EMULSION-ELECTROSPINNING OF NANOCRYSTALLINE CELLULOSE REINFORCED NANOCOMPOSITE FIBRES

by

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Abstract

Cellulose is the most abundant renewable and biodegradable natural polymer. Cellulose can release nanocrystalline cellulose (NCC). NCC is light-weight, biodegradable and strong. The strength of NCC is about 10 GPa, which is almost three times stronger than commercial high performance fibre such as Kevlar® and Spectra®. In order to realize the utmost translation of NCCs’ extraordinary properties to higher order structures, it is necessary to accomplish a well-controlled alignment and tailored distribution of NCCs within a matrix. However, it is challenging to achieve this goal since NCCs tend to agglomerate in matrix materials.

To address this problem in the present study, NCC water-in-oil (W/O) emulsions were prepared, consisting of a drop phase of NCC aqueous suspension and a continuous phase of immiscible poly (lactic acid) (PLA) solution. NCC W/O PLA emulsions were electrospun into NCC reinforced nanocomposite fibres. The concept of emulsion electrospinning of NCCs is based on that (1) NCC can be stably and uniformly dispersed in the intermediate medium water; (2) NCC aqueous suspension can be dispersed in the form of droplets into the immiscible solutions of PLA solution system; and (3) the well dispersed NCC / PLA emulsion can be electrospun into fibres.

In this work, to better control electrospinning of NCC/PLA emulsions, we started with electrospinning of W/O PLA emulsions consisting of a drop phase of distilled water and a continuous phase of hydrophobic PLA solution. This emulsion formulation for electrosp-
pinning was optimized using response surface methodology (RSM) to identify the optimal conditions for W/O PLA electrospinning. After optimizing the W/O PLA emulsion electrospinning process, the feasibility of the emulsion electrospinning of NCC W/O PLA was confirmed and the emulsion electrospun 5% NCC/ 8% PLA random fibre mats and aligned fibre yarns were collected. The distribution and alignment of NCCs in fibres were verified. The morphology, structure and properties of resultant fibres were characterized. The mechanism of the formation of fibre structure (core-shell and hollow) was also proposed and validated by the study of emulsion droplet size effect on fibre structure.
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Chapter 1  Introduction

It is imperative to decrease the world’s dependency on petroleum-based products, and reduce the pressure of waste degradation. As growing awareness of the need for sustainable development, bio-based materials are poised to become an important global research topic. A typical biomaterial is cellulose, which is the most abundant renewable and biodegradable natural polymer. It is well known that native cellulose, when subjected to strong acid hydrolysis can be hydrolyzed into nanocrystalline cellulose (NCC). NCC is light-weight and extremely strong. The strength of NCC has been reported to be 10 GPa [1]. As nanofiller, NCC has extraordinary potential to improve the strength, modulus and toughness of polymer composites. [2, 3]

The reported diameter of NCCs is generally only a few nanometers, and the length of NCCs is about hundreds of nanometers. The extreme fineness of NCC creates major challenges in handling, processing, dispersing, as well as providing favorable orientation in the composites. These issues hinder maximum translation of NCC properties to the meso- and macrostructure levels. In order to maximize the translation of properties to higher order structures, an effective dispersion process and a carrier media to assemble the NCC into higher order continuous structure is required, which allows the well-controlled alignment and tailored distribution of NCCs.

One of the effective means to carry nanoparticles and construct them into one dimensional fibrous material is electrospinning. Through this technique, nanofibres or nanoparticle-
filled nanofibres can be obtained from the polymer solutions or nanoparticle polymer suspensions. These fibres can be subsequently fabricated into yarns, fabrics, and composites for industrial use. Furthermore, the size of an electrospun fibre can be on the nanoscale, thus allowing the fibres to possess nanoscale surface texture. This leads to different modes of interaction with other materials when compared with macroscale materials. In addition to this, the ultrafine fibres produced by electrospinning are expected to have two main characteristics, a very high surface to volume ratio, and a relatively defect-free structure at the molecular level. The second property allows electrospun fibres to approach the theoretical maximum strength of the spun material, opening up the possibility of making high mechanical performance composite materials.

The electrospinning process has been used to process carbon nanotubes to overcome agglomeration and problematic alignment. [4] It has been demonstrated that the carbon nanotubes could be dispersed and aligned in a polymer solution to form nanocomposite fibres using electrospinning techniques. Results have shown that a significant reinforcing effect can be achieved with the electrospun composite nanofibres. This method effectively overcomes the concomitant problems of agglomeration and alignment associated with the nano-sized carbon tubes, problems which are similar to those encountered during the processing of NCCs. Therefore, a similar approach should be applicable to the processing of NCCs into nanofibre.

Nevertheless, although it has been confirmed that NCC/polymer can be electrospun into nanocomposite fibres in the literature [5-13], the electrospinning of NCC nanocomposite
fibres with well-controlled alignment and tailored distribution of the NCCs has not been fully achieved. The reason is that the abundant hydrogen bonds of cellulose draw the cellulose nanocrystals together to pose significant problems in their dispersion for effective processing.

Meanwhile, detailed structural examination is essential to obtain knowledge about the effect of processing route on the distribution of NCCs in the matrix. However, limited insight into the structure of NCC based nanocomposite fibre is found in the literature. This reflects the challenge in applying techniques with sufficient nanometer scale resolution capabilities on non-electroconductive, light elements and soft materials. Structural analysis of NCC nanocomposites will put strong demands on both sample preparation and instrumentation.

Accordingly, the purpose of this study is directed towards demonstrating the possibility of electrospinning NCC with an appropriate matrix to form continuous NCC nanofibres with well-controlled alignment and tailored distribution of the NCCs. In this work, the matrix polymer is poly (lactic acid) (PLA), which is biodegradable martial and from renewable sources. NCC reinforced PLA fibres will be prepared through the electrospinning of NCC water in oil (W/O) PLA emulsions. The first application of emulsion electrospinning has been focused on drug or protein delivery. Previous work has shown that emulsion electrospinning facilitates the encapsulation of hydrophilic drugs or bioactive materials inside nanofibres to avoid burst release and prolong the release duration in the drug delivery process. The emulsion system fundamentally favors the uniform distribu-
tion of one phase into another immiscible phase. We are inspired by this concept so that NCC W/O PLA emulsions will be prepared, which consist of a drop phase of NCC aqueous suspension and a continuous phase of an immiscible PLA solution. We hypothesize that the presence of an immiscible phase will not affect the stable and uniform dispersion of NCC within water, and this NCC aqueous suspension can be uniformly dispersed in the form of droplets into the immiscible solutions of PLA solution system. Therefore, we suppose that the dispersion of NCCs in the matrix can be enhanced through the uniform dispersion of emulsion droplets in the polymer solution system. We expect that NCC emulsions will be amenable to fibre-producing electrospinning with a tailored distribution and controlled alignment of NCCs to realize the reinforcing effect of NCCs. Another purpose of this thesis is to reveal the feasibility of analyzing the structures and properties of NCC/PLA nanocomposite fibres.
Chapter 2  Literature review

2.1  Structure and property of cellulose

Cellulose is the most abundant renewable and biodegradable natural polymer. Cellulose is widely found in higher plants (woods, flax, cottons and so on), in several marine animals (for example, tunicates), and to a lesser degree in algae, fungi, bacteria, invertebrates and even amoeba (protozoa). [14]

The hierarchical structure of cellulose in wood is displayed in Figure 1. In nature, cellulose is synthesized as individual molecules, which undergo spinning in a hierarchical order at the site of biosynthesis. Typically, approximately 36 individual cellulose molecules assemble and are brought together into larger units known as elementary fibrils (micelles in Figure 1), which pack into larger units called microfibrils. These microfibrils are, in turn, assembled into the familiar cellulose fibres. [15] The main function of cellulose is to act as a reinforcement material in cell wall.
The chemical structure of cellulose is shown in Figure 1. Cellulose is an organic compound with the formula \((\text{C}_6\text{H}_{10}\text{O}_5)_n\), a polysaccharide consisting of a linear chain of several hundred to over ten thousand \(\beta-(1, 4)-\text{D}-\text{glucose residues}\). This linkage pattern contrasts with that for \(\alpha-(1-4)-\text{glycosidic bonds}\) present in starch, glycogen, and other carbohydrates. All \(\beta\)-d-glucopyranose rings adopt a chair conformation, and as a consequence, the hydroxyl groups are positioned in the ring (equatorial) plane, while the hydrogen
atoms are in the vertical position (axial). This structure is stabilized by an intramolecular hydrogen bond network. Figure 2 displays the intramolecular hydrogen-bonding network in a representative cellulose structure. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen molecules on the same or on a neighboring chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength. This strength is important in cell walls, where the microfibrils are meshed into a carbohydrate matrix, conferring rigidity to plant cells.

![Intra-molecular hydrogen-bonding network in a representative cellulose structure.](image)

Figure 2 Intra-molecular hydrogen-bonding network in a representative cellulose structure.[17]

For the supermolecular structure, cellulose displays six different polymorphs namely I, II, III₁, III₂, IV₁ and IV₂ with the possibility of conversion from one form to another. The native cellulose, namely cellulose I, is constituted by a mixture of two crystalline forms called Iα and Iβ lattices. The latter, predominant in cotton and ramie, is characterized by two polysaccharide chains in a parallel arrangement resulting in a monoclinic unit cell [18, 19]. The lattice Iα is generally present in all algae and bacteria and has a triclinic unit cell structure with one polysaccharide chain. [20-22] In these two lattices, Iα and Iβ, the
conformation of the polysaccharide chains is the same although the hydrogen-bonding pattern is different. The Iα may be converted into the Iβ form by annealing the cellulose chains in the solid state. This process is irreversible because the Iβ form is more stable. [23, 24] Cellulose in regenerated cellulose fibres is cellulose II. The conversion of cellulose I to cellulose II is not reversible, suggesting that cellulose I is metastable and cellulose II is stable. With various chemical treatments it is possible to produce the structures cellulose III and cellulose IV.

Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule. Cellulose from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant fibres as well as bacterial celluloses, have chain lengths ranging from 800 to 10,000 units. Molecules with very small chain length resulting from the breakdown of cellulose are known as cellodextrins; in contrast to long-chain cellulose, cellodextrins are typically soluble in water and organic solvents.

Regarding its physical properties, cellulose is odorless, hydrophilic with the contact angle of 20–30 degree, insoluble in water and most organic solvents, and biodegradable.

