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Fabrication and Characterization of Multi-Layered Nanofibre Structures for Potential Applications in Agriculture

A Thesis submitted by

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Abstract

Recently electrospinning technique has attracted a lot of interest as a versatile and low-cost technique to manufacture sub-micron fibres and nanofibres from polymer solutions or melts. Nanofibre based structures represent new materials with potential to encapsulate and release molecules as well as biological cells for potential applications in agriculture, medical and the engineering fields. The advent of composite hollow nanostructures, including electrospun webs, offering controlled pore volume and distributions, has extended the impact of particles by bonding their functionality with the feasible processability of synthetic polymers.

In addition, recent studies have reported that using nanofibres to encapsulate agrichemicals may allow different chemical additives to be used together through separate nanofibres and can prolong agricultural additive release during several months.

Fertilisers are used broadly to promote plant growth, increase crop production, and improve quality of products. Application of large quantities of nitrogen fertilizer is common across many agricultural industries and these volumes may vary to compensate for losses through ammonia volatilization, denitrification, and leaching which may result in cause environmental pollution and health problems.

Recent investigations have focused on the development of slow-release fertilizers systems using different materials, which prolong the release of fertilizers. These systems aim to minimise soil contamination and obtain a controlled fertiliser release system at low cost, using material that is 100% natural and biodegradable, inexpensive and highly available.

To the best knowledge of the candidate, no research project has attempted to encapsulate and release chemical additives of agriculture by 3D nanofibre yarns. Although nanofibre webs are ideal porous membranes because of open pore structure and high surface area, continues nanofibre yarns bring advantages in terms of appropriate mechanical strength and being integrated into desired assemblies in one-step.

This research aims to develop a novel method to encapsulate and release molecules of agrochemicals by using electrospun nanofibre yarns as a carrier. At first, this thesis introduces a new technology to encapsulate and release molecules of agrochemicals by using multi-layered electrospun nanofibre as a carrier for the controlled release of urea. Single-layer poly L-lactic acid (PLLA) nanofibres loading urea fertiliser were fabricated using a basic electrospinning apparatus. Triple-layer nanofibrous structures were produced by electrospinning Polyhydroxybutyrate (PHB) nanofibres as upper and lower layers with PLLA nanofibres impregnated with urea fertiliser as the middle layer.

Furthermore, a new approach is introduced to fabricate double-layered hollow nanofibrous yarns by engineering a quad-layered nanofiber yarn. This yarn consists of a Polyvinyl Alcohol (PVA) multifilament core covered by a layer of PVA nanofibres, Poly L-lactic Acid (PLLA) nanofibres loaded with urea fertiliser, and Polyhydroxybutyrate (PHB) nanofibres respectively, during two different stages of electrospinning.

This PhD thesis concludes that triple-layer urea-impregnated nanofibrous structures, and hollow double-layered urea-impregnated nanofibre yarns are effective alternatives for the slow release of nitrogenous urea.

Keywords

Double-layered hollow nanofibre yarn; electrospinning; multi-layered nanofibres; slow release fertilisers; urea; urea encapsulation; urea slow release.

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Certification of Thesis

This Thesis is the work of **Leila Javazmi,** except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Dr Tobias Low

Associate Supervisor: Professor Gavin Ash

Associate Supervisor: Dr Anthony Young

Student and supervisors' signatures of endorsement are held at the University.

Statement of Authorship

Chapter 1 and 2. Introduction and Literature Review of Electrospinning and Nanofibres for Potential Applications in Agriculture.

Leila Javazmi was responsible for 65% of conception and design of this chapter, 65% of analysis and interpretation of literature, 65% of drafting and writing and 65% of final important editorial input; **Tobias Low** was responsible for 15% of conception and design, 15% of analysis and interpretation of literature, 15% of drafting and writing and 15% of final important editorial input; **Anthony Young** was responsible for 10% of conception and design, 10 % of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input; **Gavin Ash** was responsible for 10% of conception and design, 10 % of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input.

Chapter 3. Kinetics of a Slow Release Nitrogen Fertiliser from Multi-Layered Nanofibrous Structures. Leila Javazmi, Anthony Young, Gavin Ash, and Tobias Low.

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Chapter 4. Fabrication and Characterization of PLLA/PHB Hollow Double-layered Nanofibre Yarn for Encapsulation of Urea. Leila Javazmi, Tobias Low, Gavin Ash, and Anthony Young.

Leila Javazmi was responsible for 60% of conception and design of this chapter, 60% of analysis and interpretation of literature, 70% of drafting and writing and 70% of final important editorial input; **Tobias Low** was responsible for 30% of conception and design, 30% of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input; **Gavin Ash** was responsible for 5% of conception and design, 5 % of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input: **Anthony Young** was responsible for 5% of conception and design, 5 % of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input.

Chapter 5. Investigation of Slow Release Urea from Biodegradable Single- and Double-Layered Hollow Nanofibre Yarns. Leila Javazmi, Tobias Low, Gavin Ash, and Anthony Young.

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Chapter 6. Conclusions and Future Directions

Leila Javazmi was responsible for 65% of conception and design of this chapter, 65% of analysis and interpretation of literature, 65% of drafting and writing and 65% of final important editorial input; **Tobias Low** was responsible for 15% of conception and design, 15% of analysis and interpretation of literature, 15% of drafting and writing and 15% of final important editorial input; **Anthony Young** was responsible for 10% of conception and design, 10 % of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input; **Gavin Ash** was responsible for 10% of conception and design, 10 % of analysis and interpretation of literature, 10% of drafting and writing and 10% of final important editorial input.

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Chapter 1. Introduction

1.1. *Background*

In recent years, electrospinning has attracted a lot of interest as a versatile and inexpensive technique to manufacture sub-micron fibres and nanofibres from polymer solutions or melts[1]. Polymer nanofibres provide a platform for a number of diverse applications, including drug delivery, tissue engineering scaffolds sensor devices and electronics (Srivastava *et al.*, 2007). The advent of composite hollow nanostructures, including electrospun webs, offering controlled pore volume and distributions, has extended the impact of particles by bonding their functionality with the facile process of synthetic polymers. In many cases, the particles can be used as fillers or rheological modifiers and their impacts can be quantified completely in terms of total features, such as size, density, volume fraction, and shape (Xiong et al. 2008).

In addition, hollow nanostructures have been successfully used to encapsulate and control release of sensitive materials such as drugs, cosmetics, and DNA due to the large fraction of their void space (Xiong et al. 2008). Such hollow nanostructures have much higher surface to volume ratio compared to their solid counterparts with the same sizes, which would be beneficial when adsorption or storage of chemicals are required (Skrabalak et al. 2008). Processing methods, such as self-assembly, template synthesis, and electrospinning have been employed to make hollow, porous, core-sheath fibres and tubes from natural and synthetic polymers (Wan, Wei and Zhang, 2003). Coaxial electrospinning opens a route to design new functionalized composite nanofibres with core/sheath and hollow structures (Song, Zhang and Zhou, 2005). Different methods such as electrified coaxial liquid jets, co-electrospinning, and electrospinning with a two-capillary spinneret have been employed to generate hollow and core/sheath nanofibres (Sun *et al.*, 2003).

To date a few methods have been proposed to obtain hollow yarns by using the electrospinning process. These methods bring some advantages together with special, unique structure (low packing density and porous structure) of hollow yarn. Low volume and weight of electrospun nanofibres with large surface area on three dimensional nanofibrous architectures of hollow yarn can provide potential applications in materials delivery (Merati and Okamura, 2000).

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[1] In melt only one substance is involved and the liquid and solid are the same material.

1.2. ***Research motivation of the project***

Fertilisers are used broadly to promote plant growth, increase crop production, and improve quality of products. However, there are major challenges for the industry and the agricultural sector. Application of large quantities of fertiliser is common across many agricultural industries and these volumes may vary to compensate for losses through volatisation in atmosphere and leaching in soils (Al-Zahrani, 2000). Recent investigations have focussed on the development of systems using different materials which prolong the release of fertilisers. These systems aim to minimise soil contamination and obtain a controlled fertiliser release system at low cost, using material that is 100% natural and biodegradable, inexpensive and highly available. Nanofibre based structures represent new materials with potential to encapsulate and release molecules as well as biological cells for potential applications in agriculture. Therefore, high surface area and ease of incorporation of active ingredients have prompted research groups to investigate using electrospun nanofibres in agricultural applications (Castro-Enriquez et al. 2012).

In a recent study, Castañeda et al. 2014 reported that using nanofibres to encapsulate fungicide may allow different chemical additives used together through separate nanofibres. Further work by Kampeerapappun & Phanomkate (2013) introduced a coaxial electrospinning system with fertiliser loaded in the core component which can control the fertiliser release for a month.

To the best knowledge of the candidate, no research project has attempted to encapsulate and release chemical additives of agriculture by three dimensional solid and hollow nanofibre yarns (Ali et al. 2011).

This thesis aims to explore a novel concept to fabricate urea-impregnated triple nanofibrous structures and double-layered hollow nanofibre yarns by manipulating electrospinning apparatus and changing electrospinning parameters to improve prolonged-release of urea nitrogens. At first a triple-layered nanofibrous non-woven mat loading urea is fabricated, and the urea release in water is investigated. Following this, quad-layered nanofibre yarns impregnated with urea are produced by a new design of electrospinning apparatus. Double-layered hollow nanofibre yarns loading urea is achieved by dissolving the inner layer after putting the yarn in water at room temperature. Characterization and mechanical properties of core-sheath nanofibre yarns and hollow nanofibre yarns are investigated. Finally, urea releasing behaviour from core-sheath and hollow nanofibre yarns are studied.

1.3. *Research gap*

Although nanofibre webs are ideal porous membranes, continues nanofibre yarns bring further advantages in terms of mechanical strength and integration into desired assemblies in one-step. Moreover, hollow nanofibre yarns have much higher surface to volume ratio, and low weight three-dimensional structures in comparison to non-woven mats with the same sizes or solid nanofibre yarns.

There have been significant advances in the production of nanofibre yarns; however, to date, no research reports have been published which demonstrate the encapsulation and release of agricultural chemical additives using three-dimensional nanofibre yarns. Therefore, hollow nanofibre yarn with large surface area on three-dimensional nanofibrous architectures could, therefore, improve materials delivery.

1.4. *Objectives of the Thesis*

The aim of this thesis was to understand the effect of the electrospinning apparatus and parameters on the morphology and behaviour of nanofibres and hollow nanofibre yarns to deliver agrichemicals. The specific objectives of the research were :

1. Optimisation of electrospinning parameters for the production of core-sheath and hollow nanofibre yarns.
2. Characterization of nanofibres, core/sheath and hollow nanofibre yarn (nanofibre diameter and void space).
3. Investigation of urea release dynamics from nanofibre mats and hollow nanofibre yarns as a proof of concept for slow release delivery of agrichemicals.

1.5. *Thesis outline*

- Chapter 2 entitled " Literature Review of Electrospinning and Nanofibres for Potential Applications in Agriculture" reviews and summarizes studies and researches regarding nanofibre production and its applications in agriculture.
- Chapter 3 entitled " Kinetics of a Slow Release Nitrogen Fertiliser from Multi-Layered Nanofibrous Structures" addresses how a developed nanofibrous structure improves the encapsulation and release of agricultural chemical additives.
- Chapter 4 entitled " Fabrication and Characterization of PLLA/PHB Hollow

Double-layered Nanofibre Yarn for Encapsulation of Urea" refers to fabrication of a three-dimensional nanofibre yarns with proper mechanical strength in one-step. Different electrospinning parameters are optimised to manufacutre multi-layered nanofibre yarns and hollow double-layered nanofibre yarns.

- Chapter 5 entitled "Fabrication and Characterization of PLLA/PHB Hollow Double-layered Nanofibre Yarn for Encapsulation of Urea" demonestrates how a three-dimensional hollow nanofiber yarn structure is used to encapsulate and release urea as a proof of concept.
- Chapter 6 discusses the results of three chapters and concludes how multi-layered nanofibre structures and hollow double-layered nanofibre yarns are effective alterntives for the slow release of urea as a proof of concept.

Chapter 2. Literature Review of Electrospinning and Nanofibres for Potential Applications in Agriculture

2.1 Introduction to Nanotechnology

Nanotechnology encompasses a variety of technologies in nanoscales (billionths of a metre) which have novel and developing applications across a range of fields. Nanotechnology involves imaging and manipulating materials ranging from individual atoms and molecules to submicron dimensions, as well as integrating the formed nanostructures into larger systems (Bhushan, 2008). The nanotechnology era not only includes the technical innovations but also demonstrates an upward trend of using nanomaterials in people's daily lives. Construction of tiny objects atom-by-atom or molecule-by-molecule offers the prospect of the highly tailored structures that support an extensive functionality. This ability of nanoscience promises to provide many breakthroughs across a wide range of new applications in the near future (Arivalagan *et al.*, 2011). The properties of nanomaterials differ from the other materials because of two principal factors: increased relative surface area, and quantum effects.

2.2 Classification of Nanomaterials

The main classification of nanomaterials are: carbon-based materials; nanocomposites; metals and alloys; nanoceramics; and nanopolymers (Leng and Lau, 2010).

2.2.1 Carbon-based Nanomaterials

This type of nanomaterial includes fullerenes, carbon black, graphene, carbon nanotubes, and other carbides. Graphene with exceptionally high surface area is the world's strongest, thinnest, and stiffest material, as well as being an excellent conductor of electricity and heat. It is the basic building block of 0D fullerene, 1D carbon nanotubes, and 3D (Paulchamy, Arthi and Lignesh, 2015). Carbon nanotubes, introduced by S. Iijima in 1991, are hollow cylinders made of graphite sheets (Rajapakse and Hui, 2004). Carbon nanotubes (CNT) can be single-walled or multi-walled and have surprising mechanical properties because of the strength of sp2 carbon-carbon bonds. The Young's modulus[2] and tensile strength of the best nanotubes can reach 1000 Gigapascal (**GPa**) and 63 **GPa** in sequence; which are approximately five and fifty times higher than the Gpa of steel respectively. Carbon nanotubes are

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[2] Young's modulus is a mechanical property that measures the stiffness of a solid material (Leng and Lau, 2010).

being considered as a hydrogen storage medium due to their large surface area. CNTs can show various electrical properties due to the graphite arrangement around the tube resulted in being conducting, semiconducting, or insulating (Leng and Lau, 2010).

2.2.2 Nanocomposites

Nanocomposites are combined from multiphase materials where one of the component phases has one dimension less than 100 nm. These materials are typically composed of two or more inorganic/organic phases in some nano-dimensional form, where the combined properties are different from the properties of the individual components (Ajayan et al. 2003). Nanocomposites can be classified into polymer matrix nanocomposites, ceramic metal nanocomposites and metal matrix nanocomposites. In most polymer matrix nanocomposites glass fibre is used, but occasionally Kevlar, carbon fibre, or polyethylene are used as reinforcement and matrices are based on a thermostat such as an epoxy resin, polydicyclopentadiene, or polyimide (Leng and Lau, 2010). Ceramic metal nanocomposites are generally made of ceramics constituting oxides, nitrides, borides, and silicides. A metal as a second component often is composed into ceramic matrix nanocomposites (Attaf, 2011). A nanocomposite made of the metal and the ceramic matrix can improve nanoscopic properties, including optical, electrical, and magnetic properties (Hvizdoš *et al.*, 2010). In metal matrix nanocomposites, ceramics are generally used as the reinforcing phase and the main volume is occupied by matrices of engineering metals, including aluminium, magnesium, zinc, copper, titanium, nickel, cobalt and iron. This type of nanocomposite can provide the properties of low density, increased specific strength and stiffness, improved wear-abrasion resistance and increased high-temperature performance limits. By contrast, they are more expensive and difficult to make than polymer or matrix ceramic metal nanocomposites (Leng and Lau, 2010).

2.2.3 Metals and Alloy Nanomaterials

Gold, silver, magnetic ion-based alloys and magnesium-based alloys are usually defined as metal and alloy nanomaterials. Nanoparticles of gold are readily prepared and have been used as electron-opaque coatings in electron microscopy for decades, while colloidal silver is being investigated for its antibacterial features (Ahamed *et al.*, 2010). Although fluorescent organic dyes can be destroyed by exposure to through light, gold and silver nanoparticles do not burn out after long exposure to light (Frederix *et al.*, 2003). By controlleng

the chemical structure and synthesis method of magnetic nanoparticles including iron, cobalt, and their alloys, their magnetic performance can be modified (Leng and Lau, 2010). Alloying of nanomaterial results in emerging new materials that exhibit better high-tech expediency than their parent metal nanoparticles. Alloy nanomaterials show different properties from their single metallic components while creating another dimension in amending their properties besides the usual size and manipulated shape (Abdul Salam et al., 2015).

2.2.4 Nanoceramics

Ceramics are a class of materials with the largest range of functions in comparison with metals and polymers. Recently, the use of ceramic materials has significantly increased in various applications due to the unique characteristics and advantageous properties of ceramic materials including hardness, rigidity, abrasive toughness and low density. Nanoceramics consist of nanoparticles composed of ceramic materials, which are generally classified as inorganic, heat-resistant and non-metallic solids. Macroscale ceramics are brittle and rigid, however nanoceramics show enormous potential for unique functions based on a range of properties, including dielectric, electronic, piezoelectric, electro optical, ferromagnetic, superconducting, ionic, and ferroelectric, magneto resistive, pyroelectric, and gas-sensing. Dense nanoceramic materials are usually obtained by some pressing methods of nanopowders, such as hot pressing, hot isostatic pressing, and sinter forging (Khalil, 2012).

2.2.5 Nanopolymers

A polymer is a large molecule (macromolecule) composed of many repeated units (monomers) bound by covalent chemical bonds. Both synthetic (such as nylon) and natural (such as cellulose) polymers play widespread and key roles in everyday life because of their various properties. The term ‘nanopolymer’ is a single polymer molecule in the nanoscale range. The nanostructured polymers can be modified and the desired properties can be achieved when inorganic and organic nanomaterials are dispersed in the polymers. Polymer nanocomposites are generally made of nano inorganic or organic powders or films with special physical properties combined with polymers to form polymer nanocomposites.The unique characteristics of polymer nanocomposite material with optimized structures, in comparision to other conventional composites, are high strength-to-weight ratio, corrosion resistance, and more design flexibility. Instead of using traditional materials in heavy-duty installation equipment, polymer nanocomposites can provide a robust alternative to the highly corrosive properties of concrete and steel (Leng and Lau, 2010).

2.3 Nanotechnology Applications

Nanotechnologies and nanomaterials are helping to develop our understanding of matter and have major implications for an increasing number of industries, including energy production and efficiency, computing, medical appliances and drugs, agriculture and food, the automotive industry, cosmetics and household appliances (Arivalagan *et al.*, 2011). Developing nanotechnology applications have implications for the global market for agricultural, mineral, and other industrial commodities. Research shows the capability of nanomaterials in improving seed germination and growth, pathogen detection, pesticide/herbicide residue detection, and plant protection (Khot *et al.*, 2012). Increasingly, many research fields and several potential applications involve nanotechnology as an interdisciplinary research tool (Asim Kumar, 2013).

2.4 Nanofibre

Nanofibres are practically considered as one-dimensional (1D) nanomaterials owing to their diameter in the range of 100-1000 nm or less, and an aspect ratio (length/diameter) greater than 100:1. This intrinsic feature of nanofibres increases surface to volume ratio and the high aspect ratio greatly. There are several methods to prepare nanofibres, encompassing top-down (melt-blown, melt electrospinning, islands-in-the-sea, and electrospinning), and bottom-up (interfacial polymerization, self-assembly, and phase separation) approaches (Ali *et al.*, 2011).

Structures created by each of these methods are quite different and thus have their own unique properties.

Nanofibres produced from electrospinning have many features that make them useful for chemical delivery systems. These include greater control on morphology and a high aspect ratio, variable pore-size distribution, and high porosity (Ravandi et al. 2015). The electrospun nanofibres with open pore structures in the range between tens and thousands of nanometres width have high surface area, along with the high gas permeability, making them ideal porous membranes. Compared with other 1D nanostructures (e.g. nanotubes or nano-rods), continuous nanofibres are advantageous due to fabrication cost and the ability of being integrated into other desired assemblies in a single step (Ali *et al.*, 2011). Nanofibre non-woven webs have been used in areas as diverse as nanocomposites, filtrations, biomedical, medical prostheses, fuel cells, sensors, and protective clothing (Bhardwaj and Kundu, 2010)(Huang *et al.*, 2003). Figure 2.1 illustrates nanofibre size in comparison to a human hair (Raghavan, 2006).

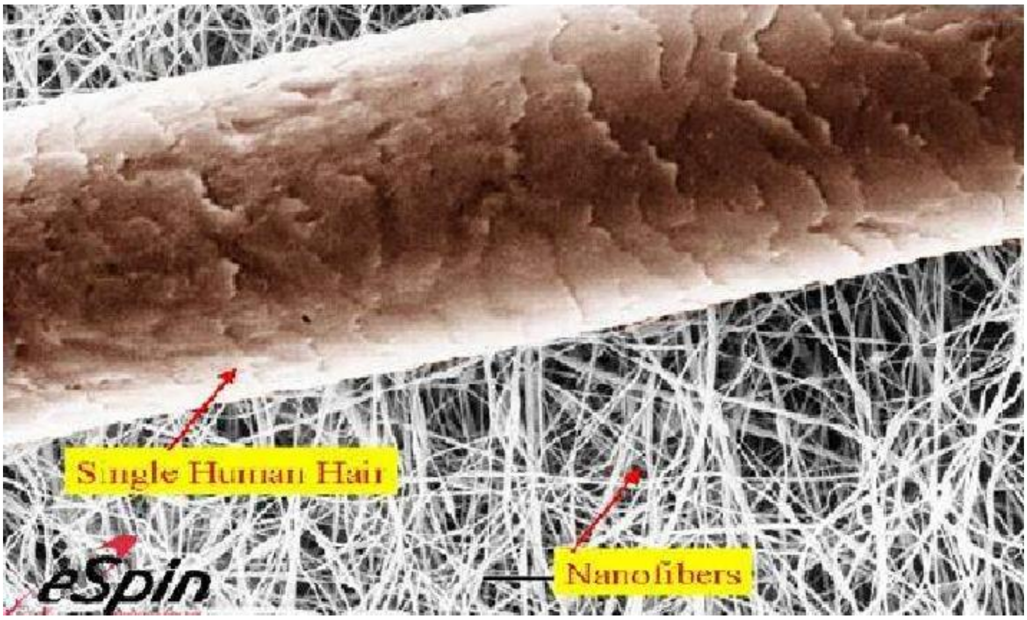


Figure 2.1: Comparison between human hair and nanofibre web (Raghavan, 2006)

2.4.1 Properties of nanofibres

The most significant properties of nanofibres are:

- Small diameter
- High aspect ratio (length to diameter ratio)
- Flexibility in surface functionalities
- Large surface area to volume ratio (can be as high as 1000 times for nanofibres compared to microfibers)
- Significant directional strength
- Chemical and thermal stability
- Reduced ohmic resistance compared to fibres
- Efficient control of pore size in webs
- Superior mechanical stiffness and tensile strength along the fibres by highly oriented crystalline structures compared to microfibres (Raghavan, 2006).

The unique properties of nanofibres have many applications ranging from clothing to reinforcement structures (He and Liu, 2008). The high aspect ratio of length to nanofibre diameter ratio is considered as the main reason for the large surface area, mechanical properties, electronic properties and bio-reactivity of the nanofibres (Raghavan, 2006).

2.4.2 Techniques to produce nanofibres

They are different techniques to produce nanofibres. The most common methods are:

- Phase separation

"Phase separation consists of dissolution, gelatine, and extraction using a different solvent, freezing, and drying resulting in nanoscale porous foam." (Huang *et al.* 2003). There are long time frames are required to form the solid polymer into the non-porous foam and (Huang *et al.* 2003).

- Self – assembly

In self-assembly processes, the components organize themselves into some preferential shapes and designs. However, this process is time-consuming to produce continuous polymer nanofibrrs like the phase separation process (Huang *et al.*, 2003).

- Drawing

The drawing process can pull viscoelastic materials, which can forgo large deformation, into nanofibres. The disadvantage of the drawing process is that the viscoelastic material should be able ductile enough and without failure can undergo large deformations(Huang *et al.*, 2003).

- Template synthesis

The template synthesis method cannot make continuous nanofibres as a template of nanoporous membranes are used to make nanofibres of hollow (a tubule) or solid (a fibril) shape (Huang *et al.*, 2003)

- Electrospinning

The electrospinning method is analgous to the drawing method, but uses an electric field instead of mechanical forces to pull the polymer and produces nanofibres. Electrospinning is a cheap and applicable approach to produce continuous nanofibres quickly and with a possible scale-up (Raghavan, 2006).

