences in crystallinity, crystal orientation/alignment along the fiber axis and molecular alignment. It’s not clear which of these factors is dominant in determining the mechanical properties or what combinations lead to higher mechanical properties.

Summary:

In order to understand the structural changes caused by the processing steps in the electrospinning process, the conformational transitions from random coil to β-sheet characterized by the crystallinity index was determined by FTIR and the relative amounts of amide I to amide III by Raman spectroscopy. The conformational changes that occur during the various processing steps prior to and during the electrospinning process are as follows:

- Degummed fibroin: the crystallinity index of the pristine and degummed fibers was the same. Degumming does not alter the secondary structure of the silk fibroin.
- Dissolution in calcium chloride: the crystallinity index of the silk from the fibroin solution was higher than degummed fiber indicating that calcium ion facilitates the formation of β-sheet.
- Dialyzed fibroin: removal of calcium ion causes a decrease in crystallinity index. The aqueous environment also facilitates the transition to random structure.
• Dried fibroin sponge: removal of water leads to a slight increase in the crystallinity index due to an increase in intermolecular hydrogen bonding of the fibroin.

• Dissolution in formic acid: formic acid increases the $\beta$-sheet crystallization and reduces the hydrodynamic radius of the fibroin molecules by increasing intramolecular hydrogen bonding of the fibroin.

• Electrospun fibers: the removal of formic acid and the fibroin molecular alignment, which occurs during electrospinning result in the highest observed crystallinity index.

The characterization results show that the electrospun fiber has a non-negligible increase in the $\beta$-sheet content in comparison with the pristine fiber. Dissolution of fibroin in formic acid enhances $\beta$-sheet crystallization and may facilitate $\beta$-sheet formation in the electrospun fiber. However, the mechanical properties of the electrospun fibers are not fully realized due to lower crystallinity, degree of orientation/alignment of crystals and loss of orientation on the molecular and fibril level due to relaxation processes.
CHAPTER 8: EVALUATION OF MECHANICAL PROPERTIES OF ELECTROSPUN SILK NANOFIBERS

The results of the mechanical tensile tests of the electrospun silk fibers [160, 161] show that, the fibers are lacking in comparison with the natural fiber. This may be due to the fact that the mechanical properties of the electrospun fibers were tested in yarns or nonwoven mat forms, which do not give accurate values of the nanofiber properties. The discrepancy in mechanical properties of the electrospun and natural silk fiber may also be as a result of the processing steps involved in the nanofiber formation, differences in crystallinity, crystal orientation and molecular orientation of chains along the fiber axis. There is therefore a need to improve on the mechanical properties of the electrospun silk fibers.

8.1 Carbon nanotube reinforced electrospun silk nanofibers

8.1.1 Background and Significance

The availability of SWNT which have exceptional mechanical (elastic modulus and tensile strength of 1TPa and 200 GPa respectively) [162] and electrical properties make them suitable candidates for potential applications as polymer reinforcements for composites, materials for energy storage [163], electronics [164], catalysis [165] and vaccine delivery [166]. Utilizing SWNT’s will provide design options of tailoring the mechanical properties of polymers for various applications. An enormous reinforcement effect with the use of only a small portion of SWNT can be anticipated. However, the challenges involved in the fabrication of SWNT reinforced polymers
such as homogeneous dispersion of the SWNT’s in the polymer, efficient load transfer, aggregation and poor solubility in water and organic solvents, acts as limitations for practical applications. Many strategies have been employed to separate and disperse the SWNT’s which include sonication [167], covalent [168] and ionic functionalization [169, 170], steric [171] and electrostatic repulsion [172].

In recent years, SWNT’s have been utilized as nanofillers to enhance the mechanical properties of polymeric materials [173-175]. Andrews et al dispersed SWNT’s in isotropic petroleum pitch matrices to form nanotube composite carbon fibers with enhanced mechanical and electrical properties [176] (5 wt % loading of SWNT’s led to an increase in modulus by 150%). Ko and coworkers have fabricated carbon nanotube–polyacrylonitrile composite fibers by the electrospinning process [105, 107]. They utilized both SWNT’s and multi-wall carbon nanotubes and studied the reinforcement effects of the nanotubes on the nanofibers. A two-stage rupture behavior of the composite fibers under tension, including crazing of polymer matrix and pull-out of carbon nanotubes was observed. They concluded that carbon nanotubes reinforce the polymer fibers by hindering crazing extension, reducing stress concentration, and dissipating energy by pullout.

To date, no one has studied the reinforcement effects of SWNT’s on natural biopolymers. Spider silk is known as the toughest natural material; however, efforts to produce it on a commercial scale have not been successful. On the other hand, silkworm silk which is produced on a large commercial scale lacks the mechanical properties of spider silk. It is hypothesized that by reinforcing the silk fibers with SWNT’s, a significant improvement may be made in its mechanical properties. It is
envisioned that new tailorable properties can be achieved for the nanofibers such as reduction in static electricity, higher tensile modulus and strength.

In this section, the reinforcement effects of SWNT’s on the morphology, structure and mechanical properties of electrospun silk nanofibers shall be studied. The silk fibroin was employed as the dispersing agent for the SWNT’s with a net gain effect of having the electrospun nanofibers reinforced by the latter. The dispersion should be driven by both steric and hydrophobic effects between silk and SWNT. Van der Waals interaction forces also provide cohesion between the SWNT’s and silk. The rigidity and cylindrical shape of SWNT’s make their surfaces good supports for protein crystallization [177] which can improve the mechanical properties of the electrospun nanofibers. Consequently, by co-electrospinning SWNT with silk fibroin, a nanocomposite fiber may be fabricated to yield multifunctional strong and tough fibers, which may open the gateway to producing multifunctional fibers that may be suitable for weaving into textiles.

8.1.2 Materials and methods

The lyophilized silk fibroin sponge was prepared as mentioned in earlier chapters and sections. Purified SWNT’s were produced by high-pressure disproportionation of CO (HiPCO) [178].

The spinning dope was prepared by sonicating 0.5-5 wt % SWNT’s in formic acid (98-100 %) for 2 hrs. Regenerated silk fibroin (12 wt %) was added to the SWNT-formic acid mixture and further sonicated for an hour and mechanically stirred for another hour. The SWNT-silk-formic acid solutions were electrospun utilizing an 18-
G needle at a 45° spinning angle, a voltage of 30 kV and spinning distance of 7 cm as described previously. Random fibers were collected on an aluminum foil covered metallic plate and aligned fibers were collected to make nonwoven mats and yarns respectively.

The diameter and morphology of the gold-sputtered electrospun SWNT reinforced nanofibers were determined and examined by SEM. The average fiber diameter and its distribution were determined based on measuring 100 randomly selected fibers obtained from each spinning condition. The transmission electron microscopy (TEM) images were obtained using a JEOL-2010F (200 kV) with a point-to-point resolution of 0.23 nm. The samples were prepared by drawing out small fibrils from the silk assemblies (yarns) with the aid of tweezers and placed on lacey carbon coated copper grids. Figure 42a-b show a photograph of the Joel JEM-2010F field emission microscope and the copper grid employed in preparation of the nanofibers for viewing.
Raman spectroscopy, FTIR and wide angle x-ray diffraction were used to elucidate the secondary structure, chemical composition and crystallinity of the SWNT reinforced silk fibers. At least five spectra were recorded per sample and data are based on averages. The laser spot size and power employed in the Raman measurements were one µm and 50-100 % respectively. Deconvolution of the Raman spectra was done by peak fitting application provided by GRAMS-32 software, which uses an iterative fitting of Gaussian functions with the data minimized by the chi-squared criterion. The mechanical properties of the co-electrospun SWNT-silk fibers were measured on the KES-G1 Kawabata microtensile tester at room temperature. A strain rate of 0.02 sec$^{-1}$ was used in the tensile tests.
8.1.3 Results and Discussion

8.1.3.1 Fiber morphology and diameter distribution

The ESEM micrographs of electrospun 1% wt SWNT reinforced fibers (Figure 43) show that the fibers have smooth surfaces. Analysis of the cross sections of the fibers shows that they have near circular cross-sections. Random structures (nonwoven mat), aligned fibers and web-like structures can be obtained (Figure 43b).

![Figure 43. ESEM micrographs of 1% SWNT reinforced fibers: a) aligned and b) random with web like structure.](image)

The mechanism for the web formation is not yet known. It is postulated that it may be as a result of the inclusion of carbon nanotubes into the fibers. Due to the methods of dispersion of the SWNT’s by sonication and mechanical stirring, the SWNT clusters break down into small ropes. The addition of silk forms a coating around each individual rope and keeps them from aggregating or binding together. During
electrospinning, the bundles are expelled from the polymer jet under extremely high force and velocity, which causes opening of the bundles to form the web-like structure.

Fiber diameter distribution at fibroin concentration of 12 wt %, electric field of 3kV/cm and spinning distance of 7 cm is shown in Figure 44. The average fiber diameter is $147 \pm 41$ nm. The average fiber diameters for all loadings of SWNT in the silk fibers are all below the 200 nm range as shown in Table 10.

![Histogram](image)

Figure 44. Fiber diameter distribution of 1% SWNT reinforced aligned silk fibers at spinning conditions of 12 % silk fibroin concentration, 7 cm spinning distance and electric field of 3 kV/cm.
Table 10. Average fiber diameters of SWNT reinforced silk fibers.

<table>
<thead>
<tr>
<th>SWNT content (%)</th>
<th>Type of Fiber</th>
<th>Average diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>random</td>
<td>54 ± 26</td>
</tr>
<tr>
<td>0.5</td>
<td>aligned</td>
<td>83 ± 30</td>
</tr>
<tr>
<td>1</td>
<td>random</td>
<td>153 ± 99</td>
</tr>
<tr>
<td>1</td>
<td>aligned</td>
<td>147 ± 41</td>
</tr>
<tr>
<td>2</td>
<td>random</td>
<td>70 ± 33</td>
</tr>
<tr>
<td>2</td>
<td>aligned</td>
<td>55 ± 20</td>
</tr>
<tr>
<td>5</td>
<td>random</td>
<td>81 ± 33</td>
</tr>
<tr>
<td>5</td>
<td>aligned</td>
<td>35 ± 15</td>
</tr>
</tbody>
</table>

There is a general trend of the aligned reinforced fibers of having lower average fiber diameters than their random counterparts. It is believed that this is because during the electrospinning and fiber formation process, electrostatic forces stretch the fibers; and upon collection on a plate, the fibers become relaxed and tend to expand in diameter. The aligned fibers on the other hand, were collected between two points under tension and hence their relaxation and expansion values are lower, leading to lower average fiber diameters.