Regarding the chemical properties of cellulose, it can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. Compared to starch, cellulose is also much more crystalline. Whereas starch undergoes a crystalline-to-amorphous transition when heated beyond 60-70°C in water (as in cooking), cellulose
requires a temperature of 320°C and pressure of 25 MPa to become amorphous in water.

2.2 Nanocrystalline cellulose (NCC)

2.2.1 Preparation of NCCs

Ränby reported for the first time that colloidal suspensions of cellulose can be obtained by controlled sulfuric acid-catalyzed degradation of cellulose fibres. [25-27] For the first time, the presence of aggregates of needle-shaped particles in the dried cellulose suspensions was revealed, and they had the same crystalline structure as the original fibres. [28, 29] Simultaneously, the development by Battista [30, 31] of the hydrochloric acid-assisted degradation of cellulose fibres derived from high-quality wood pulps, followed by sonification treatment, led to the commercialization of microcrystalline cellulose (MCC). MCC is stable, chemically inactive, and physiologically inert with attractive binding properties, offering a significant opportunity for multiple uses in the pharmaceutical industry as a tablet binder, and in food applications as a texturizing agent and fat replacer, and also, as an additive in paper and composites applications.

After the acid hydrolysis conditions were optimized, Marchessault et al. [32] demonstrated that colloidal suspensions of NCCs exhibited nematic liquid crystalline alignment. The main procedure for the isolation of NCCs from cellulose fibres is based on acid hydrolysis. The microfibrils consist of crystalline cellulose domains linked by amorphous domains. Upon acid hydrolysis the microfibrils undergo transverse cleavage along the amorphous regions and release crystalline cellulose, as seen in Figure 3.

Sulfuric and hydrochloric acids have been extensively used for NCC preparation. [33-36].
Phosphoric and hydrobromic acids have also been reported for such purposes, but the resultant NCC aqueous suspensions tend to flocculate. [37] On the other hand, when sulfuric acid is used as a hydrolyzing agent, it reacts with the surface hydroxyl groups of cellulose to yield charged surface sulfate esters that promote dispersion of the NCCs in water. However, the introduction of charged sulfate groups compromises the thermostability of the nanocrystals. [38]

![Figure 3 Schematic presentation of the crystalline structure of cellulose](image)

2.2.2 Dimensions of NCCs

Despite the previous observation showed that the particles were rather flat objects, NCCs have usually been treated as cylinders with mean diameters and lengths in models used to describe their dimensions. The geometrical dimensions (length, L, and diameter, D) of NCCs are found to vary widely, depending on the source of the cellulosic materials and the conditions under which the hydrolysis is performed. The morphological characteristics are usually studied by microscopy (TEM, AFM, FESEM, environmental SEM [40] etc.) or light scattering techniques, including small angle neutron scattering (SANS) [41] and polarized and depolarized dynamic light scattering (DLS, DDLS). [42]
Typical geometrical characteristics for NCCs originating from different cellulose sources are summarized in Table 1. The reported width is generally approximately a few nanometers, but the length of NCCs has a larger window, from tens of nanometers to several micrometers. Notably, NCCs from wood are only 3–5 nm in diameter and 100–200 nm in length.

<table>
<thead>
<tr>
<th>Sources</th>
<th>L (nm)</th>
<th>D (nm)</th>
<th>Refs</th>
<th>Sources</th>
<th>L (nm)</th>
<th>D (nm)</th>
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<td>bacterial</td>
<td>100−1000</td>
<td>5−10×30−50</td>
<td>[38, 43, 44]</td>
<td>ramie</td>
<td>50−150</td>
<td>5−10</td>
<td>[45]</td>
</tr>
<tr>
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<td>5−10</td>
<td>[46]</td>
<td>sisal</td>
<td>100−500</td>
<td>3−5</td>
<td>[47]</td>
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<tr>
<td>sisal</td>
<td>70−170</td>
<td>~7</td>
<td>[48]</td>
<td>150−280</td>
<td>3.5−6.5</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td>200−300</td>
<td>8</td>
<td>[50]</td>
<td>8.8×18.2</td>
<td>[41]</td>
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<td>15</td>
<td>[42]</td>
<td>1160</td>
<td>16</td>
<td>[42]</td>
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<td></td>
</tr>
<tr>
<td>150−210</td>
<td>5−11</td>
<td>[40]</td>
<td>500−1000</td>
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<td>28</td>
<td>[54]</td>
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</tr>
<tr>
<td>MCC</td>
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<td>3−48</td>
<td>[54]</td>
<td>Valonia</td>
<td>&gt;1000</td>
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<td>[56]</td>
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<tr>
<td>250−270</td>
<td>23</td>
<td>[57]</td>
<td>soft</td>
<td>100−200</td>
<td>3−4</td>
<td>[37, 58]</td>
<td></td>
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<tr>
<td>wood</td>
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<td>[59]</td>
<td>100−150</td>
<td>4−5</td>
<td>[33]</td>
<td></td>
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<tr>
<td>ramie</td>
<td>150−250</td>
<td>6−8</td>
<td>[60]</td>
<td>hard</td>
<td>140−150</td>
<td>4−5</td>
<td>[33]</td>
</tr>
</tbody>
</table>
Nanocrystalline celluloses are often referred to as microcrystals, whiskers, nanocrystals, nanoparticles, microcrystallites, or nanofibres. In this work, they are called “nanocrystalline celluloses” (NCCs).

2.2.3 Properties of NCC

2.2.3.1 Mechanical properties

Table 2 Mechanical properties of NCC and other high performance fibres

<table>
<thead>
<tr>
<th>Materials</th>
<th>Strength (GPa)</th>
<th>Modulus (GPa)</th>
<th>Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kevlar 49 [61]</td>
<td>3.6-4.1</td>
<td>131</td>
<td>2.8</td>
</tr>
<tr>
<td>Spectra 1000 [62]</td>
<td>3</td>
<td>172</td>
<td>3.5</td>
</tr>
<tr>
<td>NCC [63]</td>
<td>10</td>
<td>150</td>
<td>6.7</td>
</tr>
<tr>
<td>Multi-wall carbon nanotube [64]</td>
<td>63</td>
<td>800</td>
<td>16</td>
</tr>
<tr>
<td>Single-wall carbon nanotube [64]</td>
<td>150</td>
<td>1054</td>
<td>16</td>
</tr>
</tbody>
</table>

The nanoscale dimensions and extremely attractive mechanical properties of NCCs make them ideal candidates to improve the mechanical properties of a targeted host material. A summary of the mechanical properties of NCC and its comparison with other high performance fibres are shown in Table 2. The strength of NCC is about 10 GPa, which is almost three times stronger than commercial high performance fibres and is on the same level as carbon nanotube. In fact, the axial Young’s modulus of a NCC is theoretically
higher than steel and similar to that of Kevlar 49®. For NCCs, the theoretical value of Young’s modulus for the native cellulose perfect crystal has been estimated to be 167.5 GPa.[65] Recently, the Raman spectroscopy technique was utilized to measure the elastic modulus of native cellulose crystals from tunicate and cotton to yield values of 143 GPa [66] and 105 GPa,[67] respectively.

2.2.3.2 Solution properties

When sulfuric acid is used as the hydrolyzing agent, it also chemically reacts with the surface hydroxyl groups of NCCs to yield negatively charged (surface) sulfate groups that promote a perfectly uniform dispersion of the whiskers in water via electrostatic repulsions.[68] By inference, continuous removal of the water phase should therefore tend to cause the nanocrystals to adopt configurations that minimize the existing electrostatic interactions. An investigation into these systems reveals that NCCs are randomly oriented in the dilute regime (isotropic phase). A nematic liquid crystalline alignment is adopted when the NCC concentration increases because they coalesce to form an anisotropic phase, which is characterized by a unidirectional self-orientation of the NCC rods. When the suspension reaches a critical concentration of NCCs, it forms a chiral nematic ordered phase displaying lines that are the signature of cholesteric liquid crystals (Figure 4) [69]. Above the critical concentration of chiral nematic phase formation, aqueous NCC suspensions produce shear birefringence, and on standing, they can spontaneously separate into an upper isotropic and a lower anisotropic phase (Figure 5) [44, 70].
2.3 Dispersion and alignment of NCCs

2.3.1 Dispersion

Good dispersibility of the NCCs in the polymer matrix, as well as in the processing sol-
vent, is a prerequisite for creating polymer/NCC nanocomposites that display a significant mechanical reinforcement. NCCs without surface modification have intrinsically strong interactions and have been reported as notoriously difficult to disperse. Moreover, this issue worsens when the NCC dispersions are dried before nanocomposite processing, which generally implies that drying and redispersion of NCCs without aggregation is challenging.

So far, NCCs have been incorporated into a wide range of polymer matrices. The polymers summarized in Habibi's review [14], include polysiloxanes, polysulfonates, poly(caprolactone), styrene–butyl acrylate latex, poly(oxyethylene), poly(styrene-co-butyl acrylate) (poly(S-co-BuA)), cellulose acetate butyrate, carboxymethyl cellulose, poly(vinyl alcohol), poly(vinyl acetate), poly(ethylene–vinyl acetate) (EVA), epoxides, polyethylene, polypropylene, poly(vinyl chloride), polyurethane, and water-borne polyurethane. Incorporating NCCs into biopolymers, such as starch-based polymers, soy protein, chitosan and regenerated cellulose, and biopolymer-like poly (lactic acid), poly(hydroxyoctanoate) and polyhydroxybutyrates has also been reported.

There are four methods currently being used to disperse NCCs into the above polymer systems.

1. Direct mixing method - Aqueous suspensions of NCCs obtained from hydrolysis process are used as solvents for dissolving water-soluble polymers (for instance, poly (ethylene oxide) and poly (vinyl alcohol)). The dispersion of NCC in
aqueous suspension is very stable due to the presence of electrostatic repulsion in water between the individual rigid cellulose crystals achieved by sulfuric acid hydrolysis, which introduces negatively charged sulphate groups on the NCC surfaces. Due to the hydrophilic nature of NCCs, the easiest method to disperse NCCs into a polymer matrix is to incorporate NCCs into a water-based polymer. Moreover, the combination of THF/water solutions of polymers (subject to moderately strong hydrogen bonding, in miscible solvents such as THF) with aqueous NCC suspensions has been reported. However, the direct mixing method can only work for water soluble polymers, which severely limits the application field of NCCs. Moreover, the polymer dissolving process might induce NCC aggregations during the mixing process.