2.4.3 History of Electrospinning

The origin of electrospinning process is traced back to 1930s. In 1934, Anton Formhals patented a process for the production of polymer filaments using electrostatic force. He reported spinning of cellulose acetate fibres from wood cellulose (Formhals, 1934). Experimental and theoretical activities of liquid jets in electric fields revitalized by several important contributions by Geoffrey Taylor in 1964. He reported useful experimental evidence, and calculated the conical shape, often referred as Taylor cone, of the protrusion from which a charged jet sometimes travelled for a few centimetres in a straight line, and then bent into a complex path. This bending instability developed a series of lateral excursions that grew into spiralling loops (Reneker *et al.*, 2000). Taylor, in 1964, specified that the conical shape of polymeric solution exists in equilibrium when the semi-vertical angle is 49.30°. The Taylor cone marked the onset of extensional velocity gradients in the electrospinning process (Taylor, 1969). Peter K Baumgarten, in 1971, investigated the relation between the fibre diameters to the viscosity of the polymer solution, and found the fibres obtained from the solution were lower in diameter than those obtained from the melt (Bhargava, 2007). Hayati et al. (1987) studied the experimental conditions, the effects of electric field and jet stability. The study showed that semi-conducting and insulating liquids like paraffin oil resulted in stable liquid jets (Hayati, Bailey and Tadros, 1987). Following Hayati's work, some researchers including Doshi & Reneker (1993), and Jaeger et al. (1998) explored how changing electrospinning parameters, such as polymeric concrentartion and distance between collector and needle, affected the formation of nanofibres. Deitzel et al. (2001) found that applied voltage strongly correlated with the shape of bead defects on the nanofibres surface. Shin et al. (2001) demonestrated a systematic experimental and theoretical analysis of electrospinning process that described the jet instabilities with the use of operating diagrams of electric field versus flow rate

2.4.4 Electrospinning

Electrospinning is a very simple and inexpensive technique that utilizes an external electrostatic field to manufacture fibres with diameters ranging from several nanometres to micrometres. This method has gained much attention in recent years (Bhardwaj and Kundu, 2010)(He and Liu, 2008). The electrospinning process applies high voltage to create an electric field between the tip of a needle and a collector plate. A droplet of polymer solution exuded from the needle is drawn to the collector (Figure 2.2). Positive electrode of the voltage source is usually placed into the solution and the negative electrode is connected to the collector. This electrical potential creates an electrostatic force. When the voltage is increased, the pendant drop of polymer solution at the tip of the needle is built up by the electric field, which intensifies causing a force. This force deploys on the opposite direction of the drop due to surface tension. The increasing electrostatic force causes the drop to elongate forming a conical shape known as a Taylor cone (Figure 2.3). While the electrostatic force overcomes the surface tension of the drop, a continuous charged jet of solution is ejecting from the cone, and accelerates towards the collector, whipping and bending drastically (Raghavan, 2006). As the solution comes out from the needle and toward the collector, the jet rapidly thins and dries as the solvent evaporates. On the surface of collector, a nanofibrous mat of random orientation is deposited (Subbiah, G. S. Bhat, *et al.*, 2005).

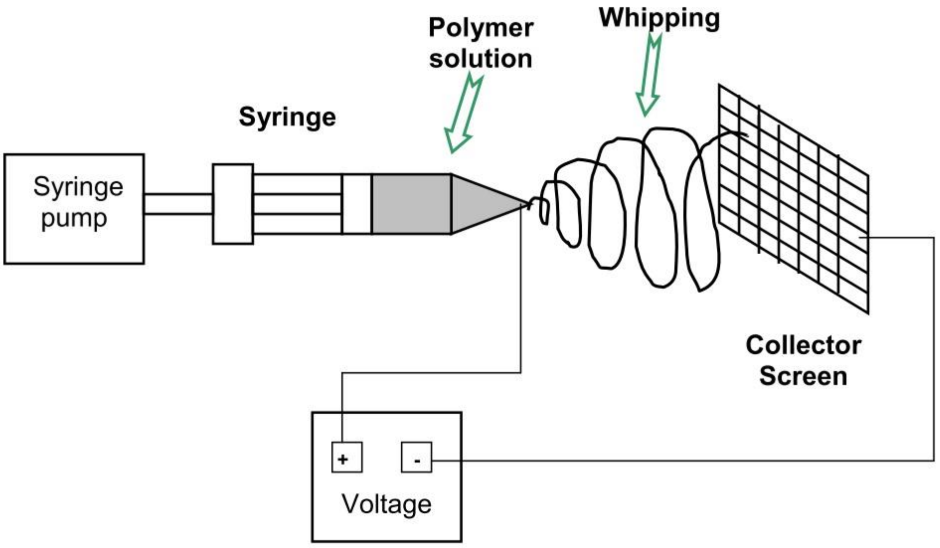


Figure 2.2: Basic electrospinning apparatus (Raghavan, 2006).

Figure 2.3: Formation of the Taylor cone. A) Voltage starting, B) Voltage increasing, C) Equilibrium between surface tension resulted in electrostatic force (Zufan, 2005).

2.4.5 Electrospinning Parameters

Many operating parameters can influence the transformation of polymer solutions into electrospun nanofibres and fibre diameters. Numerous studies examined the effect of changing electrospinning parameters and the experimental apparatus (Correia et al., 2014)(Sawawi et al., 2017). Previous research made it clear that characteristics such as fibre morphology, fibre diameter and the amount of beading are dependent upon many variables (Doshi and Reneker, 1993)(Deitzel et al., 2001). These variables include solution concentration, conductivity, viscosity, surface tension and process variables, such as voltage, needle diameter, flow rate and needle-to-collector distance (Ramakrishna et al., 2005). Thompson et al. (2007) showed there are five important parameters significantly affecting the jet radius including distance from nozzle to collector, volumetric charge density, initial jet/orifice radius, viscosity and relaxation time. The other parameters such as initial polymer concentration, solution density; perturbation frequency, electric potential and solvent vapour pressure have moderate effects on the jet radius. Parameters such as relative surface tension, humidity, and vapour diffusivity have minor effects on the jet radius. To control electrospinning process and predict electrospun fibre production, knowing the relative effects of parameters on jet radius should be useful (Leach et al., 2011).

2.4.6 Mechanism of Bending Instability of Electrically Charged Jets

During the electrospinning process, the electrically charged jet travels from the orifice in a straight line for few centimetres. At the end of this straight segment, it starts bending into a complex path and other changes in shape occur. Because of the bending instability the jet rearranges into a sequence of connected loops seen when proper illumination is provided. The part of the jet with loops is called the cone of the envelope. Due to repulsion, the charge jet in each loop gets longer and thinner as loop diameter increases. The part of the jet inside the envelope undergoes bending instability due to electrical driven bending instability (Figure 2.4) (D.H. Reneker *et al.*, 2000).

There are different forces acting on the continuous flow of the charged jet including:

1. Downward electrical force from external field to the collector.
2. Repulsive force between charges carried by the jet – trying to elongate the jet acting along the length of the jet.
3. Viscoelastic force along the length of the jet acting – opposite to repulsive force
4. Surface tension force – acting along the length of the jet.
5. Downward gravitational force (Bhargava, 2007).

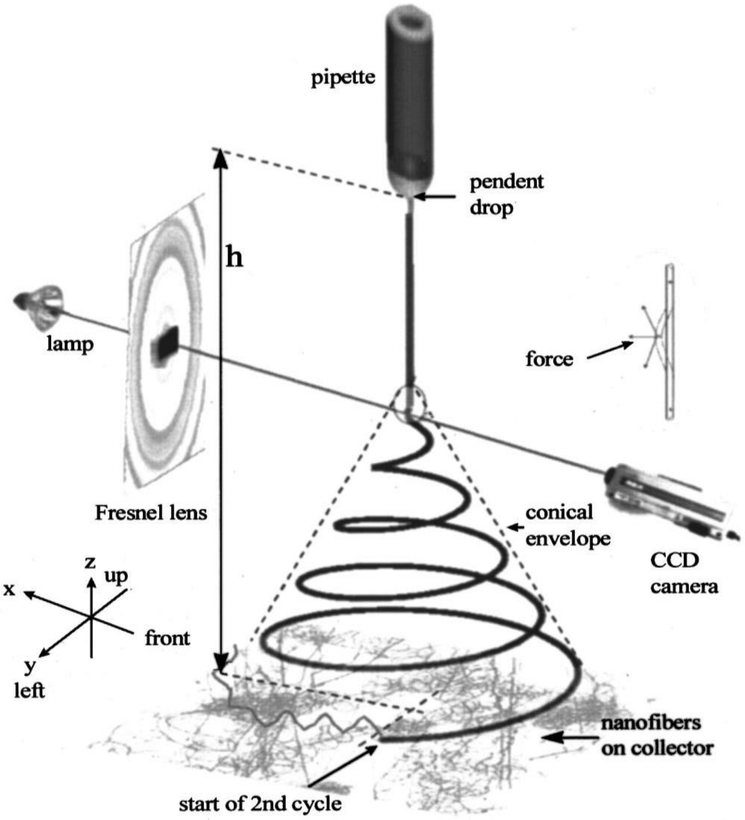


Figure 2.4: Schematic drawing of the electrospinning process, showing the jet path, reference axes, relative arrangement of parts of the apparatus at different scales, and the region where the bending instability grew rapidly (Reneker *et al.*, 2000).

Reneker et al. (2000) investigated three-dimensional paths of continuous jets, both in the region where the bending dominated the path of the jet and in the nearly straight region where the instability grew slowly. The region near the vertex of the envelope cone (Figure 2.5) was imaged at 2000 frames per second and in three dimensions.

These images illustrated the timed shape of the jet clearly and in detail. The bending instability cycle can be explained in three steps (Reneker *et al.*, 2000).

Step1: A smooth segment that includes straight or slightly curved jets that suddenly grew into an array of bends.

Step 2: The segment of each bending jet that was elongated and the array of bends became a series of spiralling loops while diameters grew.

Step 3: When the perimeter of the loops grew larger, the cross-sectional diameter of the jet forming the loop decreased, meanwhile the conditions for step 1 were established on a smaller scale, and the next cycle of bending instability started (Reneker *et al.*, 2000).

This instability cycle repeated at an even smaller scale. It was finalized that more cycles occur, and the jet diameter decreases even more as well as nanofibres are produced (Reneker *et al.*, 2000).

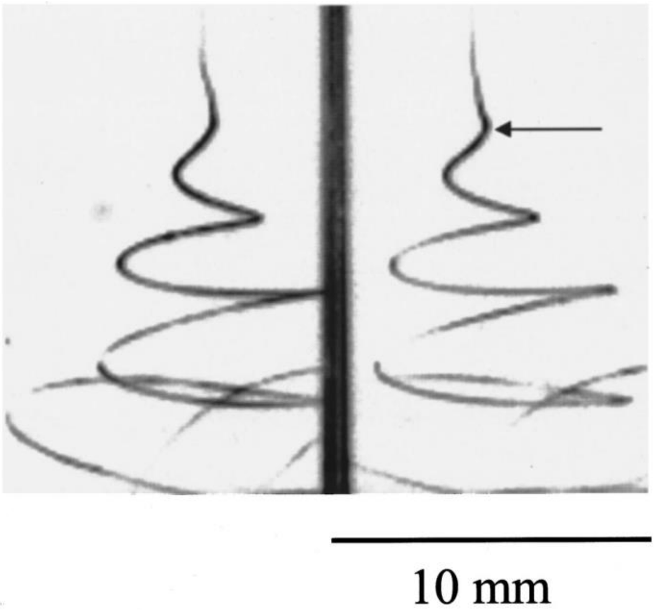


Figure 2.5: Stereographic images of an electrically driven bending instability. The exposure time was 0.25 ms. The arrow marks a maximum lateral excursion of a loop (Reneker *et al.*, 2000).

2.4.7 Electrospinning Techniques for Producing Nanofibres

Electrospinning techniques are creating interest as a functional fabrication method for producing organic, inorganic and hybrid nanofibres with controlled dimensions. Oriented continuous nanofibres are generated with possibilities of ordered internal morphologies such as hollow, core–sheath or porous fibre, or even multi channelled micro tube arrangements. These various types of nanofibres can be produced by different electrospinning fabrication techniques. Some of the important techniques are briefly described below (Kiyak and Cakmak, 2014).

2.4.7.1 Basic Electrospinning

Basic electrospinning involves a melt or solution of polymer fed through a narrow needle or nozzle (Ramakrishna *et al.*, 2005)(Zufan, 2005). The nozzle or needle performs as an electrode simultaneously applying typically high voltage from 20 to 100 kV (Patanaik *et al.*, 2014). While high voltage reaches critical value, electrostatic force overcomes the surface tension and the charged polymer solution is forced to come out of the tip of the nozzle or needle. When the pulled polymeric solution jet passes through an elongation and instability phase, solvent vaporizes, and solidification takes place. Finally, an interconnected layer of nanofibres on the surface of the collector is created (Reneker *et al.*, 2000)(He and Liu, 2008).

2.4.7.2 Melt Electrospinning

There has been a desire to produce nanofibres that are environmentally safe by concentrating on a less hazardous and environmental safe process (Lyons *et al.*, 2004). Most of the electrospun nanofibres are based on solution spinning, and hazardous chemicals used to dissolve plentiful polymers. Thus, the use of melt electrospinning system to produce electrospun webs has attracted great attention (Larrondo and Manley, 1981). Figure 2.6 demonstrates the design of typical melt electrospinning (Kiyak and Cakmak, 2014). During melt electrospinning, there must be a constant heat supply to the reservoir containing the polymer solution so that the polymer remains in a molten state. At the critical voltage, electro static forces overcome the surface tension. A fine fibre extrudes from the orifice due to viscoelastic behaviour of the melt. As the polymer is in a molten form, greater charge is required for jet initiation, the distance from nozzle to collector is typically smaller than basic electrospinning (Lyons *et al.*, 2004).

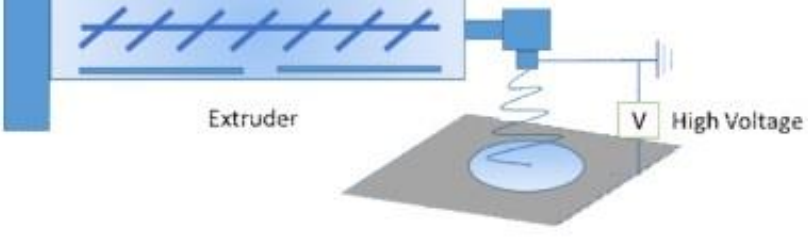


Figure 2.6: Melt electrospinning technique (Kiyak and Cakmak, 2014).

2.4.7.3 Bubble Electrospinning

The bubble electrospinning system is an innovative technique to mimic the spider spinning and minimize the surface tension of solution (Liu, He and Yu, 2008). The bubble electrospinning system includes a vertical solution reservoir with a gas tube feeding from the bottom with a metal electrode fixed along the middle of the tube. A grounded collector is fixed above the reservoir (Figure 2.7)(Yang *et al.*, 2009). The aeration system produces various bubbles on the surface during electric field absence. While an electric field is created, it induces charges into the bubble surface. A tangential stress of surface charge and external electric field results in the transformation of the little bubbles into protuberance induced upward-directed re-entrant jets. At the critical value, a fluid jet distracts from the zenith of the conical bubble. As this novel technology is independent of the solution properties such as viscosity, it has critical importance for the new generation of electrospinning (Kiyak and Cakmak, 2014).

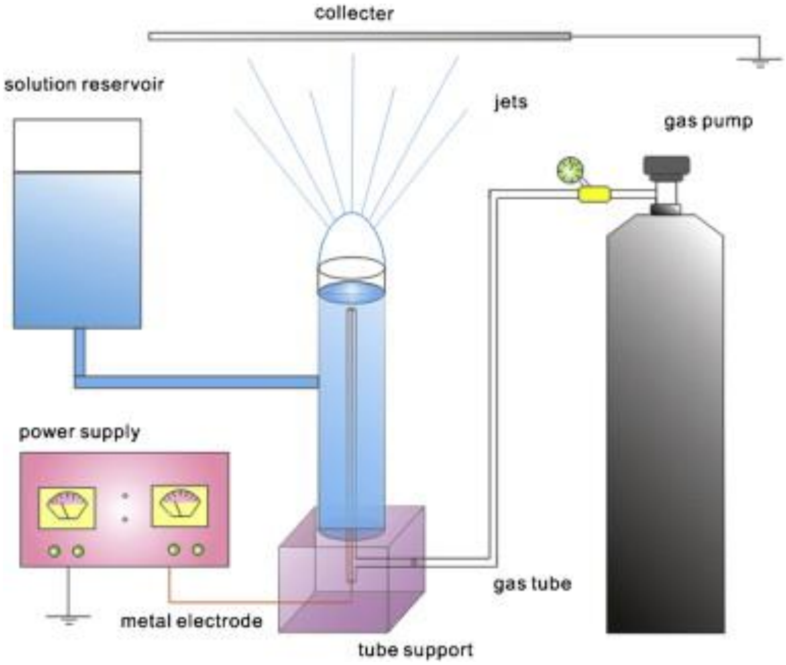


Figure 2.7: Bubble Electrospinning Technique (Yang *et al.*, 2009).

2.4.7.4 Gas-Jacket Electrospinning

Due to excessive surface tension of the solution or high viscosity, electro static force may not be enough alone to stretch the solution for producing nanofibres. Therefore, a gas jacket can be used at the spinneret tip to initiate the spinning that brings into play a stretching force on the solution (Figure 2.8) (Teo *et al.,* 2011). A heated gas can be used in fibre fabrication because the higher temperature decreases the viscosity of the solution. The inert gas is brought up from the basin and passed through the buffer. The gas flow additionally assists to stretch the jet of the polymer solution, which was slowly injected into a capillary by injector and flowed through a capillary with a definite length (Um *et al.*, 2004).

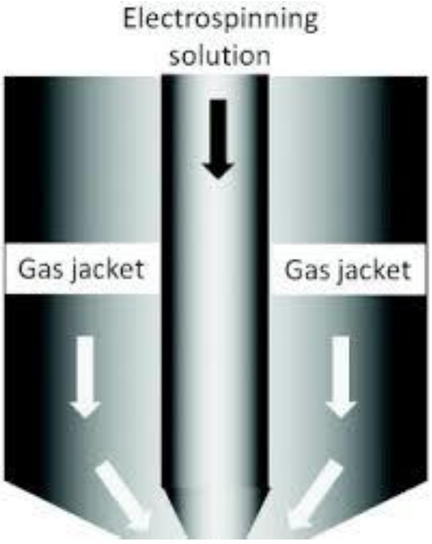


Figure 2.8: Gas Assisted Electrospinning System (Teo *et al.*, 2011).

2.4.7.5 Co-axial Electrospinning

Co-axial electrospinning technique includes two liquids that distract from the core and surrounding concentric nozzles in the form of polymer solution or melt. The liquids contact each other only at the tip of the coaxial needle or capillary (Figure 2.9) (Kiyak and Cakmak, 2014). Solvents evaporate and compound jet solidify during the spinning process, moreover core shell nanofibre is produced. The critical point in coaxial electrospinning is to prevent the mixing of polymer solutions. By this technique, polymer that cannot be electrospun by basic electrospinning is preferred to obtain core-shell nanofibre, which have potential applications in medicine, microelectronics, and optics (Andrady, 2008).

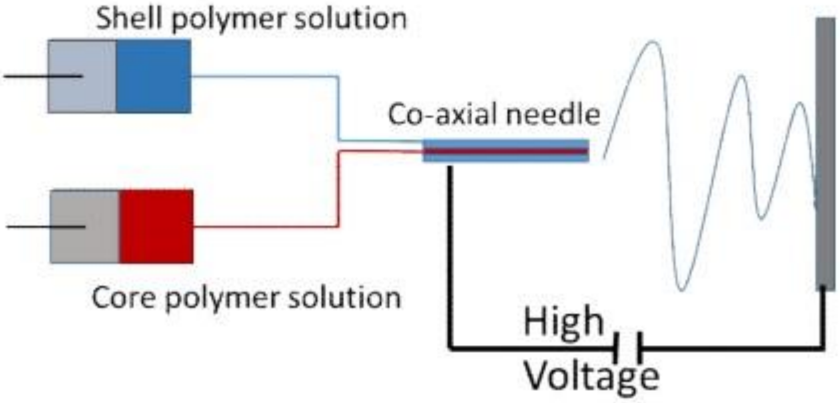


Figure 2.9: Co-axial Electrospinning (Kiyak and Cakmak, 2014).

2.4.7.6 Centrifugal Electrospinning

The centrifugal electrospinning technology is the combination of electrospinning and centrifugal spinning (Figure 2.10). A high-speed rotating spinning disc is applied to gather polymer solution centrally. The centrifugal forces overcome the surface tension of polymer solution that moved radial towards the rim of the rotating disc. The electric field stretches the jets to very small dimensions under simultaneous evaporation of the solvent, and dry nanofibres are left on the substrate. This technique is independent of ambient conditions such as humidity and temperature. The centrifugal electrospinning is a definite advantage in comparison to other electrospinning processes, which sometimes are affected severely by relative humidity. The possible defects in this method are bead and holes (Engström and Hagström, 2009).

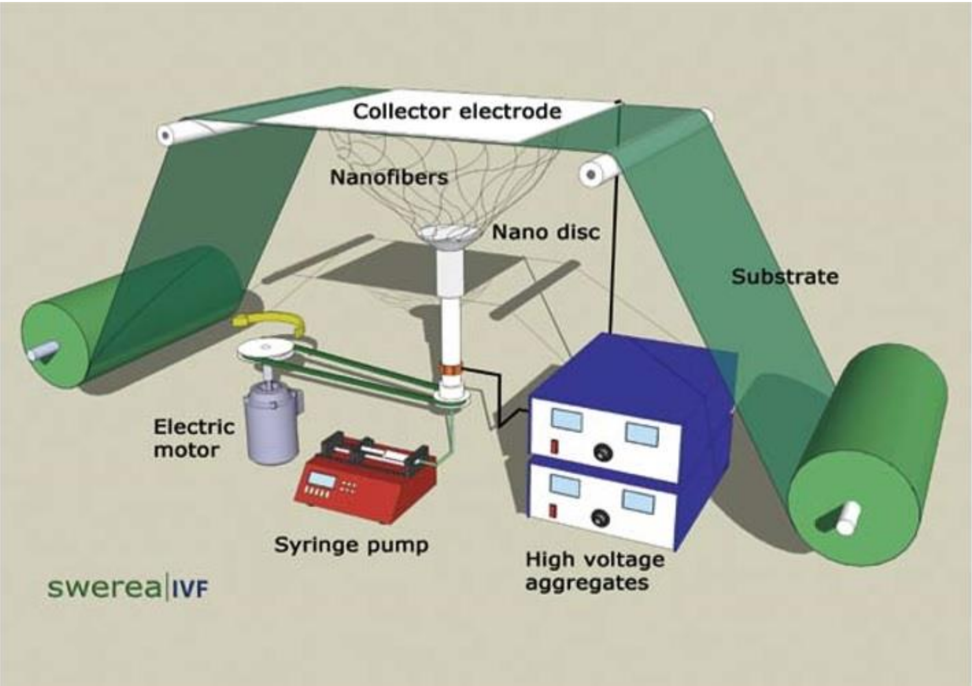


Figure 2.10: Centrifugal Electrospinning (Engström and Hagström, 2009).

The random orientation of nanofibres is applied for special applications including composite reinforcement, filtration, and biomedical applications e.g., tissue engineering scaffolds, wound dressings, drug delivery materials. For other applications, such as electronic, optoelectronic, photonic, sensors, etc., it is more desirable to obtain nanofibre yarn samples in which the fibre axis are aligned in the same direction which is referred to as "axial orientation."(Lotus *et al.*, 2008).

2.5 Electrospinning of Nanofibre Yarn

Several methods have been developed to align electrospun nanofibres and some breakthroughs have been achieved (Ali *et al.*, 2011). A simple and versatile set-up is shown in Figure 2.11. It consists of two high electrical voltages with opposite polarities applied respectively to a dual spinneret. An electric field is formed between the two spinnerets, and under the action of the electrical field, liquid jets on the tips of the spinnerets are stretched out and do whipping movements, resulting in the formation of charged nanofibres. The oppositely charged nanofibres attract each other to composite nanofibres, and then are drawn out and stretched by a drum, triggering continuous nanofibre yarn (Pan *et al.*, 2006).

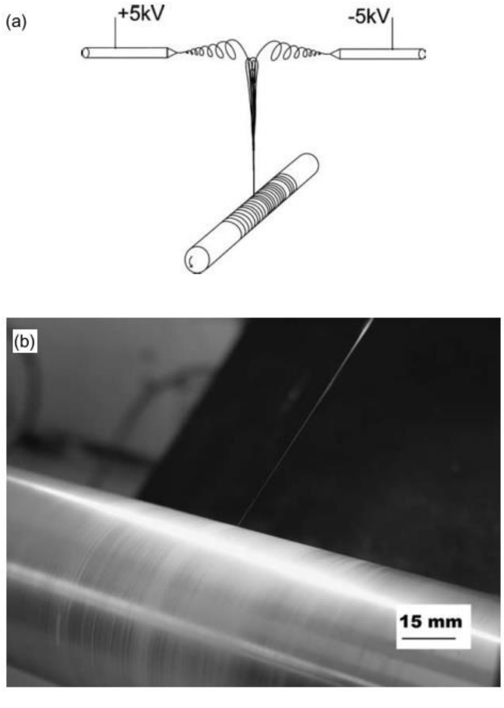


Figure 2.11: (a) Schematic electrospinning apparatus for collection continuous aligned fibres. (b) A yarn of fibres is formed between the needles and a rotating aluminium shaft (Pan *et al.*, 2006).

2.6 Electrospinning of Hollow Nanofibre Yarn

To date, only a few methods have been proposed to obtain hollow yarn by using the electrospinning process. Bhargava (2007) used a hollow rotating metal rod with attached hollow metal hemisphere at one end and two different polymer solutions to electrospin a core-sheath yarn. Subsequently, by removing the inner core yarn (PVA electrospinning yarn) in boiling water, hollow nanofibre yarn could be obtained (Bhargava, 2007). A schematic diagram of Bhargava apparatus and SEM image of Nomex hollow nanofibre yarn has been shown in Figure 2.12, and Figure 2.13, respectively.