8.1.3.2 Raman spectroscopy

The presence of SWNT in the fibers and the secondary structure of the reinforced silk fibers were determined by Raman spectroscopy. Figure 45 shows the spectra of 0.5, 1, 2 and 5 % SWNT reinforced aligned fibers with characteristic bands at 1593, 1570
cm$^{-1}$ (Tangential Mode [TM]), 195-270 cm$^{-1}$ (Radial Breathing Mode [RBM]) and 1299 cm$^{-1}$ (Disorder Induced Mode [D]); and some minor bands attributed to the silk fibroin. The RBM bands have a diameter-dependent frequency that can be used to determine the nanotubes diameters. The diameters of the SWNT range from 0.84-1.13 nm as determined from their RBM bands [179]. The TM band is derived from the in-plane Raman-active mode in graphite and can be used to differentiate metallic from semi conducting nanotubes. The Raman spectra show that the SWNT are semi conducting in nature. The D band on the other hand, originates from double resonance mechanism that couples electrons and phonons [180]. The D band provides information about the crystalline quality of the samples i.e. disorder and defects in the sp$^2$ carbon material and is a useful probe for measuring degrees of structural disorder in the nanotubes [181].

Figure 45. Raman spectra of pristine SWNT and aligned electrospun silk nanofibers with 0.5% to 5% of SWNT
The D-band occurs in the pristine SWNT at 1294.9 cm\(^{-1}\) and can be assigned to defects and tube curvature. It may also originate from small amounts of amorphous carbon remaining on the tube surface after purification, because it was not present in the double-walled nanotubes samples subjected to oxidative cleaning [182]. The D-band positions are dependent on the SWNT incorporation in the silk fiber as shown in Figure 46a. As the concentration of SWNT increases from 0.5%, there is a shift of D-band peak positions to higher frequencies. The D-band increases up to 2% SWNT loading (1299.8 cm\(^{-1}\)) after which further increases in the % of SWNT leads to slight decreases in D-band frequency at 5% (1298.4 cm\(^{-1}\)). In comparison with the pristine SWNT, there is a downward shift in the D-band by 4.9 cm\(^{-1}\). It is not clear if the shifts observed in the D-band positions are due to scattering effects.

The RBM peaks shown in Figure 46b occur in the range of 205-267 cm\(^{-1}\). These peaks are attributed to the radial breathing mode of the nanotubes, and its frequency depends on the inverse diameter and on interaction with the surrounding media (silk). The RBM peak positions appear constant when the concentration of SWNT is increased from 0.5 to 5%. The pristine SWNT have somewhat lower RBM values in comparison with the reinforced fibers, which can be attributed to the silk-SWNT interaction.

The TM bands shown in Figure 46c also depict similar increases in peak positions as with the D-bands. As the concentration of SWNT increases from 0.5% wt, there is a shift of TM peak positions to higher frequencies. There is an increase up to 1% (1568.2 cm\(^{-1}\)\(_{\text{TM1}}\) and 1595.0 cm\(^{-1}\)\(_{\text{TM2}}\)) after which increasing the loading level of SWNT does not affect the peak position.
Figure 46. Raman spectroscopy measurements of band positions in SWNT reinforced silk fibers: a) D-band positions b) RBM1–RBM3 band positions c) TM1 and TM2 band positions.

In comparison with the pristine SWNT, there is an upward shift in the TM1 and TM2 bands by 12.7 cm\(^{-1}\) and 6.6 cm\(^{-1}\) respectively. Whether these small shifts are related to the interactions between silk and SWNT loadings or as a result of heating of the nanotubes during measurements needs further investigation.

The observed changes in the TM (1595 cm\(^{-1}\))-peak intensities of the 0.5-2% SWNT reinforced silk fibers, might suggest that the quantity of silk intercalated between the nanotubes increases with increase in SWNT content. As silk is a poor electrical conductor it may act as an insulator to the nanotubes hence reducing the TM peak
intensities. This may lead to better dispersion of the nanotubes and weaker interactions between the nanotubes. For higher concentrations (~5 %), the peak intensities do not change with increase in SWNT content, which may be an indication of saturation of the composite. It seems that higher concentrations of SWNT lead to higher aggregates, which may prevent the silk from being intercalated. While measured changes on peak positions are very small and may result from data scattering or sample heating with the laser beam, their systematic nature suggests that the observed shift might result from SWNT-polymer interactions.

Polarized Raman spectroscopy was carried out to determine the alignment of the nanotubes in the reinforced fibers. Figure 47A displays the polarized Raman spectra of 1% SWNT-reinforced fibers taken at various angle ($\theta$) relative to the incident light. When the light is polarized along the fiber axis, the intensities of all peaks are high ($\theta = 0^\circ$) and they decrease to a minimum as the value of $\theta$ increases to $90^\circ$ i.e. the polarized light is perpendicular to the fiber axis.

The decrease in intensity of the Raman signal as a function of the orientation of the polarized laser beam relatively to the fiber orientation depicts local alignment of the SWNT’s in the fibers. The ratio of the intensity of the disorder-induced (D) line to Raman-allowed (TM) line, $R = \frac{I_D}{I_{TM}}$, provides a sensitive characterization of the disorder in the fibers. Figure 47B shows a decrease in the intensity ratios of the D and TM bands with increase in orientation angle.
Figure 47. A) Raman spectra of 1% SWNT reinforced silk fiber as function of orientation of the polarized laser beam relative to the fiber orientation B) intensity ratios of D and TM bands.
Tuinstra and Koenig [183] have also shown that this relationship is inversely proportional to the in-plane crystal size \((L_a)\), an important characterization parameter for polycrystalline or disordered graphite. Figure 48a illustrates this ratio \((I_D/I_{TM})\) as function of SWNT loadings in the fibers. Figure 48a shows that the degree of disorder increases with SWNT loadings in the fibers, while Figure 48b illustrates the dependence of the crystal size \((L_a)\) on the disorder. The crystal size can be determined by the following formula:

\[
L_a = 4.4 \left( \frac{I_D}{I_{TM}} \right)^{-1}
\]

(17)

where \(L_a\) is the in-plane crystallite size in nm. It’s seen that the crystal size decreases with increase in disorder.
Introduction of nanotubes into silk leads to a shift in the D-band positions from 1294.8 to 1295.8 cm\(^{-1}\) (0.5 % SWNT). There is an increase in the shift up to 2 % nanotube loading (1299.8 cm\(^{-1}\)), after which further increases in the content of SWNT leads to a decrease in the D-band positions. When the nanotubes are incorporated into
the silk, the bands are shifted towards higher frequencies, especially the lower frequency peaks (RBM). The shifts observed can be explained by the intercalation of the silk into the SWNT bundles. The silk exerts pressure on the individual tubes, thereby increasing the peak positions. Further, the quantity of silk intercalated between nanotubes could lead to further opening of the SWNT bundles, thereby enhancing the formation of nucleating sites to favor the crystallization process. These results may suggest that there is some reduced vibrational freedom of the silk polymer chains as a consequence of the intercalation of the silk matrix.

8.1.3.4 Transmission electron microscopy

The presence of SWNT in the silk fibers and their orientation can be observed by TEM. Analysis of a large number of fibers shows that tubes were aligned along the fiber axis, as previously reported for PAN fibers [105]. Figure 49 shows the TEM image of a broken reinforced fiber. The micrograph shows that the SWNT’s are imbedded in the silk fibers and not deposited on the surface during the electrospinning process. The high-resolution TEM image shows that the nanotubes are in the form of bundles and the average diameter of the nanotubes is ~ 1 nm.

The SWNT’s may be aligned along the silk fiber axis by three mechanisms: 1) charge 2) confinement and 3) flow. Due to the large electrostatic fields used in the electrospinning process, a favored orientation of the SWNT along the fiber axis is achieved. As a result of the large aspect ratio of the nanotubes ($L/d = 1 \mu m / 1 \text{ nm} > 1000$) and the nanoscale diameter of the electrospun silk fibers, a relative orientation is further attained due to confinement as there are not too many permissible
orientations in the fiber that will allow movement of the nanotubes other than along the fiber axis.

Figure 49. a) TEM micrographs of a SWNT reinforced silk fiber; (b) is a high-resolution TEM image of the area squared in (a) showing two single wall nanotubes protruding out of the silk fiber.

In addition, during the fiber formation stage in the electrospinning process, the silk matrix undergoes a drawing effect, which partially induces alignment of the SWNT along the flow direction (fiber axis). A coupling of these three mechanisms leads to a preferred alignment of SWNT’s along the electrospun silk fibers. Figure 50 shows a schematic of the flow and confinement induced alignment of SWNT’s in a silk fiber.
8.1.3.5 FTIR spectroscopy

The conformation of the aligned SWNT reinforced fiber was determined by FTIR. The absorption spectra were carried out on as-spun nanofibers and SWNT reinforced fibers at different nanotubes loading levels (concentrations). The spectra in Figure 51, shows the fibers have random coils with strong absorption bands in the 1640-1690 cm\(^{-1}\) range (amide I), 1520-1570 cm\(^{-1}\) range (amide II), and an amide (III) band at 1260 cm\(^{-1}\). The \(\beta\)-sheet bands of the amide I group are noticeable at 1620, 1632 and 1651 cm\(^{-1}\) respectively [151]. The spectra indicate that there are no significant
conformational differences between 0.5, 1, 2 and 5 % SWNT reinforced fibers. There are no significant differences in the major bands; however there are slight differences in peak intensities, which indicate changes in the amount or concentration.

![FTIR spectra](image)

Figure 51. FTIR spectra of silk fibers: a) as-spun b) 0.5 % SWNT-silk c) 1% SWNT-silk d) 2% SWNT-silk and e) 5% SWNT-silk.

8.1.3.6 Structural analysis by WAXD

An XDS 2000 WAXD was employed to determine the crystalline structure of the electrospun-unreinforced fibers and SWNT reinforced fiber. Figure 52 shows the WAXD patterns of the non-reinforced and SWNT reinforced fiber. The SWNT reinforced fiber as well as unreinforced fiber were characterized by peaks at 15.4,
18.3, 19.9, 26.9 and 29.7° corresponding to silk (I) and silk (II) crystalline d-spacings of 0.57(I), 0.49(II), 0.45(I), 0.33(I) and 0.30(I) nm respectively.

Figure 52. WAXD patterns of a) as-spun silk b) 2% SWNT-silk fibers c) 1% SWNT-silk fibers d) 0.5% SWNT-silk fibers.

Figure 53 depicts the crystallinity of the SWNT-silk and as-spun plotted against the volume fraction of SWNT in each fiber. The volume fraction was calculated from the
mass fraction using the densities, $\rho = 1500 \text{ kg/m}^3$ for SWNT and $\rho = 1300 \text{ kg/m}^3$ for silk. It can be seen that there is a linear increase in crystallinity by reinforcing the silk fibers with 0.5-2 % volume of SWNT’s. This might suggest that SWNT may induce nucleation and crystallization of silk.

Figure 53. Crystallinity increase of reinforced silk fibers versus volume fraction of single wall carbon nanotubes.

8.1.3.7 Mechanical analysis by tensile tests

The mechanical properties of the electrospun SWNT-silk and as-spun fibers were determined utilizing a KES-G1 Kawabata microtensile tester. Aligned fibers were collected as shown in previous sections, as previous research [160] have shown that random fibers do not possess satisfactory mechanical properties. The aligned fibers
were carefully rolled into yarns with as few twists as possible and mounted on a paper sample holders (3 cm gauge length). A strain rate of 0.02 sec\(^{-1}\) was used in the tensile tests. Figure 54 a-b shows the typical stress-strain curves for SWNT reinforced fiber and the average plots respectively, while Table 12 shows the averages of the mechanical properties.

