Novel Chemical and Physical Approaches for Sustainable Drug Release from Biodegradable Electrospun Nanofibres

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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March, 2016
Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

Javid Jalvandi

30 March 2016
dedicated to my beloved parents
ana & ata
Declaration

I, Javid Jalvandi, certify that:

a. except where due acknowledgement has been made, the work is that of the candidate alone;

b. the work has not been submitted previously, in whole or in part, to qualify for any other academic award;

c. the content of the thesis is the result of work which has been carried out in (or under the direction of) the School of Fashion and Textiles, RMIT University;

d. any editorial work, paid or unpaid, carried out by a third party has been acknowledged;

e. ethics procedures and guidelines have been followed.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>Levofloxacin</td>
<td>LVF</td>
</tr>
<tr>
<td>Mesoporous silica nanoparticles</td>
<td>MSN</td>
</tr>
<tr>
<td>Poly(ε-caprolacton)</td>
<td>PCL</td>
</tr>
<tr>
<td>Poly(vinyl alcohol)</td>
<td>PVA</td>
</tr>
<tr>
<td>Chitosan</td>
<td>CS</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>Mw</td>
</tr>
<tr>
<td>Nanometre</td>
<td>nm</td>
</tr>
<tr>
<td>Millimetre</td>
<td>mm</td>
</tr>
<tr>
<td>Milligram</td>
<td>mg</td>
</tr>
<tr>
<td>Millilitre</td>
<td>mL</td>
</tr>
<tr>
<td>Minute</td>
<td>min</td>
</tr>
<tr>
<td>Hours</td>
<td>h</td>
</tr>
<tr>
<td>Gram</td>
<td>g</td>
</tr>
<tr>
<td>Gauge</td>
<td>G</td>
</tr>
<tr>
<td>Kilovolt</td>
<td>kV</td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td>SEM</td>
</tr>
<tr>
<td>Transmission electron microscopy</td>
<td>TEM</td>
</tr>
<tr>
<td>Barrett-Joyner-Halenda</td>
<td>BJH</td>
</tr>
<tr>
<td>Brunauer-Emmett-Teller</td>
<td>BET</td>
</tr>
<tr>
<td>Fourier transform infrared spectroscopy</td>
<td>FTIR</td>
</tr>
<tr>
<td>Differential scanning calorimetry</td>
<td>DSC</td>
</tr>
<tr>
<td>Thermo gravimetric analysis</td>
<td>TGA</td>
</tr>
<tr>
<td>Lysogeny broth</td>
<td>LB</td>
</tr>
<tr>
<td>Centigrade</td>
<td>°C</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>DCM</td>
</tr>
<tr>
<td>N,N-dimethylformamide</td>
<td>DMF</td>
</tr>
<tr>
<td>1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
<td>EDC</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>DMSO</td>
</tr>
<tr>
<td>Phosphate buffered saline</td>
<td>PBS</td>
</tr>
<tr>
<td>Weight percent</td>
<td>wt%</td>
</tr>
<tr>
<td>Nuclear Magnetic Resonance</td>
<td>NMR</td>
</tr>
<tr>
<td>Micrometre</td>
<td>um</td>
</tr>
<tr>
<td>Centimetre</td>
<td>cm</td>
</tr>
<tr>
<td>Deionised water</td>
<td>DI</td>
</tr>
</tbody>
</table>
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Drug delivery systems have great importance for medical application. There have been wide ranging studies on drug delivery systems up to date. Among them, the fabrication of nano-size drug delivery systems and utilizing them for the effective and controlled delivery of drugs is an emerging and promising field of research. Various studies on drug nanocarriers have been reported such as electrospun nanofibres and nanoparticles; however the main drawback of nearly all is that they lack the control over the release profile of the drug. The burst release of drug is the main issue reported seen in almost all cases.

The purpose of this work was to develop composite biodegradable nanofibres fabricated using the electrospinning technique with different chemical and physical approaches to slow down the fast burst release and control the release profile. This thesis exploits electrospun biodegradable nanofibres as the main carrier of drug/nanoparticles complexes and drug-conjugated polymers to obtain sustained release of drug. To achieve this, mesoporous silica nanoparticles (MSN) acting as a nano sized vehicle as a drug delivery system were first investigated. Then, the adsorption of the drug into mesoporous structure was fabricated and characterized. The drug loaded nanoparticles were confined to the core section of core-shell nanofibres by means of co-axial electrospinning. This method provided a reduction in burst release compared to regular drug-nanofibres and a sustained release. In the next stage, a model drug was covalently bound to the silica nanoparticles via a cleavable bond. In the first two experiments the MSN was used as nanocarrier of levofloxacin (LVF). LVF-MSN complexes were prepared using different approaches. In the first approach, LVF was physically adsorbed into the porous structure of the MSN. The LVF-MSN complex was then confined into the core section of core-shell PCL nanofibres. In the second approach, LVF was covalently bound to MSN via a cleavable bond
which can be hydrolysed in release buffered solution. LVF-MSN conjugated complexes were then blended with PCL and composite nanofibres fabricated by electrospinning techniques.

In the final experiment, polyvinyl alcohol (PVA) was used as the main polymer matrix. The purpose of this experiment was to achieve sustained release of drug from a fast dissolving polymer. To achieve this goal, chitosan (CS) as a naturally derived polymer, was used as main carrier of LVF. LVF was covalently bound to the chitosan via a cleavable bond. The conjugated LVF-CS was blended into a PVA solution and electrospun.

*In vitro* drug release profiles were measured in all cases. The research confirmed that release profiles can be affected by chemical and physical factors. First, the core-shell structure of nanofibres can reduce burst release behaviour. This can be further reduced by employing LVF encapsulated MSN. Second, cleavable bonds between LVF and carriers played a significant role in reducing the burst release behaviour; where LVF was released first by hydrolysis of the cleavable bonds between LVF and carriers, and then from the polymer matrix.

The results indicated that burst release of the drug significantly reduced in all studies and the release profiles of drug were all slowed. The physical and chemical approaches used in the thesis to fabricate drug loaded composite nanofibres showed slow release of LVF with sustained release over a sustained period of time.
Chapter 1

LITERATURE REVIEW
1.1 Drug Delivery Systems

Drug delivery systems (DDS) are designed to consistently introduce therapeutic agents into the body at a required dose level and rate. The first development appeared in the 1960s when Folkman [1] circulated rabbit blood inside a silicone rubber arteriovenous fistula and discovered that if he exposed the tubing to anaesthetic gases on the outside, the rabbits would fall asleep. It was then proposed that short, sealed segments of such tubing containing a drug could be implanted and could become a constant rate drug delivery device [2]. Drug delivery systems are designed to alter the pharmacokinetics and distribution of associated drugs to function as drug carriers (i.e., as sustained release systems). Pharmacological properties of conventional drugs can be improved through the use of drug delivery systems. Drug delivery systems are expected to create novel therapeutics for successful treatment due their advantages. The main advantages of drug delivery systems include enhanced drug solubility, reduced toxicity, protecting the drug from undergoing rapid decomposition and degradation and reduced side effects in non-target tissues. Table 1-1 shows examples of the problems faced by free drug administration that can be improved by the use of drug delivery systems [3].

The concept of drug delivery and controlled drug delivery devices has been considered by the chemist Zaffaroni in California USA. He founded a company in the late 1960s focused on the concept of controlled drug delivery [4]. This term is used to define the encapsulation or attachment of drugs to enhanced carriers, which alter their localization in the body and their uptake into different tissues [5]. Subsequently, controlled release systems for drugs have been widely explored due to their advantages such as improving therapeutic efficiency by delivering the drug at a controlled release rate consequently overcoming adverse side effects [6, 7].
### Table 1-1 Effects of drug delivery systems on drugs and their therapeutic implications [3]

<table>
<thead>
<tr>
<th>Problem</th>
<th>Implication</th>
<th>Effect of DDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor solubility</td>
<td>A convenient pharmaceutical format is difficult to achieve, due to the possibility of precipitation of hydrophobic drugs</td>
<td>DDS provide both hydrophilic and hydrophobic environments, enhancing drug solubility</td>
</tr>
<tr>
<td>Tissue damage due to the leakage of the drug</td>
<td>Unintentional leakage of cytotoxic drugs lead to tissue damage</td>
<td>Regulated drug release from the DDS can reduce/eliminate tissue damage on accidental extravasations</td>
</tr>
<tr>
<td>Fast collapse of the drug in vivo</td>
<td>Loss of activity of the drug follows administration, e.g., loss of activity of drug at certain pH</td>
<td>Protected drug from premature degradation and functions as a sustained release system</td>
</tr>
<tr>
<td>Unfavourable pharmacokinetics (PK)</td>
<td>Drug is cleared too rapidly, by the kidney, for example, requiring high doses or continuous infusion</td>
<td>DDS can substantially alter the PK of the drug and reduce clearance. Rapid renal clearance of small molecules is avoided</td>
</tr>
<tr>
<td>Poor biodistribution</td>
<td>Drugs that have widespread distribution in the body can affect normal tissues, resulting in dose-limiting side effects</td>
<td>The particulate nature of DDS lowers the volume of distribution and helps to reduce side effects in non-target tissues</td>
</tr>
<tr>
<td>Lack of selectivity for target tissues</td>
<td>Distribution of the drug to normal tissues leads to side effects. Adverse therapeutic effects due to low concentrations of drugs in target tissues</td>
<td>DDS can increase drug concentrations in target tissues such as tumors</td>
</tr>
</tbody>
</table>

Various advantages of controlled drug release systems compared to other methods of drug administration have been reported [8]. These advantages include reducing the side effects of systemic administration by local administration from a controlled release system, improved
drug administration in areas where good medical supervision is not available, continuous sustained release of small amounts of drug instead of painful large doses, and improvement of patient compliance. In order to reach these goals, several factors should be considered in a controlled drug delivery system; such as delivery vehicle materials, the drug properties, route of administration, mechanism of drug release, ability of targeting, and biocompatibility (Figure 1-1) [9].

![Figure 1-1 Design requirements for drug delivery systems](image)

In the past few decades a variety of biocompatible drug delivery systems have been explored with enhanced properties for encapsulation and release kinetics. It was demonstrated that when a pharmaceutical agent is encapsulated within, or attached to, a carrier, drug efficacy and safety may be improved [10]. The concept encouraged active and intensive researches for the design of biodegradable materials as drug delivery systems. Recent efforts have led to the development of a new approach in the field of controlled drug delivery with the creation of nano-sized biodegradable polymeric drug delivery systems [11]. The key requirement of a controlled drug release system is to allow sustained release of the active agent continuously over an extended period of time to achieve a consistent therapeutic drug release profile which
results in extended therapeutic advantages over the duration of action by reducing problems associated with multiple dosing [12].