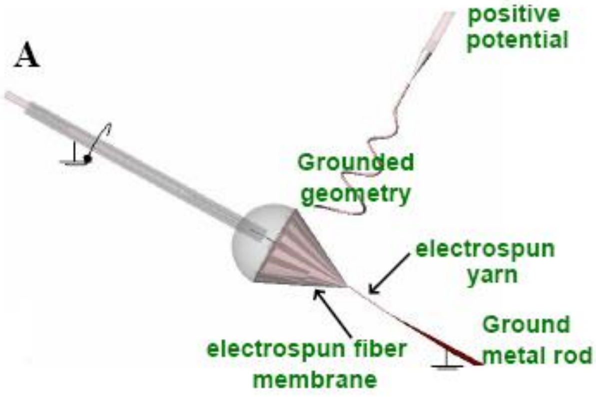


Figure 2.12: Schematic diagram of Bhargava apparatus to produce core-sheath nanofibre yarn by rotating a hemispherical collector (Bhargava, 2007).

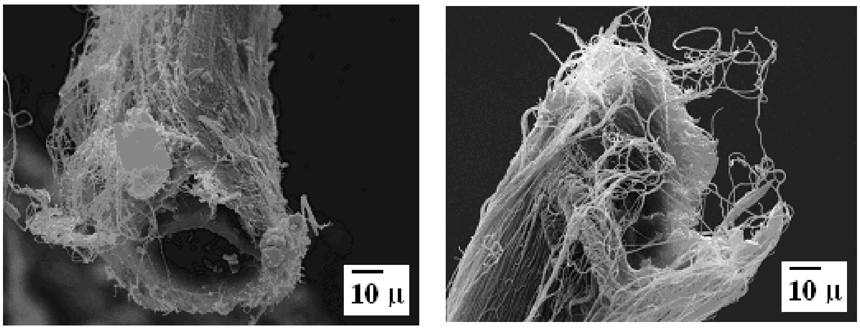
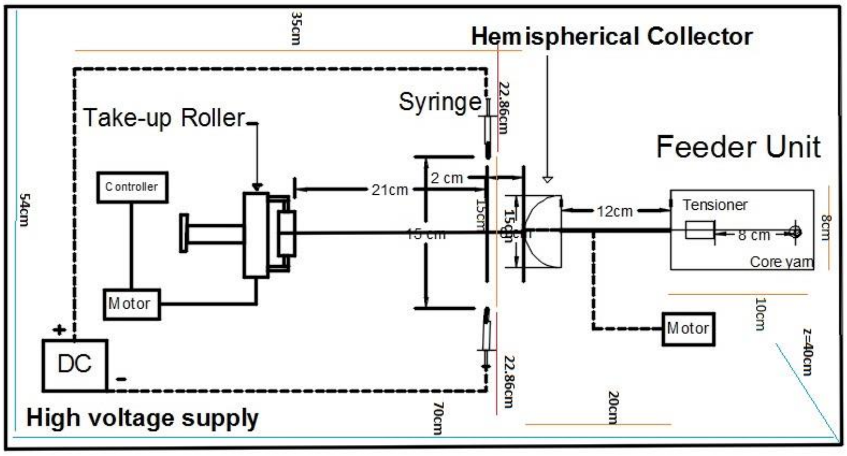
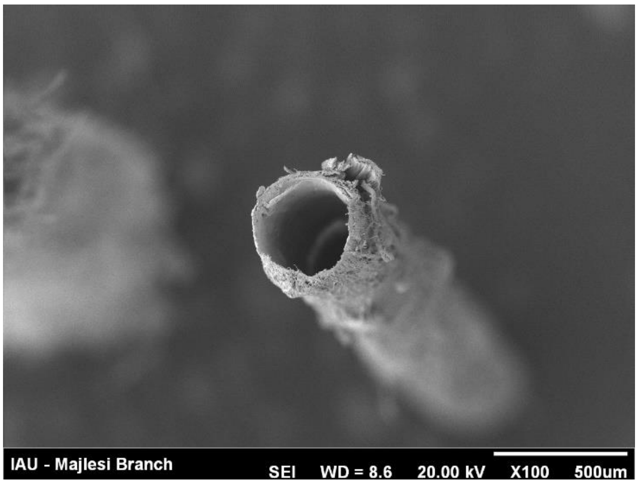


Figure 2.13: Scanning Electron Microscope images of Nomex hollow nanofibre yarn (Bhargava, 2007)

Following this work, a modified electrospinning apparatus was used to manufacture uniaxial aligned nanofibre yarns by Wu and Qin (2013). They employed two oppositely placed metal needles and applied positive and negative voltages. Meanwhile, they placed a neutral metal disc and hollow metal rod oppositely in the middle of the two needles. Nanofibres ejected from two needles were oriented in the gap between the metal disc and the tapered sharp end of hollow metal rod. A nanofibre bundle was obtained and twisted by rotating the metal disc and fastening the hollow metal rod. After passing through the hollow metal rod, a yarn was continuously wound by a rotating take-up roll (Wu and Qin, 2013). Javazmi et al. (2014) applied a two nozzle conjugated electrospinning method to fabricate a core sheath yarn from nanofibres of polyester as sheath and PVA multifilament as core yarn. Then by dissolving the core yarn in hot water for 60 minutes, a hollow nanofibre yarn was produced, however using hot water resulted in yarn shrinkage (Javazmi, *et al.*, 2014). A schematic diagram of an electrospinning apparatus to produce core-sheath nanofibreyarn and an SEM image of the cross-section of hollow nanofibre yarn are illustrated in Figure 2.14 (a, b)(Javazmi, Ravandi and Ghareaghaji, 2014). Recently, Fakhrali et al. (2014) developed a novel method of fabricating core sheath nanofibre yarns, which consisted of PVA nanofibres as a core and nanofibres of Nylon 6 as a sheath. They achieved a PVA/Nylon6 nanofibrous structure by applying changes into nozzles placement angles. Then by immersing fabricated core sheath yarn in boiling water boiling, PVA nanofibres was dissolved and hollow nanofibre yarn of Nylon 6 was produced. Although extracting the core yarn is easier by this technique, the hollow zone volume is restricted and so producing larger inner diameters is not possible in this process (Fakhrali, *et al.*, 2014).



(a)



(b)

Figure 2.14: (a) Schematic diagram of electrospinning apparatus to produce core-sheath nanofibre yarn and (b) SEM image of cross-section of hollow nanofiber yarn (Javazmi et al., 2014).

2.7 Application of Nanofibres for Drug Delivery Systems

In the fields of medicine and agriculture it is often desirable to maintain an effective concentration of an active agent, for example a pesticide, herbicide, fertiliser or drug, at some site of action for a prolonged time (Baker, 1972). The methods used to achieve these goals are diverse and complex and depend on the particular application. In fact, several mechanisms may operate simultaneously or at different stages of a delivery process. An understanding of these mechanisms is important when designing and manufacturing controlled release systems, and in identifying potential failure modes (Siegel and Rathbone, 2012). One method of achieving this goal is to deliver a large excess of the agent so that even though it is metabolized, excreted, or degraded, sufficient amount remains to maintain the effective dose. This approach is not only wasteful of the active agent but maintaining such a large excess during the early portion of the delivery period often leads to overdose-related side effects. A better pattern of delivery is to dispense the agent from a sustained release delivery system which releases the active agent at a slow controlled rate throughout the delivery period (Baker, 1976). Electrospinning is a very simple and inexpensive technique that utilizes an external electrostatic field to manufacture fibres with diameters ranging from several nanometres to micrometres (Ali *et al.*, 2011)(He and Liu, 2008)(Subbiah *et al.*, 2005). The inherently high surface to volume ratio of electrospun fibres and ease of incorporation of active ingredients show great promise for developing many types of novel drug delivery systems (DDS), enhancing cell attachment, and mass transfer properties (Hu *et al.*, 2014)(Yu *et al.*, 2009). This paper provides an overview of electrospun nanofibres used as agrochemical delivery systems.

2.7.1 Comparative Table for Material delivery systems

A comparison of nanofibre delivery systems is shown in Table 2.1.

Table 2.1: Comparative table for nanofibre material delivery systems

Method	Reference	Application	Characteristics	Advantages
Chitosan nanoparticle based delivery systems	(Kashyap, Xiang and Heiden, 2015)	Agrochemicals or genetic materials	• Biocompa-tibility • Biodegrad-ability • Non-toxicity • adsorption abilities	• Encapsulating agrochemicals and genetic material • Protective reservoir for the active ingredients • Protecting the ingredients from the surrounding environment • Controlling their release • Allowing them to serve as efficient gene delivery systems for plant transformation
Polyurethane (PU) and polycaprolactone (PCL) nanofibres based drug delivery	(Kenawy *et al.*, 2009)	The delivery of ketoprofen as non-steroidal anti-inflammatory drug (NSAID)	• Mixed nanofibres of biodegradable such as (PCL) and non-biodegradable polymers such as (PU)	• Improving visual mechanical properties of nanofibres encapsulating Ketoprofen in comparison to previous methods
Core/Shell nanofibres by co-electrospinning system for drug delivery	(Mickova *et al.*, 2012)	For the delivery of susceptible biomolecules to heat and light such as enzymes inside liposomes	• The hydrophilic core of PVA nanofibres facilitates the loading and preservation of protein bioactivity. • The hydrophobic poly-ε caprolactone shell nanofibres allow fibre formation.	• Preserve the enzymatic activity of encapsulated horseradish peroxidase in contrast to blended electrospinning. • Coaxial electrospinning provides homogeneous protein distribution throughout the fibres, and proteins can be delivered in a controlled manner. • Coaxial electrospinning enabled the retention of an aqueous environment inside intact liposomes embedded in nanofibres
PLLA nanofibres based delivery system	(Zeng *et al.*, 2003)	For encapsulating and delivering rifampicin (a drug for tuberculosis)	• Reducing the diameter size and distribution of PLLA electrospun fibres by adding surfactants (anionic, cationic, non-ionic).	• Along with degradation of the fibres, the drug was released constantly. • No burst release was observed.

Polymer coated materials	(Lubkowski *et al.*, 2015)	For controlled-release fertiliser	• Using biodegradable copolymer of (PBS/DLA)	• Environmental friendly fertiliser in comparison to all of market products with prolonged nutrient release, including commercially successful ones such as Osmocote, Multicote, Meister, Nutricote, Basacote, or Polyon which are manufactured with the use of polymers that are not biodegradable. • Decreasing nutrient release from the polymer-coated materials because of increasing layer thickness • Presenting a conceptual model to describe the mechanism of nutrient release from the materials.
PVA and PEO electrospun nanofibres based urea delivery	(Hassounah et al. 2013)	For nanoagriculture applications where the fertiliser must be released slowly over a month	• Non-toxic biodegradable water soluble polymers • The PVA and PEO nanofibres can remain for up to 34 days	• Containing high concentration of urea because of three-dimensional network of PVA and PEO electrospun nanofibres, which may have an application as means to safely distribute urea fertiliser in nanoagriculture
Polyvinylpyrr--olidone nanofibres based urea delivery to seeds	(Krishnamoorthy et al. 2016)	Seed coating Seed pelletizing	• PVP nanofibre water solubility • Non-toxicity • Adhesive nature	• Despite the coating materials like polyvinyl chloride and polysulphones that aren't degraded easily in the soil and get accumulated over time after the release of fertilisers, Polyvinylpyrrolidone nanofibres containing urea are completely biodegradable. • PVP electrospun fibers have advantages over the PVP film due to the ease of processing, no residual solvent and high surface area.
Wheat gluten electrospun membranes-based fertiliser delivery	(Castro-Enríquez et al. 2012)	Potential application in prolonged-release system of urea in agriculture.	• 100% natural • Biodegradable • Inexpensive • Highly available High surface area of nanofibres	• Providing a potential solution to the problem of loss by leaching of this fertiliser in agricultural crops. • Because of their porosity and the hydrogen bonding interactions formed between urea and WG proteins, the membrane has capability to encapsulate and prolonged release of urea. • Obtained membranes with very small thicknesses not previously reported using other techniques.

Polyvinyl alcohol (PVA) electrospun nanofibres based rhizobium delivery	(Damasceno et al. 2013)	Rhizobia immobilization incorporated in PVA nanofibres for soybean seed growing.	• High viscosity • Power of scattering.	• Efficient for the incorporation of rhizobia in PVA coating when applied to soybean seeds. • Electrospinning technique presents an excellent alternative for the encapsulation of microorganisms to protect them from dehydration and the effects of toxic chemicals
PLA/PHB core-shell nanofibres based fertiliser delivery	(Kampeerapappunet al. 2013)	Prolonged release fertiliser delivery.	• Biodegradable polymers • Preventing soil pollution	• Released fertiliser for a month and were biodegradable within 3 months.

2.8 Applications of Electrospun Nanofibres in Agriculture

High surface area and ease of incorporation of active ingredients have motivated some researchers to investigate using electrospun nanofibres in agricultural applications (Noruzi, 2016). By comparison with other fibre spinning techniques, electrospinning-based nanofibre dispenser can be made portably and used on farm and it is cost efficient for agricultural applications. Active ingredients including pheromones and pesticides are usually incorporated into electrospun nanofibres (Teo 2015).

2.8.1 Rhizobium

Bacteria and microbes play a main role in the soil ecosystem. In many important crops, the presence of special bacteria and microbes such as Pseudomonas, Rhizobium, Azospirillum and Bacillus in soil are necessary for crop health as they enhance both above and below ground biomass and therefore have a great impact in achieving sustainable agriculture outcomes (Igiehon and Babalola, 2018). Rhizobium lives in the soil or in nodules on the roots of legumes and establishs a symbiotic relationship with them. The rhizobium convert atmospheric nitrogen into ammonia acting as natural fertiliser for the plant. This nitrogen fixation by natural means is always the best and healthy option; however, fixed nitrogen by Rhizobia has often been limited by environmental stress such as climate change. Research activities have been intensified to find methods for improving the bacteria survival rate in view of efficient plant growth (Shridhar, 2012).

A previous study showed that the survival of rhizobia of the species Bradyrhizobium japonicum, used in commercial inoculants in soybean cultivation, is extended by immobilizing in PVA nanofibres. According to the study, PVA polymer increases the absorption of water resulted in suitable environment for the bacteria and minimizing the effect of cellular desiccation (Mugnier and Jung, 1985).

Brazilian scientists from Federal University of Rio Grande do Sul, and Tecnano Products and Services Company verified an innovative method to extend soil bacteria life and therefore its agricultural benefits using nanofibre. They immobilized the Rhizobia by introducing nanofibres, which proved to be a promising alternative for decreasing the harmful effects of environmental stresses on bacteria. The team used electrospinning technique firstly to fabricate polyvinyl alcohol (PVA) nanofibres. The PVA is an effective polymer in cell encapsulation owing to its physical characteristics including scattering and viscosity. The incorporated Rhizobia into PVA nanofibres were then applied to soybean seed. They observed that soybean seedlings from seeds inoculated with Rhizobia-PVA nanofibre form a larger number of nodules. Their observation illustrated that at different storage times no significant differences in the cell survival were shown even after 24 hrs of storage but there was an obvious difference in the bacterial cell concentration after 48 hrs. The team mentioned electrospinning technique is a cost effective and satisfied way to immobilize Rhizobia and suggested it as outstanding achievement compared to the usual protector inoculants because of its great capacity to control the release of Rhizobia (Damasceno *et al.*, 2013).

2.8.2 Pest Attractants

Pheromones have been used extensively for controlling insect pests by leading them to traps (Kloosterman and Mager, 2014). Large surface area of electrospun nanofibre is used to effectively release the pheromones for this purpose. Bansal (2010) used electrospun nanofibres incorporated with an insect pheromone for protecting grapevine against Lobesia botrana (grapevine moth). He prepared a complex emulsion mixture containing 1 wt. % OLA/pheromone/Brij S20 dispersion/16 wt. % PHA-b-MPEG dispersion/PEO.

More recently, a research showed that it is possible to release dispensers of active ingredients, such as Trimedlure (synthetic attractant) by electrospun nanofibres for controlling Medfly population. Ethylcelluose, polyethylene glycol (PEG- PCL), polyvinyl acetate-vinyl pyrrolidine and polycaprolactone were blended with Trimedlure separately. Subsequently, the solution was transformed to nanofibres through electrospinning process. Up to 10% w/v of the active ingredient was added (Bisotto-de-oliveira *et al.*, 2014).

2.8.3 Fertiliser Application

Fertilisers are used broadly to promote plant growth, increase crop production, and improve quality of products. Application of large quantities of fertiliser is common across many agricultural industries and quantities applied varies to compensate for losses through volatilization and leaching (Al-Zahrani 2000). Recent investigations have focused on the development of systems using different materials that prolong the release of fertilisers (Ni et al. 2009)(Trenkel, 1997). These systems aim to minimise soil contamination and obtain a controlled fertiliser release system at low cost, using material that is 100% natural and biodegradable, inexpensive and highly available (Al-Zahrani 2000). Chen *et al.*, (2008) used a membrane-encapsulated urea with starch-g-PLLA as a biodegradable carrier for slow release fertiliser. A solution-casting method was applied to prepare starch-g-PLLA/urea composite films. The release behaviour of encapsulated urea study concluded that starg-g-PLLA/urea showed relatively low swellability, large encapsulating capacity, and slow-releasing rate, from several hours to one day. Electrospun fibre has a large surface area, however nanoparticles have an even larger surface area, which may make it easier to produce on a large scale compared with electrospinning. In contrast, as fertiliser release control is more related to the chemistry and encapsulation as opposed to the carrier structure, electrospun nanofibres have an advantage over particulate carriers, as they are less likely to be washed away than particles. As a result, the farmer can decrease the amount of fertiliser loss and prevent potential environmental contamination as well as fertiliser run-off by using nanofibres. During hot and dry seasons, a layer of fertiliser with electrospun fibres is used directly over the top soil to hold down the soil with the seed in place while waiting for rain to arrive in an exposed dry field. The method used to load fertilisers into the electrospun fibres and the degradation rate of the nanofibres can affect the releasing control of fertilisers (Teo, 2014). Castro-Enriquez *et al.*, (2012) produced wheat gluten elecrospun fibre membrane and immersed into urea solution. Since the urea was coated on the surface of the fibre membrane, the prolonged-released systems of urea in water was studied. The release of urea during the first 10 min was very fast; then, the rate of release decreased as it reached equilibrium at 300 min, with a total of ≈98% urea released.

Kampeerapappun and Phanomkate, (2013) used coaxial electrospinning with the nitrogen fertiliser loaded in the core component. Biodegradable polyhydroxybutyrate (PHB) and polylactic acid (PLA) were used as the sheath and the core, respectively. After optimizing the material composition (eg. flow rate, loading amount) in core-shell structure, the core-shell electrospun mats released fertiliser for a month and were biodegradable within three months. Application of electrospun nanofibres as fertiliser careers is still at its initial stage. Electrospun nanofibre with fertiliser loading can be applied onto the soil either as ready nanowoven patch or by direct electrospinning (Teo, 2014).

2.9 Methods for Determining Nitrogen Release from Controlled-release Fertilisers

There are three subgroups of Enhanced-efficiency fertilisers (EEF) with different characteristics for agricultural production systems:

1. Slow-release fertilisers (SRFs): contain nitrogen (N) in a less-soluble, plant unavailable form that usually needs microbial degradation to provide plant available N (Trenkel, 1997).
2. Stabilized fertilisers: have a chemical inhibitor to either stop the oxidation of ammonium (NH4+) to nitrate (NO3-) by bacteria or to slow the enzymatic transformation of urea to NH4+ (Trenkel, 1997).
3. Controlled-release fertilisers (CRFs): urea, ammonium nitrate, potassium nitrate, or other soluble fertiliser materials coated with a polymer (polyethylene and ethylene-vinyl-acetate or thermoplastics), resin, sulphur, or a hybrid of sulphur-coated urea (SCU) coated with a polymer or resin (Trenkel, 1997).

There are three methods for measuring nitrogen release in controlled-release fertilisers (CRFs): laboratory; growth chamber, greenhouse, or both; and field methods (Trenkel 2010). The European Union has developed both standard and accelerated laboratory procedures for measuring N release from CRFs; however, researchers in the United States are still developing a universal test for CRFs and SRFs for commerce purposes. Growth chamber and greenhouse methods are used to evaluate or compare how CRFs will act in a particular controlled environment. Lastly, field methods are used to measure N release in commercial vegetable field conditions. Each research method has its own advantages and disadvantages (Carson & Ozores-hampton 2012).

2.10 Procedure to Measure Nitrogen

N concentration in leachate or in the CRF prills[3] needs to be measured after incubation with all CRF research methods (laboratory, growth chamber, greenhouse, and field methods). Methods to measure N include TKN, combustion, prill weight loss, calorimetrically with an auto analyser, and ion specific electrodes to measure NH4 + or NO3. The standard and most popular method, TKN, is a time-consuming laboratory procedure, which includes concentrated sulphuric acid and sodium hydroxide. All CRFN sources and research methodologies may use TKN (Carson & Ozores-hampton 2012). The term Total Kjeldahl Nitrogen refers to the combination of ammonia and organic nitrogen. The ammonia is then analysed by a modified Nessler method test. The measurement wavelength is 460 nm for spectrophotometers or 420 nm for colorimeters (Instruments 2009). Plant laboratories usually select the easy handling and quite robust volumetric and spectrometric methods. Spectrometric method mostly determines ammonium with hypochlorite and salicylate, by the ionic selective electrode and by titration with hydrochloric acid. Nitrates are analysed by the methods based on the reaction of nitric acid with 2, 6-dimethylphenol, 4-fluorophenol and sulfosalicylic acid. Nitrites are quantified by the spectrometric methods based on their ability create diazonium salts which react with N (1-naphtyl) ethylenediamine dihydrochloride. According to Kjeldahl, the determination of organic nitrogen is based on the transformation of N-III into ammonium. Total nitrogen is determined as ammonium after the digestion according to Kjeldahl, as nitrates after oxidation with peroxodisulfate and by catalytical ignition in the oxygen atmosphere at about 1000 ºC forming nitrogen oxides (Vody 2002).

2.11 Summary of the Literature

The literature review reveals that with comparison to the other fibre spinning method, the electrospinning is a versatile, unique and inexpensive technique using electrostatic forces to produce ultrafine fibres with diameters in the range of ten nanometres up to several micrometres. Using this technique, interesting results have been reported regarding the potential applications of nanofibres in agriculture sector. These nanofibres offer increased surface to volume ratio and high aspect ratio which can form

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[3] A prill is a small aggregate or globule of a material, most often a dry sphere, formed from a melted liquid.

porous structure with excellent pore interconnectivity to encapsulate and release chemical materials in agriculture.