The average initial Young’s modulus of the as-spun aligned fibers as calculated from the slope of the initial part of the stress-strain curve was \(~312\) MPa at a breaking strain of \(~4.6\)% . The 0.5 and 2 % SWNT reinforced fibers have a lower modulus in comparison with the aligned as-spun fibers. However, the 1 % SWNT reinforced fibers show a much more promising result. Reinforcing the fibers with 1 % SWNT can lead to increases in the initial modulus in the range of 110-460 %. We hypothesize that the lower mechanical properties of the 0.5 and 2 % SWNT reinforced fibers are due to poor dispersion (distribution) and imperfect alignment of the nanotubes along the fiber axis within the nanofibers. Nanotube agglomerates can act as critical flaws decreasing the ultimate strain and strength of the fibers. Better elastic properties for the 1 % SWNT reinforced fibers could be due to a more uniform distribution and alignment of SWNT along the nanofibers. This fiber also had the maximum diameter (Table 10).
Figure 54. Stress-strain curves of aligned SWNT reinforced silk fibers: a) typical samples b) average plots.
Table 11. Mechanical properties of aligned reinforced and as-spun fibers. Values are means of 5 measurements.

<table>
<thead>
<tr>
<th>Type of Fiber</th>
<th>Initial Young’s Modulus (MPa)</th>
<th>Max Stress (MPa)</th>
<th>Max Strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aligned silk fibers</td>
<td>312 ± 193</td>
<td>19.1 ± 10.3</td>
<td>5.8 ± 1.7</td>
</tr>
<tr>
<td>0.5% SWNT reinforced</td>
<td>180 ± 108</td>
<td>2.84 ± 1.1</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>1% SWNT reinforced</td>
<td>705 ± 698</td>
<td>7.4 ± 2.5</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>2% SWNT reinforced</td>
<td>285 ± 112</td>
<td>3.7 ± 1.3</td>
<td>1.8 ± 0.5</td>
</tr>
</tbody>
</table>

Summary:

In conclusion, it has been shown for the very first time the feasibility of fabricating a nanocomposite fiber comprising *Bombyx mori* silk and SWNT through the electrospinning process. The conformation of the silk is conserved as shown by the FTIR results. The WAXD results show evidence of the possible nucleating effect of the SWNT’s on the silk by the linear increase in crystallinity in the low volume fraction region. The mechanical properties of the SWNT-reinforced fibers show an increase in Young’s modulus up to 460% in comparison with as-spun fibers, but at the expense of the strength and strain to failure.

Despite these promising results, it is well understood that the mechanical properties of the SWNT reinforced silk fibers are still not fully realized due to the nanotubes not being evenly distributed and aligned throughout the fibers. Further study is needed for improving the dispersion, achieving a uniform distribution of
SWNT’s in the electrospun fibers, having a better understanding of the SWNT- silk interactions, and identifying the optimal interfacial stress transfer conditions of the SWNT-silk composite in order to fully realize their properties and potential applications.

8.2 Chemical treatment of electrospun fibers

8.2.1 Background and Significance

Another method of improving the mechanical properties of the electrospun silk fibers is by inducing conformational transition of random coils to β-sheets (crystallites) through post processing techniques. The highly organized β-sheet crystalline regions are suggested to confer strength and toughness of the silk fibers, while the amorphous regions (random coils) confer elasticity. Such post processing techniques include mechanical (drawing), chemical (immersion in methanol) and thermal (annealing) treatments. On the other hand studies have shown that heating is not an effective method for modifying structural changes in silk fibroin solutions [184] as upon recooling, the fibroin regains 90% of the random coil structure which were converted into β-sheets, which may also be the case for annealed fibers. This reversibility is not observed for chemically induced conformational transitions. Also since the fibers are collected in random mat or yarn forms, drawing of the nanofibers results in the individual fibers having different draw ratios. Chemical treatment of silk fibroin with organic solvents such as methanol is highly effective in the crystallization of fibroin from random coils to β-sheet [185, 109, 137].
The goal of this section was to determine the mechanical properties of electrospun silk fibers in a random (mat) and aligned (yarn) form and to further evaluate the influence of the methanol treatment on the morphology, secondary structure, crystallinity and mechanical properties of the electrospun nanofibers.

8.2.2 Materials and methods

8.2.2.1 Preparation of regenerated silk fibroin and electrospinning process
Methanol and all other materials used in this study were purchased from Sigma-Aldrich. The regenerated silk was prepared and electrospun into nanofibers as mentioned in the previous chapters. Electrospinning was carried out utilizing an 18-G needle at a 45° spinning angle. The tip-to-collection plate was 7cm and voltage of 30 kV was employed. Random fibers and aligned fibers were collected to make mats and yarns respectively.

8.2.2.2 Post processing treatment (methanol immersion)
Electrospun random mats and yarns were immersed into a 90/10 (v/v) methanol/water solution for 30 minutes at room temperature. The electrospun samples were then dried at ambient conditions and characterized.

8.2.2.3 Characterization
The morphology and fiber diameters of the gold-sputtered as-spun and methanol treated fibers were examined and determined by SEM. Measurements of 100 random
fibers were used to determine the fiber diameter and distribution. Raman spectroscopy, WAXD and FTIR spectroscopy were used to elucidate the secondary structure, chemical composition and crystallinity of the fibroins. The mechanical properties of the as-spun and methanol treated mats and yarns were measured on a KES-G1 microtensile tester. The samples were tested at room temperature (23 °C).

8.2.3 Results and Discussion

8.2.3.1 Fiber morphology and diameter distribution

The morphology and diameter of the as-spun fibers before and after methanol treatments are shown in the SEM micrographs in Figure 55. The fibers are uniform and appear circular in cross-section (not shown in the figure). The average fiber diameters of the as-spun random mats (AsR), as-spun yarn (AsY), methanol treated mat (MR) and methanol treated yarn (MY) are 72 ± 25, 195 ± 60, 231 ± 70 and 232 ± 66 nm respectively. The difference in average fiber diameters of AsR and AsY is due to the electrospinning parameters. In order to collect aligned fibers for the yarn assembly, the spinning distance was altered (shortened) in comparison with the random mat, therefore the aligned fibers comprising the yarn, are larger in diameter in comparison with the random mat fibers. In addition, the aligned fibers were not collected on a plate but between two points. It’s seen from the ESEM micrographs, that immersion of the fibers in methanol leads to swelling of the fibers, thus increasing their diameters. The methanol treated fibers are no longer smooth in comparison with the as-spun fibers and have a grainier or rougher surface. The
change in fiber diameter for AsR after methanol treatment is larger than AsY. An explanation for this may be due to AsR being made up of random fibers (having a lower molecular orientation) and once their formed and collected on the plate, the molecular chains (and hence fibers also) have the ability to relax, which leads to further misalignment of chains and higher amount of voids in the fiber.

The AsY’s on the other hand, as mentioned earlier are collected in a fashion between two points which lead to higher molecular orientation (due to stretching between the points) that is maintained, and hence have less voids in the fibers. On immersion in methanol, the fibers swell probably due methanol diffusing into the voids of the fibers. Since there are more voids in the AsR fibers, there is more swelling and hence a larger change in fiber diameter after immersion in methanol.
Figure 55. ESEM micrographs of electrospun fibers before and after methanol treatment.

8.2.3.2 FTIR spectroscopy

FTIR spectroscopy was utilized to follow the conformational changes that occur during methanol treatment of the as-spun fibers. The as-spun fibers show strong absorption bands in the 1640-1690 cm\(^{-1}\) range (amide I), 1520-1570 cm\(^{-1}\) range
(amide II), and an amide (III) band at 1260 cm⁻¹. The β-sheets bands of the amide I group are noticeable at 1621, 1632 and 1651 cm⁻¹ respectively as depicted in Figure 56. The spectra of the methanol treated fibers are similar to that of the as-spun fibers with slight shifts in absorption bands.

Figure 56. FTIR spectra of as-spun and methanol treated fibers.

However, there are significant differences in the spectra, with the methanol treated fiber bands having higher intensities and sharper peaks as can be seen for the β-sheet bands of the amide I group. The amide I and amide II crystallinity indexes of the as-spun and methanol treated yarns, calculated as the intensity ratio of the 1632 β-sheet /1680 cm⁻¹ random and 1520 β-sheet /1539 cm⁻¹ random bands, are 33% (I) (AsY), 48% (I)
(MY), 28% (II) (AsY) and 37% (II) (MY) respectively (Figure 57.). These results indicate an increase in the $\beta$-sheet content of the methanol treated fibers.

![Comparison of amide groups](image)

Figure 57. Crystallinity indexes of as-spun and methanol treated yarns, calculated as the intensity ratio of the 1632 $\beta$-sheet /1680 cm$^{-1}$ random, 1520 $\beta$-sheet /1539 cm$^{-1}$ random and 1261 $\beta$-sheet /1289 cm$^{-1}$ random bands.

8.2.3.3 Structural analysis by WAXD

WAXD was used to determine the crystalline structure of the as-spun and methanol treated fibers. Figure 58 shows the diffraction patterns for the as-spun and methanol treated fibers. All the fibers are characterized by the presence of five diffraction peaks at 15.4, 18.3, 19.9, 26.9 and 29.8°, corresponding to silk (I) and silk (II) crystalline d-spacings of 5.8 (II), 4.8 (II), 4.4 (I), 3.3 (I), 3.0 (I) Å respectively.
Figure 58. WAXD of silk fibers: a) methanol treated silk b) as-spun silk.

This indicates that the fibers are comprised of mixtures of random (silk (I)) and β-sheet (silk (II)) crystallites. The diffraction pattern for the as-spun fiber show that the fiber is semi-crystalline in structure, consisting of β-sheet crystallites dispersed throughout an amorphous matrix (45 % crystallinity). This result is contrary to earlier reports by Kaplan et al [161] that the as-spun fibers are amorphous in nature due to the slow rate of crystallization of the fibroin from water. The difference in the observed crystallinity of the fibers is probably as a result of the different solvents employed in the electrospinning process. Our process utilizes formic acid which is known to reduce the formation of fibroin aggregates or entanglements and induces long range ordered crystallites (β-sheet crystallization) [151].
The peak intensities of the bands indicate that silk II structure is the predominant conformation particularly after methanol treatment as shown by the fibers crystallinity in Table 12. A comparison of the crystallinity of the as-spun fiber and methanol treated fibers shows a 10% increase, indicative of higher β-sheet content. This increase in crystallinity is 5% higher than in the as received (natural) fibers.

Table 12. WAXD pattern results for silk fibers.