1.1.1 The Economics of Drug Delivery Systems

Drug delivery technology can bring both therapeutic and commercial value to healthcare products. Major pharmaceutical companies have recently started losing their market share to their competitors after their patents expired. Therefore they have started to recognize the importance of drug delivery systems. The drug delivery technology market is segmented on the basis of route of administration into different categories such as oral, pulmonary, transdermal, injectable, ocular, nasal, topical, implantable, and transmucosal [13]. The value of the global market was $142.5 billion in 2012; with North America as the largest market, followed by Europe, and Asia. The market is expected to rise at a compound annual growth rate of 5% and reach nearly $175 billion by 2016. Key players in the market are 3M (U.S.), Alkermes (Ireland), Aptalis Pharma, Inc. (U.S.), Baxter International, Inc. (U.S.), Becton Dickinson & Co. (U.S.), Catalent Pharma Solutions (U.S.), Dow Chemical Company (U.S.), Endo Pharmaceuticals (U.S.), Johnson & Johnson (U.S.), Nektar Therapeutics (U.S.), SkyePharma PLC (U.K.), and Ypsomed Holding AG (Switzerland) [14]. The following are some examples in the market now. The Atridox® is a controlled-release product for delivering tetracycline antibiotics to the infected tooth pockets for a period of 7 days. ARESTIN® contains tetracycline antibiotic loaded materials used for treating gum problems that occur after certain dental procedures. Estrasorb™ is designed to deliver estradiol to the blood circulation by absorbing through the skin and enters into the bloodstream.
1.2 Impact of Nanotechnology

Nanotechnology is about the engineering and manufacturing of materials at the molecular scale. It refers to a structure with a size of generally 1-100 nanometers (nm) in at least one dimension. Nanotechnology, regardless of the mentioned size restriction, commonly refers to structures that are up to several hundred nanometers and generally less than a micrometer (10^{-6} meter) in size.

The role of nanotechnology in the application of drug delivery is widely expected to transform the landscape of pharmaceutical and biotechnology industries in the near future [15]. Drug delivery is one of the most demanding areas in nanotechnology with engineering of nano-sized material specially for cancer therapies and diagnostics [16]. The development of nanocarriers may have an important role in adding new therapeutic achievements to the drug delivery industry and disease therapy. It is expected to achieve improved delivery of water insoluble drugs, targeted delivery of drugs in to tissues, delivery of large macromolecule drugs to intracellular sites of action, simultaneous delivery of two or more therapeutic agents for combination therapy and improved in vivo efficacy of a therapeutic agent [16].

The first nanosize drug delivery systems were lipid based carriers, which were introduced in the 1960s and were later known as liposomes [17]. Subsequently, a variety of other synthetic and natural biomaterials were developed as drug delivery systems. Various nanomaterials such as nanoparticles and nanofibres were designed with abilities to encapsulate and release the drugs to targeted tissue [18]. The field has grown rapidly. A wide range of drug delivery nanocarriers have now been investigated such as liposomes or lipid-based materials [19, 20], biocompatible polymeric micro- and nanoparticles [21-25] and nanofibres [26-29].
1.3 Recent Material Designs for Drug Delivery Systems

Design and synthesis of various biocompatible materials has driven the progress of drug delivery systems over the past few decades. The field has quickly grown since to include a wide variety of drug delivery nanocarriers such as liposomes (lipid-based materials) [30, 31], biodegradable polymeric nanocarriers including block copolymers and polymer-drug conjugates [23-25, 32]. Liposome has been the most successful candidate in clinical applications. Most of food and drug administration (FDA) approved drug delivery systems in the market are based on liposome or lipid-based material. Moreover, polymer–drug conjugates, especially those conjugated with polyethylene glycol (PEG), is now a standard method to increase circulation and bioavailability of bio-macromolecules such as antibodies. A summary of FDA approved systems is listed in Table 1-2.

Liposomes are composed of a lipid bilayer separating an aqueous internal compartment from the bulk aqueous phase. Micelles are closed lipid monolayer with a fatty acid core and polar surface, or polar core with fatty acids on the surface (Figure 1-2). They are small vehicles with a spherical shape that can be produced from cholesterol, non-toxic surfactants, lipids, glycolipids, long chain fatty acids and even membrane proteins. They can be loaded with a great variety of molecules such as small drug molecules, proteins and even plasmids. Liposomes can be formulated and processed to different size and compositions. Many anti-tumor and antifungal drugs loaded into liposomes have been commercialized to date (Table 1-2).
<table>
<thead>
<tr>
<th>Products</th>
<th>Approval Year</th>
<th>Technology</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposome &amp; micelle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxil</td>
<td>1995</td>
<td>PEGylated liposomal doxorubicin</td>
<td>Various types of cancer</td>
</tr>
<tr>
<td>Daunoxome</td>
<td>1996</td>
<td>Liposomal daunorubicin</td>
<td>Advanced HIV-associated</td>
</tr>
<tr>
<td>Ambisome</td>
<td>1997</td>
<td>Liposomal amphotericin B</td>
<td>Fungal infections</td>
</tr>
<tr>
<td>Depocyt</td>
<td>1999</td>
<td>Liposomal cytarabine</td>
<td>Lymphomatous meningitis</td>
</tr>
<tr>
<td>Visudyne</td>
<td>2000</td>
<td>Liposomal verteporfin</td>
<td>Age-related macular degeneration</td>
</tr>
<tr>
<td>Estraspore</td>
<td>2003</td>
<td>Estradiol micellar nanoparticles</td>
<td>Moderate to severe vasomotor symptoms of menopause</td>
</tr>
<tr>
<td>DepoDur</td>
<td>2004</td>
<td>Liposomal morphine sulfate</td>
<td>Postoperative pain</td>
</tr>
<tr>
<td>Oncaspar</td>
<td>1994</td>
<td>PEGylated L-asparaginase</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>PEG-intron</td>
<td>2001</td>
<td>PEGylated interferon alfa-2b</td>
<td>Chronic hepatitis C</td>
</tr>
<tr>
<td>PEG-ASYS</td>
<td>2002</td>
<td>PEGylated interferon alfa-2a</td>
<td>Chronic hepatitis C &amp; B</td>
</tr>
<tr>
<td>Neulasta</td>
<td>2002</td>
<td>PEGylated granulocyte colony</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>Somavert</td>
<td>2003</td>
<td>PEGylated</td>
<td>Acromegaly</td>
</tr>
<tr>
<td>Macugen</td>
<td>2004</td>
<td>PEGylated anti-VEGF aptamer</td>
<td>Age-related macular degeneration</td>
</tr>
<tr>
<td>Mircera</td>
<td>2007</td>
<td>PEGylated erythropoetin receptor activators</td>
<td>Anaemia associated with chronic kidney disease</td>
</tr>
<tr>
<td>Cimzia</td>
<td>2008</td>
<td>PEGylated tumor necrosis factor alpha inhibitor</td>
<td>Crohn's disease</td>
</tr>
<tr>
<td>Krystexxa</td>
<td>2010</td>
<td>PEGylated urate oxidase</td>
<td>Gout</td>
</tr>
<tr>
<td>Omontys</td>
<td>2012</td>
<td>PEGylated peginesatide</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>Zoladex</td>
<td>1989</td>
<td>PLGA/goserelin acetate</td>
<td>Prostate and breast cancer</td>
</tr>
<tr>
<td>Lupron Depot</td>
<td>1989</td>
<td>PLGA/leuprolide acetate</td>
<td>Prostate cancer and endometriosis</td>
</tr>
<tr>
<td>Gliadel</td>
<td>1996</td>
<td>Polifeprosan /carmustine</td>
<td>High-grade &amp; recurrent glioblastoma multiforme</td>
</tr>
<tr>
<td>Sandostatin LAR</td>
<td>1998</td>
<td>PLGA-glucose/Acetate</td>
<td>Acromegaly</td>
</tr>
<tr>
<td>Atridox</td>
<td>1998</td>
<td>PLA/doxycycline hyclate</td>
<td>Periodontal disease</td>
</tr>
<tr>
<td>Nutropin depot</td>
<td>1999</td>
<td>PLGA/recombinant human growth hormone</td>
<td>Growth hormone deficiency</td>
</tr>
<tr>
<td>Trelstar</td>
<td>2000</td>
<td>PLGA/triptorelin pamoatea</td>
<td>Advanced prostate cancer</td>
</tr>
<tr>
<td>Arestin</td>
<td>2001</td>
<td>PLGA/minocycline</td>
<td>Adult periodontitis</td>
</tr>
<tr>
<td>Eligard</td>
<td>2002</td>
<td>PLGA/leuprolide acetate</td>
<td>Advanced prostate cancer</td>
</tr>
<tr>
<td>Risperdal Consta</td>
<td>2003</td>
<td>PLGA/risperidone</td>
<td>Schizophrenia &amp; bipolar Disorder</td>
</tr>
<tr>
<td>Vivitrol</td>
<td>2006</td>
<td>PLGA/naltrexone</td>
<td>Alcohol dependence &amp; opioid dependence</td>
</tr>
<tr>
<td>Somatuline</td>
<td>2007</td>
<td>PLGA/lanreotide</td>
<td>Acromegaly</td>
</tr>
<tr>
<td>Ozurdex</td>
<td>2009</td>
<td>PLGA/dexamethasone</td>
<td>Macular edema</td>
</tr>
</tbody>
</table>
The lipid-based carriers, also have significant disadvantages including production cost and leakage of encapsulated drug. This has led to the development of alternative carriers such as biodegradable polymeric nanoparticles including polymer-drug conjugated nanoparticles. The advantages and disadvantages of liposome systems are tabulated in Table 1-3. Biodegradable polymeric nanofibres are other nanocarriers and are discussed later in the next section of this Chapter.

**Table 1-3 Advantages and disadvantages of liposomes as DDS [34]**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased efficacy and therapeutic index of drug</td>
<td>Low solubility</td>
</tr>
<tr>
<td>Increased stability via encapsulation</td>
<td>Short half-life</td>
</tr>
<tr>
<td>Non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations</td>
<td>Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction</td>
</tr>
<tr>
<td>Reduce the toxicity of the encapsulated agent</td>
<td>Leakage and fusion of encapsulated drug</td>
</tr>
<tr>
<td>Site avoidance effect</td>
<td>Production cost is high</td>
</tr>
<tr>
<td>Flexibility to couple with site-specific ligands to achieve active targeting</td>
<td></td>
</tr>
</tbody>
</table>
1.3.1 Biodegradable Drug Nanocarriers

Biodegradable polymers are often considered as base materials of nanoparticles and nanofibres for drug delivery systems. Various natural and synthetic biodegradable polymers have been studied and developed for drug delivery. Biodegradable polymeric nanoparticles have been used to deliver various bioactive agents, such as small drugs [35, 36], peptides and proteins [37], and plasmid DNA [38]. Poly (lactic acid) (PLA), poly(glycol acid) (PGA), poly (lactide-co-glycolide) (PLGA), poly (-ε-caprolactone) (PCL) are the most widely used biodegradable polymers for DDS development because of their biodegradability, biocompatibility and ease of processing [39, 40]. It has been demonstrated that the drug release profile is related and therefore can be controlled by the polymer degradation rate. For instance, the drug release profile of PLGA encapsulated drug could be controlled by varying the molecular weight of PLGA [41].