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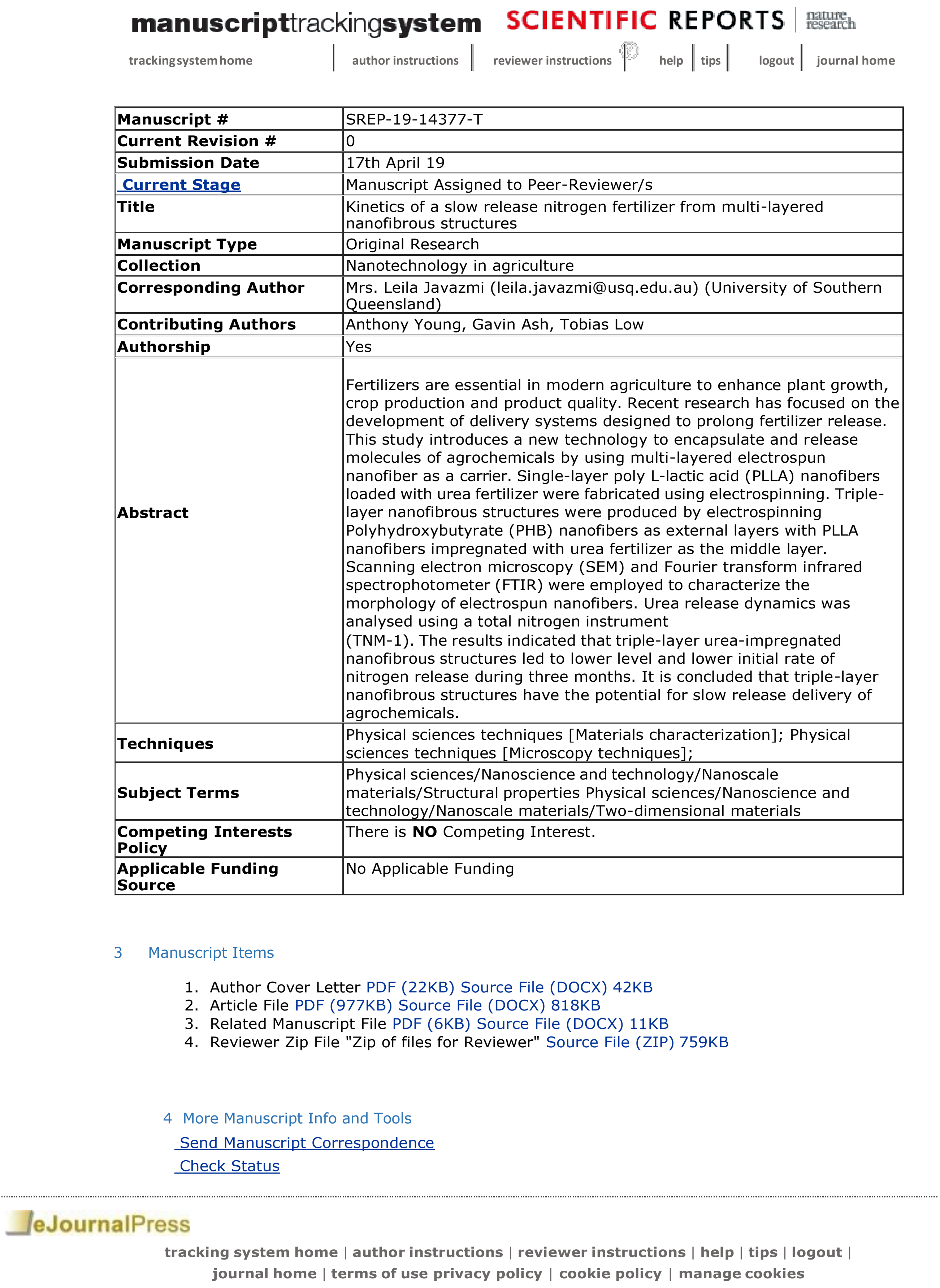
Chapter 3. Kinetics of a Slow Release Nitrogen Fertiliser from Multi-Layered Nanofibrous Structures



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Abstract	Fertilizers are essential in modern agriculture to enhance plant growth, crop production and product quality. Recent research has focused on the development of delivery systems designed to prolong fertilizer release. This study introduces a new technology to encapsulate and release molecules of agrochemicals by using multi-layered electrospun nanofiber as a carrier. Single-layer poly L-lactic acid (PLLA) nanofibers loaded with urea fertilizer were fabricated using electrospinning. Triple-layer nanofibrous structures were produced by electrospinning Polyhydroxybutyrate (PHB) nanofibers as external layers with PLLA nanofibers impregnated with urea fertilizer as the middle layer. Scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometer (FTIR) were employed to characterize the morphology of electrospun nanofibers. Urea release dynamics was analysed using a total nitrogen instrument (TNM-1). The results indicated that triple-layer urea-impregnated nanofibrous structures led to lower level and lower initial rate of nitrogen release during three months. It is concluded that triple-layer nanofibrous structures have the potential for slow release delivery of agrochemicals.
Techniques	Physical sciences techniques [Materials characterization]; Physical sciences techniques [Microscopy techniques];
Subject Terms	Physical sciences/Nanoscience and technology/Nanoscale materials/Structural properties Physical sciences/Nanoscience and technology/Nanoscale materials/Two-dimensional materials
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Kinetics of a slow release nitrogen fertiliser from multi-layered nanofibrous structures

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Fertilisers are essential in modern agriculture to enhance plant growth, crop production and product quality. Recent research has focused on the development of delivery systems designed to prolong fertiliser release. This study introduces a new technology to encapsulate and release molecules of agrochemicals by using multi-layered electrospun nanofibre as a carrier. Single-layer poly L-lactic acid (PLLA) nanofibres loaded with urea fertiliser were fabricated using electrospinning. Triple-layer nanofibrous structures were produced by electrospinning Polyhydroxybutyrate (PHB) nanofibres as external layers with PLLA nanofibres impregnated with urea fertiliser as the middle layer. Scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometer (FTIR) were employed to characterize the morphology of electrospun nanofibres. Urea release dynamics was analysed using a total nitrogen instrument (TNM-1). The results indicated that triple-layer urea-impregnated nanofibrous structures led to lower level and lower initial rate of nitrogen release during three months. It is concluded that triple-layer nanofibrous structures have the potential for slow release delivery of agrochemicals.

Introduction

Nanofibres are filaments with a diameter in the nanometre range, and an aspect ratio (length: diameter) larger than 100:1[1,2]. This intrinsic feature of nanofibres increases surface area to volume ratio and decreases spaces between individual nanofibres compared to regular fibres[3,4]. There are several methods used to prepare nanofibres, encompassing top-down (melt-blown, melt electrospinning, islands-in-the-sea, and electrospinning), and bottom-up (interfacial polymerization, self-assembly, and phase separation) approaches[5].

Electrospinning provides more control over morphology, aspect ratio, pore-size distribution, and porosity in comparison to other approaches to forming continuous nanofibres[6]. Furthermore, electrospun nanofibres with an open-pore structure in the range between tens of nanometres to one micrometre, high surface area, along with high permeability to gas make them ideal porous membranes. Such nanofibres have been used in areas as diverse as filtrations, nanocomposites, drug delivery, biomedical, medical prostheses, fuel cells, sensors, and protective clothing[5,7]. However, the use of electrospun nanofibers in agriculture field is novel and still in its infancy[8].

Urea is a cost effective solid nitrogen fertiliser used to promote plant growth and increase crop production[9]. However, uncontrolled use of urea fertiliser is harmful to plants and can lead to soil and water pollution[10]. Thus, significant research efforts have focused on minimizing any possible adverse environmental impact and increasing the efficiency of urea fertiliser use by prolonged-released systems[11].

Electrospun nanofibres, especially naturally based ones such as PHB and PLLA, when used as a matrix for the controlled release of a variety of agrichemicals have a very high specific surface area[10, 12, 13]. Therefore, electrospun nanofibrous networks have an advantage over particulate carriers to control nanofibres morphology resulting in more control of fertiliser encapsulation and prolonged- release[14,15].

In a study by Castro-Enriquez *et al*. (2012), wheat gluten electrospun-fibre membranes were immersed into a urea solution, and then the prolonged-release mechanism of urea in water was studied. Since the urea was coated on the surface of the fibre membrane, urea release during the first 10 minutes was rapid, after which the rate decreased until it reached equilibrium at 300 minutes (5 hours), with 98% of urea released[11]. However, this release rate is not acceptable for preparation of slow-release fertilisers[16]. In 2013, Kampeerapappun & Phanomkate used coaxial electrospinning with the urea loaded in the core component. Biodegradable PHB and PLLA were employed as the sheath and the core, respectively. After optimizing the electrospinning parameters including flow rate and loading amount in the core-sheath structure, the electrospun mats released urea fertiliser for a month and were biodegradable within 3 months[17]. Thus, the use of core–sheath encapsulation resulted in a sharp decrease in release rate from 5 hours in the previous study[11] to 1 month in this research. However, as the urea release from core-sheath electrospun nanofibrous matrices was rather fast and stopped in 1 month[17], a more delayed release of urea is favoured in the field of agriculture. The focus of this study aimed to assess the capability of an innovative method to encapsulate urea fertiliser within multi-layered nanofibrous matrices resulting in prolonged urea release rate.

Results and Discussion

Electrospinning of Single-Layered and Triple-Layered Nanofibre Mats Loaded with Urea: A custom electrospinning setup was used to produce single-layered PLLA nanofibres loaded with different percentages of urea. PLLA solution concentration was fixed at 5% (w/w), while urea fertiliser was loaded at 10%, 20%, and 40% (w/w), relative to the mass amount of

PLLA, and transferred to a 1 mL syringe with an attached needle. Single-layered PLLA nanofibres loading different urea concentration were collected onto an aluminium foil surface and stored in a desiccator under vacuum for 24 hr before further use.

A triple-layer nanofibre mat was introduced to protect the single-layered PLLA nanofibre loaded with urea by two distinct layers of PHB nanofibres as outer layers whereas the PLLA nanofibre was the middle layer. To fabricate this triple-layer nanofibre mat, firstly PHB polymeric solution with concentration of 7% (w/w) was fed to the electrospinning setup using a 1 mL syringe to produce the first PHB nanofibre layer on the surface of aluminium foil. Subsequently, 5% PLLA solution with 10% (w/w) urea was electrospun onto the PHB nanofibre layer. Finally, the outer surface of PLLA nanofibre layer was electrospun by another layer of PHB nanofibre resulting in a PHB/PLLA/PHB triple-layer nanofibrous structure. The process was repeated for 5% PLLA solution loaded with 20% and 40% (w/w) urea.

Nanofibre Characterization:

SEM (FEI Quanta 200 SEM 2002) with 30 kV accelerating voltage and 10 mm working distance at Royal Melbourne Institute of Technology (RMIT) Microscopy and Microanalysis Facility (RMMF) and benchtop SEM (JEOL JCM-6000PLUS) with 5 kV accelerating voltage, 35 mm working distance, and high-vacuum mode at the University of Southern Queensland (USQ) were used to determine the morphology of electrospun nanofibres. Image J processing software was used to measure PLLA and PHB nanofibre diameters from high magnification SEM images.

The morphology of electrospun nanofibres is dependent on electrospinning parameters including applied voltage, solution composition, solution concentration, solution feed rate and collection distance[18]. When the concentration of a polymeric solution increases, the solution viscosity increases[19]. By keeping all electrospinning parameters constant, and increasing the concentration of a polymeric solution, nanofibres are formed thicker as the higher viscosity solution prevents the polymer jet from stretching further and electrospinning thinner fibres[20].

In this study, the effect of urea concentration on nanofibre morphology was investigated. The diameter of PLLA electrospun nanofibres increased from 496.18 nm to 782.23 nm as urea concentration increased from 0 to 40% (Figure 3.1; Table 3.1). The morphology and diameter distribution of 7% PHB electrospun nanofibres are shown in Figure 3.1(I, J). The average diameters of PHB nanofibres, as the outer layers of PLLA nanofibre loading urea layer, was 417.58 ± 64.27 nm.

A typical SEM micrograph cross section of triple-layer PHB/PLLA/PHB containing 10% urea is illustrated in Figure 3.1 (K, L). The thickness of the triple-layer nanofibrous structure is approximately 21 μm. The micrograph illustrates nanofibre arrangement and entrapped air in the triple-layered structure.

Brunauer–Emmett–Teller (BET) analysis of the 5% PLLA electrospun nanofibres without loading urea indicated that the porous fibrous mat was successfully fabricated with a surface area of 188 m^2/g, a mean pore diameter of 37 nm, and a total pore area of 10 m^2/g. Thus, the structure satisfies the definition for nanofibrous structures[21].

Figure 3.2 shows the FTIR spectrograph of urea powder and single-, triple-layered PLLA nanofibres loaded with 10%, 20% and 40% urea. According to the Figure 3.2, the characteristic peaks of PLLA form at 1090–1190 cm^{-1} (ester bond), a middle peak at 1362 cm^{-1} (–CH3 symmetric bending vibration), and two peaks at 2997 and 2947 cm^{-1} (methyl groups)[22]. A characteristic strong peak of PLLA at about 1750 cm^{-1} is related to the stretching vibration of the carbonyl group (C=O) shifted slightly from 1755 cm^{-1} [23]. In the FTIR spectra of urea powder, the peak in the region of 1629–1680 cm^{-1} is due to the C=O stretching bond of urea and the presence of absorption peaks at 3344, 3447 and 1157 cm^{-1} can be associated to the stretching vibration of N–H bonds[24]. The characteristic peaks of urea at 1458 cm^{-1} and at around 1596 cm^{-1} reflect the stretching vibration of the C–N bond and N–H bonding, respectively[25]. The urea characteristic peaks at 1458 cm^{-1} (C–N bond), 1157 cm^{-1} (N-H bond) and between 1629–1680 cm^{-1} (C=O stretching) are all present in the FTIR spectrums of single- and triple-layered nanofibrous structures, although peak positions of C=O and N-H bonds in PLLA nanofibres have been shifted due to changes in vibrational frequency which can come about either because of changes in bond strength or in reduced mass of urea[26].

Urea Release Characteristics:

A TNM-1 total nitrogen instrument (Shimadzu) was used to determine the cumulative percentage of nitrogen released from single- and triple-layer nanofibres containing 10%, 20%, and 40% urea, respectively (Table 3.2).

Increasing urea percentage from 10% to 40% in both single-layered PLLA nanofibre mat, and triple-layered PHB/PLLA/PHB nanofibrous structures resulted in initial nitrogen release at a higher rate (P value=0.000) (Table 3.2). The results in Figure 3.3 (A) showed that the nitrogen release rate of single-layered nanofibres was not significantly affected by various urea concentration (P value =0.361); however, increasing urea concentration into triple-layered nanofibres resulted in significant higher nitrogen release content (P value =0.000).

One-way ANOVA statistical analysis of the results for cumulative nitrogen release showed that at different urea concentrations, triple-layered nanofibres containing 10% urea had a strong significant decrease in releasing rate compared with other samples (P value =0.000). Results revealed that after 39 hours, the accumulative nitrogen release for 10% triple-layered nanofibres was less than 50% while by this time; other samples had achieved over 80% release. Based on these results, the experiment was repeated for single- and triple-layered layered nanofibrous structure containing 10% urea. Figure 3.3 (B) shows cumulative nitrogen release from single- and triple-layered nanofibres loaded with 10% urea as well as the control sample for first 12 hours. Results revealed that triple-layer nanofibrous structure containing 10% urea release nitrogen at a much slower rate compared to single-layer nanofibrous structure encapsulating urea by either electrospinning fabrication or immersing deposition. The physical barrier of PHB nanofibre layers coating PLLA nanofibre layer containing urea resulted in slower rate of urea release. So, fabricating multi-layered nanofibres can be considered as a means of delaying urea release from PLLA nanofibrous matrix.

Conclusion

Single- and triple-layer nanofibrous structure were fabricated successfully and loaded with urea using a custom-built electrospinning device. Fourier transform infrared Spectrophotometer analysis showed that urea was encapsulated in all samples. As expected, increased urea concentration led to larger nanofibre diameters. Increasing the percentage of urea loaded into nanofibres from 10% to 40% increased the initial rate of nitrogen release.

Furthermore, both single- and triple- layered nanofibres samples released nitrogen for three months. Although high urea concentration did not affect the nitrogen release rate for single-layer nanofibres significantly (P value=0.361), increasing urea content into triple-layered nanofibres resulted in increasing nitrogen release content significantly (P value =0.000). Triple-layer nanofibrous structures containing 10% urea exhibited a significantly lower level and lower initial rate of nitrogen release compared to single-layer nanofibres loading 10% urea (P value=0.012). In conclusion, triple-layered electrospun PLLA nanofibres containing urea may be an effective carrier to control the release of urea fertiliser in agriculture applications.

Methods

Material: Poly (L-lactide) (PLLA) with a molecular weight of 282kD was purchased from Vorina Biomaterials Company in Ireland (CAS Number: 33135-50-1). Poly [(R)-3-hydroxybutyric acid] (PHB) was provided from Sigma Aldrich (Product Number: 363502). Solvents, N-Dimethylformamide (DMF); Reagent Plus®, ≥99%, Chloroform (CF); anhydrous, ≥99%. Acetone (AC) for HPLC, ≥99.8% were obtained from Australia Sigma Aldrich. Urea (N: P: K; 46-0-0) was provided from Richgro Garden Products. The amount of nitrogen is equal to 46% urea mass.

Electrospinning apparatus: The schematic setup for nanofibre electrospinning is shown in Figure 3.4. It consists of a high voltage power supply, model 73030, DC input 30 kV @ 1 mA, (Genvolt, Ireland), and a New Era NE-300 “Just Infusion” syringe pump. A metal frame 14cm × 16cm with attached aluminium foil is located 15cm from the syringe needle to collect the nanofibres. The positive terminal of the power supply is connected to the needle and the ground terminal is attached to the collector (metal frame). The electrospinning process occurs between the needle tip and the aluminium collector and nanofibres gather on the surface of the aluminium foil.

PLLA solution with optimum concentrations of 5% (w/w) in chloroform: acetone (3:1 v/v) was prepared and mixed with 10, 20, and 40% (w/w) urea powder relative to the weight of PLLA. Polyhydroxybutyrate polymeric solution in dimethylformamide: chloroform (30:70 v/v) solvents was prepared at concentration of 7% (w/w). Electrospinning was conducted at 32 ºC with 12 (+kV) high voltage, and 20 (+kV) high voltage for PLLA and PHB solutions, respectively. Feed rate and needle size for both solutions were selected at 1 mL/hr and 18 G. The morphology of electrospun nanofibres were determined by varying the urea concentration ranging from 0 to 40% in a fixed 5% PLLA solution.

Accelerated Surface Area and Porosimetry System (ASPA) 2400 Micropore Data Reduction was used to study BET analyses for the 5% PLLA electrospun nanofibre to evaluate surface areas, pore diameters and total pore area.

FTIR measurements of urea powder and single-, triple-layered PLLA nanofibres loaded with 10%, 20% and 40% urea were carried out using IRAffinity-1S Fourier transform infrared Spectrophotometer (Shimadzu). Aluminium foil was used to place the samples under spectrophotometer.

The single- and triple-layered nanofibrous mats with a thickness of approximately 7.05 ± 1.40 and 21.41 ± 4.12 μm, respectively, were cut into 2 cm × 6 cm pieces. These pieces were accurately weighed ± 0.00001g and placed in a plastic tube with 20 mL of milli-Q water to soak for 2 min to wash out any deposited urea fertiliser on the surface of the nanofibrous mats. Subsequently, each sample was immersed in a 50 mL plastic tube with 20 mL milli-Q water and placed in a shaker at 70 RPM and 30 °C[17]. The samples were removed from the shaker and immersed in the next tube containing 20 mL milli-Q water at different periods, ranging from 0 up to 3000 h. A TNM-1 total nitrogen instrument (Shimadzu) was used to measure nitrogen release from the nanofibre mats. The data were reported as percent nitrogen. Cumulative nitrogen release from each sample was calculated for the determined period. A 5% PLLA nanofibre mat without loading urea immersed in 0.5% urea solution for 24 h, was used as the control sample. All experiments were carried out three times and results are reported as average ± one standard deviation.

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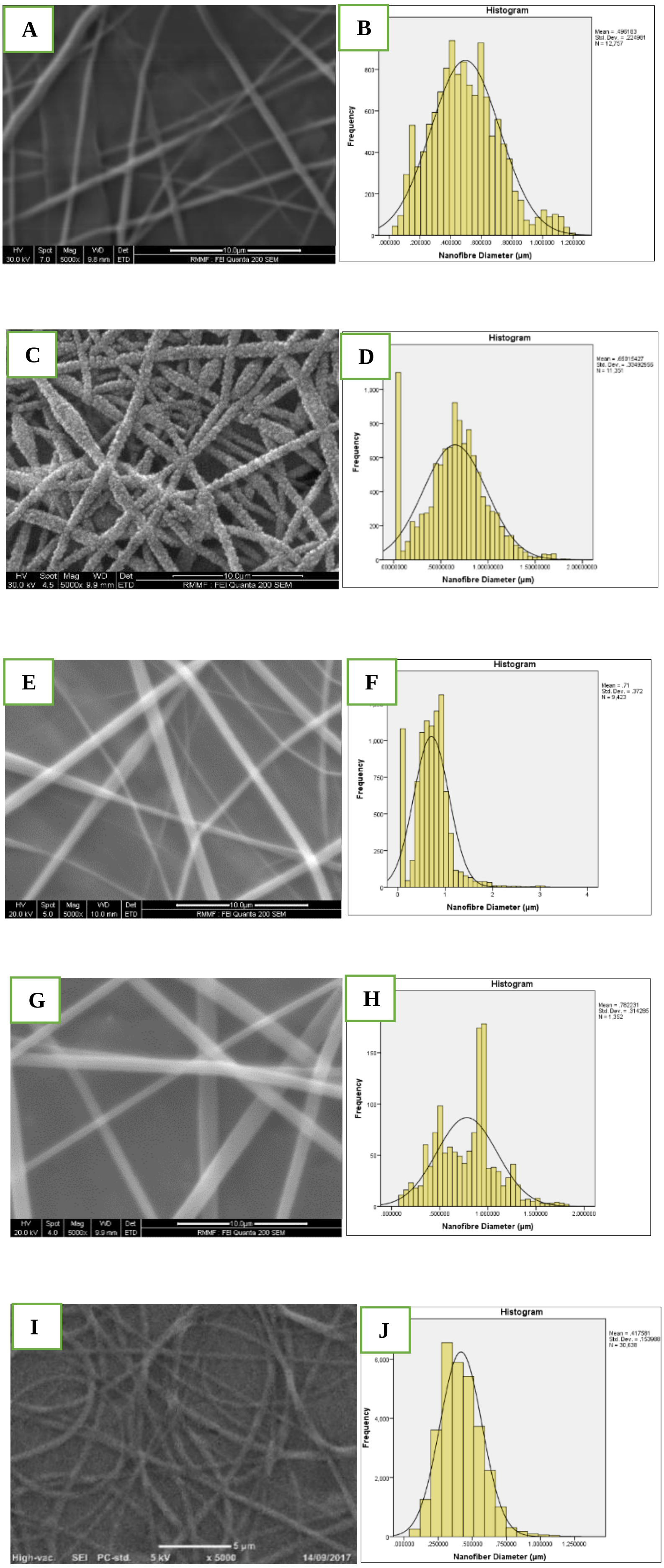
Author contributions statement

L.J. designed and performed the experiments, L.J. with T.L. analysed the data. L.J. wrote the manuscript with input from all authors. T.L., A.Y. and G.A. conceived the study and were in charge of overall direction and planning.

Additional information

Competing interests:

The authors declare no competing financial and/or non-financial interests in relation to the work described.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Std. Dev. = .224981
N = 12,757
Frequency
Nanofibre Diameter (µm)
C
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D
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Nanofibre Diameter (µm)
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Nanofibre Diameter (µm)
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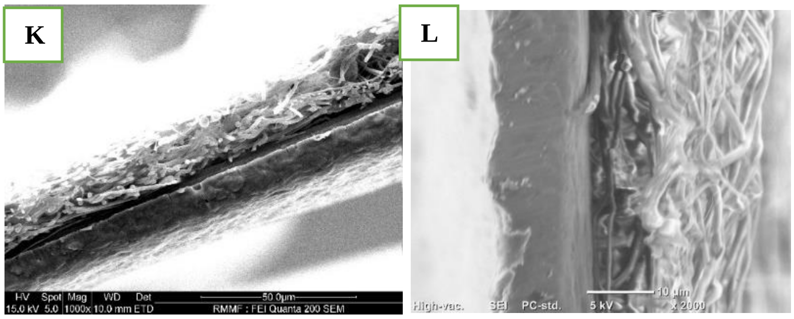


Figure 3.1: SEM images and related diameter histograms of PLLA electrospun nanofibres containing urea at concentrations of 0% *(A, B), 10% **(C, D), 20% *(E, F), and 40% *(G, H). SEM images and diameter distribution of 7% PHB electrospun nanofibres (I, J).

SEM image of cross section of PHB/PLLA/PHB triple-layer nanofibrous structure containing 10% urea at (K) 1000x magnification, and (L) 2000x magnification.

* Gold coating   ** Carbon coating

Table 3.1: Effect of urea concentration on electrospun nanofibre diameter.

Urea Concentration (*% w/w)	Nanofibre diameter (nm)	**CV%
0	496.18 ± 109.12	22%
10	650.15 ± 214.50	33%
20	710.00 ± 262.70	37%
40	782.23 ± 242.42	31%

*% w/w based on amount of PLLA used

**Coefficient of Variation (CV)

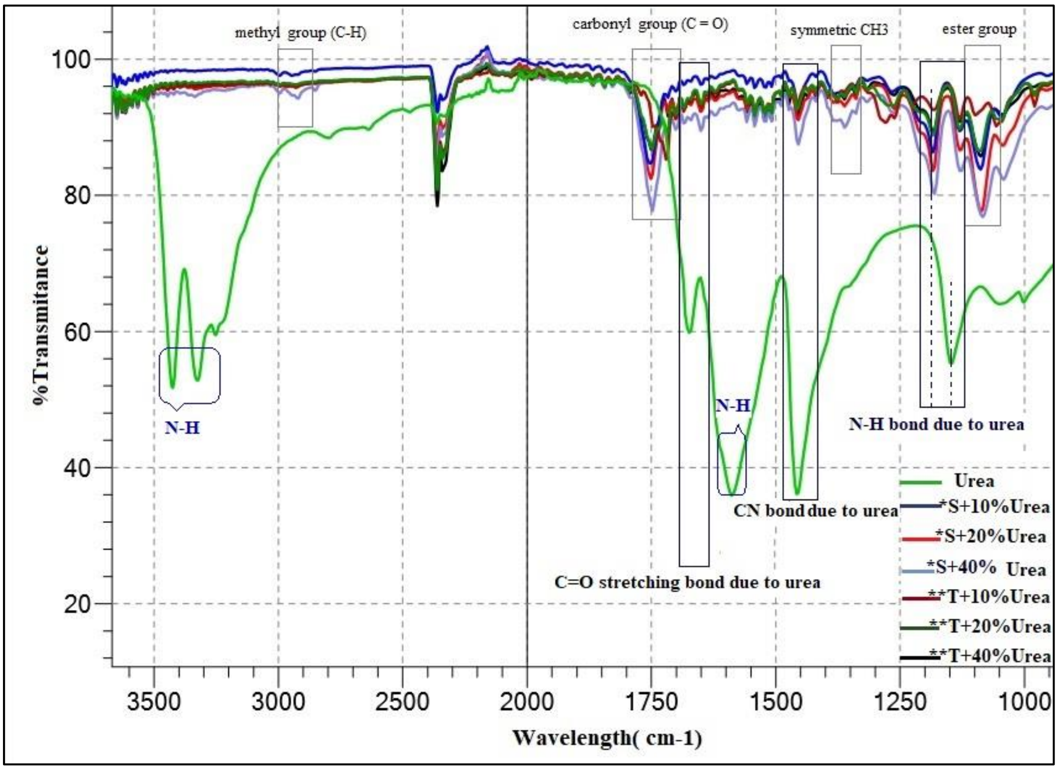


Figure 3.2: FTIR spectra of urea and single-, triple-layered PLLA nanofibres loaded with different urea percentage.