<table>
<thead>
<tr>
<th>Type of silk fibers</th>
<th>2θ (degrees)</th>
<th>d-spacings (Å)</th>
<th>Crystallinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As received</td>
<td>15.5 18.4 20.0 26.9 29.8</td>
<td>5.7 4.8 4.4 3.3 3.0</td>
<td>49.3</td>
</tr>
<tr>
<td>Degummed</td>
<td>15.4 18.3 19.9 26.9 29.8</td>
<td>5.8 4.8 4.5 3.3 3.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Electrospun (aligned)</td>
<td>15.4 18.3 19.9 26.9 29.7</td>
<td>5.8 4.8 4.5 3.3 3.0</td>
<td>45.3</td>
</tr>
<tr>
<td>Methanol treated</td>
<td>15.5 18.4 20.0 27.0 29.8</td>
<td>5.7 4.8 4.4 3.3 3.0</td>
<td>55.1</td>
</tr>
</tbody>
</table>

8.2.3.4 Raman spectroscopy

The conformational changes of the secondary structure of the as-spun fibers, which occur during the methanol treatment, were analyzed by Raman spectroscopy. The spectra for as received, degummed, as-spun and methanol treated fibers are shown in Figure 59. All the fibers display characteristic conformational bands in the range 1650-1667 cm⁻¹ and 1241-1279 cm⁻¹ which correspond to amide I and complex
amide III respectively [157]. The amide I band is a result of the stretching of $C=O$ bonds, while amide III band is a result of stretching of the $C-N$ bonds.

The amide I (random coil) pristine band was observed at 1669-1652 cm$^{-1}$ and the amide III ($\beta$–sheet) pristine band at 1232 cm$^{-1}$. These well defined bands were chosen because they give a clear indication of changes in the secondary structures from random to $\beta$–sheet. The peak position of the amide I band, because of its sensitivity to local protein structure, is used to reveal the most abundant secondary structure present in the fibers. An increase in amide I intensity is an indication of decrease in
alignment of the polypeptide backbone, while an increase in amide III intensity is an indication of increase in alignment of the polypeptide backbone and bonds.

The Raman spectra of the as-spun fibers are essentially the same as that of the methanol treated fibers with some shifts in peak positions and intensities. A comparison of the intensities of the amide I (1666 cm$^{-1}$, random) to amide III (1232 cm$^{-1}$, β-sheet) ratio of the as-spun fibers and the methanol treated fibers shows a slight decrease of 6%, which is an indication that the methanol treated fibers have a higher β-sheet content than the electrospun fibers. The increase in the amide III band intensity due to the methanol treatment of fibers is also a strong indication of a preferred alignment of the polypeptide backbone.

8.2.3.5 Mechanical analysis

The mechanical properties of the as-spun and methanol treated fibers were determined utilizing a Kawabata KES-G1 microtensile tester. The test samples were in mat (random fibers) and yarn (aligned fibers) forms. The mat fibers were measured in strips of 0.5 by 4 cm while the yarns were 4 cm in length. Both types of samples were mounted on paper sample holders (3-cm gauge length). A strain rate of 0.02 / sec, sensitivity setting of 5 and frequencies of 50 Hz were used in the tensile tests. The stress-strain curves were recorded under ambient conditions. The results of the experiment were computed in load (gram force) vs. displacement. Figures 60a-b shows the average stress-strain curves for methanol treated mats and yarns.

A comparison of the as-spun mat with the methanol treated mat shows a tremendous increase in initial Young’s modulus from an average value of ~ 560 MPa to 2390
MPa (a 4 fold increase). After methanol treatment of the mats, there is a reduction in the strain by 7 fold (86%).

A comparison of the yarns shows the same trend. There is an increase in the maximum stress and a 12-fold increase in initial Young’s modulus from ~312 to 3800 MPa. As in the case of the methanol treated mats, there is a reduction in the strain by nearly 4 fold (74% reduction). Table 13 shows a summary of mechanical properties of the tested silk fibers.

Table 13. Mechanical properties of electrospun silk fibers.

<table>
<thead>
<tr>
<th>Type of Fiber</th>
<th>Initial Young’s Modulus (MPa)</th>
<th>Max Stress (MPa)</th>
<th>Max Strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random silk fibers</td>
<td>561</td>
<td>7.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Aligned silk fibers</td>
<td>312 ± 193</td>
<td>19.1 ± 10.3</td>
<td>5.8 ± 1.7</td>
</tr>
<tr>
<td>Methanol treated silk (random)</td>
<td>2390 ± 1553</td>
<td>7.9 ± 2.0</td>
<td>0.6 ± 0.27</td>
</tr>
<tr>
<td>Methanol treated silk (aligned)</td>
<td>3806 ± 2990</td>
<td>21.9 ± 5.9</td>
<td>1.5 ± 0.8</td>
</tr>
</tbody>
</table>
A

Average stress-strain plot of methanol treated aligned fibers

B

Average stress-strain plot of methanol treated electrospun silk mat

Figure 60. Stress strain curves of silk fibers post methanol treatment: a) aligned fibers b) random mat.
It is evident that methanol treatment of the fibers leads to increases in the modulus and strength of the fibers; however, there is also a loss in the elasticity of the fibers leading to brittleness. It should be noted that the stress-strain curves for each set of tests have a large divergence resulting in a large standard deviation of the mechanical properties (modulus and ultimate tensile strength). This is a result of the high variability from sample to sample shown by silk fibers due to variations in fiber diameter along the fiber axis and slight differences in cross-sections of the fibers (oval to circular shapes) [186]. Furthermore, the mechanical properties of the mats and yarns are affected by the fiber orientation within the samples tested, slip of fibers between each other and bonding between fibers, which all lead to variations in the mechanical properties.

Summary:

The morphology and fiber diameter distribution of electrospun and methanol treated silk fibers were determined by SEM. Treatment of the as-spun fibers with methanol leads to a 1-3 fold increase in fiber diameter with grainier or rougher surfaces. The conformational changes of the secondary structure of the as-spun fibers, which occur during the methanol treatment, were characterized by FTIR and Raman Spectroscopy. The spectra indicate a higher degree of molecular alignment along the fiber axis and increases in $\beta$-sheet content of the methanol treated fibers in comparison with the as-spun fibers. These results are consistent with the diffraction patterns obtained by WAXD, which depict a 10 % increase in the crystallinity of the
methanol treated fibers in comparison with the as-spun. The mechanical tests of the samples show a 4-12 fold increase in Young’s modulus and significant increases in ultimate tensile strength after methanol treatment of the fibers. The higher mechanical properties of the methanol treated fibers are probably due to a preferred alignment of the silk polypeptide backbone along the fiber axis and an increase in the $\beta$-sheet crystallites.

8.3 Heat treatment of electrospun nanofibers

8.3.1 Background and Significance

Experiments have shown that electrospinning of nanofibers does indeed lead to certain degrees of molecular orientation of the chains that comprise the fibers; however some of this orientation is lost due to relaxation processes that occur immediately after the fiber formation. The fibers need to be kept under some form of tension in order to retain the highly oriented molecular structures that form during the electrospinning process. A means of eliminating or reducing the relaxation process which leads to lower molecular orientation of chains along the fiber axis is by annealing of the fibers under tension. If the fibers are annealed under tension, it’s assumed that the molecular orientation will have a better linear pattern along the fiber axis, hence leading to better fiber mechanical properties i.e. higher modulus and strength.
8.3.2 Materials and methods

To measure the thermal properties of the electrospun silk fibers, a differential scanning calorimeter (Perkin Elmer DSC 7) and a thermogravimetric analyzer (Perkin Elmer TGA 7) attached to a thermal analysis controller TAC7/DX were utilized. The DSC and TGA were used at a heating rate of 10 °C min\(^{-1}\) under nitrogen atmosphere. The DSC and TGA experiments were carried out at temperature ranges of 30 - 300°C and 30 - 500°C respectively. In addition, the DSC was used in conjunction with an aluminum hermetic sample pan.

Aligned electrospun silk yarns were annealed under tension in an oven in air, vacuum and argon atmosphere respectively for a period of 2 hours at 183 °C.

The mechanical properties of the annealed yarns were measured using a Kawabata KES-G1 microtensile tester operating at room temperature and humidity.

8.3.3 Results and Discussion

Thermogravimetric curves of natural, electrospun, SWNT reinforced silk and methanol treated silk are shown in Figure 61. The initial weight loss observed for all samples at \(\approx 80\) °C is due to loss of moisture. The second weight loss occurs in the range of 230 to 376 °C. This can be attributed to the break down of the side chain groups of the amino acid residues, as well as cleavage of the peptide bonds.
Figure 61. Thermogravimetric curves of silk fibers: a) electrospun b) 1% SWNT-silk c) 2% SWNT-silk d) natural e) electrospun silk dried in vacuum oven.

The SWNT reinforced silk fibers showed the fastest degradation curve, which is an indication that they have lower thermal stability than the other fibers. The initial degradation temperature is at 220 °C and full degradation occurs at 345 °C (1% SWNT) and 375ºC (2% SWNT). From their degradation curves, it seems like the degradation of the SWNT reinforced fibers occurs in two distinct phases. The electrospun fibers on the other hand, have an initial degradation temperature of 230ºC and final degradation temperature of 340ºC. The natural fiber has the highest thermal stability, with onset degradation at 326 ºC and full degradation occurring at 376 ºC.

The effect of fiber size reduction and methanol immersion were evaluated on the basis of DSC measurements. Figure 62 shows characteristic DSC thermograms
(2^nd heating cycle) of electrospun, degummed and methanol-treated electrospun fibers.

![Figure 62. DSC thermograms of silk fibers: a) electrospun b) degummed and c) methanol-treated electrospun fibers.](image)

No transitions were observed upon heating to the degradation point. There were no exothermic transitions were observed for all the samples, indicating that further crystallization (β-sheet formation) induced by heat, did not occur in the fibers. There were also no detectable glass transition temperatures ($T_g$) for any of the fibers which might be an indication of either a lack of backbone flexibility of the molecular chains or loss of free volume of the large side chain groups, thereby leading to a higher $T_g$. The degradation temperatures (not shown in Figure 62) can be observed at higher temperatures (> 300 °C).
Mechanical analysis of annealed fibers:

The electrospun silk yarns were annealed under tension in an oven at 183ºC under vacuum, argon gas and air respectively. A strain rate of 0.02 sec\(^{-1}\) was used in the tensile tests. Figure 63 shows the typical stress-strain curves for the annealed yarns. The initial Young’s modulus of the yarns annealed in air as calculated from the initial slope of the stress-strain curve is about 570 MPa. This value is much higher than the as-spun yarns with an average modulus of 312 MPa; however, it’s at the expense of strength and strain to failure (1.2%). The improved, albeit low mechanical properties of these fibers may be due to oxidation of the silk in air in the oven.

The yarns that were annealed in the oven under argon gas or vacuum showed a significant increase in initial Young’s modulus with values as high as 1560 MPa and strengths of 21.9 MPa. As noted previously, this was also at the cost of extendibility. The significant improvement in the mechanical properties may be due to an increase in molecular orientation of the fibers due to annealing under tension.
Figure 63. Typical stress-strain curves of electrospun aligned silk yarns annealed at 183 °C in air, vacuum and argon atmosphere.

Summary:

The microstructure of electrospun silk yarns was characterized by TGA, DSC and microtensile testing.

The TGA results show that the natural fiber has the highest thermal stability, with full degradation occurring at 376 °C. The SWNT reinforced fibers have the lowest thermal stability which is a result of the thermal conductive properties of the nanotubes. Electrospun fibers show an intermediate thermal stability in comparison with the natural and SWNT reinforced fibers.