(2). Re-dispersion method - NCC organic solvent suspension is obtained by drying the aqueous suspension of NCCs and re-dispersing the dried NCCs into organic solvent, followed by dissolving the polymer into the suspension. NCC suspensions of N, N-dimethylformamide (DMF) [6], dimethyl sulfoxide (DMSO) [8, 71], tetrahydrofuran (THF) [72], ethanol [8], toluene [73], and formic acid [74] have been investigated. Although there is some possibility that freeze-dried NCCs were successfully re-dispersed in dipolar aprotic solvents, such as DMSO and DMF, that contained small amounts of water (0.1%), it was reported that the re-dispersion is incomplete, and there was evidence of NCC aggregation in suspensions [71]. Freeze-drying and re-dispersing of NCCs from tunicate in toluene was accomplished to integrate these fillers into atactic polypropylene, but strong ag-
aggregation occurred [73]. When formic acid was used to re-disperse dried NCCs, very satisfactory dispersions were achieved [74]. Van den Berg et al. [75] investigated the factors limiting the dispersibility of NCCs extracted from tunicate via hydrochloric or sulfuric acid hydrolyses in a series of polar protic and aprotic organic solvents. HCl-generated NCCs, which typically have a pronounced tendency to aggregate, did not disperse in polar aprotic solvents after being dried. Only protic solvents such as formic acid and m-cresol were shown to effectively disrupt the hydrogen bonds in aggregated NCCs, dispersing both types of NCCs generated from hydrochloric or sulfuric acid hydrolyses.

(3). Solvent exchange method - The combination of aqueous solutions of polymers (subject to moderately strong hydrogen bonding, in miscible co-solvents such as THF) with aqueous NCC suspensions has been reported.[76] The use of polar solvents, most commonly N,N-dimethylformamide (DMF), with NCCs with no surface modification has been explored.[77] Solvents nonmiscible with water and with low polarity, such as toluene, have also been widely used. Owing to the fact that acetone is miscible with water; it usually serves as carrier to transfer NCCs from water to organic solvents.

(4). Surface modification method - NCCs are dispersed into polymer solution by surface modification. A variety of methods for soluble NCCs have been explored by researchers to improve the dispersability of NCC in organic media, including methods that use surfactants, (2,2,6,6-Tetramethylpiperidine-1-oxyl)-mediated
(TEMPO-mediated) oxidation reaction, silylation, grafting of PEO or maleated polypropylene, and acylation [75].

Non-covalent surface modifications of NCCs have been conducted through adsorption of surfactants. Using non-ionic surfactant sorbitan monostearate to improve the dispersion properties of NCC in the solvent tetrahydrofuran (THF), Rojas et al. mixed NCCs in a hydrophobic matrix-polystyrene (PS) and produced electrospun PS fibres [78]. An anionic surfactant was also used by Bondeson et al. [79] to enhance the dispersion of NCCs in poly(lactic acid) (PLA).

TEMPO-mediated oxidation of NCCs has been used to convert the hydroxymethyl groups present on their surface to the carboxylic form [80]. The results showed that only the hydroxymethyl groups of polysaccharides were oxidized, while the secondary hydroxyls (which are highly discriminative among primary hydroxyl groups) remained unaffected.

Wang et al. [81] improved the dispersibility of NCC through using three different grafted reactions of acetylation, hydroxyethylation and hydroxypropylation to modify the surfaces of NCCs. The reaction conditions were easy to control, and the dispersibility of NCCs was distinctly improved. However, the amount of the hydrogen bonds was perhaps significantly reduced. It should be noted that the existence of a stiff and continuous network held together by hydrogen bond in the NCC is the premise for the high mechanical performance of NCC. Hence, this...
method is still arguable for the preparation of NCC reinforced composites.

Chemical modifications (discussed above) have been explored to improve dispersibility of NCCs among a wide range of organic solvents, from medium to low polarity. This approach also allows manipulation of dried NCCs because it facilitates freeze-drying and redispersion. However, such approaches can break the percolating hydrogen-bonded network and affect the macroscopic mechanical properties of the resulting nanocomposite.

In summary, it is challenging to obtain a uniform NCC dispersion for manufacturing high quality NCC nanocomposites.

2.3.2 Alignment

Orientation of NCC by various methods, such as shear force [82], to magnetic fields [1, 83-86] or to electric fields [87] has been reported.

Under shear flow, NCCs orient vertically in a plane perpendicular to the shear direction for low shear rates and along the shear direction for higher rates. [82] Oriented submonolayer surfaces of 10–15 nm diameter NCCs were prepared by spin-coating with a speed of 500, 3000 or 6000 rpm, respectively. [88]

Under a magnetic field with strength of 7 tesla, dilute aqueous suspensions of NCCs were able to orient [83]. Films were obtained in which NCCs were oriented with their long axes perpendicular to the magnetic field [83]. Each NCC orients with its long axis per-
perpendicular to the field, owing to the negative diamagnetic anisotropy of cellulose. Thus, when a magnetic field is applied to chiral nematic suspensions, an overall orientation is achieved where the cholesteric axis becomes parallel to the magnetic field. The resulting helical structure becomes unwound if the magnetic field is rotated. [53] Kvien and Oksman attempted to align NCCs in a polymer matrix (e.g., polyvinyl alcohol) by using a strong magnetic field to obtain a unidirectional reinforced nanocomposite, and interestingly the results showed that the dynamic modulus of the nanocomposite was higher in the aligned direction compared with the transverse direction. [85]

NCCs orient parallel to the electric field, in contrast to their perpendicular orientation in a magnetic field. Aqueous suspensions of NCCs extracted from ramie fibres and tunicate dried under an AC electric field and have shown a high degree of orientation along the field vector in the films. [89] Colloidal suspensions of NCCs from ramie fibres in cyclohexane have also been oriented in an AC electric field [87].

2.4 NCC reinforced nanocomposite

Processing techniques of NCC nanocomposites have an important impact on the final properties of the composites. The techniques that are adopted should take the intrinsic properties of NCCs, the nature of the polymeric matrix (solubility, dispersibility, and degradation), and the desired final properties such as geometrical shape into consideration. So far, NCCs have been processed into fibres, films, and composites. The following section is focused on NCC reinforced nanocomposite fibres.

2.4.1 Electrospinning of NCC reinforced nanocomposite

Electrospinning, a versatile method to manufacture fibres with diameters ranging from
several micrometers down to 100 nm or less through the action of electrostatic forces, has emerged as an alternative processing method for NCCs in polymer matrices. There are two types of electrospinning of NCC nanocomposite fibres.

(1) Direct electrospinning

A blend of polymer solution and NCC has been electrospun into fibres as listed in Table 1. Electrospinning of NCCs aqueous suspension with water-soluble polymers such as PEO [90] and PVA [11, 12] produced fibres with diameters in the nanoscale range. There are various ways to electrospin polymer solutions containing NCCs in organic media: several successful ones that have been reported include surfactant-coating [78] and polymer grafting [11, 91] to disperse NCCs in polystyrene dissolved in THF and polycaprolactone in DMF–dichloromethane. The use of polar solvents, such as DMF, has also been studied in the case of electrospinning PLA.[6]
Table 3 Summary for the direct electrospinning of NCCs/polymer

<table>
<thead>
<tr>
<th>No</th>
<th>NCC</th>
<th>Polymer Matrix</th>
<th>Processing conditions</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>aqueous suspension</td>
<td>PEO in water</td>
<td></td>
<td>[90]</td>
</tr>
<tr>
<td>2</td>
<td>α-chitin whiskers aqueous suspension</td>
<td>PVA in water</td>
<td></td>
<td>[7]</td>
</tr>
<tr>
<td>3</td>
<td>re-dispersed in ethanol</td>
<td>PAA in ethanol</td>
<td>crosslinking</td>
<td>[8]</td>
</tr>
<tr>
<td>4</td>
<td>redispersed in DMF</td>
<td>PLA in DMF</td>
<td>70 °C</td>
<td>[6]</td>
</tr>
<tr>
<td>5</td>
<td>redispersed in THF and sorbitan monostreate</td>
<td>Polystyrene in THF</td>
<td></td>
<td>[78]</td>
</tr>
<tr>
<td>6</td>
<td>grafted with PCL diol and then dispersed in DMF; unmodified NCC solvent-exchanged from water to DMF</td>
<td>PCL</td>
<td></td>
<td>[10]</td>
</tr>
<tr>
<td>7</td>
<td>aqueous suspensions</td>
<td>Aqueous solutions PVA with two different concentrations of acetyl groups</td>
<td></td>
<td>[11]</td>
</tr>
<tr>
<td>8</td>
<td>aqueous suspensions</td>
<td>PVA in water</td>
<td></td>
<td>[12]</td>
</tr>
<tr>
<td>9</td>
<td>redispersed in DMF/THF</td>
<td>PMMA in DMF/THF</td>
<td></td>
<td>[13]</td>
</tr>
</tbody>
</table>

Note: the structure characterization and mechanical property of the above electrospun fibres is listed in Table 4 and 5.
(2) Core-shell electrospinning

Another technique to produce NCC electrospun fibres is the "core-shell" electrospinning approach in which an aqueous dispersion of sulfated NCCs constitutes the discrete “core" component surrounded by a cellulose "shell" [92] In this study, the electrospinning device with two concentric capillary needles and one screw-type pump syringe was built. Eucalyptus-derived cellulose was dissolved in N-methylmorpholine oxide (NMNO) at 120 °C and diluted with dimethyl sulfoxide (DMSO) which was used in the external concentric capillary needle as the shell solution. A cellulose nanocrystal suspension obtained by the sulfuric acid hydrolysis of bleached sisal and cotton fibres was used as the core liquid in the internal concentric capillary needle. Although a great effort was made to overcome the issue of orientating cellulose nanocrystals in the polymer matrix, many fibres were condensed to form a film due to the water in the core diffusing into the shell solution. Therefore, the drawback of this co-annular nozzle core-shell electrospinning method is the difficulty to control good concentricity of the core and shell materials in the spun materials. This means that the core entrainment is not guaranteed even with a co-annular nozzle, and it's likely that the core material protrude into the shell, or even from the shell.

In the coming section, the structures and mechanical properties of the above electrospun fibres are summarized and compared.

2.4.2 Structure characterization

In Table 4, the structures characterization of electrospun NCC reinforced nanocomposite fibres is summarized. TEM images and SEM images showed that NCCs were entrapped
into fibres. However, given that NCC is nonconductive and of light element and the NCC nanocomposite fibres are soft materials, nanometer scale resolution capabilities of conventional instrumentation (TEM and SEM) are inadequate to provide detailed structural examination of NCC within nanocomposite fibre.