*S: Single-layered PLLA nanofibre mat,

**T: Triple-layered PHB/PLLA/PHB nanofibrous structure

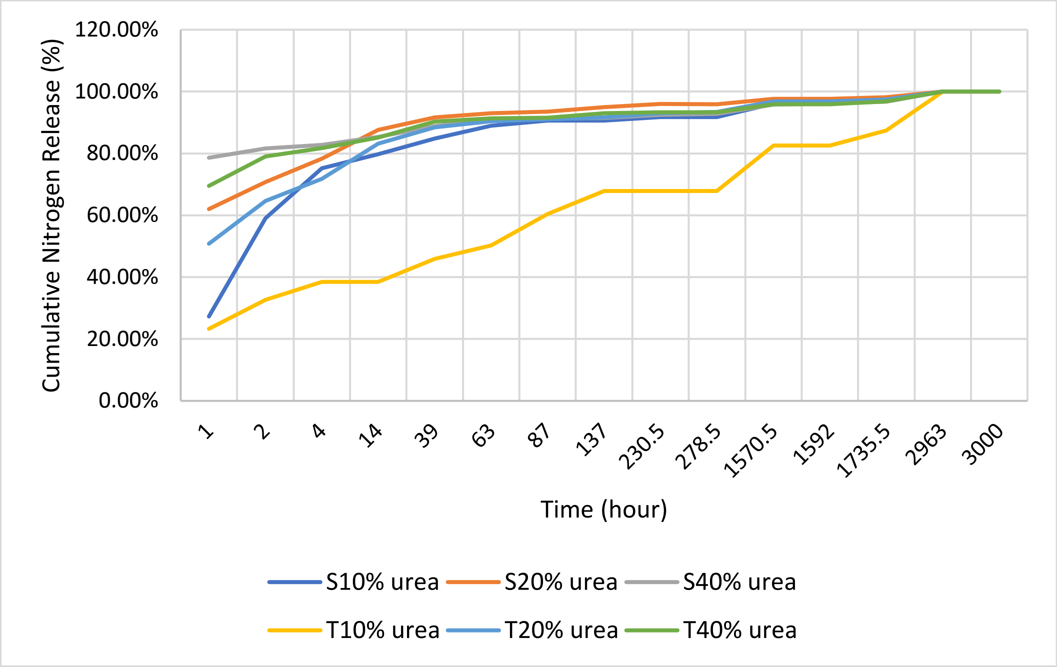
Table 3.2: Nitrogen release rate from single- and triple-layer nanofibre structures from first hour until 3000 hours.

Time (h)/ Sample	S10% urea	S20% urea	S40% urea	T10% urea	T20% urea	T40% urea
1	27.30%	62.00%	78.60%	23.30%	50.80%	69.50%
2	59.10%	70.70%	81.60%	32.70%	64.60%	79.00%
4	75.20%	78.30%	82.80%	38.50%	71.80%	81.70%
14	79.70%	87.60%	85.30%	38.50%	83.20%	85.10%
39	84.80%	91.60%	88.90%	45.90%	88.40%	90.30%
63	89.00%	93.00%	91.00%	50.30%	90.40%	91.30%
87	90.60%	93.50%	91.20%	60.40%	91.00%	91.50%
137	90.60%	95.00%	91.40%	67.80%	91.60%	93.00%
230.5	91.80%	96.00%	92.50%	67.80%	93.00%	93.30%
278.5	91.80%	95.90%	92.80%	67.80%	93.40%	93.30%
1570.5	96.10%	97.60%	95.80%	82.50%	96.80%	95.90%
1592	96.10%	97.60%	96.20%	82.50%	96.80%	95.90%
1735.5	97.20%	98.20%	96.70%	87.40%	97.40%	96.80%
2963	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
3000	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%

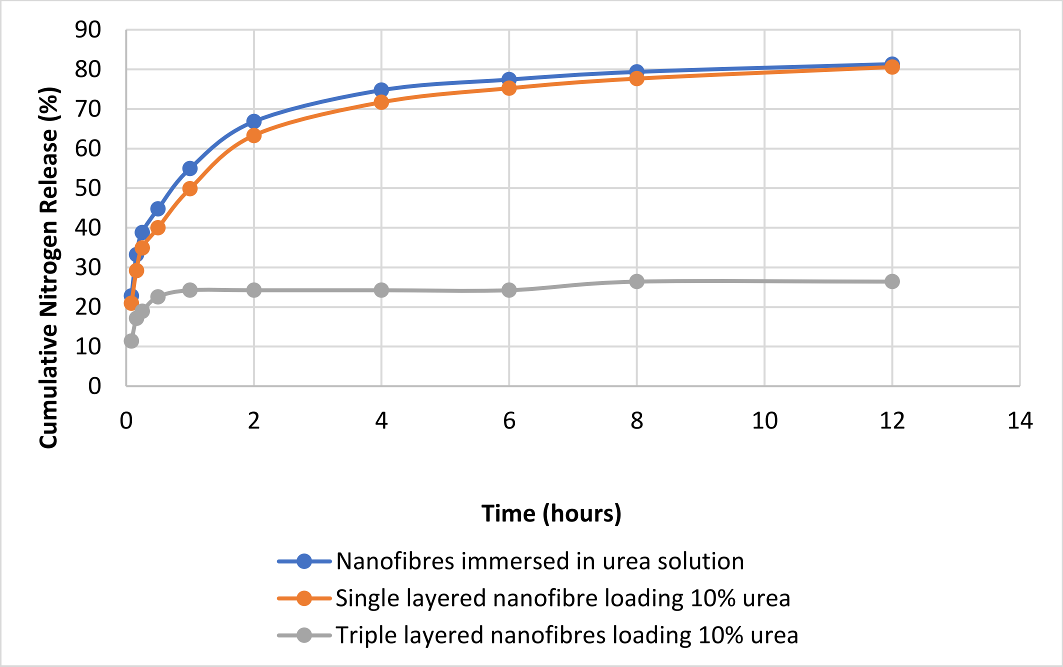
P value for initial nitrogen release	0.000	0.000

S: Single-layered PLLA nanofibre mat, T: Triple-layered PHB/PLLA/PHB nanofibrous structure

P value: Probability value



A



B

Figure 3.3: A) Cumulative nitrogen release from single- and triple-layer nanofibre structures from first hour until 3000 hours, and **B)** Cumulative nitrogen release from immersed nanofibres in urea solution and single-, triple-layer nanofibrous structure containing 10% urea first hour until 12 hours.

S: Single-layered PLLA nanofibre mat; T: Triple-layered PHB/PLLA/PHB nanofibrous structure

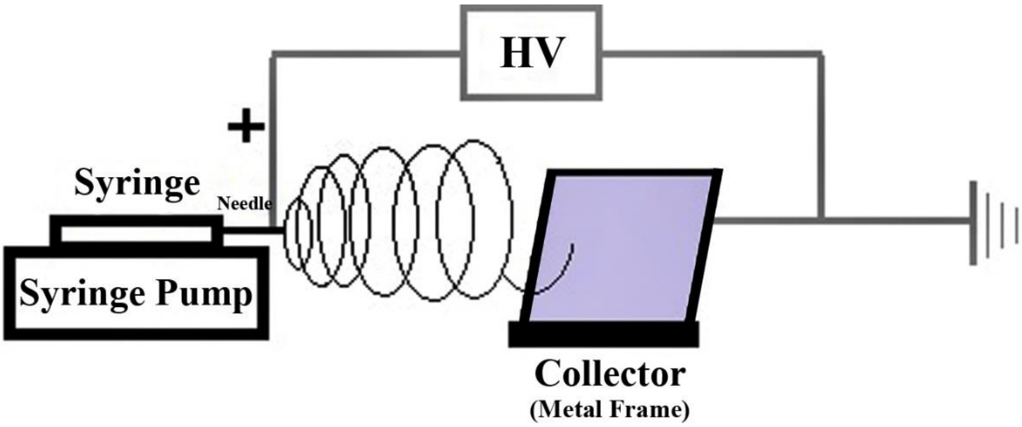
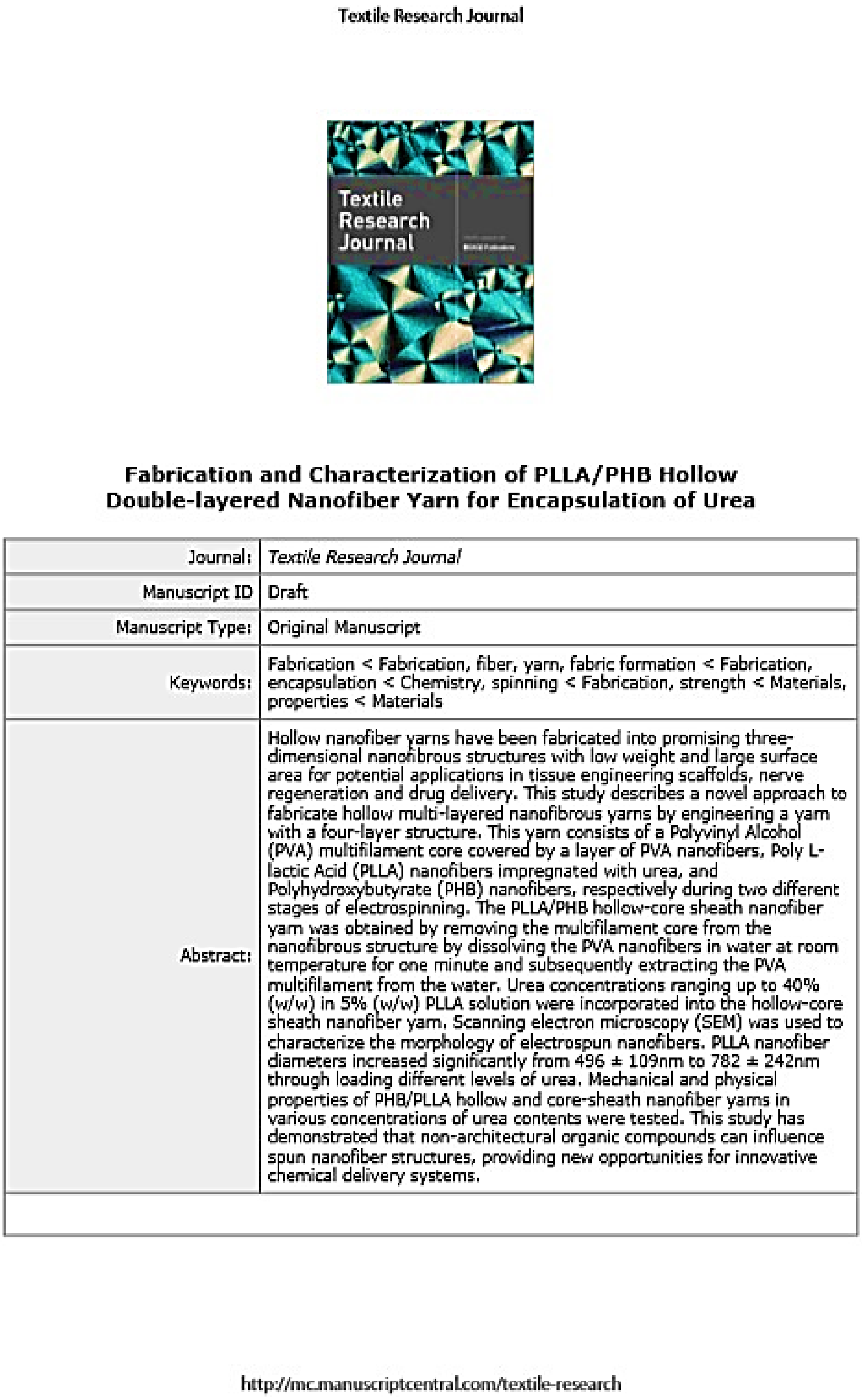


Figure 3.4: Schematic diagram of the electrospinning setup

Chapter 4. Fabrication and Characterization of PLLA/PHB Hollow Double-layered Nanofiber Yarn for Encapsulation of Urea



Textile Research Journal

Fabrication and Characterization of PLLA/PHB Hollow Double-layered Nanofiber Yarn for Encapsulation of Urea

Journal:	*Textile Research Journal*
Manuscript ID	Draft
Manuscript Type:	Original Manuscript
Keywords:	Fabrication < Fabrication, fiber, yarn, fabric formation < Fabrication, encapsulation < Chemistry, spinning < Fabrication, strength < Materials, properties < Materials
Abstract:	Hollow nanofiber yarns have been fabricated into promising three-dimensional nanofibrous structures with low weight and large surface area for potential applications in tissue engineering scaffolds, nerve regeneration and drug delivery. This study describes a novel approach to fabricate hollow multi-layered nanofibrous yarns by engineering a yarn with a four-layer structure. This yarn consists of a Polyvinyl Alcohol (PVA) multifilament core covered by a layer of PVA nanofibers, Poly L-lactic Acid (PLLA) nanofibers impregnated with urea, and Polyhydroxybutyrate (PHB) nanofibers, respectively during two different stages of electrospinning. The PLLA/PHB hollow-core sheath nanofiber yarn was obtained by removing the multifilament core from the nanofibrous structure by dissolving the PVA nanofibers in water at room temperature for one minute and subsequently extracting the PVA multifilament from the water. Urea concentrations ranging up to 40% (w/w) in 5% (w/w) PLLA solution were incorporated into the hollow-core sheath nanofiber yarn. Scanning electron microscopy (SEM) was used to characterize the morphology of electrospun nanofibers. PLLA nanofiber diameters increased significantly from 496 ± 109nm to 782 ± 242nm through loading different levels of urea. Mechanical and physical properties of PHB/PLLA hollow and core-sheath nanofiber yarns in various concentrations of urea contents were tested. This study has demonstrated that non-architectural organic compounds can influence spun nanofiber structures, providing new opportunities for innovative chemical delivery systems.

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Fabrication and Characterization of PLLA/PHB Hollow Double-layered Nanofiber Yarn for Encapsulation of Urea

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Abstract

Hollow nanofiber yarns have been fabricated into promising three-dimensional nanofibrous structures with low weight and large surface area for potential applications in tissue engineering scaffolds, nerve regeneration and drug delivery. This study describes a novel approach to fabricate hollow multi-layered nanofibrous yarns by engineering a yarn with a four-layer structure. This yarn consists of a Polyvinyl Alcohol (PVA) multifilament core covered by a layer of PVA nanofibers, Poly L-lactic Acid (PLLA) nanofibers impregnated with urea, and Polyhydroxybutyrate (PHB) nanofibers, respectively during two different stages of electrospinning. The PLLA/PHB hollow-core sheath nanofiber yarn was obtained by removing the multifilament core from the nanofibrous structure by dissolving the PVA nanofibers in water at room temperature for one minute and subsequently extracting the PVA multifilament from the water. Urea concentrations ranging up to 40% (w/w) in 5% (w/w) PLLA solution were incorporated into the hollow-core sheath nanofiber yarn. Scanning electron microscopy (SEM) was used to characterize the morphology of electrospun nanofibers. PLLA nanofiber diameters increased significantly from 496 ± 109nm to 782 ± 242nm through loading different levels of urea. Mechanical and physical properties of PHB/PLLA hollow and core-sheath nanofiber yarns in various concentrations of urea contents were tested. This study has demonstrated that non-architectural organic compounds can influence spun nanofiber structures, providing new opportunities for innovative chemical delivery systems.

Keywords: Electrospinning, hollow nanofibre yarn, core-sheath nanofibre yarn, urea encapsulation, mechanical properties

Short title: Hollow nanofibre yarn fabrication to encapsulate urea

Introduction: Electrospinning has attracted significant interest as a versatile and low-cost technique to manufacture sub-micron fibres and nanofibres from polymer solutions or melts [1]. Polymer nanofibers are a versatile material and can be used in a number of diverse applications, including drug encapsulation, biosensing, filtration and electronics [2]. In contrast to solid nanostructures, hollow nanocomposite structures, including electrospun webs that offer control over pore volume and distribution, have extended the impact of particles by bonding their functionality with the feasible process of synthetic polymers. Hollow nanostructures have been successfully used to encapsulate and control release of sensitive materials such as drugs, cosmetics and DNA due to their relatively large proportion of void space [3]. Likewise, such hollow nanostructures have much higher surface area to volume ratio compared to solid counterparts of the same dimensions. This is beneficial when adsorption or storage of chemicals or charges are required [4].

To date, few methods have been proposed to obtain hollow yarns by using the electrospinning process. The unique electrospun hollow yarn structure brings together advantages including high volume and low weight of electrospun nanofibers due to low packing density and increasing the entrapped air and porosity in the yarn. The qualities of hollow yarn combined with the large surface areas of three-dimensional nanofibrous architectures have potential applications in tissue engineering scaffolds and materials delivery [5]. One method used a rotating metal rod attached to a metal hemisphere at one end to produce hollow nanofiber yarn [6]. This method was used to produce a hollow core-sheath yarn using two different polymer solutions. Subsequently, hollow nanofiber yarn was obtained by removing the inner yarn core [6]. Wu & Qin (2013) introduced an electrospinning apparatus that fabricated uniaxial aligned nanofiber yarns [7]. In another study, Javazmi et al. (2014) applied a two-nozzle conjugated electrospinning method to fabricate a core sheath yarn from nanofibers of polyester as the sheath and PVA multifilament as the core of the yarn. Subsequent dissolution of the core yarn in hot water produced a hollow nanofiber yarn [8]. Fakhrali et al. (2014) developed a novel method of fabricating core-sheath nanofiber yarns. This core sheath nanofiber consisted of PVA nanofibers as core and nanofibers of Nylon 6 as the sheath. Such yarn has potential applications in various fields such as loading drugs into PVA solution as a core with variable content of the drug [9]. Nanofibrous webs also can be used to encapsulate different agrichemicals including nitrogen fertilizers through separate nanofibers to prolong agricultural additive release by several months [10, 11, 12, 13]. Among nitrogen fertilizers, urea is relatively low cost being used in the worldwide [11].

This study aimed to explore a new technique to encapsulate molecules of urea as a proof of concept by introducing electrospun hollow PLLA/PHB nanofiber yarn as a carrier. A new electrospinning apparatus with two asymmetric opposite nozzles was adjusted in a two-step method to fabricate four-layered nanofiber yarn loaded with urea. Furthermore, by dissolving PVA nanofibers in water and extracting the PVA multifilament, hollow PLLA nanofiber yarn loaded with urea surrounded by PHB nanofibers was produced.

Experimental

Materials

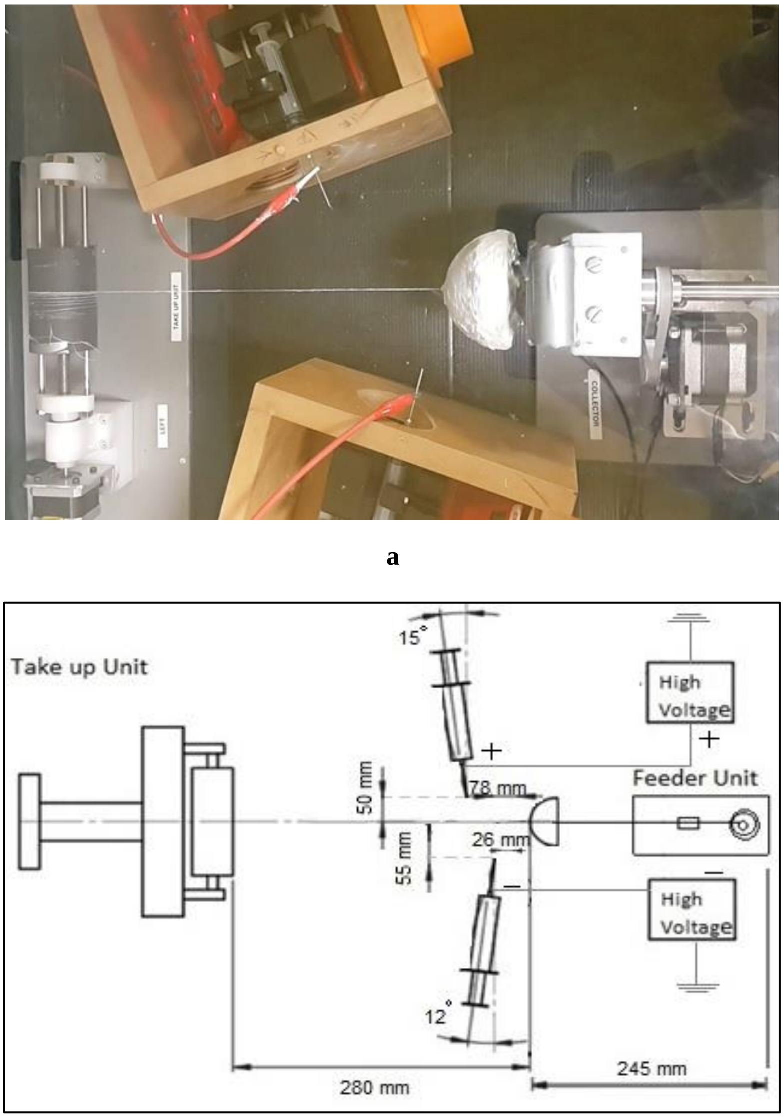
PVA with an average molecular weight (MW) ranging from 89,000 g/mol to 98,000 g/mol was obtained from Sigma Aldrich Chemical Company (Product Number: 341584). PLLA with a MW of 282 kD was purchased from Vorina Biomaterials, Ireland (CAS Number: 33135-50-1). Polyhydroxybutyrate was provided from Sigma Aldrich (Product Number: 363502). Solvents, N-Dimethylformamide (DMF); Reagent Plus®, ≥ 99%, Chloroform (CF); anhydrous, ≥ 99%. Acetone (Ace) for HPLC, ≥ 99.8% were obtained from Australia Sigma Aldrich. Urea (% N:P:K; 46:0:0) was provided from a commercial supplier. A 300/84 denier PVA multifilament yarn was purchased from Dongguan Cocou Textile Materials Company, China.

An aqueous PVA solution with a concentration of 10% (w/w) was prepared by adding Milli-Q water to PVA powder and heating to 80 °C with stirring for 20 minutes at 80 rpm. PLLA solution with a concentration of 5% (w/w) in chloroform: acetone (3:1 v/v) was prepared and mixed with 10, 20, and 40% (w/w) urea powder relative to the weight of PLLA. Polyhydroxybutyrate polymeric solution in N-dimethylformamide: chloroform (30:70 v/v) solvent was used by concentration of 7% (w/w).

Electrospinning Apparatus

A custom electrospinning apparatus was used to produce four-layered nanofiber yarn in two different steps with two series of electrospinning parameters. The image and schematic diagram of the modified apparatus are shown in Figures 4.1(a) and 4.1(b). The apparatus consisted of three main sections including feeder, middle and take up units. The feeder unit was a negative feeder system that fed a PVA multifilament core yarn from a bobbin using take up unit tension. Electrospinning was conducted in the middle unit, which consists of two high voltage power supplies (HV), model 73030

DC input 30kV@1mA, from Genvolt, Ireland, Two NE-300 ‘Just Infusion’ syringe pumps from New Era Pump Systems Company, Australia, and a rotating conductive hemisphere with a hollow metal rod attached. The take up unit, which draws at a speed of 1.62 cm/min, includes a 5cm×8cm cylindrical roller, and a stepper motor to control the speed of uptake using an ATMEGA328P Pro Mini 328 Mini 16 MHz microcontroller.



a

b

Figure 4.1: (a) Custom electrospinning apparatus, (b) schematic diagram of the modified apparatus.

The 8 cm diameter hemispherical collector can rotate clockwise and anti-clockwise with an angular velocity ranging from 32 to 150 rpm, with yarn twisting from a rate between 1850 Twist Per Meter (TPM) to 5000 TPM. The positively and negatively charged electrodes were connected to the nozzles with angles of 15º and 12º, respectively. The horizontal distance between positive charged nozzle and neutral hemispherical collector was 78 mm while the vertical distance was 50 mm. The negative charged nozzle was positioned 26 mm horizontally and 55 mm vertically from the hemispherical collector. The hemispherical collector was located 280 mm, and 245 mm away from the take-up unit and feeder unit, respectively. The angular placement of the nozzles in the apparatus facilitated fabrication of two distinctive sheaths of nanofibers surrounding the core yarn during two different stages of the electrospinning process.

In the first stage, the PVA multifilament was fed through the hemispherical collector by the feeder unit, and the take-up roller collected the other end of the folded PVA multifilament. Polyvinyl Alcohol solution with concentration of 10% (w/w), and 5% PLLA solutions loaded with 10%, 20%, and 40% (w/w) urea relative to the total weight of dissolved PLLA were introduced into the electric field at a feeding rate of 0.5 mL/h and 1 ml/h, respectively. Once jets of charged solution ejected from the nozzles, the solvents vaporized, and fibres formed. Figure 4.2 illustrates the electrospun nanofibers formed triangular shapes between two nozzles on the rotating hemisphere, bearing electrical charges of the opposite polarity. This occurred at a rotational speed of 32 rpm, with a difference in electrical potential between the two nozzles of 21 kV. Therefore, the PVA multifilament was firstly coated by PVA nanofibers fed through the negative charged nozzle and then by PLLA nanofibers loaded with 10% urea formed from the positive charged nozzle.

Figure 4.2: Triangular spinning of electrospun nanofiber.