Biodegradable block copolymers have also captured the imagination of researchers since the early days of drug delivery because of their chemical flexibility [4]. Depending on the choice of building blocks, they can be assembled in different nanostructured formats such as nanoparticles and nanofibres. A triblock copolymer, poly(propylene oxide)- poly(ethylene oxide)- poly(propylene oxide) (PEO-PPO-PEO) as polymeric micelles has been developed by Kabanov et al. to deliver doxorubicin [42]. Yang et al. has synthesized core–shell nanoparticles from a biodegradable amphiphilic copolymer, comprising of poly(N-methyldietheneamine sebacate) (PMDS) as the cationic block for Ribonucleic acid (RNA) encapsulation, and a cholesterol pendant group to increase the hydrophobicity of the core for efficient drug loading [43]. Another biodegradable triblock copolymer nanoparticle of poly(ethylene glycol)-b-poly(ε-caprolactone)-b-poly(2-aminoethyl ethylene phosphate) (PEG-b-PCL-b-PPEEA) was
developed by Wang et al to deliver paclitaxel [44]. More such block (or triblock) copolymers
delivery systems have been investigated by various groups [45-48].

Another DDS approach involves the conjugation to a polymeric carrier to a drug via a cleavable
bond. Various polymer–drug conjugates have been exclusively studied for the delivery of anti-
cancer drugs [49]. Some of reported anti-cancer drug-polymer conjugates are including
paclitaxel [50], doxorubicin [51] and camptothecin [52]. Drug-polymeric conjugated carriers
improve solubility of hydrophobic drugs and extend drug circulation in vivo although they still
show unexpected release behaviour and side effects in clinical testing [53, 54]. Drugs were
conjugated to polymers via a hydrolysable ester linker and controlled release was observed
under defined conditions. The polymer-conjugate displays good aqueous solubility and the
released drug shows similar pharmaceutical effect as the free drug. The major drawback of
these polymeric nanoparticle based systems is their relative instability in blood upon systematic
administration which leads to rapid dissociation and subsequently the burst release behaviour
[55].
1.4 Biodegradable Polymers

Polymeric materials are rapidly growing for use as biomaterials due to their flexibility [23]. Both synthetic and natural polymers have been extensively investigated as biodegradable polymeric biomaterials [30, 56]. Biodegradable polymers are classified into hydrolytically degrading and enzymatically degrading polymers. The majority of natural biopolymers are degraded by enzymatic attack. Hydrolytically degradable polymers are polymers that have hydrolytically labile chemical bonds in their backbone. The functional groups susceptible to hydrolysis include esters, anhydrides, carbonates, amides, urethanes, ureas, etc [57]. The majority of biodegradable polymers are bulk eroding where degradation takes place through the whole of the polymer samples over time (Figure 1-3). The erosion of the polymer starts with the intrusion of water into the polymer bulk so the whole polymer matrix is affected by erosion.

![Figure 1-3 Schematic illustration of the changes in a polymer matrix undergoing bulk erosion](image)

Polyglycolic acid (PGA), polylactic acid (PLA), and poly-ε-(caprolactone) (PCL); polyurethane (PU), polyvinyl alcohol (PVA), chitosan and collagen are the polymers most...
widely used in clinical applications including tissue engineering, DDS etc [58, 59]. Table 1-4 shows the most common biodegradable polymers and their degradation properties [60, 61].

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Degradation time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly glycolide (PGA)</td>
<td>6 to 12</td>
</tr>
<tr>
<td>Poly-L-lactic (LPLA)</td>
<td>&gt;24</td>
</tr>
<tr>
<td>Poly-D-lactic (DPLA)</td>
<td>12 to 16</td>
</tr>
<tr>
<td>Poly-ε-caprolactone (PCL)</td>
<td>&gt;24</td>
</tr>
<tr>
<td>Poly dioxanone (PDO)</td>
<td>6 to 12</td>
</tr>
<tr>
<td>85/15 PLA-co-PGA</td>
<td>5 to 6</td>
</tr>
<tr>
<td>75/25 PLA-co-PGA</td>
<td>4 to 5</td>
</tr>
<tr>
<td>65/35 PLA-co-PGA</td>
<td>3 to 4</td>
</tr>
<tr>
<td>50/50 PLA-co-PGA</td>
<td>1 to 2</td>
</tr>
</tbody>
</table>

1.4.1 Poly-ε-caprolactone (PCL)

PCL is semicrystalline polyester which undergoes hydrolytic degradation due to the presence of cleavable aliphatic ester linkages (Figure 1-4). PCL is soluble in a wide range of organic solvents and has the ability to form miscible blends with variety of biodegradable polymers for used in clinical applications such as PLA, PEO and chitosan [62-64] polymers, with a low melting point (55–60°C) and glass transition temperature (-60°C). PCL is degraded by hydrolysis of its ester linkages in physiological conditions [56, 65].

![Figure 1-4 Chemical structure of PCL](image)
It has a very slow rate of degradation (>24 months) compared to the other aliphatic polyesters such as PLA, PGA, and PLGA. In vivo degradation of PCL was observed for 3 years in rats by Sun et al [66]. It was observed that the shape of PCL products with molecular weight (MW) of 66000 remained undamaged for 24 months after implantation. They then broke into low Mw pieces at the end of 30 months. A linear relationship was observed between log MW and time and as a result MW of PCL deceases with time (Figure 1-5).

PCL has received a great deal of attention for use in biomedical applications due to its remarkable properties especially in drug delivery systems [67], scaffolds for tissue engineering [58], and bone tissue engineering [68]. In the case of drug delivery systems, various studies of drugs incorporated in different PCL based formulations have been reported [69]. For example, Chawla et al [70] have synthetised Tamoxifen loaded PCL nanoparticles with maximum tamoxifen loading efficiency for treatment of positive breast cancer; however due to the fact that most of the drug was located on the surface of the nanoparticle instead of being uniformly dispersed throughout the nanoparticle volume resulting in burst release behaviour. (Figure 1-6).
1.4.2 Polyvinyl Alcohol (PVA)

PVA is the only carbon-carbon backbone polymer that is biodegradable. PVA is known to be a biodegradable synthetic polymer that can be degraded by single microorganisms and symbiotic mixed cultures. It has been reported that the polymer is oxidized by enzymatic systems with formation of carbonyl groups along the polymer chain [71]. PVA is a linear synthetic polymer produced via partial or full hydrolysis of polyvinyl acetate to remove the acetate groups (Figure 1-7).

![Chemical structure of PVA](image)

**Figure 1-7** Chemical structure of PVA

The amount of hydroxylation determines the physical characteristics, chemical properties, and mechanical properties of the PVA [72]. The resulting PVA polymer is highly soluble in water but insoluble in most organic solvents. The higher the degree of hydroxylation of the PVA, the lower the solubility in water and the more difficult it is to crystallize [73]. FDA has approved
PVA to be in close contact with food products; in fact, PVA films exhibit excellent barrier properties for food packaging systems. PVA is used as a biomaterial due to its biocompatible, nontoxic, non-carcinogenic, swelling properties, and bioadhesive characteristics [72, 74]

1.4.3 Chitosan (CS)

Natural polymers derived from biological systems including protein and polysaccharides are biocompatible and biodegradable polymers. They possess low toxicity and offer potentially favourable pharmacokinetics in the circulatory system [75, 76]. For example, Lee et al. summarized the characteristics of various polysaccharides such as alginate, cellulose, chitin, chitosan, hyaluronic acid, and starch that can be used for biomedical applications including tissue engineering, wound dressings, drug delivery, and enzyme immobilization [77]. Chitosan is FDA approved and has extensively been investigated for biomedical applications such as wound and burn dressing material due to its easy applicability, air permeability, water absorption ability and non-toxicity, etc [56]. The N-deacetylated derivative of chitin is chitosan. Typically, commercial chitosan is approximately 85% deacetylated, which leads to a \( \text{–NH}_2 \) functionality of the D-glucosamine residues with some randomly distributed N-acetyl glucosamine groups (Figure 1-8) [78, 79]. It is a semicrystalline linear polymer of linked d-glucosamine and is completely soluble in aqueous acidic solutions [80]. It undergoes biodegradation \textit{in vivo} enzymatically to nontoxic products such as glucosamine and oligosaccharides. The rate of degradation of chitosan depends inversely on the degree of acetylation and crystallinity of the polymer [80]. Cross-linked chitosan products form attractive materials for drug delivery applications where the rate of drug release can be controlled by varying the cross-linking density [81, 82]. Chitosan has also shown good anticancer activity, mainly due to its polycationic nature.
Many studies have been reported on chitosan used for drug delivery systems. The primary amine functional group in chitosan structure makes it a natural choice to conjugate with other drugs [83]. Chitosan–thioglycolic acid conjugated hydrogels for in situ gelling was synthetised via amide bond formation between the primary amino group of chitosan and the carboxylic acid group of thioglycolic acid by Sakloetsakun et al.[28]. Chitosan–thiobutylamidine and chitosan–thioethylamidine conjugates were also reported to be used as hydrogel to treat wounds [29].
1.5 Nanoparticles

Biodegradable and biocompatible nanoparticles have attracted considerable attention as a potential new mode for drug delivery. Their significant advantages over other drug delivery systems include high surface area to mass ratio and ease of surface modification which is used to bind, adsorb or carry other compounds [84]. They are employed as a strategy to enhance solubility of hydrophobic therapeutics, protect the drug from early degradation, enhance absorption of the drugs into a targeted tissue, prolong circulation time, prevent undesirable side effects, to improve intracellular penetration and control the drug release profile [85]. The term nanoparticle is used for both nanospheres and nanocapsules. Nanospheres have a matrix type structure which drugs may be absorbed at the particle surface or encapsulated within the particle. Nanocapsules are vesicular systems in which the drug is confined to a cavity consisting of an inner core surrounded by a membrane [86]. Nanocarriers used for drug delivery have to be biocompatible and nontoxic. It is generally accepted that nanoparticles with a smaller size have a greater surface area and as a result have better pharmacokinetic properties for in vivo applications [87].