TEM analysis of the NCC nanocomposite fibre structure was challenging for several reasons. The major problem was lack of contrast between the NCCs and the matrix. However, in some areas of the fibres it appeared to be more concentrated allowing some insight into the structure. These areas typically have a slightly higher concentration of NCCs, i.e., local areas with a tendency of agglomeration (e.g. case 5). In areas where the NCCs were more evenly dispersed, the contrast between the whiskers and the PLA matrix was low and insufficient for imaging.

SEM has been utilized for structure determination of NCC nanocomposite fibres. In all the above studies, fractured surfaces were generated and coated to avoid charging. SEM could allow information about the dispersion and orientation of NCCs in the matrix, in addition to the presence of aggregates and voids. For example, in the case 9, a very good SEM image displayed the distribution and orientation of NCCs within fibres. But it doesn't mean SEM applies for the characterization of all NCC nanocomposite fibres. The matrix in case 9, PMMA, is a very brittle material, giving excellent fractured surface of fibres. However, the cleavage of most samples will most likely occur in areas where agglomerates are present, and therefore those might be overrepresented in the studies. In addition, the resolution of a conventional SEM is limited (e.g. case 7), and the coating
will most probably oversize the nanosized structures.

**Table 4 Structure characterization of electrospun NCC fibres.**

<table>
<thead>
<tr>
<th>No</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>TEM images of electrospun microfibres from neat PS (A) and from PS filled with 9% NCCs in the presence of equivalent amount of nonionic surfactant (B, C). Reprinted with permission from ref [7]</td>
</tr>
<tr>
<td>7</td>
<td>Cryo-SEM images (in the top) and variable pressure, ultrahigh resolution FE-SEM images in transversal cross-sections (in the bottom) of electrospun PVA-98 loaded with 15% of NCCs. (NCC : D=3−10nm L=100 ~ 250nm) Reprinted with permission from ref [78]</td>
</tr>
<tr>
<td>9</td>
<td>(a) SEM micrographs of fracture surfaces of hybrid NCC−PMMA fibres (7 wt % NCC, top left) with NCCs pointing outward from the fracture interface, and PMMA fibres (top right) with smooth fracture interface. (b) Fracture surfaces of hybrid NCC−PMMA fibres (14 wt % NCC) showing bundles of NCCs pointing outward from the fracture surface in parallel alignment with the hybrid fibre axis. (NCC dimensions: D=15−20nm and L=300~8000nm) Reprinted with permission from ref [10]</td>
</tr>
</tbody>
</table>

Although the lack of aggregates in samples could be an indication of good dispersion of NCCs, it is still hoped to characterize the detailed structure of the NCC nanocomposite fibres. Therefore, the use of electron microscopes will, in particular, require special attention to electron dose, contrast, and methods to assess the bulk structure without signifi-
cantly affecting the interesting morphology

2.4.3 Mechanical properties

Incorporating nanoparticles into polymers enables the exploitation of nanocomposites with synergistic effects from multiple constituents. One important technological motivation is the development of structural materials with improved mechanical properties. The mechanical properties of the electrospun fibres filled with NCC are listed in . The results showed that mechanical properties of NCC reinforced nanocomposite fibres are better than that of the pristine polymer fibres. However, since NCCs are not uniformly distributed within these fibres, the agglomeration of NCCs would lead to stress concentration when these nanocomposite fibres are under load. Therefore, it is essential to uniformly disperse NCC into the polymer matrix.
Table 5 Mechanical properties of the electrospun fibres filled with NCC

<table>
<thead>
<tr>
<th>No</th>
<th>Mechanical properties</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>194% increase in modulus; 74% increase in tensile strength; 233% increase in elongation; maximum strength : 1.74 MPa</td>
<td>[90]</td>
</tr>
<tr>
<td>2</td>
<td>maximum strength : 5.7 MPa; 4-8 times increase in modulus</td>
<td>[7]</td>
</tr>
<tr>
<td>3</td>
<td>maximum strength : 16.7 MPa; 77 fold increase in modulus; 58 fold increase in stress; 87.4% decrease in elongation</td>
<td>[8]</td>
</tr>
<tr>
<td>4</td>
<td>maximum strength : 5.6 MPa; 1 wt% NCC; 37% increase in modulus; 31% increase in strength; 10% NCC; 29% increase in modulus; 7% increase in strength</td>
<td>[6]</td>
</tr>
<tr>
<td>5</td>
<td>glassy modulus increased (no concrete value);</td>
<td>[78]</td>
</tr>
<tr>
<td>6</td>
<td>Maximum strength: 1.71 MPa; 1.5 fold increase in Young’s modulus; 1.5 fold increase in the ultimate strength.</td>
<td>[10]</td>
</tr>
<tr>
<td>7</td>
<td>3-fold increase of the storage modulus; Maximum storage modulus: 57.3 MPa</td>
<td>[11]</td>
</tr>
<tr>
<td>8</td>
<td>6.6 wt% NCC 2.4 fold increase in strength</td>
<td>[12]</td>
</tr>
<tr>
<td>9</td>
<td>No mechanical test</td>
<td>[13]</td>
</tr>
</tbody>
</table>

Note: the electrospinning materials and conditions are listed in Table 1

2.5 Electrospinning

2.5.1 Regular electrospinning

Electrospinning is a technique that utilizes electric force to produce ultrafine fibres. Generally, polymer solutions or melts are fed by a syringe pump through a capillary. When a sufficiently high voltage is applied to a liquid droplet, the body of the liquid becomes charged, and electrostatic repulsion counteracts the surface tension and the droplet is stretched. At a critical point a jet erupts from the surface. This point of eruption is known as the Taylor cone. As the jet elongates by a whipping process, the solvent evaporates and
ultrafine fibres are finally deposited on the grounded collector. In the whipping process, due to the bending instability, elongation and thinning of the fibres lead to the formation of uniform fibres with nanometer-scale diameters.

Numerous researchers have explored electrospinning of polymer solutions that have a solid phase such as carbon nanotube, nanoclay. This technique can be considered as "suspension electrospinning". Usually the solid phase is dispersed into the polymer phase, thus the resultant fibres have uniform structure. However, if the second phase can be not dispersed or its property is easily affected by the polymer phase, modifications for the conventional electrospinning are needed.

2.5.2 Coaxial electrospinning

To fabricate fibres from two separate immiscible solutions, coaxial electrospinning has been developed which is a technique using a spinneret composed of two coaxial capillaries by which two different solutions can be electrospun simultaneously into core/shell structured ultrafine fibres [93]. The core/shell structured composite fibres can fulfill special application purposes, for instance, to load drugs or growth factors in the core component of the fibres.[94, 95] This technique allows for physical separation of aqueous-based biological molecules from the organic solvents essential for scaffold fabrication and minimizes the interaction between the two to the order of microseconds [96, 97]

It was revealed that influencing factors including miscibility, diffusion rate, viscosity and flow rate of the outer and inner solutions were crucial in coaxial electrospinning.
Processing parameters, such as electric field strength, capillary size, the protrudent degree of the inner outside the outer one, and flow rate ratio of the inner and outer solutions, have significant effect on the formation of core/shell fibre structure.

2.5.3 Emulsion electrospinning

To fabricate fibres from two separate immiscible solutions, emulsion electrospinning has been developed. Emulsions are able to be electrospun via an ordinary device. An emulsion is a mixture of two immiscible liquids: one liquid forms a continuous phase in which the other liquid is dispersed in the form of small drops. Phase separation occurs to emulsions via three mechanisms: coalescence whereby drops merge to form larger drops, coarsening in which larger drops grow and smaller drops shrink due to the larger internal pressure of the smaller drops, and creaming or sedimentation in which drops sink or rise out of the bulk. Surfactants are additives that inhibit coalescence and coarsening by keeping drops from aggregating and/or reducing the interfacial tension between the liquids. Most emulsions are formed by ripping droplets apart with shear forces (usually by stirring) or inertial forces (usually by impact). These processes are usually not well controlled and consequently the emulsions have a wide drop size distribution.

With respect to the development of emulsion electrospinning, Sanders et al [98] were inspired by the process that the small droplets of water in oil can be prepared by electrospraying using coaxial jets, which could be extended to electrospinning in principle. In their work, water-in-oil emulsions were electrospun into fibres. The aqueous drops contained solutes e.g., protein, sugar and salt, and the oily continuous phase was poly (ethylene-co-vinyl acetate) in dichloromethane. The diameters of the resulting fibres were
about 20 um, much larger than typical for electrospinning, and the resulting fibres contained droplets of the aqueous phase. Xu et al [99] electrospun water-in-oil emulsions, in which the water drops contained anti-cancer drugs, and the continuous organic phase was a poly (ethylene glycol)-poly (L-lactic acid) diblock copolymer in chloroform. However, in Xu's work [99], almost all the water from the drop phase evaporated during electrospinning and hence the anti-cancer drug was incorporated into the electrospun fibres in dry form.

Another remarkable achievement was made in the field of emulsion electrospinning by Xu et al. [100] They found that emulsion electrospinning facilitates the preparation of ultrafine core-shell structured fibres. In their work, the water drops were comprised of a polymeric (poly ethylene oxide, PEO) aqueous solution, whereas the organic phase was the diblock/chloroform solution. Composite fibres with sub-micrometre diameter were obtained, but notably, the fibres had core-shell structures with PEO forming the core, and diblock copolymer forming the shell.