In the second stage, the feeder unit fed the triple layered nanofiber yarn that was produced in the first stage, and was collected by the take-up roller, again. This time the nozzles with flow rate of 1 ml/h injected 7% (w/w) PHB solution and 5% (w/w) PLLA solution loaded with 10% (w/w) urea by positive and negative charged jets respectively, and the same electrospinning apparatus and parameters were applied for the second stage. The PVA multifilament coated by PVA and PLLA nanofibers passed through the rotating collector and was firstly covered by PLLA nanofibers loaded with urea, followed by PHB nanofibers coverage. Finally, the take-up roller pulled and collected the complete four layered nanofiber yarn. Electrospinning of solutions was conducted at 32°C and 60% humidity. The process was repeated for 5% PLLA solution loaded with 20%, and 40% urea. To produce the PLLA/PHB hollow core-sheath nanofiber yarn, the core yarn was easily recovered after dissolving the PVA nanofiber in water, at room temperature for one minute [12].

Characterization

The morphology of electrospun nanofibers and hollow nanofiber yarns was characterized using a SEM (FEI Quanta 200 ESEM 2002) at RMIT Microscopy and Microanalysis Facility (RMMF) and a benchtop SEM (JEOL JCM-6000 PLUS) at the University of Southern Queensland (USQ). Image J processing software was used to measure PVA, PLLA and PHB nanofiber diameters from high magnification SEM images. To determine the linear density of the hollow nanofiber yarns, 10 pieces of 5cm length were cut from each sample and weighed by an electronic balance with a precision of 0.0001g.

For investigation of the tensile properties of hollow nanofiber yarns and core-sheath yarns, 10 randomly selected pieces of each sample were tested. Tensile tests were carried out with a tensile tester (Instron 5566, USA) based on the constant rate of elongation method (CRE). The gauge length was adjusted to 18 mm with 250 N load. Crosshead speed for examining the samples was 2.5 mm/min.

The regain moisture (the percentage of water in a sample compared to its oven dry weight) of PLLA triple-layered hollow nanofiber yarn with 10%, 20%, and 40% (w/w) urea concentrations was measured. To measure regain moisture of triple layered hollow nanofiber yarn, samples were cut randomly into 5 cm lengths and five pieces of each samples were oven dried at 110°C for 48 hours. The dried samples were then weighed and stored for 24 hours at 65% relative humidity and 20°C. Following this, the sample were weighed again and regain moisture using the formula below [13].

Moisture regain, $R = W/D \times 100$

Where
R is the moisture regain
W is the weight of water
D is the oven dry weight

Results and Discussion

Hollow nanofibre yarn morphology The SEM images of the electrospun nanofiber from the 5% PLLA solutions containing 0%, 10%, 20%, and 40% are shown in Figures 4.3(A), 4.3(B), 4.3(C) and 4.3(D), respectively. The average diameters of the PLLA nanofibers increased from 496 ± 109 to 782 ± 242nm by increasing urea concentration from 0% to 40%. Increasing urea concentration led to an increase in nanofiber uniformity and higher regular morphology. This effect is mainly attributed to the increase in solution viscosity [14].

Scanning electron microscopic images of PHB electrospun nanofibers with average diameter of 417 ± 62 and PVA nanofibers with average diameter of 198 ± 14 nm are shown in Figure 4.3(E) and 4.3(F), respectively.

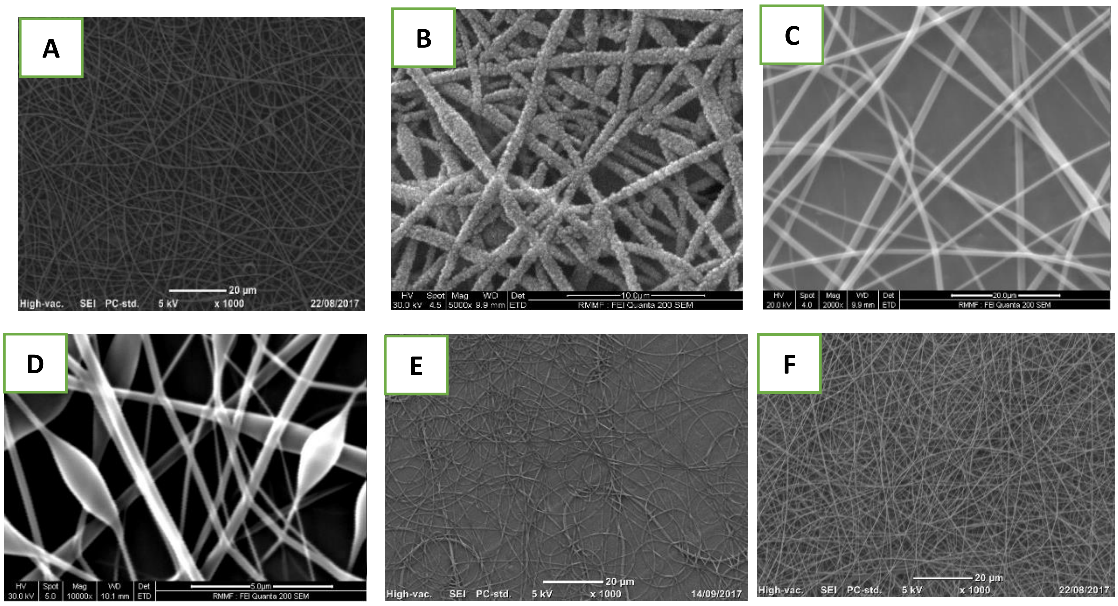


Figure 4.3: SEM images of A) PLLA nanofiber without urea; B) PLLA nanofiber loaded with 10% urea; C) PLLA nanofiber loaded with 20% urea; D) PLLA nanofiber loaded with 40% urea, E) PHB nanofibers, and F) PVA nanofibers.

Double-layered hollow nanofibre yarn was manufactured by completely removing PVA nanofibres and PVA multifilament yarn from multi-layered nanofibre yarn. The average inner diameter of fabricated hollow yarns was approximately 282.00 µm in all fabricated samples. Figure 4.4 illustrates cross sectional SEM images of A) core-sheath PLLA multi-layered nanofiber yarns, and B) hollow PLLA multi-layered nanofiber yarn at 10% urea concentration.

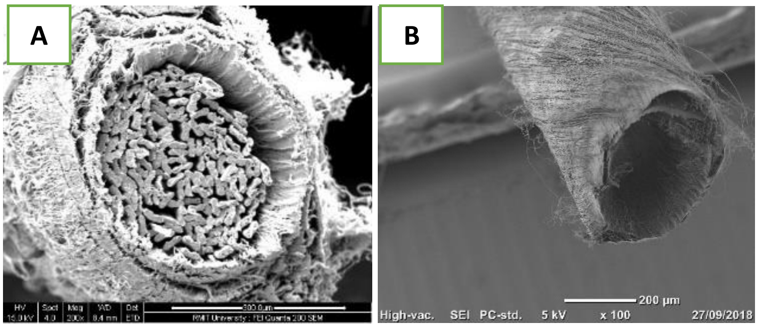


Figure 4.4: SEM image of the cross-section area of A) core-sheath B) hollow multi-layered PLLA nanofiber yarn at urea concentration of 10%.

Linear density

With increasing of concentration of polymeric solution due to increasing urea concentration (Table 4.1), there was a correspondingly significant (P <0.00) increase in the linear density of PLLA core-sheath yarns and PLLA hollow nanofiber yarns. By extracting PVA multifilament yarn from multi-layered nanofibre yarn structures, the linear density of hollow nanofibre yarns decreased.

Table 4.1. The linear density of core-sheath yarns and hollow multi-layered PLLA nanofiber yarns in different concentrations of urea.

Urea concentration relative to total PLLA weight in 5% PLLA solution	Linear density of multi-layered PLLA core-sheath yarns (tex)	The linear density of multi-layered of PLLA hollow nanofiber yarns (tex)
0	51.00 ± 2.30	24.50± 1.30
10	95.50 ± 8.03	48.90± 5.15

20	110.00 ± 8.04	61.70± 4.60
40	130.05 ± 9.10	95.90± 10.34

The regain moisture curve of PLLA hollow multi-layered nanofiber yarn at different concentrations of urea (Figure 4.5) indicated that the regain moisture of PLLA hollow nanofiber yarns decreased with increasing urea concentration. Increasing polymeric concentration led into thicker nanofiber diameters and the ratio of large surface area to volume ratio decreased.

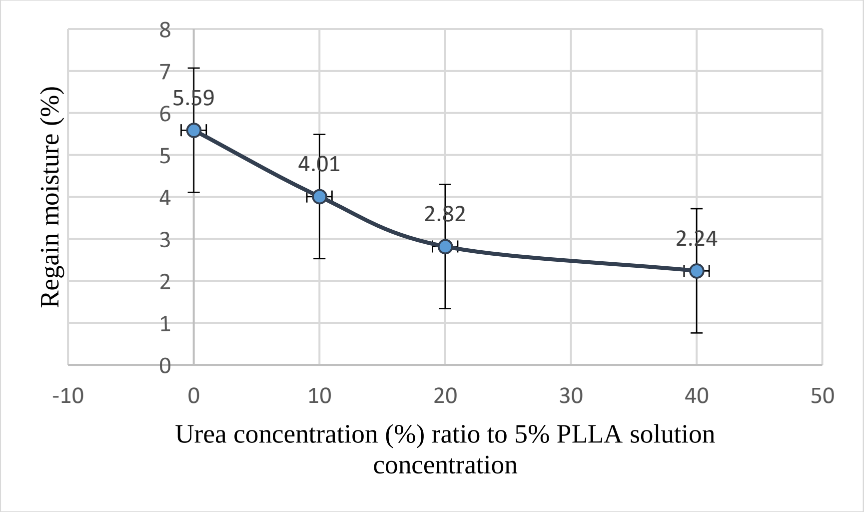


Figure 4.5: Regain moisture of hollow PLLA multi-layered nanofiber yarns at different urea concentrations

Tensile testing

Tensile properties of PVA core yarn, core-sheath yarns and hollow double-layered PLLA nanofiber yarns in different concentrations of urea are summarized in Table 4.2. The results indicated that the average strength of core sheath nanofiber yarn loading 10% urea was more than the average strength of core-sheath yarns at urea concentration of 20%, and 40%. The presence of beads in PLLA nanofiber structure increased by adding urea powder into PLLA polymeric solution and caused fewer interactions between PLLA nanofibres as well as increasing weakness of individual nanofibres (Figure 4.3). This resulted in generally lower tensile strength values. As beads act as defects in nanofibers, by increasing the urea content into core-sheath and hollow nanofiber yarns, the number of beads and weak points increased [15].

Moreover, by removing PVA core yarn from core-sheath nanofiber yarns, both stress and strain in double-layered hollow nanofiber yarn decreased.

The Tukey-test for significance showed that the difference between strength of core-sheath PLLA nanofiber yarns loading 10% and 20% urea was not meaningful, while the strength of core-sheath nanofiber yarn loading 40% urea caused a significant difference (P < 0.00) with core-sheath yarns loading 10% and 20% urea. Also, the results of the Tukey test showed that there are no meaningful differences in the elongation among PVA multifilament yarn and core-sheath nanofiber yarn (P = 0.115) while the core-sheath nanofibre yarns and hollow nanofiber yarns showed significant differences in the strength (P < 0.00). Figure 4.6 shows typical stress-strain curves of samples.

Table 4.2. Mechanical properties of the core-sheath and hollow nanofiber yarns

Sample	Tensile modules		Ultimate tensile strength		Elongation at break		Work up to break	
	MPa	%CV	MPa	CV%	%	CV%	Nmm	CV%
PVA Multifilament Yarn	85.95	17.76	109.67	5.32	16.85	5.45	37.24	6.44
Core-sheath nanofibre yarn loading 10% urea	68.48	26.79	73.70	5.01	15.39	7.74	34.37	8.32
Core-sheath nanofibre yarn loading 20% urea	54.35	20.31	69.36	6.10	16.60	17.49	36.78	12.54
Core-sheath nanofibre yarn loading 40% urea	49.52	29.51	62.42	6.73	15.71	17.92	31.26	14.04
Hollow nanofibre yarn loading 10% urea	4.5	19.35	5.60	5.94	10.75	19.59	0.68	14.83
Hollow nanofibre yarn loading 20% urea	3.9	26.72	5.23	9.66	9.25	9.20	0.58	11.96
Hollow nanofibre yarn loading 40% urea	2.18	18.84	3.89	9.51	2.66	16.81	0.11	15.02

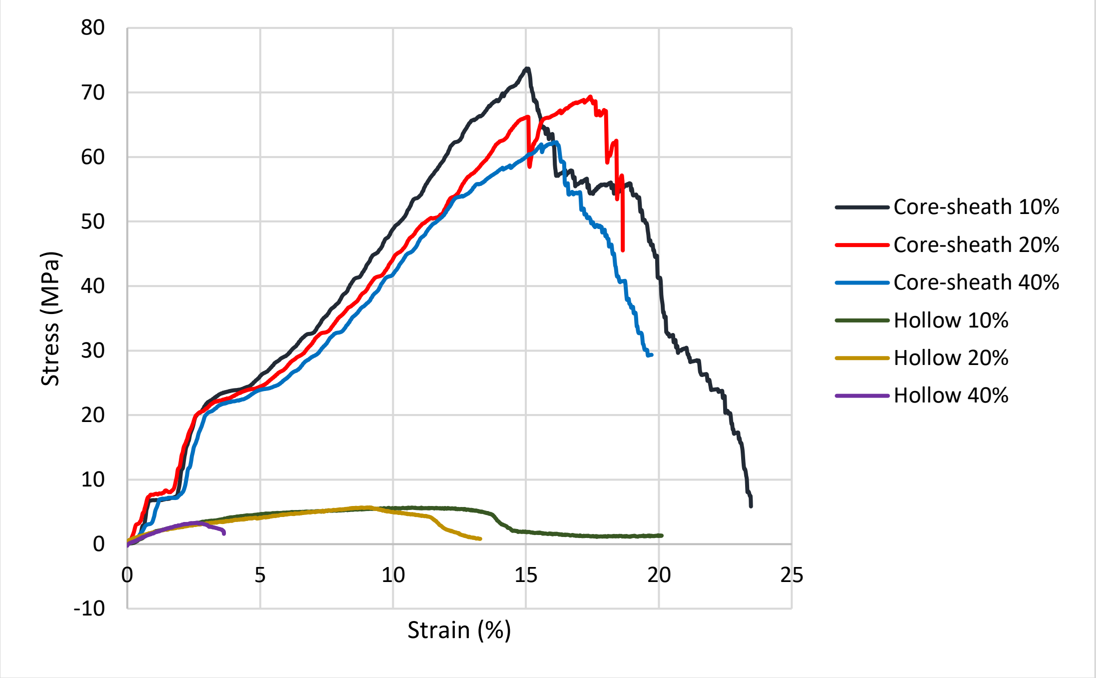


Figure 4.6: Typical stress-strain behaviour of various core-sheath and hollow nanofibrous yarns

Conclusion

In this study, a new method was developed for fabricating hollow multi-layered nanofibrous yarn. Using this method, a four-layered yarn structure was created using different angular placement of nozzles. Scanning electron microscopic images of the morphology of samples indicated that by increasing urea concentration into PLLA polymeric solutions, electrospun nanofiber diameters increased. Linear density measurement of both hollow and core-sheath multi-layered nanofiber yarns showed that by increasing urea concentration from 0% to 40%, nanofiber yarns diameters increased significantly. Examination of tensile properties of samples indicated that with increasing of the thicknesses of urea concentration into PLLA polymeric solution, stress in both core-sheath and hollow nanofibre decreased due to the presence of beads in nanofibre structure, and hollow nanofibre yarn loading 40% urea had the lowest strain and stress. Regarding physical properties, results showed that by increasing urea concentration, the entrapped air into hollow nanofibre yarn reduced; therefore, regain moisture of PLLA hollow nanofiber yarns decreased.

. This work resulted in a hollow nanofibrous yarn from PLLA and PHB nanofibers as biodegradable and biocompatible polymers, with an inner diameter large enough for the potential applications in agriculture area such as fertilizer encapsulation for prolonging release rate in delivery systems.

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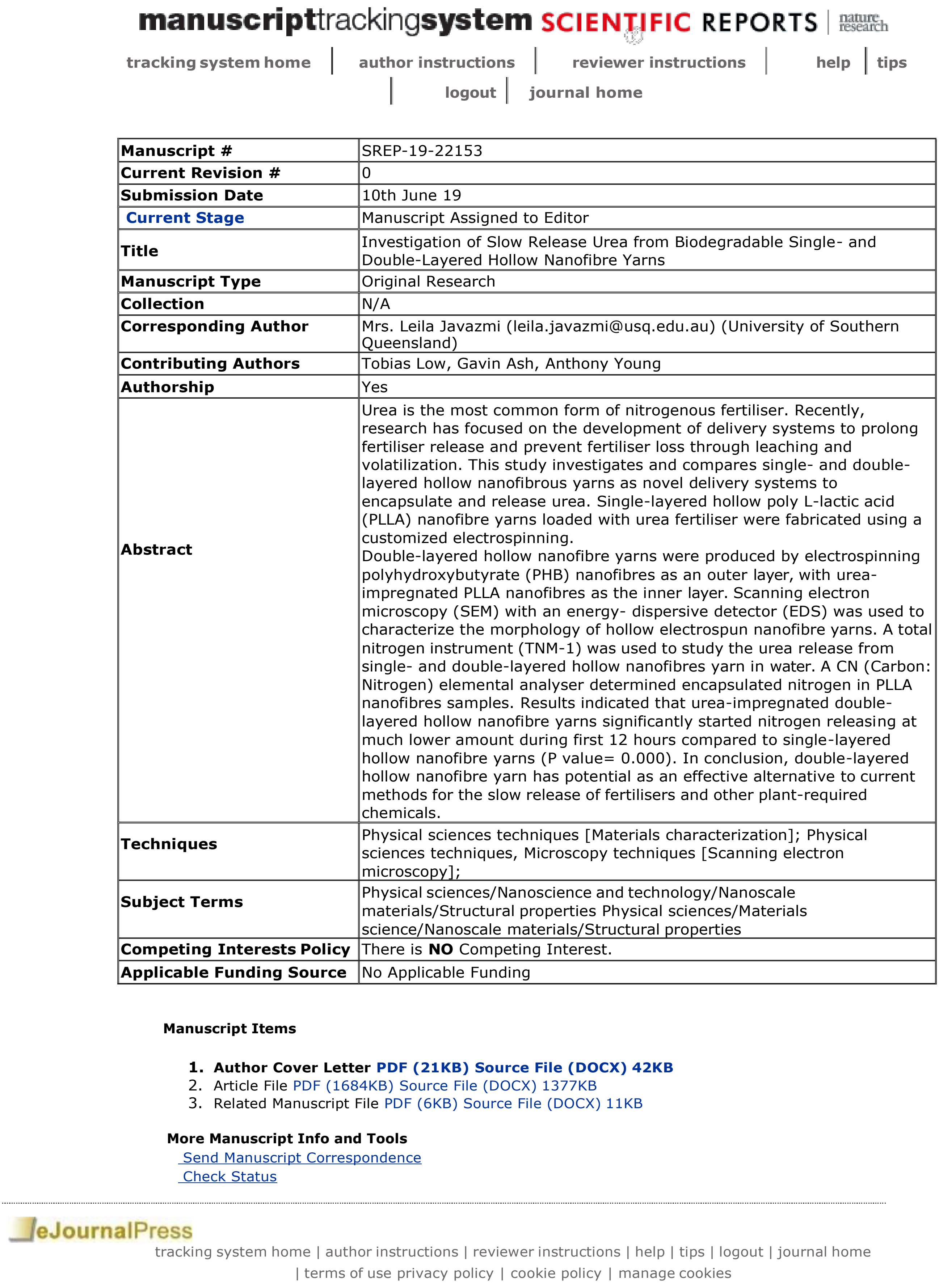
Chapter 5. Investigation of Slow Release Urea from Biodegradable Single- and Double-Layered Hollow Nanofibre Yarns



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Corresponding Author	Mrs. Leila Javazmi (leila.javazmi@usq.edu.au) (University of Southern Queensland)
Contributing Authors	Tobias Low, Gavin Ash, Anthony Young
Authorship	Yes
Abstract	Urea is the most common form of nitrogenous fertiliser. Recently, research has focused on the development of delivery systems to prolong fertiliser release and prevent fertiliser loss through leaching and volatilization. This study investigates and compares single- and double-layered hollow nanofibrous yarns as novel delivery systems to encapsulate and release urea. Single-layered hollow poly L-lactic acid (PLLA) nanofibre yarns loaded with urea fertiliser were fabricated using a customized electrospinning. Double-layered hollow nanofibre yarns were produced by electrospinning polyhydroxybutyrate (PHB) nanofibres as an outer layer, with urea-impregnated PLLA nanofibres as the inner layer. Scanning electron microscopy (SEM) with an energy- dispersive detector (EDS) was used to characterize the morphology of hollow electrospun nanofibre yarns. A total nitrogen instrument (TNM-1) was used to study the urea release from single- and double-layered hollow nanofibres yarn in water. A CN (Carbon: Nitrogen) elemental analyser determined encapsulated nitrogen in PLLA nanofibres samples. Results indicated that urea-impregnated double-layered hollow nanofibre yarns significantly started nitrogen releasing at much lower amount during first 12 hours compared to single-layered hollow nanofibre yarns (P value= 0.000). In conclusion, double-layered hollow nanofibre yarn has potential as an effective alternative to current methods for the slow release of fertilisers and other plant-required chemicals.
Techniques	Physical sciences techniques [Materials characterization]; Physical sciences techniques, Microscopy techniques [Scanning electron microscopy];
Subject Terms	Physical sciences/Nanoscience and technology/Nanoscale materials/Structural properties Physical sciences/Materials science/Nanoscale materials/Structural properties
Competing Interests Policy	There is **NO** Competing Interest.
Applicable Funding Source	No Applicable Funding

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Investigation of Slow Release Urea from Biodegradable Single- and Double-Layered Hollow Nanofibre Yarns

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Urea is the most common form of nitrogenous fertiliser. Recently, research has focused on the development of delivery systems to prolong fertiliser release and prevent fertiliser loss through leaching and volatilization. This study investigates and compares single- and double-layered hollow nanofibrous yarns as novel delivery systems to encapsulate and release urea. Single-layered hollow poly L-lactic acid (PLLA) nanofibre yarns loaded with urea fertiliser were fabricated using a customized electrospinning. Double-layered hollow nanofibre yarns were produced by electrospinning polyhydroxybutyrate (PHB) nanofibres as an outer layer, with urea-impregnated PLLA nanofibres as the inner layer. Scanning electron microscopy (SEM) with an energy-dispersive detector (EDS) was used to characterize the morphology of hollow electrospun nanofibre yarns. A total nitrogen instrument (TNM-1) was used to study the urea release from single- and double-layered hollow nanofibres yarn in water. A CN (Carbon: Nitrogen) elemental analyser determined encapsulated nitrogen in PLLA nanofibres samples. Results indicated that urea-impregnated double-layered hollow nanofibre yarns significantly started nitrogen releasing at much lower amount during first 12 hours compared to single-layered hollow nanofibre yarns (P value= 0.000). In conclusion, double-layered hollow nanofibre yarn has potential as an effective alternative to current methods for the slow release of fertilisers and other plant-required chemicals.

Introduction

Nitrogenous fertilisers improve crop yield and quality by promoting plant growth[1]. Among nitrogen fertilisers, urea is commonly preferred as it is cost effective and has a high nitrogen content of 46% by weight. However, as a neutral organic molecule, urea is not readily absorbed by charged soil particles and can volatilize before hydrolysis is achieved[2]. This results in only a fraction of applied urea nitrogen being absorbed by plants, which may result in large quantities of urea being lost in agricultural runoff, contributing to groundwater pollution[3]. Therefore, the application of surplus nitrogen fertiliser is routine across many agricultural industries to ensure that at least nitrogen use is partially effective. The quantity applied may increase further to compensate for losses through volatilization, denitrification, and leaching which may result in further serious environmental hazards[4]. While some examples of losses greater than 40% of applied urea exist, most investigations report losses of approximately 10%[5]. Consequently, the use of slow- or controlled-release fertilisers has been suggested to improve the efficacy of nitrogen fertilisers and overcome these problems[6]. In parallel to this, researchers have aimed to develop systems which control fertiliser release at low cost and minimise soil contamination, while using biodegradable, inexpensive and readily available material[5].

Nanofibre electrospinning techniques have attracted interest as versatile, low cost techniques to manufacture sub-micron fibres and nanofibres from polymer solutions or polymer melts [7]. Nanofibre-based structures represent novel materials that can encapsulate and release molecules, as well as biological cells, for applications in agricultural, medical and engineering fields[8].