Lipid-based materials, biodegradable polymers and silicon materials are all examples of nanocarriers that have been studied as drug delivery systems. The most commonly and extensively used polymeric nanoparticles are poly(lactic acid) (PLA), poly-L-lactide-co-glycolide (PLGA), poly(ethylene glycol) (PEG) and poly(-ε-caprolactone) (PCL), poly(alkyl-cyanoacrylates) (PAC), chitosan and gelatine [88-93]. In general, at the preclinical stage, these nanocarriers were successfully applied to the treatment of various diseases, mainly for cancer and for infectious diseases [94]. Some representative examples of therapeutic nanocarriers on the market are briefly described Table 1-5.
Table 1-5 Representative examples of nanocarrier-based drugs in the market [85]

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Type of nanocarrier</th>
<th>Active ingredient/drug</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmBisome®</td>
<td>Liposomes</td>
<td>Amphotericin B</td>
<td>Fungal infections</td>
</tr>
<tr>
<td>Doxil®</td>
<td>Liposomes</td>
<td>Doxorubicin</td>
<td>Ovarian cancer, and breast cancer</td>
</tr>
<tr>
<td>Caelyx®</td>
<td>Liposomes</td>
<td>Doxorubicin</td>
<td>Ovarian cancer, and breast cancer</td>
</tr>
<tr>
<td>Depocyt®</td>
<td>Liposomes</td>
<td>Cytarabine</td>
<td>Lymphomatous meningitis</td>
</tr>
<tr>
<td>Daunoxtome®</td>
<td>Liposomes</td>
<td>Daunorubicin</td>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>Adagen®</td>
<td>Polymer–drug conjugates</td>
<td>Adenosine deaminase</td>
<td>Adenosine deaminase enzyme deficiency</td>
</tr>
<tr>
<td>Onscaspar®</td>
<td>Polymer–drug conjugates</td>
<td>I-asparaginase</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Pegasys®</td>
<td>Polymer–drug conjugates</td>
<td>PEGylated IFN-α-2a</td>
<td>Hepatitis C</td>
</tr>
<tr>
<td>Genexol-PM®</td>
<td>Polymeric micelles</td>
<td>Paclitaxel</td>
<td>Cancer chemotherapy</td>
</tr>
<tr>
<td>Abraxane®</td>
<td>Protein(albumin) nanoparticles</td>
<td>Paclitaxel</td>
<td>Metastatic breast cancer</td>
</tr>
<tr>
<td>Amphotec®</td>
<td>Lipid colloidal dispersion</td>
<td>Amphotericin B</td>
<td>Fungal infections</td>
</tr>
</tbody>
</table>

Despite the impressive progress made in the design of drug loaded nanoparticles with improved specificity, only a few nanoparticles based medicines have reached the market (Table 1-7). Except those mentioned in Table 1-5, currently-available nanoparticle technologies have not been able to improve the activity of a great number of drugs. The major disadvantages of nanoparticles as DDS [94] include:

- Poor drug loading, as a result, either the quantity of the drug administered is not sufficient to reach a pharmacologically active concentration in the body, or the amount of the carrier material required is too great, leading to toxicity or undesirable side-effects.
- Burst release of the encapsulated drug, generally corresponding to the release of the drug which is adsorbed at the surface of the nanocarriers. As a consequence, a large portion of the drug will be released before reaching its pharmacological target in the body, leading to lower activity and more side-effects.

Mesoporous silica nanoparticles (MSNs) have attracted lots of attention for their potential biomedical applications. Due to the customized mesoporous structure and high surface area, MSNs as drug delivery systems (DDSs) show significant advantages over traditional drug nanocarriers [95].

1.5.1 Mesoporous Silica Nanoparticles (MSN)

Inorganic nanomaterials have special properties that can be exploited for use as nanocarriers. Since the discovery of methods for the synthesis of mesoporous silica materials in 1992 [96], a large number of them have been explored for biomedical application [97]. Mesoporous silica nanoparticles (MSN) are solid materials comprised of a honeycomb-like porous structure with thousands of empty channels (mesopores) that are able to absorb/encapsulate relatively large amounts of bioactive molecules. MSN’s have become a good alternative for controlled drug release applications due to their unique properties such as a large surface area, easily modified pore size and volume, as well as being chemically inert and allowing easier chemical functionalization of their surface [95, 98, 99]. MSNs, in order to be effective and universally applicable nanocarriers, must simultaneously demonstrate multiple characteristics including [100]: dispersibility, ability to load and deliver large concentrations of drugs, biocompatibility and low toxicity.

Importantly, silica has good biocompatibility, is accepted by the US FDA and has been widely used in cosmetics and as FDA-approved food additives [101]. The biocompatibility of MSN with and without surface functionalization has been tested by different methods in a report by
Slowing et al [102]. They showed that the viability and proliferation of various mammalian cells were not affected by the internalization of MSN. As a result MSNs are used to enhance the biocompatibility of several drug delivery systems, such as magnetic nanoparticles [103] biopolymers [22], and micelles [104].

Another advantage of MSNs is their ability to be further functionalised ie reacting the silanol groups present both within the pore interiors and on the outer surface. Chemical moieties can also be adsorbed onto MSNs resulting functionalised MSNs. For example, the amine functionalized MSNs were prepared by Manzano et al [105]. First the MSNs were synthetised and then the MSNs were treated with 3-aminopropyltriethoxysilane (APTES) as follows. Template-free MSNs were dehydrated under a nitrogen flow and then refluxed with 5 mmol of APTES/g SiO$_2$ in toluene. The final products were filtered, washed with a mixture of diethyl ether and dichloromethane (1:1) and finally dried.

Tsai et al [106] synthesized mesoporous silica nanoparticles (MSN) for a surfactant assisted controlled release of the hydrophobic drug, Resveratrol. The MSN were used as drug carrier for Resveratrol by taking advantage of the hydrophobic environment created by the phosphate monoester surfactant (PMES) micelle inside the nanoparticles pores (Figure 1-9).

![Figure 1-9](image_url) TEMP image of surfactant assisted MSN (left), Schematic illustration of the Resveratrol loading and release. Reported by Tsai et al [106]
This delivery technique showed an increase of the drug loading efficiency and a steady release of resveratrol compared to the MSN without surfactant. The hydrophobic back of the surfactant helped to adsorb the drug and the surfactant molecules come out together with drug.

**Figure 1-10** Release profiles of resveratrol-loaded (▲) PMES–MSN; (■) MSN [106]

PMES–MSN exhibited a sustained release during the first 4h before reaching the maximum release. On the other hand, non-surfactant assisted MSN showed a burst release in the first 5–10min where almost 80% of the drug was released (Figure 1-10). This indicates that Resveratrol was physically adsorbed on the surface of the MSN material compared to that in PMES-MSN where Resveratol interacted with PMES as well. This result, in addition to other studies reported, show that MSN materials are able to store and gradually release therapeutic agents [102, 107-111].

Numerous studies have been reported of MSN as nanocarriers of drugs. Series of MSNs with different pore sizes were loaded with a poorly water-soluble telmisartan (TEL) [112]. The release profiles (Figure 1-11) showed that MSNs could retain the drug for up to 60 min due to the weak hydrogen bonds between TEL and silanol groups on the surface of MSNs. It was
observed that pore sizes affected the release profiles of TEL. As shown in Figure 1-11 the release of TEL was faster where the MSN has the larger pore diameter [113, 114].

![Figure 1-11](image)

**Figure 1-11** Release profiles of TEL from TEL-MSNs with pore diameters of MSNs for the sample A, B and C were 12.9, 7.8 and 3.6 nm, respectively.

In another study, thiol functionalized MSNs were synthetized for controlled release of doxorubicin (DOX) [115]. As shown in Figure 1-12, DOX loaded MSNs could retain drug from long period of time (over 50 h) in PBS (pH 7.4) as it just released 13% of the loaded Dox after 48 h. Conversely, a faster release of Dox was observed when loaded MSNs were incubated in artificial urine (pH 6.1) with 63% of DOX content was released after 48 h. This was because of the stronger interaction between DOX and thiol group at pH 7.4 is weaker in an acidic environment (pH 6.1).
1.6 Nanofibres

Nanofibres have attracted much attention for various applications such as filtration [116, 117], nanocomposite reinforcing for nanotechnology,[118, 119], for substances recovery [120] and medical applications [121-123] The advantages of nanofibres are derived from their high specific surfaces area and formation of high porous surface [124]. Medical applications of nanofibres are included to fabricate wound dressings [125, 126] tissue engineering scaffolds [126, 127] and nanofibrous drug (pharmaceutical agent) delivery systems [27, 122, 128].

1.6.1 Electrospinning Process

There are different techniques to fabricate nanofibres including: phase separation [129], melt-blown [5], self-assembly [130], phase template [131], and electrospinning [124, 132]. Electrospinning has received much attention lately since it is a simple technique to fabricating nanofibres from a large variety of polymers in a relatively easy and simple way.

The setup for electrospinning consists of three major components: a high-voltage power supply, a spinneret (a metallic needle), and a collector (a grounded conductor) as shown in Figure 1-
The spinning mechanism is rather complicated due to complex electro-fluid mechanical forces involved. In the process of electrospinning the polymer solution housed in a syringe is fed through the spinneret at a constant and controllable rate. The electrostatic field exists in the space of the electrospinning device when a high voltage (in the range of 1–40 kV) is applied. The electrostatic field charges the solution drop at the nozzle of a spinneret and the induced charges are evenly distributed over the surface (Figure 1-23). Under the action of electrostatic interactions, the drop is distorted into a conical object that is called a “Taylor cone” [134]. The electrified jet can be attracted by the collectors and it is directed towards the collector with the opposite electrical charge. As it travels to the collector the solvent is evaporated and the solid polymer nanofibre is deposited as a nonwoven mat on the surface of the collector.

Figure 1-13 Schematic view of electrospinning process [135]
Several parameters can influence the electrospinning as well as the morphology of the nanofibres such as polymer type, polymer MW, polymer concentration, solvent, viscosity, etc [136]. These variables are summarized in Table 1-6.