One of the applications of emulsion electrospinning which is similar to coaxial electrospinning is to deliver water soluble drugs or bio-molecules in water-in-oil (W/O) emulsion. [101]. The emulsion-electrospun core/shell fibres have hydrophilic drugs inside the core to avoid burst release and prolong the release time in the drug delivery process. Similarly, emulsion electrospinning can also be used to encapsulate growth factors or DNA for tissue engineering. [101]
In summary, emulsion electrospinning and coaxial electrospinning are advantageous to conventional suspension electrospinning insofar as the emulsion composition comprises an aqueous phase and an oily phase wherein these two immiscible phases are not influenced by each other. Therefore, the activities of the functional materials in one of the two phases are unaffected by the other phase. Comparatively, emulsion electrospinning is more convenient than coaxial electrospinning. In addition, it is difficult to achieve good concentricity of the core and shell materials in coaxial electrospinning; this may result in protrusions of long segments of the core material from the shell [102].
Chapter 3  Materials and methods

3.1 Materials
Poly (lactic acid) (PLA) was purchased from Nature Works ®. Nanocrystallite cellulose (NCC) aqueous suspensions (3.25%) and fluorescent isothiocyanate (FITC) labeled NCCs were provided by FP Innovation. Span 80 was purchased from Sigma Aldrich. Chloroform and toluene were purchased from Fisher Scientific. The properties of solvents used in experiments are listed in Table 6

<table>
<thead>
<tr>
<th></th>
<th>Chloroform</th>
<th>toluene</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface tension@ 20 °C in mN/m</td>
<td>27.5</td>
<td>28.4</td>
<td>72.8</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>61.2</td>
<td>110</td>
<td>100</td>
</tr>
</tbody>
</table>

3.2 Methods
Firstly, the dimensions of NCC and the stability of its aqueous suspensions were characterized. In addition, PLA water emulsions were prepared and electrospun, which consist of a drop phase of distilled water and a continuous phase of immiscible PLA solution. This emulsion formulation was optimized using Response Surface Methodology (RSM). RSM is an empirical mathematical modeling method which utilizes experimental data to create contour plots and to explore the processing window [103] and the optimum condition for PLA emulsion electrospinning in order to produce specific desired responses, fibre average diameter or fibre diameter standard deviation. This will allow for an understanding of the effect of water content and surfactant content on fibre diameter. Secondly,
after optimizing the emulsion electrospinning process, validation of emulsion electrospinning of NCCs will be confirmed. The morphology, structure and properties (physical, mechanical and chemical) of NCC/PLA nanocomposite fibres will be characterized using SEM, FM, CLSM, FESEM, TEM, XRD, DSC and tensile test. The presence, location, distribution and alignment of NCC will be also verified. Finally, it is necessary to gain an understanding of the mechanism for the structure formation of emulsion electrospun NCC/PLA nanocomposite fibres. A summary of research plan is shown in Figure 6.

**Figure 6 Flow chart of thesis research plan**

### 3.2.1 NCC characterization

The morphology of NCCs was characterized using TEM (Hitachi H-7600) which is a 120 kV tungsten filament TEM capable of 0.35 nm point-to-point resolutions when utilizing the liquid nitrogen cold finger. The H-7600 is equipped with a side mount AMT Advantage (1 mega-pixel) CCD camera (Hamamatsu ORCA). Before imaging, a droplet of di-
lute NCC suspension was mounted on carbon coated Formvar grids and subsequently air-dried. The samples were then examined at an accelerating voltage of 80 kV. The dimensions of NCCs were measured from the TEM images using Quartz software.

The Zeta potential of the NCC aqueous suspensions was determined using the Malvern Zetasizer Nano. The term Zeta potential applies to electrical charges existing in liquid emulsions or suspensions of a colloidal nature. Zeta potential is a measure of charges carried by particles (or dispersed liquid) suspended in a liquid (mostly water). Most particles suspended in water possess negative surface charges in the neutral pH range as shown in Figure 7. The particle is said to carry a negative electrostatic surface charge. Positive ions in the solution are attracted to those which are negatively charged and form a dense layer, known as the Stern layer. A diffuse layer lies outside the Stern layer, in which positive ions outnumber negative ions and balance the excess in the Stern layer. The electrostatic potential at the particle surface A decreases through the Stern layer along AB and BC in the diffuse layer, and reaches zero at point C where the bulk of the solution is encountered. The Stern layer and diffuse layers are called double layers. They are important in determining forces between colloidal particles. The Zeta potential BC is the difference between the electric potential at the edge of the Stern layer and the bulk of the suspending liquid as shown in Figure 7. The stability of a colloidal system is dependent on the degree of ion absorption, and, therefore on the Zeta potential. Thus, measurement of Zeta potential makes it possible to control processes wherein dispersion or agglomeration is important. Practically all aqueous colloids are electronegative, with a general Zeta potential range of -14 to -30 mV. As the Zeta potential becomes more negative, the stability of
the system is increased. This can be accomplished by addition of an anionic electrolyte or polyelectrolyte. Zeta potential values more electronegative than -30 mV generally represent sufficient mutual repulsion to result in stability. Stability is assured within a Zeta potential range of -45 to -70 mV.

![Figure 7 Schematic representation of particle in suspension](image)

**3.2.2 Experimental design**

To systematically examine the effects of parameters on electrospun fibre formation and diameter, experiments were designed to use response surface methodology (RSM). The RSM approach has the advantage of taking into account the combined effects of several parameters and uses statistical methods to fit an empirical model to experimental data. Therefore, RSM gives an overview of the processing parameters and their influence on
each other. Furthermore, it helps in obtaining the surface contours. These contour plots outline the processing window and point out the direction to attain the optimum condition. These following experiments were planned according to the modified central composite design. This type of design defines the minimum number of experimental combinations in the experimental domain that are required to obtain the maximum information for adjusting the proposed model. For a quadratic model, experiments must be performed at least three levels for each factor. These levels are best chosen equally spaced.

The two factors (water content and surfactant content) and three levels resulted in nine possible combinations of factor settings. The diameter (Y1) and standard deviation of fibre diameter (Y2) were investigated by varying surfactant and water content (X1 and X2 respectively. Table 7 shows the factors and levels in the experimental design. Table 8 shows the 9 runs of experiments to investigate the possible relationships between the response and independent variables.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Factor levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant content (wt %)</td>
<td>3,5,7</td>
</tr>
<tr>
<td>Water content (wt %)</td>
<td>10,20,30</td>
</tr>
</tbody>
</table>

Water content:

Preliminary trials showed that PLA solution with 10-30% water were able to electrospun into fibres. 30% appears to be the upper bound of water content because the emulsions
clogged at the needle affecting the spinnability of fibres.

Surfactant content:

Based on preliminary results, 3% is on the lower bound because 3% surfactant can only stabilize for approximately 1 hour before separation. 7% borders on the upper boundary because too much surfactant will affect the properties of resultant nanocomposite fibres.

<table>
<thead>
<tr>
<th>Experimental No.</th>
<th>Coded value</th>
<th>Uncoded value</th>
<th>Surfactant Content (Wt %)</th>
<th>Water content (Wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi_1$</td>
<td>$\chi_2$</td>
<td>$\zeta_1$</td>
<td>$\zeta_2$</td>
</tr>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-1</td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>1</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
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<td>1</td>
<td>0.07</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>-1</td>
<td>0</td>
<td>0.03</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-1</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0.2</td>
</tr>
</tbody>
</table>
3.2.3 Preparation and characterization of emulsions

The same preparation method of W/O PLA emulsions, NCC W/O PLA emulsions and FITC-NCC W/O PLA emulsions is in the following description. Emulsions consisted of a drop phase of aqueous solutions and a continuous phase of the 8 wt% solution of PLA in chloroform and toluene (7:3). The surfactant, Span 80, was added to stabilize emulsions. This two phase system was shaken by hand and vortexed at 3000 rpm for 2 minutes to mix the emulsion components; and were subsequently sonicated in a bath sonicator for 1h (Branson Bransonic ® 3510 5.5L MT UltraSonic Bath Cleaner).

The control of the aqueous phase becoming the dispersed phase is very important. Although the higher viscosity phase tends to be continuous, the choice of surfactant is normally the controlling factor. Our surfactant selection was guided by the hydrophilic-lipophilic balance(HLB) number\cite{104}. This is defined in terms of numerical values assigned to chemical groupings in the surfactant \cite{104}, as follows:

\[
HLB = 7 + \sum (\text{hydrophilic group numbers}) - \sum (\text{lipophilic group numbers})
\]

The HLB number was developed for use with non-ionic surfactants. A higher number indicates increased hydrophillicity (water soluble), while lower numbers indicate higher lipophilicity (oil soluble). Emulsions have specific HLB requirements. Table 9 shows the classification of surfactant application by using the HLB range of the surfactant \cite{104}. Matching HLB requirements with a surfactant's HLB value yields optimum performance. In our case, HLB range of the surfactant used as water-in-oil emulsifiers should be 3~6. Following the HLB guidelines, span 80 (sorbitan monooleate) (HLB=4.3) became our preferred choice to stabilize water droplets in oil. Span 80 (Figure 8) is usually used in
the area of pharmaceuticals and food preparation.

Table 9 Classification of surfactant application using HLB range of surfactant

<table>
<thead>
<tr>
<th>Application</th>
<th>HLB range</th>
</tr>
</thead>
<tbody>
<tr>
<td>W/O emulsifier</td>
<td>3~6</td>
</tr>
<tr>
<td>Wetting agent</td>
<td>7~9</td>
</tr>
<tr>
<td>O/W emulsifier</td>
<td>8~18+</td>
</tr>
<tr>
<td>Detergent</td>
<td>13~16</td>
</tr>
<tr>
<td>Solubilizer</td>
<td>15~18</td>
</tr>
</tbody>
</table>

Figure 8 Chemical structure of Span 80

To study the effect of emulsion droplet size on fibre structure. The two phase system was shaken by hand and vortexed at 3000 rpm for 2 minutes to mix the emulsion components; and were subsequently sonicated in a bath sonicator for 0.5, 1, 1.5, 2, 2.5 and 3 hours (Branson Bransonic ® 3510 5.5L MT UltraSonic Bath Cleaner) or a cell sonicator for 5 minutes (Misonix Sonicator® 3000 Ultrasonic Cell Disruptors). The output power per volume for the bath sonicator is 20 kW/m³. The output power per volume for the tip sonicator is 225 kW/m³ and 320 kW/m³. The process of emulsion preparation is summarized in Figure 9. Emulsions were examined under an optical microscope (Nikon Eclipses LV 100) to determine the dimensions of emulsion droplets, which were measured using ImageJ software.
3.2.4 Electrospinning

For the electrospinning of fibre mat, the experiment was conducted in a Drum Electrospinning Unit (Kato Tech Inc, Japan). A schematic representation of drum electrospinning unit is shown in Figure 10. The electrospinning solutions or emulsions were placed in a horizontally positioned plastic syringe (15 ml) with a flat end metal needle. The syringe was operated by a syringe pump to feed solutions at a rate of 0.080 mm/min and the needle was subjected to a high voltage DC power of 23 kV relative to a vertically grounded drum rotating at a constant speed of 8 m/min (equal to 32 rpm). The drum was positioned 15 cm from the needle orifice, which was covered by a sheet of aluminum foil to collect fibres. The fibrous nonwoven mats were dried under vacuum and peeled off for subsequent analysis. The usual spinning atmosphere was air at 30 – 40 % relative humidity. In dry air with relative humidity less than 5%, spinning could only run for 1 - 2 minutes due to the quick dry-out of the spinning droplet. In humid air (greater than 60% humidity), the fibres did not dry properly and fused together as they accumulated on the
collection plate.