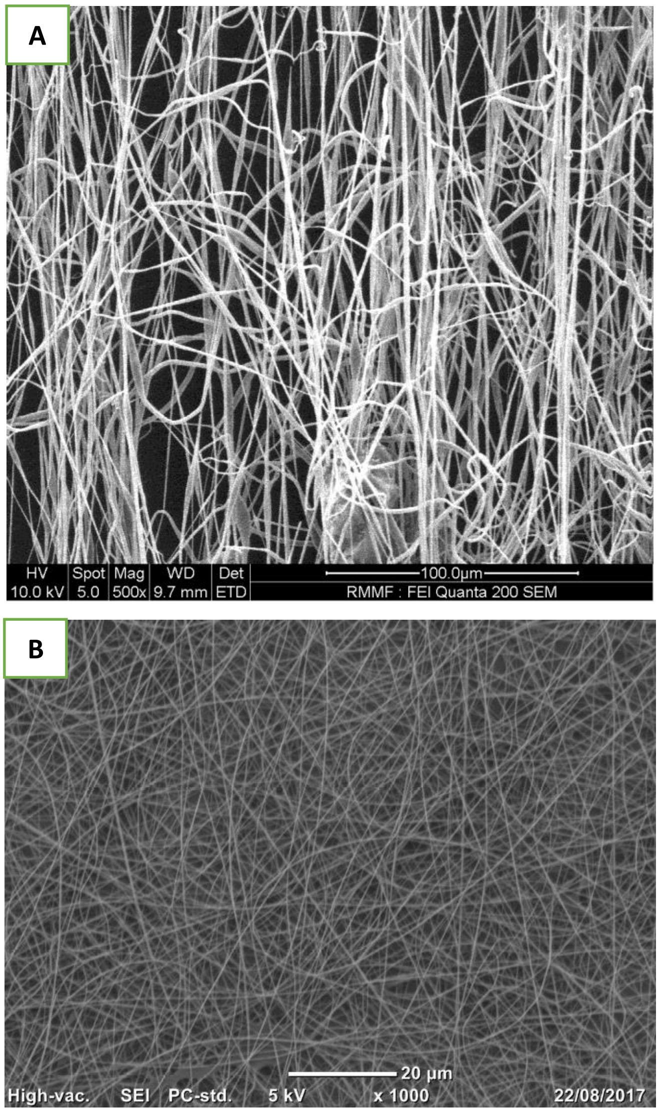
The advent of composite hollow nanostructures adds a new dimension to nanofibre applications. These have extended the impact of particles by coupling their functionality with the feasible processability of synthetic polymers [9]. Furthermore, such hollow nanostructures have much higher surface area to volume ratios compared to their solid counterparts of the same dimensions, a quality which is beneficial when adsorption or storage of chemicals or charges are required[10]. For example, they include electrospun webs that offer control over pore volume and distribution that facilitates dynamic release of target molecules. The use of nanofibres to encapsulate agrichemicals may also allow different chemical additives to be used together through separate nanofibres, and can prolong agricultural additive release by several months[4,5,11–13].

To date, few methods have been proposed to obtain hollow yarns by using the electrospinning process. This unique hollow yarn structure brings together some advantages including high volume and low weight of electrospun nanofibres, combined with the large surface areas of three-dimensional nanofibrous architectures. One such method reported by Bhargava in 2007 used a rotating metal rod attached to a metal hemisphere at one end to produce hollow nanofibre yarn. A hollow core-sheath yarn using two different polymer solutions was electrospun by this method. By subsequently removing the inner core yarn, a hollow nanofibre yarn was obtained[14]. Following this work, a modified electrospinning apparatus to fabricate uniaxial aligned nanofibre yarns was introduced by Wu & Qin[15] . In a recent study, Javazmi et al. (2014) applied a two-nozzle conjugated electrospinning method to fabricate a core-sheath yarn from nanofibres of polyester as the sheath and polyvinyl alcohol (PVA) multifilament as the core of the yarn. Subsequent dissolution of the core yarn in hot water produced a hollow nanofibre yarn[16]. Fakhrali et al. (2014) developed a novel method of fabricating core-sheath nanofibre yarns. This core-sheath nanofibre consisted of PVA nanofibres as the core and nanofibres of nylon 6 as the sheath. Such yarn has potential applications in various fields such as loading drugs into a PVA solution as a core with variable portions[17].

This research aims to investigate and compare single- and double-layered hollow nanofibrous yarn as delivery systems to encapsulate and release urea molecules.

Results and Discussion

PVA, PLLA loading urea, and PHB electrospun nanofibres were bead free and exhibited good uniformity (Figure 5.1; Table 5.1). Uniformly produced electrospun nanofibres indicated that electrospinning parameters were optimized accurately[18–21].



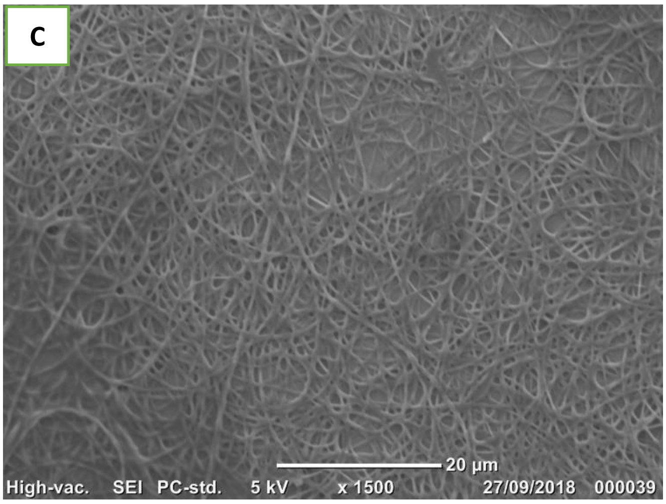


Figure 5.1. SEM micrographs of nanofibres produced during this study. (A) 5% PLLA electrospun nanofibres loaded with 20% urea, (B) 10% PVA electrospun nanofibres and 7% PHB electrospun nanofibres.

Table 5.1. Diameters of PLLA, PVA, and PHB electrospun nanofibres at specific polymeric solutions

Solution Type	**Concentration (%) (w/w) of Polymeric Solution**	**Nanofibre Diameter (nm)**
PLLA loading 20% urea in CF: Acetone (3:1)	5%	710.00 ± 262.70
PVA in Distilled water	10%	242.142 ± 22.20
PHB in DMF:CF (3:7)	7%	417.581 ± 63.80

The core-section diameters of the quad-layered yarns and double-layered hollow nanofibre yarns were 288 ± 3 and 282 ± 2 micrometers, respectively (Figure 5.2). The decrease in the diameter of the core space is due to placement of core-sheath nanofibre yarn in boiling water for one minute to dissolve the PVA nanofibres that led to lateral shrinkage of the hollow nanofibre yarn. Also, the process decreased the air gap between nanofibres resulting in nanofibre layers sticking together[17].

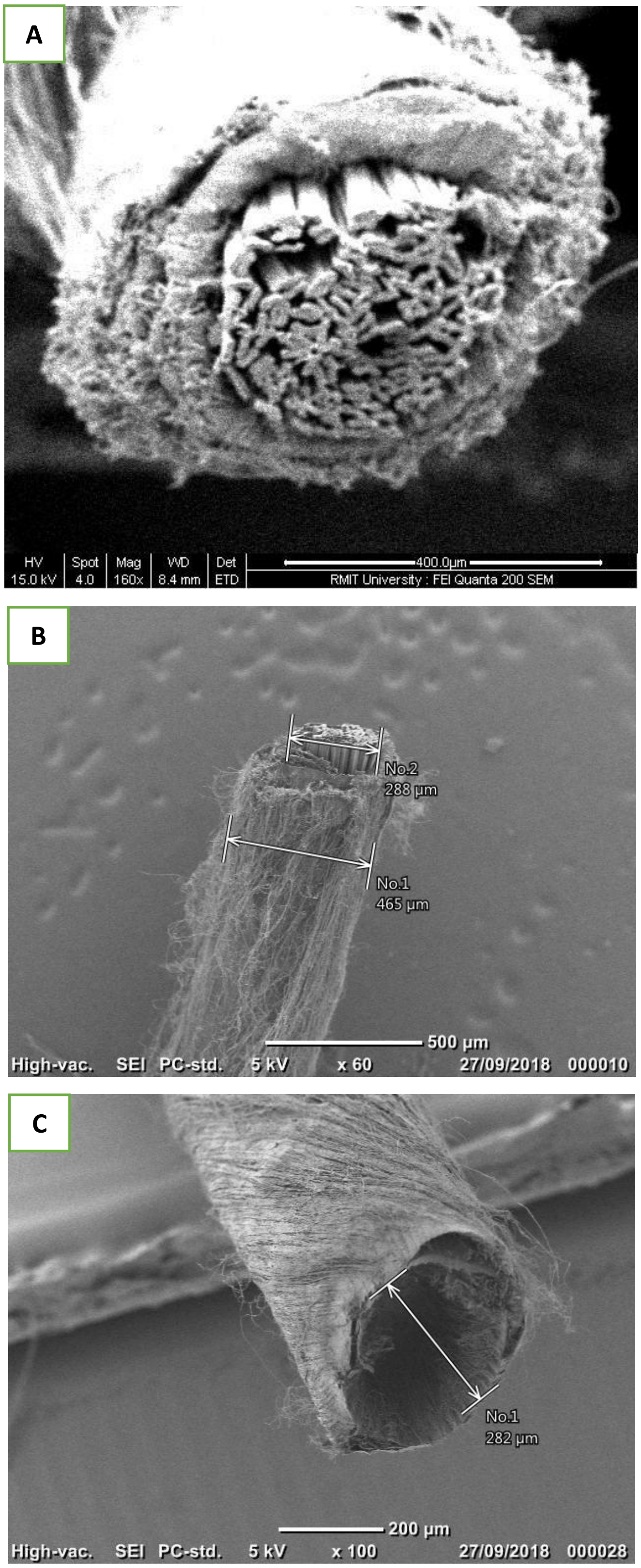


Figure 5.2. Scanning electron microscopy images of: (A) the cross section of quad-layered electrospun nanofibre yarn (B) the surface area of quad-layered electrospun nanofibre yarn, and (C) the cross-section area of hollow double-layered electrospun nanofibre yarn.

Electrospun nanofibre diameter increased with increasing urea concentration from 496nm to 782nm (Figure 5.3). This was expected as higher viscosity tends to lead to higher diameter electrospun nanofibres[22]. A typical SEM micrograph and EDS analysis of PLLA nanofibre containing 20% urea is illustrated in Figure 5.4 (A) and 5.4 (B). The EDS shows the amount of nitrogen element in spectrum 11 based on (w/w) % to PLLA mass amount. From the analysis, it is evident that nitrogen, which is equal to 46% of urea mass, was successfully loaded into all PLLA nanofibre layers. The average percentage amount of main identified elements in spectrums from 1 to 11 is shown in Table 5.2.

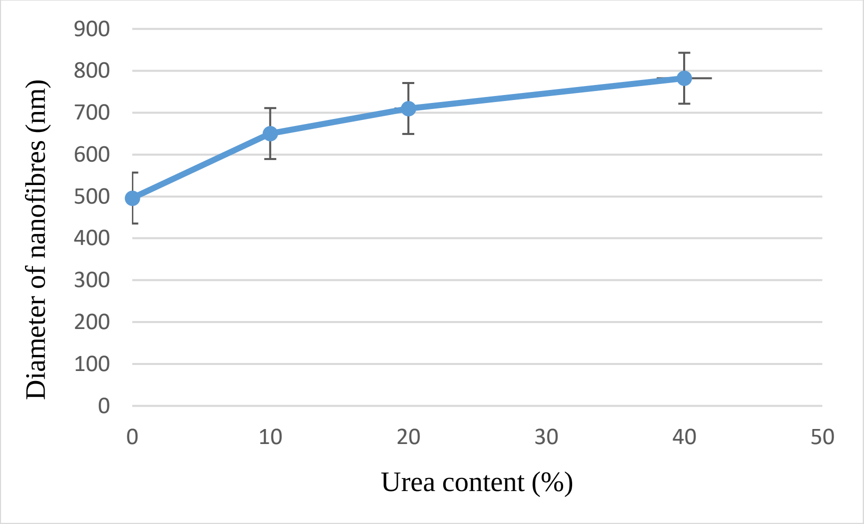
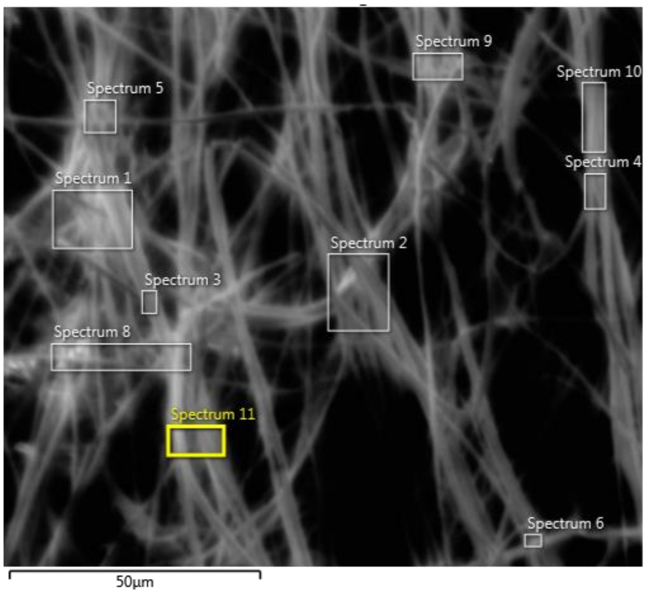


Figure 5.3: The relationship between the electrospun PLLA- urea nanofibre diameter and the urea content.



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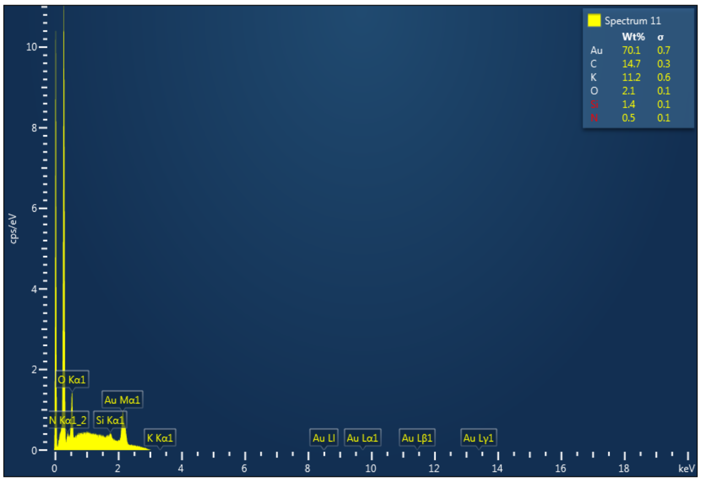

B

Figure 5.4: (A) SEM micrograph of PLLA nanofibre containing 20% urea, and (B) a typical EDS analysis of PLLA nanofibre containing 20% urea.

Table 5.2. Average percentage of identified elements in PLLA nanofibres shown in EDS spectrums as above

Statistics	C (%)	O (%)	N (%)
Max	15.80	2.50	0.5
Min	9.80	1.50	0
Average	13.43	1.87	0.13
Standard Deviation	2.47	0.31	0.13

One-way ANOVA analysis showed nitrogen release commenced at a higher rate as urea concentration increased from 10% to 40% into both single-layer and double-layer hollow nanofibre yarn structures. Thus, as may be expected, by increasing the percentage of urea loaded into nanofibres, nitrogen release rate increased. Double-layered nanofibres containing 10% urea showed a significant decrease (P value= 0.003 and 0.000) in release rate compared with double-layered samples loading 20% and 40% urea, respectively which demonstrated 10% loaded urea into nanofibres as the optimum load. The cumulative nitrogen release for double-layer hollow nanofibre yarn loading 10% urea was approximately 24% of total release while control sample and single- layer hollow nanofibre yarn containing 10% had achieved over 82% and 79% release, respectively, during first 12 hours (Figure 5.5). Double-layer hollow nanofibre yarn structures containing 10% urea release nitrogen at much lower rate compared to single-layer hollow nanofibre yarn encapsulating urea by either electrospinning fabrication or immersing deposition. PHB nanofibrous layer as a physical barrier around PLLA nanofibres in double-layer hollow yarn delayed urea release loaded in PLLA nanofibres. The release mechanism of nitrogen from PLLA nanofibre matrix followed both diffusion and degradation kinetic release in water[23].

Table 5.3 shows nitrogen content of urea- impregnated PLLA nanofibre yarns at concentrations of 0%, 10%, 20%, and 40% by CN analyser. By increasing urea content from 0% to 40%, the percentage of nitrogen increased significantly (P value= 0.03) from 0.06% to 5.23%, respectively, which indicated that nitrogen was encapsulated successfully.

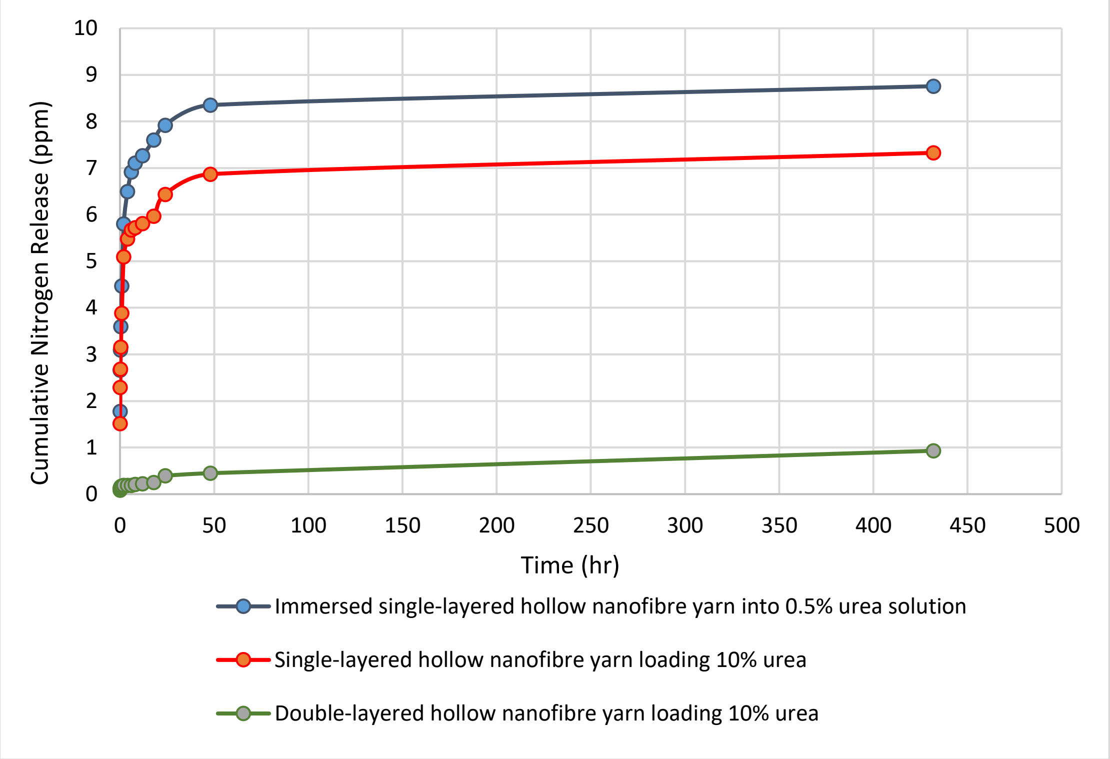

Figure 5.5: Cumulative nitrogen release from immersed nanofibre yarn in 0.5% urea solution and single-, double-layer hollow nanofibre yarns containing 10% urea.

Table 5.3. CN analysis of PLLA nanofibres loading using different percentages of urea

Sample	**Weight (mg)**	**Nitrogen (%)**	**Carbon (%)**
PLLA nanofibre loaded with 0% urea	64.02	0.06	53.66
PLLA nanofibre loaded with 10% urea	22.24	1.44	50.92

PLLA nanofibre loaded with 20% urea	30.10	2.56	51.24
PLLA nanofibre loaded with 40% urea	41.72	5.23	51.60
Pure Urea	63.60	46.32	20.12
*P Value for nitrogen percentage of different loaded urea	0.03 ≤ 0.05		

*P value=Probability value

If P value ≤ 0.05, there is significant difference between data.

Conclusion

Single- and double-layer hollow nanofibre yarn structures were fabricated successfully and loaded with urea using the electrospinning technique. Increased urea concentration produced larger nanofibre diameters. The urea concentration did not affect the nitrogen release rate efficiency within single- or double-layer hollow nanofibre yarns significantly. Moreover, double-layer hollow nanofibre yarns containing urea exhibited a significant reduction in the release amount of nitrogen compared to single-layered nanofibre yarns. Energy-dispersive detector analysis of PLLA nanofibres loading urea illustrated that nitrogen element was encapsulated into PLLA nanofibrous structure. This work has demonstrated that double-layer hollow PLLA nanofibre yarn containing urea may be an effective carrier to control the release of urea fertiliser for future research in agriculture field.

Methods

Materials

PVA with average molecular weights (MW) ranging from 89,000 g/mol to 98,000 g/mol (Product Number: 341584), PHB (Product Number: 363502), and solvents, N-dimethylformamide (DMF); Reagent Plus®, ≥ 99%, chloroform (CF); anhydrous, ≥ 99%; and acetone (Ace) for HPLC, ≥ 99.8% were obtained from Sigma Aldrich. PLLA with a MW of 282,000 g/mol was obtained from Vorina Biomaterials, Ireland (CAS Number: 33135-50-1). Urea (% N: P: K; 46:0:0) from Richgro Garden Products was used in all experiments. A 400/112 denier PVA multifilament yarn was obtained from Dongguan Cocou Textile Materials Company in China.

Preparation of PVA, PLLA and PHB solutions

An aqueous PVA solution with a concentration of 10% (w/w) was prepared by adding Milli-Q water to PVA powder and heating to 80°C with stirring for 20 minutes. A PLLA solution with a concentration of 5% (w/w) in chloroform: acetone (3:1 v/v) was prepared and mixed with 10%, 20%, and 40% (w/w) urea powder relative to the total weight of dissolved PLLA. A PHB polymeric solution in DMF: CF (30:70 v/v) solvent was used at a concentration of 7% (w/w).

Electrospinning Apparatus

To produce single- and double-layered hollow nanofibrous yarn, a customised electrospinning apparatus with two different sets of electrospinning parameters was used in the first and second stages. The schematic diagram of the modified apparatus (Figure 5.6) displays the three main sections that include a feeder unit, middle and a take up unit. The feeder unit was a negative feeder system that fed a PVA multifilament core yarn from a bobbin using take up unit tension. A friction disc tensioner was attached to the bobbin to control the PVA multifilament tension. Electrospinning was conducted in the middle unit, which consisted of two high voltage power supplies (HV), model 73030 DC input 30kV@1mA, from Genvolt, Ireland, Two NE-300 'Just Infusion' syringe pumps from New Era Pump Systems Company, Australia, and a rotating conductive hemisphere with a hollow metal rod attached. The take up unit, that drew electrospun nanofibre yarn at a speed of 1.62 cm/min, consisted of a 5 cm × 8 cm cylindrical roller, and a stepper motor to control the speed of uptake using an ATMEGA328P Pro Mini 328 Mini 16 MHz microcontroller.

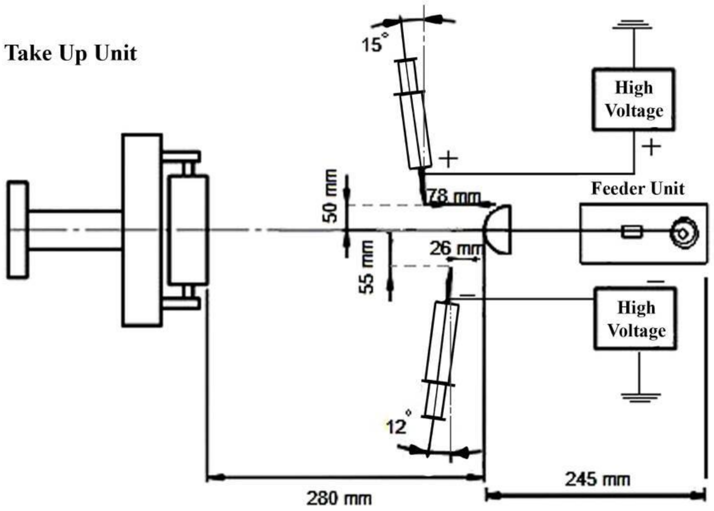

Figure 5.6: Schematic of custom electrospinning apparatus used in this study.

Positively and negatively charged electrodes were connected to the needles with angles of 15º and 12º, respectively. During the two different stages of the electrospinning process, the angular placement of the needles in the apparatus mentioned above facilitated fabrication of two distinctive sheaths of nanofibres surrounding the core yarn. The positively charged needle was spaced 78 mm horizontally, and 50 mm vertically from the hemispherical collector, while the negatively charged needle was positioned 26 mm horizontally and 55 mm vertically from the hemispherical collector.

The aluminium-covered hemispherical collector, with neutral charge and 80 mm diameter, was located 280 mm and 245 mm away from the take-up unit and feeder unit respectively. Angular velocity of the collector was 32 rpm and it could rotate both clockwise and anti-clockwise.

During the first stage, single-layered hollow nanofibrous yarn was fabricated. PVA multifilament was fed through the hemispherical collector by the feeder unit, and the take-up roller collected the other end of the folded PVA multifilament at a rate of 1.62 cm/min. The PVA solution with concentrations of 10%, and 5% (w/w) PLLA solutions loaded with urea at concentrations of 0%, 10%, 20%, and 40% were introduced into the electric field at feeding rates of 0.5 mL/h and 1 mL/h respectively. Electrospun nanofibres were formed between two needles with opposite polarities on the rotating hemispherical collector surface. The different electrical potential between the two needles was 21 kV. By this method, the PVA multifilament was firstly coated by PVA nanofibres fed through the negative charged needle and then by PLLA nanofibres loaded with 10% urea formed from the positive charged nozzle. During the second stage, the feeder unit fed the triple layered nanofibre yarn that was achieved from first stage. The same electrospinning apparatus and parameters are applied for the second stage, however both positive and negative needles are directly located 120 mm from each other and 26 mm from the hemisphere both needles with flow rates of 1 mL/h used to inject 7% (w/w) PHB solutions. PVA multifilament coated by PVA and PLLA nanofibres passed through the rotating collector and was covered by a layer of PHB nanofibres. Finally, the take-up roller collected the four distinctively layered nanofibre yarns. Electrospinning of solutions was conducted at 32°C and ambient humidity. To produce double- layered PLLA/PHB hollow nanofibre yarn, the core yarn was easily recovered after dissolving the PVA nanofibre in water at room temperature for one minute as shown in Figure 5.7[24].