<table>
<thead>
<tr>
<th>Polymer properties</th>
<th>Solution properties</th>
<th>Other properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>Viscosity</td>
<td>Solution feed rate</td>
</tr>
<tr>
<td>Glass-transition temperature</td>
<td>Viscoelasticity</td>
<td>Voltage</td>
</tr>
<tr>
<td>Solubility</td>
<td>Concentration</td>
<td>Vapor pressure of the solvent</td>
</tr>
<tr>
<td></td>
<td>Surface tension</td>
<td>Relative humidity</td>
</tr>
<tr>
<td></td>
<td>Electrical conductivity</td>
<td></td>
</tr>
</tbody>
</table>

Increasing the electrospinning voltage results in a decrease in the stability of the initiating jet, which has been correlated with an increase in the number of beads formed along the nanofibre length [137]. The properties of solutions were found to influence the size and size distribution of nanofibres significantly. The higher the concentration of polymer results in higher viscosities and surface tensions respectively. The diameter of the electrospun nanofibres was found to increase with solution concentration according to a power law relationship (Figure 1-14) [138].
Figure 1-14 Effect of electrospinning conditions on the morphology of the nanofibres

- a. PCL 11%, 25Kv, 0.4 mL, 20cm
- b. PCL 14%, 25Kv, 0.4 mL, 20cm
- c. PCL 14%, 25Kv, 0.4 mL, 10cm
- d. PCL 14%, 30Kv, 0.4 mL, 20cm
1.6.2 Co-axial Electrospinning

Many modifications have been done on conventional electrospinning in order to improve the functionality of the electrospun nanofibres. Co-axial electrospinning is one method of producing core-cell nanofibres, with high potential in various applications [139]. A process contains the spinneret by inserting inner capillary inside the outer capillary to make co-axial configuration. Both needles are attached to two different reservoirs containing the shell and core solutions. The feeding rates of the solutions are controlled separately. The co-axial set up expectedly requires careful design in order to fabricate core-shell nanofibres (Figure 1-15) [139].

![Figure 1-15 Schematic view of core-shell electrospinning technique](image)

In a conventional electrospinning (single fluid), increase in solution concentration results in an increase in the nanofibre diameter. The same effect has been observed in the case of co-axial electrospinning. It has been reported that the increase in the core solution concentration increased both the core and overall (core-shell) nanofibre diameters and subsequently an
increase in the core diameter resulted in a decrease in the thickness of the shell diameter (Figure 1-16) [140].

![Figure 1-16](image)

**Figure 1-16** The effect of core solution concentration on shell thickness, (A) Smaller core diameter and larger shell thickness; (B) Larger core diameter and smaller shell thickness due to the increased concentration of core solution.

The significant issue is the interaction between the core and shell phases which determines the electrospinnability of the polymer solutions. Numerous exotic uses of co-axial electrospinning have been reported recently.

Hollow tubes with encapsulated enzymes have been fabricated by co-axial electrospinning, have been reported by Dror et al. [141], as bioreactors. The reaction substrates would diffuse efficiently into the hollow fibres where the encapsulated enzyme would convert them into products, which would diffuse out of the tubes again. The enzymes were mixed in an aqueous core solution of poly(ethylene oxide) (PEO) for the core while poly(ε-caprolactone) (PCL) forms the shell. Different ratios of the core/shell systems produced outer shells with different morphologies [104] Figure 1-17a is when only PCL used to form the shell resulting in a rough surface due to the rapid evaporation of the solvents, and Figure 1-17b when poly(ethylene glycol) (PEG) is added to PCL and the tube walls became increasingly porous due to the PEG and PCL intermolecular polar interactions.
Figure 1-17 SEM images of PCL hollow tubes (a), and PEG/PCL hollow tubes. Reported by Dror et al. [141]

*Park et al.*, produced core-shell nanofibres from chitosan (CS) and poly(ε-caprolactone) (PCL) with levofloxacin encapsulated in the core section using co-axial electrospinning [142]. They showed that the presence of drug in core of the CS–PCL core-shell nanofibres exhibited more sustained release of levofloxacin compared to that in PCL conventional single nozzle nanofibres. As shown in Figure 1-18 the burst release of levofloxacin reduced from 70% in PCL nanofibres to 40% in CS-PCL core-shell nanofibres.

![Figure 1-18](image)

*Figure 1-18* The release profile of levofloxacin from core-shell nanofibres (Left), and TEM image of a CS–PCL nanofibrous (Right). Reported by *Park et al* [142]
1.7 Drug delivery Application and Release Kinetics of Electrospun Nanofibres

Several biodegradable polymers have been electrospun for drug delivery application; ranging from natural polymers like collagen, chitosan and silk fibroin to synthetic polymers such as poly(lactic acid) (PLA), poly(e-caprolactone) (PCL), polyethylene oxide (PEO), and copolymers such as poly (l-lactide-co-caprolactone) and poly(lactic-co-glycolic acid) (PLGA) [143]. Several drugs have been investigated; including anticancer drugs such as paclitaxel and doxorubicin [144, 145], antibiotics such as rifampicin and tetracycline hydrochloride [146, 147], DNA [148, 149], and RNA [150]. In most cases the drugs were simply mixed with a polymer and then electrospun. The drug release profiles varied depending on the polymer used. The electrospinning conditions only had a minimal effect on the release profile [27, 151-153]. Typically, the release of an encapsulated compound from polymeric nanofibres has been attributed to solid state diffusion. Drug release kinetics is affected by many factors such as dissolution of the drug, drug diffusion through the polymer matrix, polymer degradation (erosion and hydrolysis), polymer–drug interactions, drug/polymer ratio and system geometry [154]. The release profile of drug from nanofibres is due to the drug diffusion out of the polymeric matrix and drug diffusion because of polymer degradation [155, 156]. In the case of polymer degradation, the rate of degradation can be controlled to some extent by altering parameters such as polymer blend composition and ratio of amorphous to crystalline segments. For example, the hydrophobic nature of PLA and PLGA is major problem in a mainly hydrophilic biological environment. Degradation rate and hydrophilicity of PLA nanofibres were controlled and altered by adding PLGA (LA/GA= 50/50) random copolymer and PLA-b-PEG-b-PLA triblock copolymer [157]. Table 1-7 shows some electrospun drug loaded nanofibres with different biodegradable polymers (copolymers) and their blends for controlled
drug release. Table 1.7 provides examples of polymer-drug mixtures that have been formulated and electrospun are reported in the literature.

**Table 1-7** Examples of recent drugs loaded electrospun nanofibres reported in the literature [122]

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Drugs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA, PEVA and PLA/PEVA</td>
<td>Tetracycline hydrochloride</td>
<td>[152]</td>
</tr>
<tr>
<td>PLA/PCL</td>
<td>Tetracycline hydrochloride</td>
<td>[158]</td>
</tr>
<tr>
<td>PCL</td>
<td>siRNA</td>
<td>[159]</td>
</tr>
<tr>
<td>PCL-co-EEP</td>
<td>Human glial cell-derived neurotrophic factor(GDNF)</td>
<td>[160]</td>
</tr>
<tr>
<td>PCL-co-PCLLEEP</td>
<td>Human α-nerve growth factor (NGF)</td>
<td>[161]</td>
</tr>
<tr>
<td>PCLEEP</td>
<td>siRNA</td>
<td>[150]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Fusidic acid and rifampicin</td>
<td>[146]</td>
</tr>
<tr>
<td>Poly(ester urethane) (PEUU) and PLGA</td>
<td>Tetracycline hydrochloride</td>
<td>[147]</td>
</tr>
<tr>
<td>Ethyl cellulose (EC)/polyvinylpyrrolidone (PVP)</td>
<td>Ketoprofen (KET)</td>
<td>[162]</td>
</tr>
<tr>
<td>PLGA polylethylene glycol-g-chitosan (PEG-g-CN)</td>
<td>Ibuprofen</td>
<td>[163]</td>
</tr>
<tr>
<td>PVP/zein</td>
<td>Ketoprofen (KET)</td>
<td>[164]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Mefoxin</td>
<td>[165]</td>
</tr>
<tr>
<td>Poly(ethylene oxide) (PEO)</td>
<td>Bovine serum albumin (BSA)</td>
<td>[166]</td>
</tr>
<tr>
<td>PLA</td>
<td>Lysozyme</td>
<td>[167]</td>
</tr>
<tr>
<td>Poly(1-lactide-co-caprolactone)</td>
<td>Human nerve growth factor (NGF)</td>
<td>[168]</td>
</tr>
<tr>
<td>PEG–PLA</td>
<td>Doxorubicin hydrochloride</td>
<td>[145]</td>
</tr>
<tr>
<td>PEG–PLA</td>
<td>Doxorubicin hydrochloride</td>
<td>[169]</td>
</tr>
<tr>
<td>PLLA</td>
<td>Doxorubicin</td>
<td>[170]</td>
</tr>
<tr>
<td>PEG–â-cyclodextrin</td>
<td>Hydroxycamptothecin (HCPT)</td>
<td>[171]</td>
</tr>
<tr>
<td>PLA–PEG and PLGA</td>
<td>DNA</td>
<td>[149]</td>
</tr>
<tr>
<td>PLGA/gelatin</td>
<td>Fenbufen</td>
<td>[172]</td>
</tr>
<tr>
<td>PCL/PVA</td>
<td>HRP encapsulated liposomes</td>
<td>[173]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Paclitaxel</td>
<td>[174]</td>
</tr>
<tr>
<td>PLA/PLGA</td>
<td>Cisplatin</td>
<td>[175]</td>
</tr>
<tr>
<td>PLA</td>
<td>Dichloroacetate (DCA)</td>
<td>[176]</td>
</tr>
<tr>
<td>PEG–PLLA</td>
<td>1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU)</td>
<td>[177]</td>
</tr>
</tbody>
</table>


A major drawback of drug loaded nanofibres reported in the literature is the early release behaviour of the drug from the nanofibres called burst release. Burst release occurs when a large portion of drug content is released from the nanofibres rapidly after incubation in the release medium (Figure 1-19) [163, 178, 179]. Although, the burst release of drug may be favourable in some cases such as antibacterial wound dressings and inhibition of the tumour cell growth by providing sufficient initial dosage of the antibacterial/anticancer drug within a short time period, but the sustained release pattern is desired to maintain constant therapeutic concentration of drug over an extended period of time [180, 181]. The major disadvantage of the initial burst release of drugs is toxicity due to high drug dose administration, rapid loss in drug affinity, and an increase in dosing frequency [182].

![Figure 1-19 Common release profiles: burst release (…..) and desired controlled release (-----) [180]](image)

The release profile of doxorubicin from PEG-PLA nanofibres shows a burst release pattern where around 50% of the drug content was released during first 3 hr of incubation [145].