DSC results don’t indicate any significant differences between the electrospun, degummed natural and methanol-treated electrospun fibers. No transitions were observed upon heating to the degradation point. No glass transition exotherms and
crystallization endotherms were observed for any of the fibers, which may be due to
the high β-sheet content, reduction of backbone flexibility of the molecular chains
and large side chain groups comprising the fibers. The absence of crystallization
temperatures indicates that no modification occurs in the silk due to additional
crystallization of the fibers.

The mechanical properties of electrospun silk yarns annealed in air, vacuum and
argon atmosphere were investigated by microtensile testing. Silk yarns annealed in air
showed the lowest Young’s modulus and the annealed-in-argon yarns exhibited the
highest modulus. These results in comparison with mechanical properties of the as-
spun fibers show that annealing of the fibers, leads to significant increase in Young’s
modulus but at the expense of the strength and strain to failure.
CHAPTER 9: TRANSPORT PROPERTIES OF ELECTROSPUN
NANOFIBROUS SILK MEMBRANES

9.1 Background and Significance

Textile fibers and fabrics with their different constructions and geometries (yarns, knits, weaves etc) are normally treated with finishes that either act as lubricants, antistatic agents and protective coverings to facilitate their use in respective applications. A lot of studies have been carried out during the processing of these textile materials to characterize the wettability and structural properties of the fabrics as well as to determine how the finishes added to the materials affect their properties (mechanical and chemical) [187-189]. Various techniques have been developed to determine the wettability or wicking of droplets of such finishes on the fabrics [189-191]. However, nearly all these studies were conducted on fibers ranging from a few micrometers in diameter to larger sizes (millimeter). With the advent of the electrospinning process, which can produce fibers on a nanometer scale, there arises a need for a new methodology of characterizing the transport properties of the nanofibrous assemblies. Nanostructured materials interact with liquids in unique ways requiring the development of new modeling and characterization methods. The fibers produced depending on the specific material being electrospun can have a wide range of fabric properties such as strength, weight and porosity. When the electrospun fibers are collected in a random fashion (nonwoven mat), the fibers have a high porosity and very small pore sizes which have a wide range of applications in high performance membrane filters [110], chemical and biological protective clothing [79] and tissue
engineering uses such as fabrication of cell-growth scaffolds [112], vascular grafts, wound dressings [113] and drug delivery [115, 116].

In order for these applications to be viable, especially those that involve the transport of fluids around or through the nanofibrous assemblies, a detailed understanding of the absorption mechanism(s) is required.

The aim of this study is to determine a methodology of wetting and wicking of nanofibrous assemblies (nonwoven random mats, aligned fibers and yarns) and to determine the absorption mechanism(s) involved. It is also envisioned that the results of this study will help to determine the optimum thickness of a material required to ensure adequate absorption of fluids. The ability to provide answers to such questions as the optimum thickness of a fabric required to maximize fluid transport and minimization of air penetration through the fabric would be of great scientific interest. Determination of a method to control liquid retention within a fabric would have important applications in the textile industry.

The goal of this section of the study is to gain an understanding of wetting and fluid flow properties on silk-based nanofibrous materials and to develop experimental and theoretical tools to characterize the novel materials and their structural and transport properties.
9.1.1 Theoretical mechanisms of spreading of droplets over different materials

9.1.1.2 Spreading over solids

When a droplet of wetting fluid is brought in contact with a solid, it spreads over the solid and, at the end, forms a pancake-like nanometer thick fluid layer [192, 193]. The dynamics of droplet spreading is governed by the wetting force, which is proportional to the circumference of the droplet base, $2\pi R$, times the difference in surface energies of dry, wet solid surface and air/liquid interface as shown in the equation below and in Figure 64.

$$\Delta = \gamma_d - \gamma_w - \gamma \cos \theta$$ (18)

where $\theta$ is the dynamic contact angle, $\Delta$ is the driving/wetting force; $\gamma_d$, $\gamma_w$ and $\gamma$ are the surface energies of the dry solid, wet solid and air/liquid interfaces respectively. This wetting force is balanced by the friction force, which originates from the friction between the fluid layers in the droplet bulk and at the droplet edge. As first shown by Tanner [194] in most cases the contribution from the flow in the droplet bulk can be neglected.
Spreading over Solids

Driving force = $\gamma_{\text{dry}} - \gamma_{\text{wet}} - \gamma \cos \theta$

Resisting force $F = \eta AV/d$

$\eta$ - viscosity; $A$ – area;
$V$ – velocity; $d$ - layer thickness

Figure 64. Schematic of spreading of droplet over a solid.

Assuming the droplet profile has a small slope, the friction force can be calculated by the lubrication approximation. That is, the velocity profile at the edge is assumed to be linear. In the system of coordinates, attached to the contact line, the flow at the edge looks like that pictured in Figure 65.

In this system of coordinates, the substrate is moving with the velocity of contact line in the direction opposite to that observed during spreading. Employing the lubrication approximation and the geometrical relations in shown in Figure 66, we find that Tanner law states that the dynamic contact angle ($\theta$) is proportional to the velocity of the contact line to an exponential value of one third. This law has been proven experimentally by Tanner and other researchers [192].
Spreading over Solids. Tanner's law $\theta \propto V^{1/3}$

Inflection point is droplet fingerprint

wetting force $\sim$ friction force

wetting force $= \gamma_{\text{dry}} - \gamma_{\text{wet}} - \gamma \cos \theta \sim$

Assumption: $\theta \ll 1$, $\cos \theta \sim 1 - \theta^2/2$

$\gamma_{\text{dry}} - \gamma_{\text{wet}} - \gamma + \gamma \theta^2/2 \Rightarrow$ wetting force $\sim \gamma \theta^2/2$

friction force = viscous stress times area

$F = \eta \Lambda V/d \sim \eta V x / h = \eta \Lambda V / \theta \Rightarrow$

$V \propto \gamma \theta^3 / \eta$

Transition from plug-like to parabolic velocity profiles

$l \sim \rho U D^2 / 10 \eta$

$\rho$-density

$T = \tau_{\text{inertia}} / \tau_{\text{viscous}} = \eta / (\rho R \gamma)^{1/2} \ll 1$ inertia dominated resistance

$d(x dx/dt)/dt = 2(\gamma_{\text{dry}} - \gamma_{\text{wet}})/\rho R$,

(Bosanquet regime)

$T = \tau_{\text{inertia}} / \tau_{\text{viscous}} = \eta / (\rho R \gamma)^{1/2} \gg 1$ viscosity dominated resistance

$(8 \eta R^2) x dx/dt = 2 \gamma \cos \theta / R$,

(Lucas-Washburn regime)

Figure 65. Schematic of spreading of droplet over a solid material and equations for deriving the driving force.

Figure 66. Schematic of absorption by capillaries depicting two types of kinetics.
Sometimes, it is much easier to monitor the expansion of the droplet base. The relation between the radius of the droplet base and time can be obtained by analyzing the mass conservation relation. If the droplet volume was initially \( \Omega_0 \), it must be the same during spreading. Assuming that the droplet is slender and its profile is semispherical, we can calculate the volume of the droplet at any time as:

\[
\Omega_0 = \frac{\pi R^3 \theta}{4} = \frac{\pi R^3 (\eta R'/\gamma)^{1/3}}{4}
\]

(19)

, where the prime ('') denotes the time derivative, \( \eta \) is viscosity. Integration of Equation (19) leads to another representation of the Tanner law in the form \( R \propto t^{1/10} \). This relation can be checked easily with some image analysis system.

9.1.1.3 Spreading over porous membranes

This case is more complicated and only recently has it received much deserved attention [195-199]. To date, to the best of our knowledge, the study of the spreading of fluids over nanoporous substrates still remains untouched. Before proceeding to the analysis of images taken on droplet spreading over nanowebs, some physical mechanisms governing this phenomenon need to be considered.

Droplet spreading over porous materials is accompanied by absorption of some portion of liquid by the porous material. Assuming that all pores are cylindrical in cross section, the rate of absorption can be estimated as follows.

The driving force for absorption by a capillary of radius \( R \) is capillary force...
\[ F_c = 2\pi R (\gamma_d - \gamma_w) = 2\pi R (\gamma_d - \gamma_w) \cos \theta \]  

(20)

Assuming the droplet is sufficiently small to neglect the gravity, we can consider this wetting force as the only driving force. There are two types of kinetics associated with two different mechanisms of resistance as shown in Figure 66. If the ratio of characteristic time scales is less than 1,

\[ \frac{t_{\text{inertia}}}{t_{\text{viscous}}} = \frac{\eta}{(\rho R \gamma)^{1/2}} \]  

(21)

we have inertia dominated resistance (\(\rho\) is fluid density). The corresponding kinetics of meniscus propagation through pores is linear [200, 201].

In the opposite case, the resistance of fluid column moving through capillary is caused by the viscosity. The corresponding kinetics of meniscus propagation is of the Lucas-Washburn type [202, 203]. In this regime, meniscus velocity is inversely proportional to time by an exponential value of one half. The critical condition at which \(\eta/(\rho R \gamma)^{1/2} = 1\) provides an estimate of the smallest radius of pores, where the linear kinetics could be observed,

\[ R = \eta^2/\rho \gamma \]. For water, \(\eta= 0.01 \text{ Ps}, \rho = 1 \text{ g/cm}^3, \gamma = 70 \text{ dynes/cm}, \) we have \(R \sim 10^{-6}\) cm. Therefore, if the pore size of the material is smaller than a micron, the linear kinetics seems to be non-observable. Taking this into consideration, we can assume that in nanowebs the absorption proceeds through the Lucas-Washburn regime.

Typical experimental data are presented in Figure 67 for electrospun polyethylene oxide nanowebs. The solid dots correspond to the data points for droplet spread over thick substrates, while nodes correspond to the data points for droplet spread over thin
substrates. The Tanner law seems to work for spreading over thick substrates. In the case of thin substrates, the exponent is about 2/5.

At a first glance, the thickness of nanowebs correlates with the fiber density, namely, the tendency seems to favor the hypothesis that the longer the spinning process, the smaller the characteristic pore size of the product. This, in turn, decreases the permeability of the nanoweb resulting in almost wetting controlled kinetics of spreading, $R \propto t^{1/10}$ (Figure 67). Figure 68 depicts the schematic for liquid spreading in nanowebs.

Figure 67. The diameter change of a droplet as a function of time. Experiments were performed with hexadecane droplets on electrospun nanowebs (○ - thin and ●- thick).
In the wetting controlled regime, the droplet spreads as if the nanoweb would be a solid. In the absorption controlled regime, the droplet sends a precursor ahead of it. The propagation of the precursor of radius $L$ is driven by the capillary pressure $P_c$ that is built up at its edge ($P_c \sim \gamma/r$, where $r$ is the pore radius). There is a peculiar feature of this regime: one can predict where the contact line meets the substrate. This is the point where the pressure in the porous material is equal to the atmospheric pressure (red arrow in Figure 68).