For the electrospinning of aligned fibres, the experiment was conducted in a disk electrospinning unit (Nanon). A schematic representation of disk electrospinning unit is shown in Figure 11. The syringe was operated by a syringe pump to feed solutions at a rate of 0.70 ml/h. The needle was subjected to a high voltage DC power of 25 kV relative to a vertically grounded disk rotating at a constant speed of 2000 rpm. The disk was positioned 15 cm from the needle orifice. The usual spinning atmosphere was air at 30 – 40 % relative humidity.

Figure 10 Schematic representation of Drum Electrospinning Unit
3.2.5 Characterization of electrospun fibres

3.2.5.1 Microscopy

The electrospun fibres were observed using a Hitachi S-3000N scanning electron microscope (SEM) after being sputter-coated with Au for 30 seconds. The electrospun fibres were also characterized with Hitachi S-4700 field emission scanning electron microscopy (FESEM). Before FESEM imaging, electrospun fibres were freeze-fractured under liquid nitrogen environment and then sputter-coated with a 2 nm layer of Au. Micrographs of the fibre specimen were taken at various magnifications and 50 readings were recorded from each specimen.

The distribution of FITC-NCC in electrospun fibres was examined with a Nikon FV500 fluorescent microscope (FM) and Olympus BX61W1 FluoView confocal laser scanning microscope (CLSM). FITC has excitation and emission spectrum peak wavelengths of approximately 495 nm/521 nm.
The electrospun fibres were also characterized using TEM (Hitachi H 7600) operated under an accelerating voltage of 80 kV to avoid damaging the fibres and NCCs. The electrospun fibres were placed on carbon-coated Formvar grids and then stained with 2% uranyl acetate aqueous solution for 2 minutes.

3.2.5.2 FTIR

A Fourier transform infrared spectrometer (FTIR) (Perkin-Elmer 16 PC FTIR spectrometer) coupled with the attenuated total reflection (ATR) optical unit to analyze the chemical structure of NCC/PLA fibres. The spectrum was measured with an average of 16 scans and a resolution of 4 cm\(^{-1}\) over the range of 400 to 4000 cm\(^{-1}\).

3.2.5.3 WXRD

WXRD was conducted with a Rigaku (Japan) S-3000N Multiplex X-ray diffractometer at 40 kV and 20 mA with a Ni-filtered Cu K\(\alpha_1\) radiation (\(\lambda=1.542\) Å). Samples were scanned at a scan rate of 1°/min.

3.2.5.4 DSC

DSC was performed using a TA Instruments Q 1000 DSC. Approximately 10 mg of samples were loaded into the sealed pans and subsequently heated, cooled and heated in a nitrogen atmosphere at a rate of 10 °C/min from -70 °C to 200 °C. All temperatures were determined from the second heating scan.

3.2.5.5 Tensile test

Mechanical properties of the fibres were evaluated using a KES-G1 micro-tensile tester (Kato Tech). Nanofibres were tested in both random fibre mat and aligned fibre bundle methodologies. A brief description of each characterization method is given in the subse-
quent sections.

A 30 mm gauge length was used for the random fibre mat and aligned fibre assemblies. Samples were mounted on thick paper frames using double side tape to fix the ends of the samples to the paper. For the random nanofibre mat, the sample had dimensions of 5 mm x 40 mm. The aligned nanofibre bundles were slightly twisted to gain a certain degree of integrity within the yarn. Approximately one twist per centimeter was applied. The weight of each sample was recorded and used in the tensile properties calculations.

Five samples were tested in each group, due to the limited amount of NCC supplies. The load and displacement data of each sample was converted to stress-strain relation and an average curve was generated from accumulating samples within each group. The strain was determined by dividing the displacement by initial gauge length. The stress (MPa) of random nanofibres was calculated from the below equation 2. The elastic modulus was estimated from the slope of the linear portion of the stress-strain curve.

Specific stress (g/tex) = \( \frac{\text{Force (g)/specimen width (mm)}}{\text{Areal density (g/ m}^2) \)  

Equation 1

Engineering stress (MPa) = Stress (g/Tex) * 9.8 * density (g/cc)  

Equation 2

The stress (MPa) of aligned nanofibres yarn was calculated from the below equation 4. The cross-sectional area (A) based on the measured linear density (denier = g/9000 m)
was calculated by:

\[
\text{Cross-sectional area } A(\text{cm}^2) = \frac{\text{Denier(g / 9000m)}}{9 \times 10^5 \times \text{density(g / cm}^3)\text{)}
\]

Equation 3

The stress becomes:

\[
\text{stress (MPa)} = \frac{F(N)}{A(\text{m}^2)}
\]

Equation 4

The elastic modulus was calculated from the slope of the linear portion of the stress-strain curve.
Chapter 4 Results and discussions

4.1 NCC characterization

The morphology and dimensions were studied by TEM. Figure 12 shows TEM images of NCCs provided by FP innovation. The diameter is around 5~20 nm - the length is about 100~500 nm.

The stability of NCC aqueous suspension was characterized through the absolute magnitude of zeta potential of suspensions. Figure 13 displays the zeta-potential distribution of NCC aqueous suspension. The average zeta potential was determined to be -36.7± 2.72 mV, which is an indication that the suspension of NCC used in this study was fairly stable.

The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles (in this case negatively charged sulphate groups on NCC surface) in dispersion. A high zeta potential will confer stability of colloids which will resist aggregation. When the potential is low, attraction exceeds repulsion. Thus, the NCC dispersion will break and NCCs tend to flocculate. Usually the dividing line between stable and unstable suspensions is taken at either +30 mV or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable [105].
4.2 Characterization of emulsion-electrospun PLA fibres

In initial research on electrospinning of PLA, chloroform was used as the solvent to dis-
solve PLA. However, the nozzle always became blocked during electrospinning. This problem originated because the evaporation rate of chloroform (boiling point 38.9 °C) is very rapid.[106]. To solve this problem in this present study, solvents with high boiling points such as toluene (boiling point 110 °C) were added to prepare the mixed solvents, in order to slow the evaporation rate of the solvent.

The PLA solutions of 8% wt of PLA in chloroform and toluene (any ratio) cannot be electrospun into continuous fibres, while only droplets can be formed and sprayed on the collector. However, emulsions consisting of 10~30% water emulsified in the 8% wt of PLA in chloroform and toluene (7:3) and 3%~7% Span 80 were electrospun into fibres (Figure 14). This phenomenon is consistent with the observation made by V.P. Shastri and co-workers [107]. In their work, 10 wt% chloroform solutions of poly (lactic-co-glycolic acid) (PLGA) were electrosprayed under conventional electrospinning conditions, while the PLGA-emulsion was electrospun into submicron fibres.

The reason for the differing spinabilities of the PLA solution and w/o PLA emulsion system may involve the stabilization of the Taylor cone at the spinneret [108]. This is accomplished using a multiphase emulsion system: First, stabilization of the Taylor cone results from an increase in the apparent viscosity of the solution, which allows for electrospinning of a lower concentration of the polymer in the compatible solvent. Lower viscosity solutions or solutions with low polymer concentrations tend to electrospray, forming polymer droplets rather than fibres at the grounded electrode. However, by adding additional phases as an emulsion, it is possible to increase the viscosity at the needle tip.
This increase in viscosity allows for the formation of a more stable Taylor cone so that it can be elongated and evolved into a jet and thus produces fibre. Second, the stabilization of the Taylor cone results from the addition of surfactant. Surfactant can prevent droplet breakup, which promotes the transport of polymer to the water-organic solvent interface, thereby favoring chain entanglement and gelation of the polymer phase.

Figure 14 shows SEM images for fibre morphology and diameter of emulsion-electrospun PLA fibres at different water and Span 80 content levels. Figure 15 shows contour plot of average diameters of the W/O emulsion-electrospun PLA fibres with different water and surfactant content levels. Figure 16 shows contour plot of standard deviation of the W/O emulsion-electrospun PLA fibre diameter with different water and surfactant content levels. The response surfaces in Figure 15 and Figure 16 shows similar trend. Both surfaces resemble a saddle in two dimensions as seen in Figure 17. A saddle point is a point in the domain of a function which is a stationary point but not a local extremum [103]. These saddle surfaces provide two possible optimum windows to produce small and uniform fibres. These two possible optimum ranges are water content 10%-15% and span 80 content 4%-5% or water 27%-30% and span 80 4.5~6%. However, the RSM method bases on the statistical modeling which is only an approximation to reality. In reality, surfactant content should be as low as possible, otherwise the resultant fibre thermal and mechanical properties would be affect; water content should not be too high otherwise the fibre is not spinnable. Therefore, after taking into account of theoretical results and practical reasons, it was concluded that the emulsion with surfactant content 4% ~ 5% and water content 10~15% should be used to produce small and
uniform fibres.

Figure 14 Fibre morphology and diameter of emulsion-electrospun PLA fibres at varying water and Span 80 content levels
Figure 15 Contour plots of average diameters of emulsion-electrospun PLA fibres with differing surfactant and water content levels
Figure 16 Contour plots of diameter standard deviation of emulsion-electrospun PLA fibres with different surfactant and water content levels.

Figure 17 Schematic representation of saddle point [109]
4.3 Characterization of emulsion-electrospun NCC/PLA fibres

4.3.1 Fibre morphology

In the optimum region of W/O PLA emulsion electrospinning, a formulation of 13% aqueous phase and 5% Span 80 was used to prepare 5% NCC W/O 8% PLA emulsions in which the weight of NCC was 5% of PLA weight. 5% NCC W/O 8% PLA emulsions were sonicated in the bath sonicator with input power of 20 kW/m³ and subsequently electrospun into smooth random fibre mats and smooth aligned fibres.

Figure 18 and Figure 19 show SEM images and the distribution of diameters for electrospun 5% NCC / 8% PLA random fibres. Statistical analysis of fibre diameters reveals that the diameters are 591 ± 198 nm. Figure 20 and Figure 21 show the SEM images and the diameter distributions of the electrospun 5% NCC/8% PLA aligned fibres. Statistical analysis of the fibre diameters reveals that the diameters are 1008± 412 nm. As seen in the SEM images, good alignment is presented. A rotating disk with a speed of 2000 rpm was used to collect aligned electrospun fibre yarn which was taken off from the edges of the disk. The length of this yarn was the circumference of the disk, approximately 60 cm (Figure 22 ).
Figure 18 SEM images of electrospun 5% NCC/8% PLA random fibres (collector speed = 32 rpm)

Figure 19 Diameter distributions of electrospun 5% NCC/8% PLA random fibres
Figure 20 SEM images of electrospun aligned 5% NCC/ 8% PLA fibres (collector speed =2000 rpm)
4.3.2 Fibre structure

4.3.2.1 Fibre physical structure

In Figure 23, it can be seen that both the random and aligned emulsion electrospun NCC/PLA fibres had a mixture of core-shell structure and hollow cylinder structure. It was found that for thin fibres (diameter below 400~500 nm), the structure of fibres is core-shell (Figure 23 a). The core diameters were in the range of 5 nm~ 250 nm. For thick fibres (diameter larger than 500 nm), the structure of fibres is hollow cylinder (Figure 23 b). The inner diameter of the hollow cylinder varied from tens of nanometers to a few hundred nanometers. In addition, the cylindrical structure within the fibre col-
lapsed as seen in Figure 23 c. Similar results are also provided in [99, 107, 108, 110, 111].