In another study [183], the burst release of bovine serum albumin (BSA) from core-shell PCL-PEG nanofibres was observed with the 50% of BSA content released within 2 days (Figure 1-20).
Figure 1-20 Release profile of BSA from co-axial PCL-PEG nanofibres

Figure 1-21 shows the release profile of ketoprofen from tri-layered meshes polyvinylpyrrolidone/ethyl cellulose (PVP/EC/PVP) with different electrospinning time of PVP. Burst release behaviour was observed in both cases; with around 60% of drug content were released in the first 10 h. Increasing the PVP contents (by spinning time of PVP meshes) increased the drug release rate. This could be related to hydrophilic properties of PVP, increasing hydrophilicity increasing the drug release rate.

Figure 1-21 Release profiles of ketoprofen from EC/PVP nanofibres (Different spinning time of PVP) adapted from [162]
Fenbufen (FBF) release behaviour from PLGA/gelatin (9/1) nanofibres showed (Figure 1-22) the burst release behaviour of FBF with around 70% release of FBF content in the first 5 h.

![Release profile of FBF from PLGA/gelatin nanofibres](image)

**Figure 1-22** Release profile of FBF from PLGA/gelatin nanofibres adapted from [172]

*Liu et al* [170] fabricated doxorubicin (DOX) loaded poly(L-lactide) (PLLA) nanofibres with different DOX concentration to study the efficacy of DOX-loaded PLLA electrospun nanofibres as a local chemotherapy system. As shown in Figure 1-23, burst release of DOX was observed for both DOX concentrations (3 and 6% units) with around 50% of DOX content released in the first 10 h. This burst release was related to rapid diffusion of DOX, in the release medium, due to its high hydrophilicity, large specific surface area of the nanofibre complex, and to the hydrolysis and degradation of PLLA *in vivo*. 
The burst release provided high local DOX concentrations in the tumor site achieving effective inhibition of tumor within a short time period. However due to rapid release of DOX content, DOX-PLLA nanofibres cannot provide sustained drug supply to prevent the recurrence of the tumour.

Ibuprofen (IBU) was loaded into MSN and then uniformly encapsulated in PLLA electrospun nanofibres [184]. The release profile of IBA showed that electrospun PLLA/IBU-MSN composite nanofibres significantly reduced the initial burst release to 6% of IBU released in the first 12 h compared to 46% released from electrospun PLLA–IBU nanofibres at the same time (Figure 1-24) [184]. The release of IBU from PLLA nanofibres is affected by drug diffusion from the nanofibres into the release medium. In contrast, the entrapment of drug into nanoporous structure of MMSs which were further encapsulated in the PLLA nanofibres provided two stages of drug diffusion. First drug diffusion is from the MSN into the PLLA nanofibres and then drug diffusion from the PLLA nanofibres into the release medium. Such a sequential diffusion of drug resulted in a lower initial burst and longer term of sustained release of drug from composite nanofibres [185].
Similar studies of drug-nanoparticles/nanofibres composite have been reported. Doxorubicin (DOX) loaded MSNs were fabricated and incorporated into poly(L-lactic acid) (PLLA) nanofibres. The release profiles of DOX indicated that uniform DOX loaded nanofibres have an initial burst release with over 45% of DOX was released after 3 days followed by a sustained release over 20 days. In contrast, the presence of DOX loaded MSN incorporated with nanofibres, PLLA/DOX-MSNs composite nanofibres, reduced the burst release to 25% of DOX content was release after the same time [181]. The initial burst release is due to the distribution of DOX molecules close to the surface of nanofibres during the electrospinning process while the MSN/-nanofibres composite mats could be tailored to provide a slow release followed by a sustained release of the drug.

In another study, incorporating coumarin 6 (C6) loaded PLGA nanoparticles into PVA nanofibres reduced the burst release of C6 from C6-PLGA nanoparticles. The release profile showed burst release with the whole encapsulated C6 content was released from nanoparticles within 2 h due to the short diffusion of C6 in the matrix (Figure 1-25). In contrast, incorporating C6-PLGA nanoparticles into PVA nanofibres significantly decreased the burst release to approximately 70% after 2 h.
These results indicated that the inclusion of C6-PLGA nanoparticles within the PVA nanofibres implied two diffusion steps. First through the nanoparticles and then followed by a second diffusion step through the PVA matrix into the release medium [186].

1.8 A Model Drug, Levofloxacin

In order to investigate the release profiles of composite nanofibres, easy detection of the model drug (agent) is necessary. LVF was used as a model drug in this work because of its chromophore for easy detection, $\lambda_{\text{max}}$ 290 nm. It also has functional groups such as carboxylic acid group that can be used for further chemical modification (i.e. conjugation).

LVF, a fluoroquinolone antibacterial agent, is active against most aerobic gram-positive and gram negative organisms. LVF penetrates rapidly and efficiently throughout the body, achieving concentrations in tissues or body fluids which are generally higher than those observed in plasma. Within 24 h of an administration, about 80% of the drug is excreted unchanged in the urine. That means the plasma elimination half-life of LVF is 4 to 7 h. It has demonstrated antibacterial efficacy against a variety of infections, including respiratory tract, genitourinary, obstetric, and skin tissue in clinical trials. It has a broad range of in vitro activity against most aerobic Gram-negative bacteria. Inhibition of bacterial multiplication by LVF
was not influenced by growth medium types. Acidic pH may reduce the *in vitro* antibacterial activity of LVF. LVF usually has additive or indifferent effects when combined with other antibacterial agents *in vitro*, but may occasionally show synergy or antagonism [187].

![Chemical structure of Levofloxacin](image)

**Figure 1-26** Chemical structure of Levofloxacin
1.9 Research concepts and hypotheses

This thesis investigates the scope of fabricating drug loaded electrospun nanofibres. The main objective is to fabricate drug-nanofibres composite mats through physical and chemical approaches and characterise and compare the drug release profiles of composite nanofibres with uniform drug loaded nanofibres. The major challenge is to overcome the problem of burst release profile of drug from conventional and uniform nanofibres. The scope of this research is to derive a suitable means of reducing the burst release of drug. The possibilities are:

1. Introduction of nanoparticles during the electrospinning process to fabricate core-shell nanofibres. The hypothesis is to control the release of drug by combining two drug delivery systems.
2. Use of conjugation to chemically bind the drug to the nanocarriers, incorporating them into biodegradable composite nanofibres by electrospinning. The hypothesis is to reduce the burst release by hydrolysis of the cleavable bind between drug and nanocarriers.

1.9.1 Aims and objectives

The main objective of this research project is to reduce the burst release of drug from electrospun nanofibres followed by sustained release. The detailed objectives of the current study are:

1. To fabricate LVF-loaded MSN complexes as nanocarriers of LVF.
2. To fabricate the core-shell PCL/LVF-MSN composite nanofibres.
3. To investigate the loading efficiency of LVF-MSN complexes and core-shell structure of composite nanofibres.
4. To investigate the effect of MSN and core-shell structure of nanofibres in the release profiles of LVF.

5. To determine the antimicrobial activity of the core-shell composite nanofibres.

6. To synthesize the conjugated LVF-functionalized MSN.

7. To investigate the conjugation efficiency of LVF-MSN and their release profiles in different pH buffer solutions.

8. To fabricate PCL/conjugated LVF-MSN composite nanofibres via electrospinning.

9. To investigate the effect of LVF-MSN conjugation on the release profiles of different composite nanofibres.

10. To synthesize LVF-chitosan conjugation and to investigate the conjugation.

11. To fabricate composite PVA/LVF-chitosan composite nanofibres via electrospinning.

12. To investigate the effect of presence of conjugated drug-polymer on the release profile of composite PVA nanofibres.
1.10 References


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Chapter 2

MATERIALS, METHODS AND CHARACTERIZATION TECHNIQUES

This chapter presents the materials that have been utilized in the fabrication of the electrospun composite materials used in this thesis as well as the methods used to characterise them. The characterisation methods include TGA, DSC, FTIR, NMR, EDX, BET & BJK analysis, and electron microscopy (SEM and TEM). The chapter also presents the method for measuring the in vitro drug release profile of electrospun nanofibres and drug-nanoparticles complexes, and the quantitative antimicrobial assay (AATCC Test Method 100–1999) used to measure the efficacy of the electrospun composite mats.
2.1 Materials

The following is the list of the materials used in this research work.

Polymers

- Poly(ɛ-caprolactone) (PCL) with a molecular weight (MW) of 80,000g/mol, (Sigma-Aldrich, Australia)
- Polyvinyl alcohol (PVA) with an average MW of 77,000-79,000g/mol, 98% hydrolyzed, (Sigma-Aldrich, Australia)
- Chitosan (low molecular weight), 75-85% deacetylated, (Sigma-Aldrich, Australia)

Nanoparticles

- Mesoporous silica nanoparticles (MSN), with linear formula SiO₂, 200nm particle size, pore size 4nm, melting point >1600 °C, (Sigma-Aldrich, Australia)
- Propylthiol functionalized mesoporous silica nanoparticles (MSN-SH), 200nm particle size, pore size 4nm, melting point 1600-1725°C, (Sigma-Aldrich, Australia)

Drug

- Levofloxacin (LVF) (≥98.0%), with molecular weight (MW) of 361.37g/mol, (Sigma Aldrich, Germany)

Chemicals

- N,N-dimethylformamide (DMF) anhydrous, 99.8%, (Sigma-Aldrich, Australia)
- Dichloromethane (DCM) anhydrous, ≥99.8%, (Sigma-Aldrich, Australia)
- Tetrahydrofuran (THF) anhydrous, ≥99.9%, (Sigma-Aldrich, Australia)
• Acetic acid, ≥99.7%, (Sigma-Aldrich, Australia)
• Sodium hydroxide (NaOH), (Merck, Germany)
• Potassium chloride (KCl), (Sigma-Aldrich, Australia)
• Disodium hydrogen phosphate (Na₂HPO₄), (Sigma-Aldrich, Australia)
• Potassium dihydrogen phosphate (KH₂PO₄), (Sigma-Aldrich, Australia)
• Tryptone (Peptone from casein), (Sigma-Aldrich, Australia)
• Yeast extract, (Sigma-Aldrich, Australia)
• Agar, (Sigma-Aldrich, Australia)
• 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), (Pierce)
• Dimethyl sulfoxide (DMSO), (Fisher Chemical)
• 2-(N-morpholino)ethanesulfonic acid (MES), (Fluka)
2.2 Characterization Techniques

The electrospun membranes and drug-nanoparticles complexes were characterised using the following techniques, Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Infra-Red Spectroscopy (FTIR), BET & BJK surface area and pore sizes analysis, Nuclear Magnetic Resonance (NMR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).