Figure 68. Schematic of fluid distribution during droplet spread over nanowebs.
The 2/5 exponent can be explained in the following way:

If the nanoweb has a porosity \( \varepsilon \) and thickness \( H \), the mass balance equation can be written as:

\[
\Omega_0 = \pi R^3 \frac{\theta}{4} + \Omega_p = \pi R^3 \left( \frac{\eta R'}{\gamma} \right)^{1/3} \frac{1}{4} + \Omega_p,
\]

(22)

where \( \Omega_p \) is the absorbed volume. The latter is expressed as \( \Omega_p = \varepsilon \pi H L^2 \). To find \( L \), we use Darcy’s law relating the pressure drop with the length \( L \) and the velocity of the precursor propagation as:

\[
L' = kPc / [\eta \ln(L/R)L]
\]

(23)

where \( k \) is the permeability of the nanoweb, \( k \propto r^2 \), and \( r \) is the pore radius. If the difference between the precursor and droplet base is insignificant, \( L \sim R \) then Equation (23) can be integrated to give \( L = [tkPc/\eta]^{1/2} \). Substituting this result into Equation (22), we obtain:

\[
L - L_0 \propto \left[ \Omega_0 - \varepsilon \pi H (tkPc/\eta) \right]^{2/5}
\]

(24)

This result could explain the 2/5 exponent seen in the experiment, Figure 67. It is still questionable, whether the assumption that \( L \sim R \), is valid or not.

9.2 Materials and methods

9.2.1 Experimental setup

A Micro Absorb Meter device was used to measures the volume of a micro sized droplet deposited on a porous substrate as a function of time, as described earlier in Chapter 4 (section 4.1.4.7). Based on the theory of fast absorption, the method allows
us to extract the dynamic wetting properties of fluids invading micro/nanometer pores.

The wettability and rate of absorption of liquid on the silk membrane were measured using the visualization device described in the same section. Pure water and hexadecane were used as test liquids. The two liquids were chosen because they have different hydrophobic effects on the silk membrane thereby giving the ability to measure different absorption rates. The two liquids will permit the characterization of the pore structure and absorbency of the silk material.

9.2.1.1 Spinning dope preparation

Hexadecane was obtained from Sigma-Aldrich. 12-18% wt solution of *B. mori* silk in formic acid was electrospun as described in the previous chapters.

9.2.1.2 Electrospinning

The silk-formic acid solution was placed in a 5-ml syringe (18-G and 180° spinning angle). The tip-to-collection plate distance varied from 7 - 20 cm. The collection plate was covered with a Teflon fabric to facilitate easy removal of the fibers. The electric field expressed in terms of voltage/distance between the collection plate (cathode) and the needle tip (anode) ranged from 1.5 to 4 kV/cm, and the flow rate of the electrospinning process was varied from 0.8 to 1 ml/hr.
9.2.1.3 Characterization

The average fiber diameter, fiber distribution, morphology and thickness of the membranes were examined by ESEM. The wetting properties of the electrospun membranes were examined by the PC-based imaging system as described earlier in Chapter 4. Electrospun nonwoven samples of 1 cm x 0.5 cm size were mounted in front of the camera system. The focus was adjusted to the desired setting. The frame rate was adjusted during the image acquisition session accordingly, depending on the speed of the droplet motion. A droplet of solvent (hexadecane or water) was deposited on the membrane sample with a micro syringe, and the images recorded as a function of time. The volume of the droplet and wetting areas at each moment in time were obtained from the images independently. The volume of the wetted sample varies from 0.24 to 0.3 mm$^3$ as determined by the image analysis marker.

9.3. Results and Discussion

9.3.1 Fiber morphology and distribution

Typical fiber morphology for the electrospun nonwoven silk membranes at various concentrations are shown in Figure 69. The fibers are uniform and appear circular in cross section.
Figure 69. Photographs showing the structure in electrospun silk at various concentration: a) 12wt%, 3kV/cm,0.8ml/h  b) 15wt%,3kV/cm,0.8ml/h  c) 18wt%,3kV/cm,0.8ml/h d) web-like assemblies can also be formed.

It can be seen from Figure 70 that as the concentration of the silk increases, the average fiber diameter also increases. Concentrations of silk lower than 10% wt were not utilized as they resulted in bead and droplet formation. Higher concentrations than 18% wt also resulted in beads and non-uniform fibers.
Table 14 shows the average fiber diameters and thickness of the silk membranes before and after wetting with solvents, namely hexadecane and water. A general trend is noticeable for all the concentrations of spun fibers, which after wetting of the membrane with hexadecane or water, lead to swelling and increase in fiber diameter.
Table 14. Average fiber diameters and thickness of sample strips wetted with solvent.

<table>
<thead>
<tr>
<th>% wt of Silk solution</th>
<th>Wetting fluid</th>
<th>Amount of solution (ml)</th>
<th>Electrospin. flow rate (ml/hr)</th>
<th>Average diameter (nm)</th>
<th>Membrane thickness (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>none</td>
<td>0.9</td>
<td>0.8</td>
<td>46 ± 21</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>hexadecane</td>
<td>0.9</td>
<td>0.8</td>
<td>176 ± 85</td>
<td>80 ± 27</td>
</tr>
<tr>
<td>12</td>
<td>hexadecane</td>
<td>0.9</td>
<td>0.8</td>
<td>69 ± 30</td>
<td>133 ± 46</td>
</tr>
<tr>
<td>12</td>
<td>water</td>
<td>0.9</td>
<td>0.8</td>
<td>66 ± 29</td>
<td>199 ± 35</td>
</tr>
<tr>
<td>15</td>
<td>none</td>
<td>0.9</td>
<td>0.8</td>
<td>138 ± 56</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>hexadecane</td>
<td>0.35</td>
<td>0.8</td>
<td>98 ± 36</td>
<td>162 ± 176</td>
</tr>
<tr>
<td>15</td>
<td>hexadecane</td>
<td>0.9</td>
<td>0.8</td>
<td>81 ± 28</td>
<td>89 ± 24</td>
</tr>
<tr>
<td>18</td>
<td>none</td>
<td>0.9</td>
<td>0.8</td>
<td>196 ± 88</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>hexadecane</td>
<td>0.9</td>
<td>0.8</td>
<td>276 ± 79</td>
<td>31 ± 16</td>
</tr>
<tr>
<td>18</td>
<td>hexadecane</td>
<td>0.9</td>
<td>0.8</td>
<td>299 ± 86</td>
<td>24 ± 17</td>
</tr>
<tr>
<td>18</td>
<td>hexadecane</td>
<td>3</td>
<td>0.8</td>
<td>219 ± 90</td>
<td>74 ± 23</td>
</tr>
</tbody>
</table>

The fiber diameter distribution curve for the 12% wt solution spun fibers and membrane wetted with hexadecane and water are shown in Figure 71. The water wetted fibers swell less than the hexadecane wetted fibers which might be as a result of the hydrophobic nature of silk.
Figure 71. Fiber diameter distribution curves for 12% solution spun fibers: a) water wetted b) hexadecane-wetted and c) as-spun fibers.

The fiber diameter distribution of the 15% solution spun fibers and hexadecane wetted fibers are shown in Figure 72. The same trend is seen for the 18% solution spun fibers, with the diameter of the hexadecane-wetted fibers being larger than the as-spun fibers (Figure 73).
Figure 72. Fiber diameter distribution curves for 15% solution spun fibers: a) hexadecane-wetted and b) as-spun fibers.

A comparison of hexadecane wetting on thin (23.6 µm) and thick (31.2 µm) fibers is shown in Figure 74. It seems that the thinner membranes absorb more hexadecane depicted by the larger average fiber diameter (~ 22 nm increase).
Figure 73. Fiber diameter distribution curves for 18% solution spun fibers: a) hexadecane-wetted and b) as-spun fibers.

Figure 74. 18% solution spun fiber diameter distribution curve comparison of: a) thick and b) thin hexadecane wetted membranes.
9.3.2 Absorption and wetting mechanisms of electrospun nanofibrous membranes

To characterize the wettability and structural properties of the electrospun silk membranes, a liquid droplet was delivered to a certain point of the membrane, where there were the least number of beads or fused fibers (defects). The position of the liquid front was traced as a function of time and the relationship between the liquid front position (droplet radius) and the time was plotted as derived by De Gennes [192].

The droplet generated by the micro syringe is absorbed by the silk membrane. Images obtained by the high-speed digital CCD camera of the hexadecane droplet absorption process are shown in Figure 75. The images are of the changes that occur on the surface of the membrane. Figure 75(a) shows the silk membrane before a droplet of hexadecane is put on the surface i.e. the droplet is still at the tip of the micro syringe. The subsequent figures (b-d) show the droplet of hexadecane being absorbed on the membrane at different time scales. It was observed that the area/volume occupied by the liquid increases with time i.e. spreading of the droplet. The initial absorption is immediate and very fast as shown by the time scales in the figures.
Figure 75. Photographs showing absorption of hexadecane on silk membrane at different time (a) 0 s (b) 0.17 s (c) 0.33 s (d) 1s.
Figure 76 (a-d) shows the cross sectional images of the absorption of hexadecane on silk membrane captured by the high-speed camera.

Figure 76. The cross sectional view of absorption processes (a) the droplet at the end of the capillary (b) the droplet leaving the capillary (c) the droplet penetrating the membrane (d) the droplet disappearing.
The time for complete absorption of the hexadecane droplet into the depth of the membrane is very fast (few tenths of a second to a second) as shown in Figure 75 and Figure 77.

Figure 77. Photographs of wetting and spreading of hexadecane on silk membrane over time. The electrospinning parameters are: silk concentration- 18% wt, flow rate- 0.8ml/h, spin time- 180 minutes.

The rate of absorption also depends on the membrane thickness. A comparison of the rate of absorption was conducted on two membranes whose thickness was varied by
changing the collection time for accumulation of fibers. The collection time for the membranes shown in Figure 78, were 30 and 60 minutes respectively.

Plots of the liquid fronts (radius) versus time are shown in Figure 78. The relationship between the droplet radius and time as derived by De Gennes, shows that the spreading mechanism of a liquid droplet on a solid surface follows a power law relationship as shown in the following formula:

\[ R = A t^x \]  \hspace{1cm} (25)

, where \( R \) is the radius of the liquid front, \( A \) is a constant, \( t \) is the time of movement of liquid droplet front and \( x \) is an exponential number. It’s seen that for the samples electrospun for 30 minutes from a 15% wt solution (Figure 78a), that the exponential value of \( x \) is 0.1465 which suggests that the dominant mechanism for the fluid transport might be a Lucas-Washburn absorption regime.

For the membrane spun for a longer period of time (Figure 78b), which corresponds to a thicker sample, the \( x \) value is 0.0965 which suggests a Tanner spreading mechanism. This means that there is a significant difference in the transport mechanism due to changes in the membrane thickness.

Images of membranes electrospun from a higher concentration of silk (18 % wt) with collection times of 60 and 180 minutes were also taken. Plots of droplet radius versus time for the membranes are shown in Figure 79. A similar trend is observed as with the 15% wt samples. The \( x \) value for the thin and thick membrane samples are 0.1299 and 0.0689 respectively.
The transport mechanism of the thin sample exhibits a Lucas-Washburn (absorption) regime while the thick sample is governed by Tanner spreading (wetting) regime.