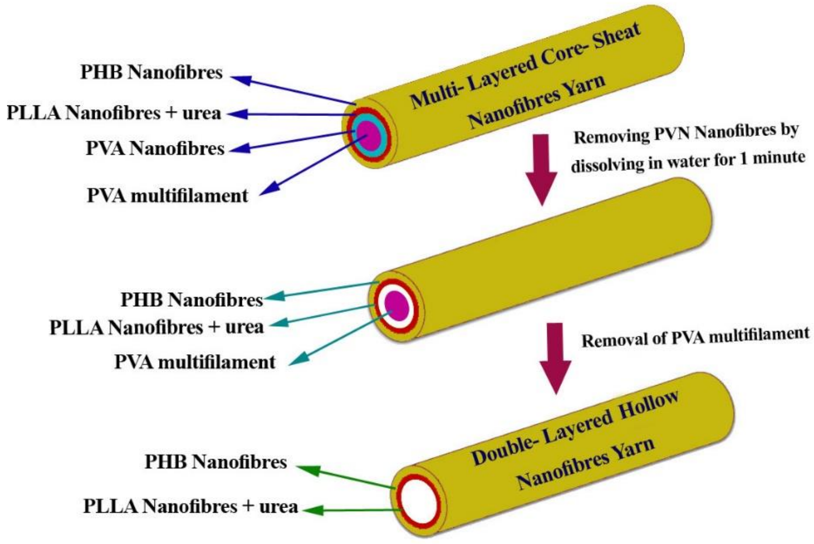

Figure 5.7: Demonstration of how double-layered hollow nanofibres loading urea are obtained from quad-layered core-sheath nanofibre yarns.

Characterization

The morphology of electrospun nanofibres, PVA, PLLA loading urea, and PHB nanofibres, and hollow nanofibre yarns was determined by SEM (FEI Quanta 200 ESEM 2002) with attached EDS at RMIT Microscopy and Microanalysis Facility (RMMF) and benchtop SEM (JEOL JCM-6000 PLUS) at the University of Southern Queensland (USQ). Image Java analysis software was used to measure PVA, PLLA and PHB nanofibre diameters from SEM images at magnifications of 1000x and 5000x.

The single- and double-layered hollow nanofibrous yarns loaded with urea were cut into 6 cm lengths. These pieces were weighed with an analytical balance accurate to ± 0.00001 g and placed in a plastic tube with 20 mL of Milli-Q water to soak for 2 minutes to dissolve any superficial urea deposited on the surface. In addition, a single-layered hollow nanofibre yarn without loaded urea immersed in 0.5% urea solution for 24 hours was selected as a control sample. Subsequently, each sample was immersed in 20 mL Milli-Q water in a 50 mL plastic tube and placed in a shaker at 70 RPM and 30°C [4]. The samples were then removed from the shaker and immersed in a tube containing 20 mL of fresh Milli-Q water for different periods, ranging from 0 to 440 h. A Shimadzu TNM-1 total nitrogen instrument was used to measure nitrogen release from these nanofibre mats. Cumulative nitrogen release of each sample was calculated for the selected period and reported as % nitrogen. Three replicates were produced for all samples and results were reported as the average ± one standard deviation. Nitrogen content of urea-impregnated nanofibres yarns was analysed by a CN analyser (LECO CN626 analyser, Michigan, USA). The single- and double-layered hollow nanofibrous yarn samples were weighed in tin containers and then shaped to little round balls. Tin was important for the correct combustion in the elemental analyser. The tin cups were then dropped into a tube where in the presence of external oxygen flash combustion occurred at a temperature of 1800 °C.

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Author contributions statement

L.J. designed and performed the experiments, L.J. with T.L. analysed the data. L.J. wrote the manuscript with input from all authors. Additionally, T.L., A.Y. and G.A. conceived the study and were in charge of overall direction and planning.

Additional information

Competing interests:

The authors declare no competing interests.

Chapter 6. Conclusions and Future Directions

Conclusions

This research investigated fabrication and characterization of multi-layered nanofibrous structures, and their potential as a delivery system for agrochemicals. This involved an extensive literature review covering electrospinning techniques and current nanofibre applications in agriculture. Experimental work involved validating characterizations of multi-layered nanofibrous structures to prolong urea release from impregnated nanofibres. Presented below is a summary of findings of each chapter, followed by a discussion of the limitations of the study and suggested future directions of the research.

Chapter 1 and 2; "Introduction and Literature Review of Electrospinning and Nanofibres for Potential Applications in Agriculture" revealed that with comparison to the other fibre spinning methods, electrospinning is a versatile, unique and inexpensive technique using electrostatic forces to produce ultrafine fibres with diameters in the range of ten nanometres up to several micrometres. These nanofibres offer increased surface to volume ratio and high aspect ratio which can form porous structure with excellent pore interconnectivity to encapsulate and release chemical materials in agriculture; however, continuous nanofibre yarns bring advantages in terms of appropriate mechanical strength and being integrated into desired assemblies in one-step compared to nanofibre mats. Moreover, hollow nanofibre yarns have much higher surface to volume ratio, and low weight three-dimensional structures in comparison to non-woven mats with the same sizes or solid nanofibre yarns. The literature review highlighted several knowledge gaps in the efforts toward developing core-sheath and hollow nanofibre yarns directly through an electrospinning process. Furthermore, to date, no research project has attempted to encapsulate and release chemical additives of agriculture by three-dimensional nanofibrous structures. Therefore, three-dimensional nanofibrous architectures and hollow nanofibre yarns with large surface area may have potential to improve materials delivery.

The aim of this research was to understand the effect of the electrospinning parameters on the morphology and behaviour of nanofibres and hollow nanofibre yarns to investigate urea release dynamics from nanofibrous structures as a proof of concept for slow release delivery of agrichemicals.

Chapter 3; “Kinetics of a Slow Release Nitrogen Fertiliser from Multi-Layered Nanofibrous Structures”, studied the development of nanofibrous structures for prolonging urea release. The research presented here demonstrated the potential of the new method to encapsulate and release molecules of agrochemicals by using multi-layered electrospun nanofibres as a carrier for the controlled release of nitrogenous fertiliser. Single-layer poly L-lactic acid (PLLA) nanofibres loading urea fertiliser were fabricated using custom-built electrospinning apparatus. Triple-layer nanofibrous structures were produced by electrospinning Polyhydroxybutyrate (PHB) nanofibres as upper and lower layers with PLLA nanofibres impregnated with urea fertiliser as the middle layer. Fourier transform infrared spectrophotometry (FTIR) was employed to show urea encapsulation in all samples and Scanning electron microscopy (SEM) was used to characterize the morphology of electrospun nanofibres. The diameters of nanofibres increased with increasing the urea concentration in PLLA solutions. Urea release dynamics were analysed using a total nitrogen instrument (TNM-1). The results indicated that in both single- and triple-layer nanofibrous structures, by increasing the percentage of urea loaded into nanofibres from 10% to 40%, an increase in the initial rate of nitrogen release was observed, with release continuing for three months. Triple-layer nanofibrous structures containing 10% urea exhibited a significantly lower level and lower initial rate of nitrogen release compared to single-layer nanofibres loading 10% urea (P value=0.012). In conclusion, triple-layered electrospun PLLA nanofibres containing urea may be an effective carrier to control the release of urea fertiliser in agriculture applications.

Chapter 4; “Fabrication and Characterization of PLLA/PHB Hollow Double-layered Nanofibre Yarn for Encapsulation of Urea”, the findings of this study demonstrated promising three-dimensional nanofibrous architectures with low weight and large surface area for potential applications in agrochemical delivery. A new method was developed for fabricating hollow double-layered nanofibrous yarn. A four-layered yarn structure was created using different angular placement of the extruder nozzles. Scanning electron microscopic images of the morphology of samples indicated that by increasing urea concentration from 0% to 40%, into PLLA polymeric solutions, nanofibre yarn diameters increased significantly. Mechanical and physical properties of PHB/PLLA hollow double-layered nanofibre yarns in various concentrations of urea contents were investigated to understand the effective electrospinning parameters on the nanofibrous structure functionality. The results indicated that by increasing the diameter of PLLA nanofibres containing urea, strength in both core-sheath and hollow nanofibre yarns decreased due to the presence of beads in PLLA nanofibre structures at urea concentration of 20% and 40%; therefore, hollow nanofibre yarn loading 40% urea had the lowest strain and stress. Furthermore, increasing urea concentration led to a decrease in regain moisture of PLLA hollow nanofibre yarns. This chapter resulted in a deep understanding of effective electrospinning parameters on nanofibre architecture to fabricate hollow nanofibrous yarn structures with PLLA and PHB nanofibres as biodegradable and biocompatible polymers, with an inner diameter large enough for potential applications in agriculture area such as fertiliser delivery systems. Chapter 5; "Investigation of Slow Release Urea from Biodegradable Single- and Double-Layered Hollow Nanofibre Yarns", reported the development and evaluation of hollow nanofibre yarns (multi-layered nanofibre yarn loading fertilisers) for improving urea release. Single- and double-layered hollow nanofibrous yarns, as novel delivery systems, were fabricated and used to investigate encapsulation and release of urea. The results in developing nanofibrous structures indicated that increasing urea concentration into PLLA polymeric solutions led to increased PLLA nanofibre diameters. However, the urea concentration did not affect the urea release rate efficiency within single- or double-layered hollow nanofibre yarns significantly (P value≥ 0.05). Moreover, 10% urea-impregnated double-layered hollow nanofibre yarns showed a significant reduction in the release amount of urea compared to single-layered nanofibre yarns ($P < 0.01$). Energy-dispersive detector analysis of PLLA nanofibres loading urea illustrated that nitrogen element was encapsulated into PLLA nanofibrous structure. A CN (Carbon: Nitrogen) elemental analyser determined encapsulated nitrogen in PLLA nanofibres samples increased significantly (P value$= 0.03$) from 0.06% to 5.23% by increasing urea content from 0% to 40%, respectively, which indicated that nitrogen was encapsulated successfully. This research has demonstrated that continuous three-dimensional double-layer hollow PLLA nanofibre yarn containing urea with appropriate mechanical strength may be an effective carrier to control the release of urea fertiliser.

In conclusion, this research introduced a new method to encapsulate and release molecules of urea. This system used a basic electrospinning apparatus to fabricate multi-layered electrospun nanofibre as a delivery system. Triple-layer nanofibrous structures were produced by electrospinning PHB nanofibres as upper and lower layers with PLLA nanofibres urea-impregnated as the middle layer. The triple-layer nanofibre prolonged the nitrogen release up to three months with lower initial rate of nitrogen release compared to single-layer PLLA nanofibres. Furthermore, a new approach was introduced to fabricate hollow double-layered nanofibrous yarns by engineering a yarn with a four-layer structure. The yarn consists of a Polyvinyl Alcohol (PVA) multifilament as core yarn covered by layers of PVA nanofibre, PLLA nanofibres loaded with urea fertiliser, and PHB nanofibres, respectively during two different stages of electrospinning. Moreover, the double-layer hollow nanofibre yarn loading urea released nitrogen for 432 hours, showing a significant reduction in the release of urea compared to single-layer hollow nanofibre yarns. The thesis concluded that triple-layer urea-impregnated nanofibrous structures, and double-layer hollow nanofibre yarns containing urea are potential candidates and effective alternatives for the slow release of urea nitrogen.

Future directions

The use of nanofibres in agriculture will likely continue in the future due to their unique nanostructures. Electrospinning process to manufacture nanofibres is still an infancy technology and has not been scaled-up for extensive commercial purposes. Examination of electrospun nanofibres for the agriculture field with industrialized portable electrospinning machines is an interesting future topic for investigation. Also, improving the use of coaxial electrospinning systems to encapsulate agrochemical materials will be great importance because this configuration provides sustained release and decreases burst release. One of the main areas where electrospun nanofibres can be developed further in the future is food packaging because antimicrobials, antioxidants, and other bioactive materials can be easily incorporated in electrospun nanofibres during electrospinning and release gradually; therefore, using nanofibres in packing materials for food industries will increase shelf life and prevent food spoilage. In addition, one of the potential applications of the double-layered hollow nanofibre yarns is to monitor releasing manner of two pesticides simultaneously according to degradation time of polymers applied to load them. Mathematical modelling of release dynamics in material delivery systems would be an interesting topic to study. It can help predict release amounts and select suitable coating materials for obtaining desirable release kinetics.

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Appendix

Poster Presentation entitled:

Encapsulation of Flutriafol Fungicide into Electrospun Biodegredable Poly (L-lactide) Nanofibers

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Encapsulation of Flutriafol Fungicide into Electrospun Biodegredable Poly (L-lactide) Nanofibers

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Introduction

- Electrospinning techniques have attracted interest as versatile, low cost techniques to manufacture sub-micron fibres and nanofibers from polymer solutions or polymer melts [1].
- High surface area and ease of incorporation of active ingredients have prompted some researchers to investigate using electrospun nanofibers in agricultural application [2].
- However, most of the reports have focused on using electrospun nanofiber delivery systems for the encapsulation of drugs and food materials [3-4], and there are few studies have reported the encapsulation of agrochemical materials into electrospun nanofibers [2].
- Flutriafol is a commonly used fungicide in plant protection in Australia, and in this study, for the first time, we aimed to encapsulate flutriafol fungicide into poly(L-lactide) (PLLA) nanofibers matrix by optimizing electrospinig conditions.

Materials and Methods

- PLLA with a average molecular weights (MW) of 282,000 g/mol was purchased from Vorina Biomaterials, Ireland (CAS Number: 33135-50-1).
- Solvents, Chloroform (CF); anhydrous, ≥ 99%; and Acetone (AC) for HPLC, ≥ 99.8% were obtained from Sigma Aldrich.
- Flutriafol PESTANAL ($C_{16}H_{13}F_2N_3O$) with molar mass of 301.29 g/Mole was purchased from Sigma Aldrich.
- The schematic setup for nanofiber electrospinning is shown in Figure 1. It consists of a high voltage (HV) power supply, model 73030, DC input 30 kV @ 1 mA, from Genvolt, Ireland, and a New Era NE-300 "Just Infusion" syringe pump. A metal frame 14cm × 16cm with attached aluminium foil was located 15cm from the syringe needle to collect nanofibers. The positive terminal of the HV was connected to the needle and the ground was attached to the collector (metal frame). The electrospinning process occurs between the needle tip and the aluminium collector, and nanofibers are gathered on the surface of the foil.
- PLLA solution with optimum concentrations of 5% (w/w) in chloroform-acetone (75–25 v/v) was prepared and mixed with 10% (w/w) flutriafol powder relative to the weight of PLLA. The PLLA/flutriafol mixture was fed with a syringe to a basic electrospinning setup. Optimum electrospinning conditions were observed at 32 °C with a flow rate speed of 1mL/ h, and 12kV high voltage.

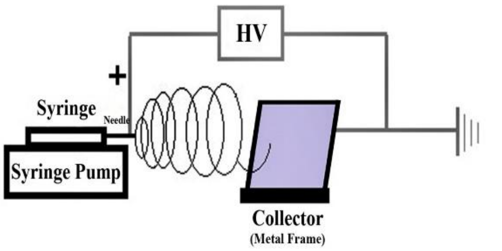

Figure 1: Schematic diagram of the electrospinning setup

Results and Discussion

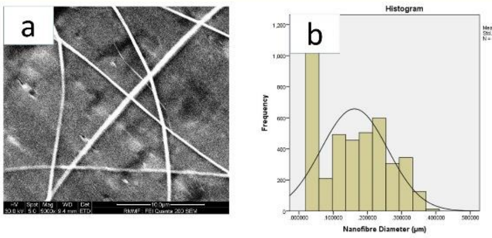

Figure 2: SEM image and related diameter histogram of the PLLA electrospun nanofibers containing 10% flutriafol

Nanofiber Characterization:

- SEM (FEI Quanta 200 SEM 2002) at the RMIT Microscopy and Microanalysis Facility (RMMF) with attached Energy-dispersive detector (EDS) was used to determine the morphology of electrospun nanofibers and identify successful encapsultion of flutrifol into PLLA nanofibers. Image J processing software was used to measure PLLA nanofiber diameters from high magnification SEM images.
- In the case of effect of flutriafol concentration on nanofiber morphology, SEM image of PLLA electrospun nanofibers loading flutriafol with related histogram of nanofibers diameter distribution are shown in Figure 2 (a, b), and nanofiber diameters are displayed in Table 1. This effect was consistent in the present study, the diameter of PLLA electrospun nanofibers decreased from 496.183nm to 162.079nm with adding 10% flutriafol to the electrospinning solution (Figure2; Table 1).
- By adding some ionic organic/inorganic compounds such as flutriafol to the PLLA polymeric solution, the increased number of electrical charge causes an increase in the elongation capacity of the solution, favoring the formation of smooth fibers, with smaller diameter [5] .

Table 1. Effect of flutriafol concentration on nanofiber diameter

Flutriafol Concentration (*% w/w)	Nanofiber diameter (nm)	**CV%
0	496.183	22.0%
10	162.079	9.7%

*% w/w based on amount of PLLA used, **Coefficient of Variation (CV).

EDS Characterization:

- A typical SEM micrograph and EDS spectrum analysis of PLLA nanofiber containing 10% flutriafol are illustrated in Figure 3(a, b). The EDS shows the amount of nitrogen, and fluorine elements in spectrums 10, and 11 based on wt. % ratio to PLLA mass amount. The percentage amount of identified elements is shown in Table 2.

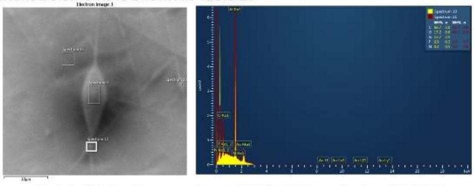

Figure 3: (a) SEM micrograph, and (b) EDS analysis of PLLA nanofiber containing 10% flutriafol.

Table 2. Percentage of identified elements in PLLA nanofibers.

Statistics	C	N	O	F
Max	72.63	1.30	44.06	2.27
Min	53.57	0.40	24.42	1.36
Average	63.10	0.85	34.24	1.81
Standard Deviation	13.40	0.74	12.76	0.64

Conclusion

- EDS analysis of PLLA nanofibers proved that flutriafol compound elements have been successfully encapsulated into PLLA nanofiber structures.

Acknowledgment

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Effect of TiO_2 nanoparticle on Wicking Phenomenon in PAN Nanofiber Yarns

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Abstract Summary: In this research, different concentrations of PAN polymeric solutions with various percentages of TiO_2 nanoparticles were electrospun to investigate vertical wicking of PAN/TiO_2 nanofiber yarns. Results showed by the presence of nanoparticles in the nanofiber structure, the capillary rise increased, although by increasing concentrations of polymeric solutions and nanoparticles, capillary rises were statistically insignificant.

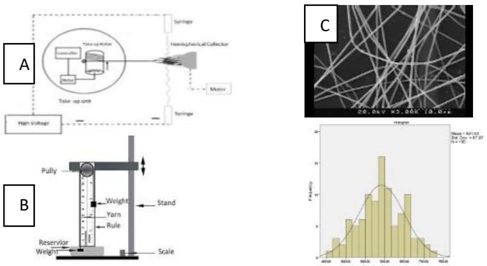
Keywords: PAN/TiO_2 Nanofiber Yarns, Electrospinning, Wicking, Nanoparticles, Capillary

Introduction: A spontaneous transport of a liquid into a fibrous system such as nanofiber yarns and mats by capillary forces is termed wicking. Capillary phenomenon is a function of liquid properties and nanofibrous structure properties such as radius of capillary space, pore size, pore size distribution, porosity, and surface chemical characteristics [1]. Recently, researchers have focused on using electrospun nanofibers for many applications due to their extremely high surface to volume ratio, highly porous structure with excellent pore interconnectivity. Nanofibers with specific characterizations of their structures show different capillary behaviour from normal spun or filament fibres [2]. The aim of this work is to study wicking behaviour in PAN/TiO2 electrospun nanofiber yarns. SEM images were taken and the nanofiber diameters were measured in different concentrations of PAN polymer solutions and Tio_2 nanoparticles.

Experimentation:

Materials: A kind of polyacrylonitrile (PAN) polymer with molecular weight (Mw) of 7000 was obtained from Iran Polyacryl Company. Anatas TiO2 nanoparticles with average nanometre sizes of 21 were purchased from Evonik Company, and Dimethylformamide (DMF) as solvent was provided from Merck Company. PAN/DMF polymer solutions with 11 %(w/v) and 15 %(w/v) concentrations were mixed with 0%, 1%, and 2% of TiO2 nanoparticles, respectively. The coloured liquid used for the wicking measurements was single-distillation water with 0.2% non-ionic detergent and 0.2 % red acid dye. *Electrospinning Set-up:* A modified schematic set-up for electrospinning is shown in Figure 1(A). It consists of a high voltage-power supply, a conductive hemisphere, two syringe needles, a take up unit and a feeder unit. The hemisphere diameter was 8 cm. Electrospinning was done between two syringe needles with opposite voltages and collected electrospun nanofibers on rotating hemisphere were pulled out by take-up roller [3]. PAN/TiO2 solutions were fed to the set-up via syringes with flow rate of 0.085 mL/h and the electrospinning was done at a voltage of 13 kV between two nozzles. The distance between two nozzles, and the distance between hemispherical neutral surface and nozzles tips were 19 and 4 cm, respectively. The distance between the hemispherical collector and take-up unit was 20 cm. Rotating speed of take-up unit was 0.088 rpm. *Capillary Tests:* Figure 1(B) illustrates the apparatus designed for capillary height measurement schematically. The nanofiber yarn with 20cm length was placed to a coloured liquid reservoir vertically while, a Sony digital handy cam (DCR-PC115E) was used to make video films of the capillary rise. The nanofiber yarn wicking measurements were observed for 24 hours in standard conditions.

Result and discussion: The effect of polymer concentration on the PAN/TiO2 nanofiber yarns morphology was investigated for five different concentrations. Figure 1(C) shows a typical SEM image and histogram of PAN/TiO2 nanofibers at the polymer concentration of 15% (w/v) PAN/DMF and 2% (w/v) TiO2/DMF. Also, the average diameters of PAN/TiO2 nanofibers ranged from 172.94 to 591.525 nm was illustrated in Figure 2. Thus, Results showed by increasing concentrations of polymer solution and nanoparticles, nanofibers diameter increased.



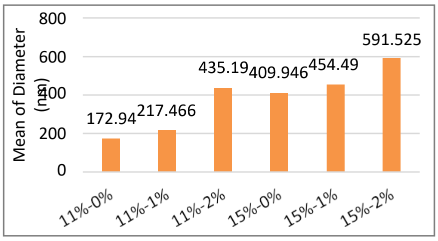


Fig.1.Electrospinning setup (A), Wicking apparatus (B), Fig.2. Average diameters of PAN/TiO2 nanofibers. SEM and histograms of diameter (C).

To analyse the kinetics of capillary flow in PAN/TiO2 nanofiber yarns, images taken during the capillary rise were processed to gain a set of points (t, h). Figure 3 (A) and 3 (B) show the effect of nanoparticles at 0%, 1%, and 2% concentrations on capillary rises of 11% PAN nanofiber yarn and 15% PAN nanofiber yarn, respectively. Figure 4 (A) and 4 (B) show the variations of height square as a function of time for 2 samples of PAN/TiO2 nanofiber yarns. The curve obtained is linear in range of 0-10 seconds, and the capillary rate coefficient of the nanofiber yarns is bigger than 0.99, so Lucas-Washburn Equation is valid in PAN/TiO2 nanofiber yarns for establishing kinetics of liquid capillary rise [4]. Thus, by the presence of nanoparticles in the nanofiber structure, the height of liquid rise in yarn increased, which indicates a reduction in the radius of the capillary space. By keeping the twist and nanoparticles in constant value and increasing in polymer solution concentration, the capillary rise furthered, but it was not noticeable and statistically significant. Moreover, during the capillary tests, the effect of gravity on liquid rise was ignored.

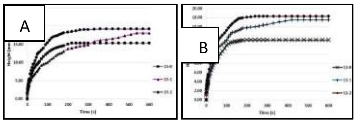


Fig. 3 (A) and (B). Wicking height of 11% and rise 15% PAN nanofiber yarn with different percentages of TiO2

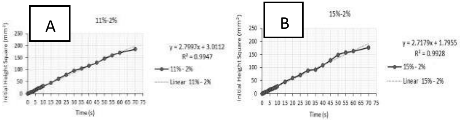


Fig. 4 (A) and (B). Square height of the capillary on PAN/TiO2 nanofiber yarns

Conclusion: The aim of this work was study of wicking behaviour in PAN/TiO2 nanofiber yarns. Results showed by adding TiO2 nanoparticles to PAN nanofiber yarns, the height of liquid rise in the nanofiber yarns increased. However, by keeping the values of twist and nanoparticles constantly and increasing in PAN polymer solution concentration, the capillary rise was not noticeable and statistically significant.

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