2.2.1 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) measures changes in physical and chemical properties by increasing temperature as a function of time. Physical properties measured includes phase transitions, including vaporization, sublimation, absorption, adsorption, and desorption. Chemical properties measured include chemisorption, decomposition and solid-gas reactions. TGA can also determine either mass loss or gain due to decomposition or oxidation [1]. Furthermore, TGA can evaluate the thermal stability of a material. In a specific temperature range; if a material is thermally stable, no mass change will be observed. The TGA instrument continuously weighs a sample as it is heated to temperatures of up to 2000°C. Usually as the temperature increases, decomposition occurs and the decomposition components can be evaluated using mass loss (reported as %loss of mass) or even techniques such as mass spectrometer or infra-red spectrophotometry. Results are plotted with temperature on the X-axis and mass loss on the Y-axis (Figure 2-1).
TGA has a wide variety of applications, including analysis of ceramics and thermally stable polymers. Samples in this dissertation were analysed using a Netzsch STA 449 F1 Jupiter Simultaneous TGA/DSC Thermal Analyser system (Figure 2-2).
2.2.2 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is another thermal analysis tool in which the amount of energy absorbed or released by a material in relation to an inert reference is measured as a function of temperature, while both sample and reference are subjected to identical temperature programs. DSC basically consists of sample and reference holders, a heat sink and thermocouples. Heat flow is delivered from the heat sink to the sample and reference holders to increase or decrease temperature (Figure 2-3) [2]. DSC records the required energy to maintain the temperature of both the sample and reference. Output from a DSC includes the glass transition temperature and melting point as well as information on crystalline structure (Figure 2-4). For one major part of this project, which deals with studying the presence of crystalline drug in conjugated products, DSC provided a means to determine the changes in drug melting point and crystallisation after conjugation and providing an indirect means confirming conjugation.

![Figure 2-3 Schematic of a DSC](image)

In order to plot the DSC endotherm or exotherm, heat is transferred to the sample and reference pans through a disc made of either constantan or silver. The heat difference between the sample and reference is measured by a thermocouple located beneath the pans. The temperature difference signal is converted to heat flow by a calibration equation [3].
Fourier Transform Infra-Red Spectroscopy (FTIR):

Any organic or inorganic substance having covalent bonds absorbs various frequencies of electromagnetic radiation in the infrared region of the electromagnetic spectrum. This region lies at wavelengths longer than those associated with visible light. Infrared (IR) spectroscopy has been widely used to characterise the chemical structure of materials. This technique is based upon the vibrations of atoms of a molecule. An infrared spectrum is obtained by exposing the infrared through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The frequency at which any peak in the absorption spectrum appears corresponds to the frequency of a part of sample molecules [4]. These frequencies are correlated to specific functional groups.
Infrared spectroscopy is usually carried out using a Fourier transform infrared (FTIR) spectrometer. A schematic diagram of an FT-IR (Figure 2-5) shows the basic components. The FT-IR uses an interferometer to process the energy sent to the sample. In the interferometer, the source energy passes through a beam splitter, a mirror placed at a 45° angle to the incoming radiation, which allows the incoming radiation to pass through but separates it into two perpendicular beams. When the two beams meet at the beam splitter, they recombine. The combined beam containing these interference patterns is called the interferogram. This interferogram contains all of the radiative energy coming from the source and has a wide range of wavelengths. Then, it is passed to the sample before reaching a detector. Data received by a computer receiver is converted to digital data and then transferred to the computer for Fourier transformation to take place. [5]. An example result obtained from the FTIR spectroscopy is shown in Figure 2-6, the spectrum of levofloxacin taken between 600-4000 cm\(^{-1}\) in transmission mode.
Figure 2-6 LVF spectrum taken between 600–4000 cm\(^{-1}\) wavelength

![LVF spectrum](image)

O-H of the carboxylic acid at 3265 cm\(^{-1}\)
Carboxyl group C=O 1720 cm\(^{-1}\)
Aromatic C-C 1620 cm\(^{-1}\)
Amines at 1294 cm\(^{-1}\)

Wavelength (cm\(^{-1}\))

Figure 2-7 Thermo Nicolet 6700 FTIR Spectrometer

FTIR was also used to determine any changes in the chemical structure of nanocarriers after loading and conjugation. Samples were carried out using Fourier transform infrared (FTIR) (Figure 2-7) spectrometer ranging between 600 and 4000 cm\(^{-1}\), with a total of 16 scans.
2.2.4 BET/BJH Surface Area and Pore Size Distribution Analysis

The specific surface area of a fine particle is determined by physical adsorption of a gas onto the surface of the solid and by calculating the amount of adsorbed gas corresponding to a monomolecular layer on the surface. The BET theory describes the changes in pressure during multi-layer adsorption of inert molecules onto a surface [6, 7]. This is usually carried out at liquid nitrogen. The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure. The data is treated using the BET adsorption isotherm equation characterisation first proposed by Brunauer, Emmett and Teller (BET) [8]:

\[
\frac{P_V}{W(P_0 - P_V)} = \frac{1}{W_m C} + \left( \frac{C - 1}{W_m C} \right) \frac{P_V}{P_0}
\]

Equation 2-1

where \(W\) is the amount of absorbed gas in moles, \(W_m\) is the amount of gas to form a monolayer in moles, \(P_V\) and \(P_O\) are the gas vapour pressure (Pa) and the equilibrium vapour pressure (Pa), respectively. \(C\) is a constant rate related to the absorption energy of the gas molecule to a solid substrate.

The Barrett-Joyner-Halenda (BJH) method is used for calculating pore size distributions and volumes. This is a method based on a model of the adsorbent as a collection of pores. The method accounts for capillary condensation in the pores using the classical Kelvin equation. In each pore the total excess adsorption is given by a surface layer thickness \(t(P)\) plus a pore-filling term [9].
\[ \ln \frac{P}{P^o} \geq -\frac{2\gamma V_L}{RT} \frac{1}{r_c} \]

Equation 2-2

Where \( r_c = r - t(P) \) and \( r \) is the radius of the pore. \( V_L \) is the molar volume of the liquid, \( \gamma \) is the surface tension, and \( P^o \) is the vapour pressure. This analysis requires independent determination of \( \gamma, V_L, P^o \), and the reference isotherm \( t(P) \).

Nitrogen adsorption-desorption isotherms were measured using a Micrometrics Tristar 3000 BET surface area analyser (Micrometrics, USA), pore volumes and pore sizes of the MSN before and after loading the drug were determined from the desorption branches of isotherms by the Barrett-Joyner-Halenda (BJH) method. The specific surface area was calculated according to the Brunauer-Emmett-Teller (BET) method [10-12].

2.2.5 Nuclear Magnetic Resonance (NMR):

Nuclear magnetic resonance spectroscopy (NMR) provides comprehensive data in relation to the structure, chemical environment of molecules, dynamics and reaction state. NMR spectroscopy is commonly used to investigate the properties of organic and inorganic materials, although it is only a relevant technique for nuclei possessing a spin [13]. NMR gives information about the number of magnetically distinct atoms. A hydrogen nucleus (1H) behaves as a small magnet in the presence of a magnetic field so for nuclei with spin \( \frac{1}{2} \) it will align with an external magnetic field or opposed to it. Again, the alignment where it is opposed to the field is less stable (at a higher energy).

The nuclear magnetic resonance phenomenon occurs when nuclei aligned with an applied field are induced to absorb energy and change their spin orientation with respect to the applied field.
Figure 2-8 illustrates this process for a hydrogen nucleus. The energy absorption is a quantized process, and the energy absorbed must equal the energy difference between the two states involved.

\[ E_{\text{absorbed}} = (E_{\text{-1/2state}} - E_{\text{+1/2state}}) = h\nu \]

Equation 2-3-2

Each proton in a molecule is shielded from the applied magnetic field to an extent that depends on the electron density surrounding it. The counter field that shields a nucleus diminishes the net applied magnetic field that the nucleus experiences. Each proton in a molecule is in a different chemical environment and consequently has a slightly different amount of electronic shielding, which results in a slightly different resonance frequency. Measuring the exact precise frequencies is very difficult; so the resonance frequency of each proton is measured relative to the resonance frequency of the protons of the reference substance. Tetramethylsilane (TMS) is the standard reference substance which is used commonly. Therefore, when measuring another compound, the resonances of its protons are reported in Hertz are shifted from those of TMS. The shift from TMS for a given proton depends on the strength of the applied magnetic field. The chemical shift in \( \delta \) units expresses the amount by which a proton resonance is shifted from TMS, in parts per million (ppm), of the spectrometer’s basic operating frequency [13].
The chemical structure of a polymer/nanoparticles/ or drug subjected to conjugation process may be substantially different from the original (due to cross linking after drug-nanoparticle/polymer conjugation). NMR was used to study the chemical composition of the drug ,nanoparticles and polymers before and after conjugation. $^{13}$C NMR spectra were collected from the solid state NMR experiments performed on an NMR spectrometer (Bruker BioSpin Av500) operating at 125.8 MHz. Samples were packed into a 4 mm ZrO$_2$ rotor and spun at 5 kHz in a standard-bore 4 mm broadband MAS probe. Data were processed in Bruker BioSpin TopSpin v3.0.

2.2.6 Scanning Electron Microscopy (SEM):

Scanning electron microscopy (SEM) is a form of electron microscopy that produces images of a sample by scanning with a focused beam of electrons. The electrons interact with atoms in the sample, contain information about the sample's surface topography and composition. SEM can achieve resolution better than 1 nm. Specimens can be observed in high vacuum, in low vacuum, in wet conditions, and at a wide range of cryogenic or elevated temperatures. The schematic diagram of SEM is shown in Figure 2-9 consisting of an electron gun, a series of electromagnetic lenses and apertures. An electron beam is emitted from the electron gun and it passes through several electromagnetic lenses, including condenser lenses and an objective lens. When the electrons hit on the sample surface, they produce inelastic scattering. During the inelastic scattering, an incident electron transfers kinetic energy to an electron in a sample. With a sufficient kinetic energy, this electron will become a secondary electron. The secondary electrons are then collected by a detector and amplified by an amplifier to produce an image [14].
Transmission Emitted Microscope (TEM):

A transmission electron microscope differs from a scanning electron microscope in that the electron beam used to create an image is passed through an ultra-thin sample instead of being reflected or emitted from the sample surface. Therefore, in contrast to SEM, this technique results in a 2D image and can be utilised to provide information about the structures and components within a sample.

TEM consists of electron source, condenser lens, objective lens, projection lens and image plane on fluroscent screen, as shown in Figure 2-10. In a TEM, the electron source, generally a tungsten filament is heated with a low voltage source. The electron source generates a beam of electrons which is then focused on the sample by condenser. The filament is held at a large negative potential and the electrons are accelerated towards the sample with less than 100 nm thickness. Similar to SEM, X-rays escape from the material surface and the X-rays detected.
After the electron beam passes through the sample, transmitted beams accordingly pass through the other lenses and finally an image is produced [2, 16].