Figure 78. Plots of radius change of hexadecane droplet versus time on: a) thin and b) thick silk membranes electrospun from 15% wt solution.
Figure 79. Plots of radius change of hexadecane droplet versus time on: a) thin and b) thick silk membranes electrospun from 18% wt solution.
These results can be explained thus: the thin membranes have Lucas–Washburn absorption regime due to their loose fiber assemblies and microstructure (large pore sizes). The membrane structure is not compact and doesn’t give much resistance to the liquid droplet movement; hence the flow of the liquid isn’t hampered in its surface (spreading) and depth (absorption) motion. This results in a high permeability of the membrane leading to absorption-controlled kinetics of wetting. The thick membrane on the other hand, has a more compact and rigid structure of fiber assemblies (more physical entanglements between fibers and hence smaller pore sizes), which gives additional resistance to the liquid droplet flow into the depth of the membrane but allows for flow across the surface of the membrane. The additional resistance may be due to friction between increased fiber layers and an increase in number of fibers, which can be fused together as shown in the SEM micrographs after wetting of the membrane (Figures 80, 81).

(a) pre-wetting                (b) after wetting

Figure 80. Micrographs showing the morphology of silk membrane: (a) surface view of as-spun membrane and (b) cross-section of membrane after wetting with hexadecane.
Therefore the governing mechanism for transport of the hexadecane droplet on the thick silk membrane is that of spreading.

As mentioned earlier, wetting of the silk membrane with hexadecane leads not only to swelling of the nanofibers, but also fusion of the fibers as shown in the above micrographs. The reason for the swelling and fusion may be due to the hexadecane penetrating the pores of the nanofibers and becoming a bonding material for the nanofibers. It may also be as a result of the high vacuum employed during characterization of the membrane by ESEM. It’s still not clear what the effect(s) of swelling and fusion of fibers might have on the transport properties of the silk membrane.

Figure 81. Micrographs showing the surface morphology of silk membrane: (a) as-spun and (b) fused fibers after wetting with hexadecane.
Wetting experiments carried out using water droplets showed that the silk membrane has poor wetting properties. A droplet deposited on the membrane did not spread nor was it absorbed and remained on the surface throughout the observation time of over 15 seconds as shown in Figure 82.

Figure 82. The cross sectional view of absorption of a water droplet on electrospun silk membrane processes depicting poor wetting properties: image taken after 1 s and 15 s.

Summary:

The kinetics of droplet spreading over nanofibrous substrates seems challenging. The traditional approaches to describe droplet spreading must be modified to include specific kinetics of absorption by nanopores, which is not yet explored. An attempt was made to shed a light on this phenomenon and to bridge the gap between the traditional Tanner’s description of droplet spreading over solid materials and
experiments done within this study with nanoporous materials. The 2/5-exponent of
droplet expansion finds its explanation within the Tanner approach augmented by
absorption equations and assumption that the precursor is very short. This idea seems
promising but it must be supported by future experimental analysis of this problem. It
is hoped that this idea can be exploited and used in the future for characterization of
nanowebs with respect to permeability and absorbency. Monitoring the change of the
droplet profile during spreading and using some model similar to afore mentioned
equations will give the ability to analyze quantitatively the physical and chemical
properties of electrospun nanowebs. Preliminary wicking experiments and theoretical
analysis, done in collaboration with TRI show that the nanowebs can be considered as
a laminated membrane with surface transport layers being composed of a loose
system of grooves and open pores, and the core with much dense pore network [204].
As shown by ESEM micrographs, the pores in the dense core are about 1 micron,
while analysis of experimental data suggests that the surface transport pores are about
27 microns. The liquid is transported mostly through surface layers driven by a high
capillary pressure built up by the core pores.

Further work still needs to be done in clarifying the effect of swelling and
fusion of the nanofibers on the transport properties. The samples used in this study
didn’t have uniform membrane thicknesses; therefore a more robust system of
fabricating uniform membrane thickness needs to be developed. It seems that there is
a transition phase at which the mechanism changes from being governed by Tanner’s
law (spreading) to a Lucas-Washburn regime (absorption). The membrane thickness
at which this transition occurs needs to be determined.
CHAPTER 10: CONCLUSIONS

- The electrospinning of *B. mori* silk fibroin in formic acid was processed and fiber diameters ranging from 12 to 1500 nm were obtained depending on the electrospinning conditions.

- Morphology of fibers and distribution of fiber diameter were investigated varying electric field, concentration of regenerated silk solution and distance between tip and collection plate. The concentration of regenerated silk solution was the most dominant parameter to produce uniform and continuous fibers.

- Uniform fibers with diameter less than 100 nm were produced at the spinning conditions of 12–15% concentration and electric field of 3 and 4 kV/cm.

- RSM analysis was applied to the experimental results to develop a processing window which will produce nanoscale regenerated silk fibers by electrospinning process. Contour plots relating fiber diameter to electric field and solution concentration were generated for spinning distances of 5 cm and 7 cm. From the RSM models, a concentration of 8 to 10% and electric field of 4 to 5 kV/cm were found to be the sufficient condition for achieving nanofibers of diameters less than 40 nm. The difference between the experimental fiber diameters and the predicted fiber diameters is less than the standard deviation of the experimental fiber diameters.

- In order to understand the structural changes caused by the processing steps in the electrospinning process, the conformational transitions from random coil
to $\beta$-sheet characterized by the crystallinity index was determined by FTIR and the relative amounts of amide I to amide III by Raman Spectroscopy. The conformational changes that occur during the various processing steps prior to and during the electrospinning process are as follows:

- **Degummed fibroin**: the crystallinity index of the pristine and degummed fibers was the same. Degumming does not alter the secondary structure of the silk fibroin.

- **Dissolution in calcium chloride**: the crystallinity index of the silk from the fibroin solution was higher than degummed fiber indicating that calcium ion facilitates the formation of $\beta$-sheet.

- **Dialyzed fibroin**: removal of calcium ion causes a decrease in crystallinity index. The aqueous environment also facilitates the transition to random structure.

- **Dried fibroin sponge**: removal of water leads to a slight increase in the crystallinity index due to an increase in intermolecular hydrogen bonding of the fibroin.

- **Dissolution in formic acid**: formic acid increases the $\beta$-sheet crystallization and reduces the hydrodynamic radius of the fibroin molecules by increasing intramolecular hydrogen bonding of the fibroin.

- **Electrospun fibers**: the removal of formic acid and the fibroin molecular alignment, which occurs during electrospinning result in the highest observed crystallinity index.
• The characterization results show that the electrospun fiber has a non-negligible increase in the $\beta$-sheet content in comparison with the pristine fiber. Dissolution of fibroin in formic acid enhances $\beta$-sheet crystallization and may facilitate $\beta$-sheet formation in the electrospun fiber. However, the mechanical properties of the electrospun fibers are not fully realized due to lower crystallinity, degree of orientation/alignment of crystals and loss of orientation on the molecular and fibril level due to relaxation processes.

• It’s been shown for the very first time the feasibility of fabricating a nanocomposite fiber comprising of *Bombyx mori* silk and SWNT through the electrospinning process. The conformation of the silk is conserved as shown by FTIR results.

• The WAXD results show evidence of the possible nucleating effect of the SWNT’s on the silk by the linear increase in crystallinity in the low volume fraction region.

• The mechanical properties of the electrospun SWNT reinforced fibers show significant improvement with increases in Young’s modulus up to 460% in comparison with as-spun fibers, but at the expense of the strength and strain to failure.

• Despite these promising results, it’s well understood that the mechanical properties of the SWNT reinforced fibers are still not fully realized due to the nanotubes not being uniformly distributed and properly aligned throughout the fibers.
• Further study needs to be carried out not only on improving the dispersion and even distribution of SWNT’s in the electrospun fibers, having a better understanding of the SWNT- silk interactions, but also identifying the optimal interfacial stress transfer of the SWNT-silk composite in order to fully realize their potential applications.

• The morphology and fiber diameter distribution of electrospun and methanol treated silk fibers were determined by SEM. Treatment of the as-spun fibers with methanol leads to an increase in fiber diameter with grainier or rougher surfaces.

• The conformational changes of the secondary structure of the as-spun fibers, which occur during the methanol treatment, were characterized by FTIR and Raman spectroscopy. The spectra indicate a higher degree of molecular alignment along the fiber axis and increases in β-sheet content of the methanol treated fibers in comparison with the as-spun fibers. These results are consistent with the diffraction patterns obtained by WAXD, which depict a 20 % increase in the crystallinity of the methanol treated fibers in comparison with the as-spun.

• The mechanical tests of the samples show a 4-12 fold increase in Young’s modulus and significant increases in ultimate tensile strength after methanol treatment of the fibers. The higher mechanical properties of the methanol treated fibers are probably due to a preferred alignment of the silk polypeptide backbone along the fiber axis and an increase in the β-sheet crystallites.

• Heat treatment:
The TGA results show that the natural fiber has the highest thermal stability, with full degradation occurring at 376 °C. The SWNT reinforced fibers have the lowest thermal stability which is a result of the thermal conductive properties of the nanotubes. Electrospun fibers show an intermediate thermal stability in comparison with the natural and SWNT reinforced fibers.

DSC results don’t indicate any significant differences between the electrospun, degummed natural and methanol-treated electrospun fibers. No transitions were observed upon heating to the degradation point.

Silk yarns annealed in air showed the lowest Young’s modulus and the annealed-in-argon yarns exhibited the highest modulus. These results in comparison with mechanical properties of the as-spun fibers show that annealing of the fibers, leads to significant increase in Young’s modulus but at the expense of the strength and strain to failure.

10.1 FUTURE WORK AND RECOMMENDATIONS

- Reinforcement of electrospun silk nanofibers with SWNT:
  
  - Improving the dispersion and uniform distribution of SWNT in fibers.
  
  - Determining a method of quantification for the degree of dispersion SWNT-silk solution.
Optimization of the co-electrospinning process of silk and SWNT i.e. what percentage of SWNT and silk give the best reinforcement effect?

Determination of the possible role of nanotubes in nucleation and crystallization of silk.

Utilization of “nano manipulator” (Zyvex) to determine properties of individual nanofibers.