Figure 23 Structure characterization of 5% NCC/8% PLA fibres: (a) TEM image of core-shell structure fibres; (b) FESEM image of hollow cylinder structure and (c) FESEM image of collapsed hollow cylinder structure

4.3.2.2 Verification of the distribution, location and alignment of NCC

To verify the presence and distribution of NCC within fibres, fluorescently labeled NCC (FITC-NCC) was used. Figure 24 displayed a fluorescent microscopy image and a CLSM image of FITC-NCC/PLA fibres. Results indicate that NCC is uniformly continually in-
corporated into fibres. Figure 25 shows CLSM images of core-sheath structured nanofibres, which is consistent with our previous TEM and FESEM results. In CLSM image, some area on the fibre is dark mainly due to the photobleaching of FITC molecules upon exposure to excitation light.

![CLSM image of 5% FITC-NCC/8% PLA fibres](image1.png)

Figure 24 Fluorescent microscopy images of the 5% FITC-NCC/8% PLA fibres

![CLSM image of 5% FITC-NCC/8% PLA fibres](image2.png)

Figure 25 The CLSM image of 5% FITC-NCC/8% PLA fibres (The scale bar is 10 um)

In order to gain further information about the interior of composite fibres, TEM and FE-
SEM observations of the fibres were carried out. For TEM characterization, nanocomposite fibres were negative stained with uranyl acetate to enhance the contrast between the matrix PLA and the NCCs. Figure 26 shows TEM and FESEM images of NCCs within core-shell and hollow cylinder structures. For thin fibres of core-shell structures, NCCs were continuously aligned in the core (Figure 26, a and b). For thick fibres of hollow cylinder structure, NCCs were aligned on the wall of these hollow cylinders (Figure 26, c and d).

![Fig 26](image)

Figure 26 Location and alignment of NCC in fibre FESEM image (c) and TEM images (a, b and d) of NCCs within core-shell and hollow cylinder structure

### 4.3.2.3 Fibre chemical structure

The presence of NCCs in PLA fibres was also verified using FTIR characterization. Fig-
Figure 27 shows the FTIR results of emulsion-electrospun NCC/PLA fibres. The band at around 1050 cm\(^{-1}\) is the most intense in the spectrum of cellulose [112]. A broad peak in the 3650–3000 cm\(^{-1}\) range is the characteristic peak of cellulose O–H stretching. Bands at 1759, 1185, 1130 and 1089 cm\(^{-1}\) represent the backbone ester group of PLA [113].

![FTIR spectrum of NCC powders and emulsion-electrospun NCC/PLA fibre](image)

**Figure 27** FTIR of NCC powders (a) (Reprinted with permission from ref [7]) and emulsion-electrospun 5% NCC / 8% PLA random fibre (b)

### 4.3.2.4 Fibre crystal structure

XRD was also used to characterize the presence of NCC in the fibres and the crystal
structures of NCC/PLA fibres. Figure 28 shows the X-ray diffraction patterns of the emulsion-electrospun NCC nanocomposite fibres, as well as the freeze-dried NCC powder and pristine PLA random fibre mat. The pristine PLA random fibre mat is nearly amorphous. The peak of NCC powders is located at $2\theta = 22.6^\circ$, which is typical of the 200 planes of the naturally occurring cellulose crystal (cellulose I). The strong peaks of NCC/PLA fibres were at $2\theta = 21.4^\circ$ and $2\theta = 23.7^\circ$ and a broad peak at $2\theta = 17^\circ$. The peak at $21.4^\circ$ was narrow and sharp, illustrating that the crystals have a high degree of regularity and crystallinity. This suggested that the first sharp peak may be caused by NCC. It also clearly showed that the NCC peak was left-shifted.

Figure 28 X-ray diffraction patterns of NCC/PLA random fibres and NCC powders and pristine PLA fibres

It was previously reported that NCC peaks were left-shifted as the dispersion of NCC im-
proved. In Wang’s paper [81], the surface of NCCs were modified by acetylation, hydroxyethylation and hydroxypropylation respectively. After ultrasonic treatment, for 120 min, the samples NCC-HEC and NCC-HPC could be dispersed in water, and the sample NCC-CA could be dispersed in dilute acetic acid. This observation indicated that the dispersibility of the samples NCC-HEC and NCC-HPC was much better than that of the sample NCC-CA. Their XRD patterns of the modified and unmodified NCC are shown in Figure 29. The peaks of the poorly dispersed samples of NCC-CA were at the same position of the unmodified NCC. The peaks of the well dispersed samples of NCC-HEC and NCC-HPC were left shifted relative to the unmodified NCC.

![Figure 29 X-ray diffraction patterns of unmodified and modified NCC samples. Reprinted with permission from ref [81]](image)

On the basis of these observations, we hypothesize that the NCC peak was left shifted
because PLA molecules may intercalate into the NCCs, leading to an increase of d-spacing and resulting in peak shifting of the NCC. The same phenomenon was also shown in the study of carbon nanotube [114] and nanoclay [115]. The sharp peak at 23.7° and the broad peak at 17° may be caused by PLA which induced crystallization by NCC. This hypothesis has been verified in the further DSC study.

![Figure 30](image)

**Figure 30** Schematic representation of PLA molecules intercalating into the NCCs

To confirm that NCC induced the crystallization of PLA, DSC experiments were carried out for NCC powders, the pristine electrospun PLA fibres and NCC/PLA nanocomposite fibres. The data are presented in Table 10 and Figure 31. Distinct cold crystallization peaks were observed in the nanocomposite fibres. The crystallization temperature corresponding to the peak of the cold crystallization exotherm decreased as the NCC incorporated, which indicated that NCCs acted as nucleating agents for PLA crystallization. In Xiang’s work [6], DSC results showed that the incorporation of NCC tends to lower the cold crystallization temperature and demonstrated that NCC also induced PLA crystallization. We speculate that the induced PLA crystallites may grow in one dimension which
is parallel to the normal surface of NCC. This special morphology is known as trans-crystalline structure. Transcrystallization is the preferential nucleation of polymer melts at crystalline surfaces. The term often refers to enhanced nucleation of spherulitic growth along fibres, and has been reported before. It has long been known that the crystallization of matrix polymers such as polypropylene may be preferentially nucleated by Cellulose I surfaces, leading to a “transcrystalline” layer around the fibre [116]. A thorough study of isotactic polypropylene transcrystallization at flax fibre surfaces also showed the enhanced interfacial adhesion phenomena [117].

Figure 31 DSC thermograph of the pristine PLA fibre mat and the NCC/PLA random fibre mat (2nd heating)
Table 10 Summary of DSC results for PLA electrospun fibres and the NCC/PLA electrospun fibres

<table>
<thead>
<tr>
<th>Samples</th>
<th>$T_g$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pristine PLA electrospun fibres</td>
<td>61</td>
<td>143</td>
<td>150</td>
</tr>
<tr>
<td>NCC/PLA electrospun fibres</td>
<td>64</td>
<td>124</td>
<td>146</td>
</tr>
</tbody>
</table>

4.3.2.5 Fibre mechanical properties

Figure 32 shows the stress-strain curves of electrospun 8% PLA random fibre mats, 8% PLA/5% NCC random fibre mats and the yarn of 8% PLA/5% NCC aligned fibres. The addition of NCCs increased the Young’s modulus, maximum tensile strength and decreased the strain at maximum tensile strength of the electrospun PLA random fibre mats (Table 11). After incorporation of 5% NCC, the strength of NCC/PLA random fibres increased by greater than 12%, its modulus increased by 163% and its strain decreased by 54%, compared to the pristine PLA random fibres; the strength of the NCC/PLA aligned fibres increased by 90%, its modulus increased by 551%, and its strain decreased by 91% compared to the pristine PLA random fibres compared to the pristine PLA random fibres. Therefore, the addition of NCC does provide the reinforcing effect to the PLA polymer matrix and also fibre alignment has significant effect on the mechanical properties of electrospun fibre assemblies.
Figure 32 Typical stress-strain curves of pristine electrospun PLA fibre mats and 8% PLA/5% NCC random fibre mats and aligned fibre yarn

Table 11 Mechanical properties of pristine electrospun PLA fibre mats and 8% PLA/5% NCC fibre mats and aligned fibre yarn

<table>
<thead>
<tr>
<th>Samples</th>
<th>Maximum tensile strength (MPa)</th>
<th>Strain at break (%)</th>
<th>Young's Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pristine 8% PLA electrospun mats</td>
<td>8.33±0.05</td>
<td>197±93</td>
<td>35.25±2.98</td>
</tr>
<tr>
<td>5% NCC/ 8% PLA random fibres mat</td>
<td>9.34±0.62</td>
<td>89±11</td>
<td>92.43±26.22</td>
</tr>
<tr>
<td>5% NCC/ 8% PLA aligned fibres</td>
<td>15.84±0.61</td>
<td>16 ±3</td>
<td>228.60 ±23.29</td>
</tr>
</tbody>
</table>
4.3.3 Structure formation mechanism investigation

The mechanism for structure formation (core-shell and hollow cylindrical structures) could be a combination of emulsion droplet coalescence in the core of fibres and phase separation during the electrospinning process (as seen in Figure 33). Alignment of NCC in the core and cylinder wall of fibres is likely due to the movement of aqueous droplets and the tendency to orient parallel to the electric field.

![Figure 33 Schematic mechanism for the formation of core-shell and hollow structure (the dark dash and dark line represents NCCs)](image)

Emulsion droplet coalescence during the electrospinning process may involve emulsion droplets moving inward and, furthermore, being stretched and merging in the fibre direction. [100]

The inward movement of emulsion droplets might originate from rapid elongation and
quick evaporation of solvents [100]. In the organic phase, solvents of chloroform and toluene evaporate faster than water and the viscosity of the polymer phase rapidly increases. The difference in evaporation speed between the dispersed phase and continuous phase induces droplets moving into center and stabilizing in the center. This helps NCCs settle into the center of fibres and allows NCCs to be aligned in the core of the fibres.