- Determination of nanofiber properties such as thermal and electrical conductivity, surface energy, degradation etc.
- Cell-scaffold interaction studies need to be carried out to determine the viability of using the scaffolds in tissue engineering applications.
- Development of a “co-spinning technology” of combining electrospun fibers with conventional spun fibers.
- Development of molecular filters for drug delivery applications.
- Transport Properties:
  - Determination of the effect that fiber diameter has on transport properties in the fiber assemblies? How does this affect cell-scaffold interaction?
  - Development of more robust system of fabricating uniform membrane thickness.
  - Determination of membrane thickness at which the dominant mechanism is governed by either spreading or absorption.
LIST OF REFERENCES


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APPENDIX A: SUPPLEMENTARY FIGURES OF EXPERIMENTS

Figure A1. Fiber diameter vs. Concentration for A) 5, B) 7 and C) 10 cm spinning distances at various electric fields of 1, 2, 3, 4, 5 and 6 kV/cm.
Figure A2. Fiber diameter vs. concentration at A) 2, B) 3, C) 4, and D) 5 kV/cm.
Figure A2. (continued)
Figure A3. Fiber diameter vs. electric field at A) 9, B) 12 and C) 15 wt % for 5, 7 and 10 cm spinning distances.
Figure A3. (continued)
Figure A4. Stress-strain plots of as received (pristine) *B. mori* silk at gauge lengths of A) 1 cm B) 2 cm C) 3 cm and D) 5 cm.
C

Tensile test of as received silk (3 cm gauge length)

D

Tensile tests of as received silkworm silk; gauge length 5 cm

Figure A4. (continued)
Determination of Weibull parameters of silk

\[ y = 1.9902x - 18 \]
\[ R^2 = 0.9012 \]

Figure A5. Determination of Weibull Parameters of Silk at gauge lengths of: A) 1 cm B) 2 cm C) 3 cm and D) 5 cm.
C

Determination of Weibull (modulus) parameters; 3 cm gauge

\[ y = 2.2892x - 21.861 \]
\[ R^2 = 0.9267 \]

\[ \ln(\ln(1/(1-F))) \]

LN (Modulus)

D

Determination of Weibull (modulus) parameters; 5 cm gauge

\[ y = 3.4677x - 33.045 \]
\[ R^2 = 0.9405 \]

\[ \ln(\ln(1/(1-F))) \]

LN (Modulus)

Figure A5. (continued)
Figure A6. Weibull plot of as received (pristine) B. mori silk

Table A1. Young’s modulus and corresponding Weibull parameter (m)

<table>
<thead>
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<th>Gauge (cm)</th>
<th>Modulus (MPa)</th>
<th>m</th>
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</thead>
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<td><strong>STDEV</strong></td>
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<td><strong>3.61</strong></td>
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</table>

\[ y = 1372.7x + 7110.5 \]
\[ R^2 = 0.7644 \]
Figure A7. Stress-strain curves of aligned electrospun silk

Table A2. Mechanical properties of electrospun aligned silk fibers

<table>
<thead>
<tr>
<th>Aligned silk fibers</th>
<th>Initial Youngs Modulus (MPa)</th>
<th>Final Modulus (MPa)</th>
<th>Max stress (MPa)</th>
<th>Max Strain (%)</th>
<th>Apparent modulus (MPa)</th>
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</thead>
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<tr>
<td>Sample 1</td>
<td>80.19</td>
<td>363.69</td>
<td>17.18</td>
<td>7.74</td>
<td>226.60</td>
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<tr>
<td>Sample 2</td>
<td>518.11</td>
<td>664.57</td>
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<td>8.04</td>
<td>483.54</td>
</tr>
<tr>
<td>Sample 3</td>
<td>414.54</td>
<td>257.02</td>
<td>14.56</td>
<td>4.72</td>
<td>307.09</td>
</tr>
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<td>Sample 4</td>
<td>630.11</td>
<td>630.11</td>
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<td>535.74</td>
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<td>11.78</td>
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<td>191.61</td>
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<td>33.28</td>
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<td>558.35</td>
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<td>Sample 7</td>
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<td>5.16</td>
<td>7.88</td>
<td>65.53</td>
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<tr>
<td>Sample 8</td>
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<td><strong>19.05</strong></td>
<td><strong>5.84</strong></td>
<td><strong>342.23</strong></td>
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Figure A8. Stress-strain plot of 0.5 % SWNT reinforced silk (aligned fibers)

Figure A9. Stress-strain plot of 1 % SWNT reinforced silk (aligned fibers)
Figure A10. Stress-strain plot of 2% SWNT reinforced silk (aligned fibers)

Figure A11. Dependence of Young’s modulus on denier of electrospun silk fiber assemblies (yarn)
Figure A12. Dependence of Young’s modulus on denier of 0.5% SWNT reinforced electrospun silk fiber assemblies (yarn)

Figure A13. Dependence of Young’s modulus on denier of 1% SWNT reinforced electrospun silk fiber assemblies (yarn)
Figure A14. Dependence of Young’s modulus on denier of 2% SWNT reinforced electrospun silk fiber assemblies (yarn)

Table A3. Mechanical properties of electrospun silk random mat

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<tr>
<th>Random silk fibers</th>
<th>Initial Young’s Modulus MPa</th>
<th>Max stress MPa</th>
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Table A4. Mechanical properties of electrospun aligned silk fiber assemblies (yarn)

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<th>Max stress (MPa)</th>
<th>Max Strain (%)</th>
<th>Apparent modulus (MPa)</th>
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Table A5. Mechanical properties of 0.5% SWNT reinforced electrospun aligned silk fibers

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Table A6. Mechanical properties of 1% SWNT reinforced electrospun aligned silk fibers.

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<th>Apparent modulus (MPa)</th>
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</thead>
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<td>635.15</td>
<td>9.68</td>
<td>1.94</td>
<td>499.22</td>
</tr>
<tr>
<td>9</td>
<td>729.68</td>
<td>1217.25</td>
<td>9.55</td>
<td>1.00</td>
<td>956.73</td>
</tr>
<tr>
<td>5</td>
<td>1903.61</td>
<td>1294.98</td>
<td>6.29</td>
<td>0.40</td>
<td>1573.77</td>
</tr>
<tr>
<td>Average of 5 samples</td>
<td>704.79</td>
<td>800.16</td>
<td>7.35</td>
<td>1.40</td>
<td>730.05</td>
</tr>
</tbody>
</table>

Table A7. Mechanical properties of 2% SWNT reinforced electrospun aligned silk fibers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Modulus (MPa)</th>
<th>Final Modulus (MPa)</th>
<th>Max stress (MPa)</th>
<th>Max Strain (%)</th>
<th>Apparent modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120.81</td>
<td>109.97</td>
<td>2.28</td>
<td>2.36</td>
<td>96.66</td>
</tr>
<tr>
<td>2</td>
<td>428.55</td>
<td>305.92</td>
<td>3.98</td>
<td>1.15</td>
<td>346.23</td>
</tr>
<tr>
<td>3</td>
<td>251.69</td>
<td>113.29</td>
<td>2.49</td>
<td>2.28</td>
<td>109.14</td>
</tr>
<tr>
<td>4</td>
<td>325.55</td>
<td>304.58</td>
<td>4.42</td>
<td>1.50</td>
<td>295.42</td>
</tr>
<tr>
<td>5</td>
<td>298.58</td>
<td>337.85</td>
<td>5.18</td>
<td>1.69</td>
<td>306.48</td>
</tr>
<tr>
<td>Average</td>
<td>285.04</td>
<td>234.32</td>
<td>3.67</td>
<td>1.79</td>
<td>230.79</td>
</tr>
</tbody>
</table>
Table A8. Mechanical properties of methanol treated electrospun random mat silk fibers.

<table>
<thead>
<tr>
<th>Methanol treated silk (random)</th>
<th>Initial modulus MPa</th>
<th>Apparent modulus MPa</th>
<th>Max Stress MPa</th>
<th>Elong at break %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>3024.50</td>
<td>1165.63</td>
<td>7.62</td>
<td>0.65</td>
</tr>
<tr>
<td>Sample 2</td>
<td>714.18</td>
<td>1182.87</td>
<td>8.36</td>
<td>0.71</td>
</tr>
<tr>
<td>Sample 3</td>
<td>4153.40</td>
<td>2488.55</td>
<td>7.30</td>
<td>0.29</td>
</tr>
<tr>
<td>Sample 4</td>
<td>3059.60</td>
<td>1495.03</td>
<td>4.58</td>
<td>0.31</td>
</tr>
<tr>
<td>Sample 5</td>
<td>222.73</td>
<td>1001.86</td>
<td>10.29</td>
<td>1.03</td>
</tr>
<tr>
<td>Sample 6</td>
<td>3162.70</td>
<td>1540.47</td>
<td>9.45</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>2389.52</strong></td>
<td><strong>1479.07</strong></td>
<td><strong>7.93</strong></td>
<td><strong>0.60</strong></td>
</tr>
</tbody>
</table>

Table A9. Mechanical properties of methanol treated electrospun aligned silk fibers.

<table>
<thead>
<tr>
<th>Methanol tested (aligned)</th>
<th>Initial modulus MPa</th>
<th>Max stress MPa</th>
<th>Max strain %</th>
<th>Apparent modulus MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1898.77</td>
<td>27.28</td>
<td>2.65</td>
<td>1029.43</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1493.24</td>
<td>15.97</td>
<td>1.41</td>
<td>1132.62</td>
</tr>
<tr>
<td>Sample 3</td>
<td>7446.48</td>
<td>27.22</td>
<td>1.06</td>
<td>2567.92</td>
</tr>
<tr>
<td>Sample 4</td>
<td>1512.92</td>
<td>23.38</td>
<td>1.82</td>
<td>1284.62</td>
</tr>
<tr>
<td>Sample 5</td>
<td>6679.50</td>
<td>15.41</td>
<td>0.67</td>
<td>2300.00</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>3806.18</strong></td>
<td><strong>21.85</strong></td>
<td><strong>1.52</strong></td>
<td><strong>1662.92</strong></td>
</tr>
</tbody>
</table>
Table A10. Mechanical properties of annealed electrospun aligned silk fibers.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Init.Mod. (MPa)</th>
<th>UTS (MPa)</th>
<th>Strain</th>
<th>Atmosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>519</td>
<td>1.3</td>
<td>0.005</td>
<td>air</td>
</tr>
<tr>
<td>Sample 2</td>
<td>617</td>
<td>9.9</td>
<td>0.020</td>
<td>air</td>
</tr>
<tr>
<td>Sample 5</td>
<td>1041</td>
<td>2.9</td>
<td>0.005</td>
<td>argon</td>
</tr>
<tr>
<td>Sample 6</td>
<td>1438</td>
<td>7.5</td>
<td>0.007</td>
<td>vacuum</td>
</tr>
<tr>
<td>Sample 8</td>
<td>1564</td>
<td>21.9</td>
<td>0.021</td>
<td>argon</td>
</tr>
<tr>
<td>Average</td>
<td>1036</td>
<td>8.7</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>STDEV</td>
<td>470</td>
<td>8.2</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>
Name: Jonathan E. Ayutsede

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Ph.D in Materials Science and Engineering, Drexel University, Philadelphia, PA, 2005.

Professional Organizations:
American Institute of Chemical Engineers
American Chemical Society
Society for the Advancement of Material and Process Engineering
Materials Research Society
The National Society of Black Engineers

Publications:
- Jonathan Ayutsede, Milind Gandhi, Sachiko Sukigara and Frank Ko. “Carbon Nanotube Reinforced Bombyx Mori Nanofibers by the Electrospinning Process” (submitted to Biomacromolecules)
- Jonathan Ayutsede, Milind Gandhi, Christopher Li and Frank Ko. “Comparative Structural Characterization of Electrospun Bombyx Mori Silk Fibers and Methanol Treated Fibers” (manuscript in progress)