Deformation of the droplets may result from the presence of a leaky dielectric [118, 119]. It is well established that dispersion of a conductive aqueous phase in an insulating fluid results in the formation of a leaky dielectric, promoting droplet deformation and elongation into an elliptical shape under an electric field.

The emergence of emulsion droplets may arise through a dielectrophoresis process under an electric field [107]. Dielectrophoresis is a phenomenon in which a force is exerted on a dielectric particle subjected to a non-uniform electric field. This force does not require that the particle being charged. All particles exhibit dielectrophoretic activity in the presence of electric fields. This has allowed, for example, the separation of cells or the orientation and manipulation of nanoparticles and nanowires. [120] In our study, in response to an electric field, the aqueous phase will coalesce into column structures within the oil phase instead of bead-in-string structures before the onset of bending instability during electrospinning.

It is therefore understandable that phase separation of aqueous and organic phases might occur, in this case the aqueous phase in the core and polymer phase in the shell.
In a phase-separated system, the balance between the elasticity of the polymer phase and the compressive forces from the aqueous phase imposes a limit on the deformation of the system [121]. Once the shell solidifies, the average diameter of the fibres does not change. Solidification of the core phase then takes place. If aqueous solution penetrates the shell, an immediate precipitation of the inner layers of the hollow cylinder embedded with NCC takes place. After water evaporation, NCCs are able to orientate within the fibre on the core or on the wall of the hollow cylinder (as seen in Figure 33). If diameters of the aqueous column in the core are small, the evaporation of the water in the core solution may be quite thorough, promoting the formation of core-shell structures. The fibre diameter should be small. If the diameters of the aqueous columns in the core are large, the phase-separation of the emulsion components may be quite extensive. The fast evaporation of the shell solvent and the existence of a slowly solidifying core may lead to the hollow cylinder structure. The fibre diameter should be large.

We suppose that the different sizes of aqueous phase columns in this phase separated system could be a consequence of the polydispersed size of emulsion droplets. To verify this idea, the diameters of about 100 emulsion droplets were measured directly from optical microscopy images of emulsions (Figure 34, a). Polydisperse emulsions consisted of 1~30 μm-diameter drops (Figure 34, b) and the average diameter of emulsion droplets is 5.87 μm, with a standard deviation of 4.048 μm. The corresponding size distribution histograms are shown in Figure 34 b. The emulsion diameter distribution is asymmetrical, extending toward larger diameter drops. The distribution was fitted using a log-normal
function whose expression is: 

\[ D = A \exp\left(\frac{-(\ln(x) - \ln(m))^2}{s^2}\right), \]

where \( m \) is the mean of \( \ln(x) \) and \( s^2 \) is the variance of \( \ln(x) \). After logarithmic transformation, the original distribution was fitted by a normal distribution (Figure 34, c). Log-normal functions are classically used to describe the size-distribution of objects obtained by fragmentation.[34, 54]
Figure 34 (a) Optical microscopy image of emulsion, (b) histogram and distribution of NCC W/O PLA emulsion droplet diameter and (c) histogram and distribution of logarithmic transformation of NCC W/O PLA emulsion droplet diameter.

The production of NCC W/O PLA polydisperse emulsions is reproducible and controllable, which was confirmed with another set of experiments. The two phase system of NCC W/O PLA was sonicated in a bath sonicator with an input power of 20 kW/m\(^3\) for 0.5, 1, 1.5, 2, 2.5 and 3 hours. The size of emulsion droplets and optical image is shown in Figures 35-42. The size of emulsion droplets didn't change much with different sonication time. According to the literature [104], the emulsion droplet size is governed by the input power, \( D \sim \dot{E}^{0.4} \gamma_{12}^{0.6} \rho_2^{-0.2} \), where \( \dot{E} = \frac{dE}{dt} \) is the rate of input power per unit volume, \( \gamma_{12} \) is the interfacial tension of the dispersed phase 1 against the continuous phase 2.
and \( \rho_2 \) is the density of the continuous phase. Thus, if our input power doesn't change, the droplet sizes are steady, but the emulsion droplet size gets smaller if input power becomes larger.

Further experiments were conducted to sonicate emulsions with much higher input power, 225 kW/m\(^3\) and 320 kW/m\(^3\) to check if only the core-shell structure will form as the initial emulsion has smaller droplet size.

When emulsions were sonicated with an input power of 225 kW/m\(^3\), the diameter of emulsion droplets is 2.69±1.175 um (Figure 43) which is less than our previous results (Figure 45). The diameter of these resultant fibres is 480±245 nm. All the fibres have core-shell structure and the diameter of the fibre core is in the range of 50 nm to 300 nm (Figure 44).

When emulsions were sonicated with an input power of 320 kW/m\(^3\), the size of emulsion droplets are even smaller than 1 um and some aggregates were found on the surface of fibres as seen in Figure 46. Some fibres have core-shell structure, but there are a lot of NCC aggregates inside the fibres and on the fibre surface (Figure 47). Therefore, the energy for emulsions sonication should be optimized in the future work.
Figure 35 Optical microscopy image and histogram of emulsion droplet size after being shaken
Figure 36 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed
Figure 37 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed and sonicated for 0.5 hour at the output power per volume of 20 kW/m$^3$. 
Figure 38 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed and sonicated for 1 hour at the output power per volume of 20 kW/m³.
Figure 39 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed and sonicated for 1.5 hours at the output power per volume of 20 kW/m$^3$
Figure 40 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed and sonicated for 2 hours at the output power per volume of 20 kW/m$^3$. 
Figure 41 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed and sonicated for 2.5 hours at the output power per volume of 20 kW/m$^3$.
Figure 42 Optical microscopy image and histogram of emulsion droplet size after being shaken and vortexed and sonicated for 3 hours at the output power per volume of 20 kW/m³
Figure 43 Optical microscopy images and diameter distribution of 5% NCC /8% PLA emulsions sonicated at 225 kW/m³ for 5 minutes
Figure 44 TEM images of emulsion-electrospun NCC/PLA fibres sonicated at 225 kW/m³ for 5 min.
Figure 45 Droplet size comparison between different processing methods for emulsions (S: Shaking; SV: Shaking and vortexing; SVS 0.5, 1, 1.5, 2, 2.5 and 3: Shaking, vortexing and sonicating at 20 kW/m$^3$ for 0.5, 1, 1.5, 2, 2.5 and 3 hours at the output power per volume of 20 kW/m$^3$; SVS 225: Shaking, vortexing and sonicating at 225 kW/m$^3$ for 5 min)
Figure 46 Optical microscopy images of NCC/PLA emulsions sonicated at 320 kW/m$^3$ for 5 min

Figure 47 TEM images of NCC/PLA random fibre sonicated at 320 kW/m$^3$ for 5 min (a) fibre with core-shell structure; (b) fibre with NCC aggregates on the surface of fibres
Chapter 5   Conclusions and significance

NCC-reinforced PLA nanocomposite fibres mats and aligned yarns were successfully prepared by emulsion electrospinning. In this emulsion system, NCC was dispersed and isolated in the aqueous phase, and thus the presence of another immiscible polymer solution phase did not affect the stable and uniform dispersion of NCC within water. The dispersion of NCCs in the polymer matrix was enhanced through the uniform dispersion of NCC aqueous droplets in PLA solution system. The use of NCC emulsions was shown to facilitate the fibre electrospinning with controlled alignment and a tailored distribution of NCCs.

The key achievement of this work is successfully demonstrated that NCC, PLA, surfactant and solvents were able to be electrospun. It was also shown that the electrospun nanofibre mats and aligned nanofibre yarn comprising of hollow and core/shell structures with NCCs continually aligned in the core and in the hollow cylinder wall of the fibres. It was further found out that NCCs act as nucleating agents inducing PLA crystallization.

It was also shown that the addition of NCC does provide the reinforcing effect to the PLA polymer matrix. With the incorporation of 5 % NCC, the strength of NCC/PLA random fibres increased by more than 12%, modulus increased by 163% and strain decreased by 54%. It was also found that fibre alignment have significant effect on the mechanical properties of electrospun fibre assemblies.
Based on the experimental observations, this study also provided an initial understanding of the mechanism for the formation of fibre structure (core-shell and hollow cylinder). It was found that the formation of hollow and core/shell fibre structures is emulsion droplet size dependent. For example, when the emulsion droplet size is in the range of 1-30 um with an average diameter of 6 um, the fibre has core-shell and hollow cylinder structure; when the emulsion droplet size is in the range of 1-6 um with an average diameter of 3 um, all the fibres have core-shell structure. But the emulsion droplet size cannot be too small (e.g. less than 1 um); otherwise, the NCCs will come out from the aqueous phase and aggregates.
Chapter 6  Recommendations

In this thesis, it was shown that emulsion-electrospun NCC/PLA hollow and core/shell random and aligned fibres can be formed. NCCs were found to continuously align in the core and in the hollow cylinder wall of the fibres. Structure and property relationships for these new electrospun materials were investigated preliminarily. In order to capitalize this initial success, further research on the fundamental properties and manufacturing technique of NCC composite fibres is recommended. The following tasks are the proposed future work.

1. In view of the statistical nature of emulsion size and the properties of electrospun fibres, a stochastic analysis of the fibre physical structure and properties needs to be carried out. Since our fibres have hollow cylinder and core-shell structures, it would be helpful to know the probability of forming core-shell and hollow structure as well as their effect on the mechanical properties of fibres.

2. To obtain a clear picture of the structure-property relationship of the emulsion electrospun NCC fibres, mechanical testing for single fibre is needed. In this thesis, the tensile test was only carried out on the fibre mat and yarn.

3. In order to improve the reproducibility and uniformity of fibres, careful control of mono/poly-dispersity of the emulsion and emulsion droplet sizes is required.

4. In order to explore the commercial value of NCC, the scalability of the emulsion electrospinning process of NCC composite fibres needs to be investigated.
References


[34] D. Bondeson, A. Mathew, and K. Oksman, "Optimization of the isolation of na-


[61] [http://composite.about.com/od/aboutcompositesplastics/l/aa050597.htm](http://composite.about.com/od/aboutcompositesplastics/l/aa050597.htm).


[76] M. Schroers, A. Kokil, and C. Weder, "Solid polymer electrolytes based on nano-


[84] N. Yoshiharu, K. Shigenori, W. Masahisa, and O. Takeshi, "Cellulose microcrystal


Nov 17 2009.


[17] N. E. Zafeiropoulos, C. A. Baillie, and F. L. Matthews, "A study of transcrystallin-
ity and its effect on the interface in flax fibre reinforced composite materials.