**Figure 2-10** Schematic diagram showing the signals used for transmission electron microscopy [17]
2.3 Methods

2.3.1 *In Vitro* Drug Release

Absorption spectroscopy in the UV region is a highly effective tool in the analysis of chemical compounds. The main emphasis in the following thesis was to monitor drug release rate from nanofibres and nanoparticles. UV/Visible spectrophotometry is widely used to determine the amount of released drug. The principle of UV/Visible spectrophotometer is shown in Figure 2.11. The wavelengths of ultraviolet and visible lights in the range of 200nm - 800nm are utilized by the equipment. The energy of waves corresponds to the difference of electron condition energy in atoms, molecules or groups within molecules such as aromatic, conjugated chromophores etc. Therefore the spectrum obtained through the detector shows intensity of absorbed light as well as the information of sample molecules. By this theory the drug release profile of the sample in buffer is determined [2].

![Figure 2-11 Principle of UV-Visible spectrophotometer](image)
The following equation applies to the solution used in the laboratory. Beer’s law provides a relationship among absorbance, molar absorptivity ($\epsilon$), path length (b) and molar concentration (c) [18]:

$$A = \epsilon bc$$

![Figure 2-12 UV/Vis spectrum of LVF, indicating the $\lambda_{max}$ at 290nm](image)

A UV/Vis spectrum of the model drug utilized in this research, LVF, strongly absorbs at 290nm (Figure 2-12). The calibration plot of UV absorbency of LVF in the concentration range of 0-20 mg/ml ($\lambda_{max} = 290$ nm) (5 data points/concentrations) was prepared in buffer solution (Figure 2-13). The linear part of the calibration plot is used to determine the concentration of the released drug in release buffer based on the UV absorbency. The percentage of the released drug was then calculated based on the initial weight of the drug incorporated in the electrospun nanofibres and nanoparticles.
In the following thesis, UV/Vis spectra were obtained on a Cary 300 Bio spectrometer shown in Figure 2-14, operating at a resolution of 1 nm over a wavelength range of 200-800 nm.

**Figure 2-13** Standard calibration plot of UV absorbency of LVF

**Figure 2-14** UV-Vis Spectroscopy (VARIAN CARY 300) used in the project
2.3.2 Determination of Total LVF Content of the Samples

A known weight of the electrospun mats or nanoparticles (e.g. 10mg) was suspended in 10mL of alkaline solution of pH 9.0 (sodium hydroxide solution) [19]. The electrospun mats or nanoparticles were stirred for 24h at 60°C and the amount LVF released was determined by sampling the solution and measuring its absorbance at 290 nm.

2.3.3 LVF Release from Electrospun Mats and Nanoparticles

In vitro dissolution testing is a requirement for drug dosage forms and is used in all phases of development for product release and stability testing based on FDA guidance on dissolution testing [20]. Dissolution testing is an in vitro method that characterizes how the drug is extracted out of a solid dosage form. The analysis is carried out at certain time points by removing aliquots of release medium and analysing for drug content by UV-Vis. In this thesis, the release of the LVF from the electrospun mats and nanoparticles the films was determined by placing a known mass of the composite in known volume (e.g. 10 mL) of phosphate buffer saline solution (PBS, 100 mM, pH 7.4 and pH 9.0) and shaken at 150 rpm at 37°C. At each time point 1mL of release medium was removed and replenished with an identical fresh release buffer. The amount of released LVF at each time point was determined by UV spectrophotometer. The UV absorbancy of LVF in buffer solutions was measured at 290 nm and converted to the LVF concentration according to the calibration curve. LVF release profiles were calculated from the LVF concentration at each time points by following equations:

\[ C_r = C_a \times V_b \]

\[ R(\%) = \left( \frac{C_r}{T_d} \right) \times 100 \]
Where \( C_r \) is the released drug in the medium, \( C_a \) is drug content in 1mL, \( V_b \) is total volume of the release medium, \( T_d \) is total drug content of the samples, and \( R \) is the percentage of total drug content released.

### 2.3.4 Antibacterial Assay

The antimicrobial activity of the core-shell electrospun composite nanofibres in chapter 3 was assessed quantitatively against Gram-negative \( E. coli \). The principle of the test is killing the bacteria by intimate contact with electrospun mats.

Antimicrobial efficacy of the electrospun composite mats were carried out as per AATCC Test Method 100–1999 using \( E. coli \) (ATCC 4352) as the test species. The cells were cultured in LB nutrient broth yeast extract consisting of 5g/L, tryptone 10g/L, and NaCl 10g/L, pH 7.2. In these assays, 0.03 mL of overnight culture (diluted to \( 4 \times 10^8 \) cells/mL with nutrient broth) was applied to four layers of electrospun mats (14.3 mm in diameter). Care was taken to ensure that the bacterial solution was fully adsorbed without any excess liquid remaining. The electrospun mats were placed in humidified sealed jars (250 mL) and incubated for 4 h at 37°C. The bacteria were eluted with 100 mL of sterile water by vigorous shaking. The total number of live \( E. coli \) cells was counted after serial dilution and plating on nutrient agar plates (Figure 2-5). The percentage of bacterial reduction was calculated from the following equation:

\[
\text{Bacterial reduction} (%) = 100 - (C - A)/C
\]

where \( A \) is the number of colonies from the test electrospun mats after 4 h of incubation and \( C \) is the number of colonies from PCL electrospun control mats (no drug) at time zero. Figure 2-5 shows the schematic view of the whole procedure.
Figure 2-15 The schematic of Antimicrobial test method – AATCC Quantitative Method 100-1999

2.4 Acknowledgement

I would like to give my special thank to Dr. Yuan Gao for his precious help during Antibacterial Assays and for the Figure 2-10.
2.5 References


Chapter 3

RELEASE AND ANTIMICROBIAL ACTIVITY OF LVF FROM COMPOSITE MATS OF PCL AND MSN CORE–SHELL NANOFIBRES


The paper published in the Journal of Materials Science in August 2015 [1] is based on the work described in this chapter. It demonstrates sustained release of LVF by combining two drug delivery systems. First, LVF was loaded into mesoporous structure of MSNs (as a biocompatible nanocarrier) and then LVF-MSN complexes were confined into the core section of core-shell biodegradable PCL nanofibres produced by co-axial electrospinning.
3.1 Abstract

Nanofibrous materials have often been reported as carriers for clinical drugs but face the limitation of releasing the drugs in a burst fashion on application. The aim of this study is to produce composite nanofibrous mats with sustained release, using the broad spectrum antibiotic levofloxacin (LVF) as a model. Sustained release was achieved through two approaches, i.e. by firstly loading LVF into mesoporous silica nanoparticles (MSN) and then incorporating the MSN in the core regions of poly(ε-caprolactone) (PCL) nanofibres via core-shell electrospinning. Uniform PCL/LVF nanofibrous mats were also produced as controls. Loading of LVF into the MSN nanopores was confirmed by FTIR, TGA, BJH and BET measurements. After electrospinning, electron microscopy revealed that the MSN was confined in the core regions of the nanofibres. Drug release profiles showed that burst release was decreased from 59 % in the uniform PCL/LVF electrospun mats to 39 % in the core–shell PCL/LVF–MSN mats after 1 day in phosphate buffer at 37 °C. Gradual release in the latter was observed over the next 13 days. Antimicrobial assays showed that the composite electrospun mats were highly effective in killing Escherichia coli even after the mats had been incubated in a phosphate buffer for 14 days while the uniform PCL/LVF mats lost the ability after only 7 days. The results show that adsorption of the drug onto MSN and confining it in the core of nanofibres is an effective way of minimizing burst release and achieving sustained release of the drug.
3.2 Introduction

Sustained release of drugs has gained much attention in recent decades [2, 3]. The rationale of developing a sustained release system includes extending the duration of action of the drug, reducing the frequency of dosing and improving therapeutic efficacy by providing a constant drug level (as opposed to the application of several high doses) therefore overcoming adverse side effects [4]. Drug delivery methods include the adsorption or incorporation of the drug into particles or polymer systems as well as grafting the drug to a polymer backbone [5]. A number of different carriers have been explored to control drug release, such as biocompatible micro- and nanoparticles, nanofibres, erodible polymer gels, liposomes and polymer micelles [6].

Electrospinning is a convenient method for fabricating nanofibres which have been used in a wide range of applications such as catalysis, filtration, nanocomposites and medical textiles [7-9]. More recently, electrospun nanofibres are being considered as a drug delivery carrier where the drug is blended with the polymer solution prior to electrospinning. In this approach ideally the drug is evenly distributed throughout the fibre volume, in order to provide continuous even release. In reality there is fast burst release of drugs. The burst release could be caused by various reasons such as the presence of the drug on the surface of nanofibres rather than even distribution throughout the fibre volume, the degradation rate of the polymer used and the larger surface area of the nanofibres due to very small diameter size [10-15]. One approach used to prolong drug release is to fabricate core-shell nanofibres via core-shell electrospinning with the drug loaded into the core structure [14, 16-18]. This prolonged release profile is probably due to the drug molecules being trapped in the core section of the fibres by the shell polymer [19, 20]. The shell must degrade first sufficiently before any drug contained in the core is released. For example, Wang et al. [20] studied the release of dimethyloxalylglycine from PLLA/PHB nanofibres and found that 25% of the drug was released over a 60 h period.
in a core-shell situation compared to 80% of the drug released in single component nanofibre structure, and attributed the reduction to the presence of the shell polymer [21-24]. In another study core-shell PVP/zein nanofibres with ketoprofen in core section were fabricated for providing biphasic drug release profiles. It was observed that the core-shell nanofibres showed a first burst release of 42.3% of the contained ketoprofen but then followed by a sustained release over a 10h period of the remaining drug [25].

Another method to achieve sustained release is to adsorb the drug onto/into nanostructure carriers such as polymeric nanoparticles and micelles, liposomes, gold nanoparticles and silica nanoparticles [7, 26-28]. Mesoporous silica nanoparticles (MSN) have been used as a nanocarrier of drugs due to their unique properties such as high specific surface area, high pore volumes and the presence of reactive groups for further functionalisation, i.e. covalent conjugation to a drug [29-33]. A recent report by Zhang et al, shows that a poorly water-soluble drug, telmisartan, was loaded into MSN at a very high drug loading of about 60% in weight [34] and the release profile was improved and controlled by decreasing the pore sizes. The burst release reduced from 96% from MSN with 12.9 nm pore diameter to 65.7% in MSN with 3.6nm pore diameter after 15min. This is due to the restricted diffusion of release medium into the small pores and relatively diffusion of telmisartan out of the pores which leads to delayed release.

Another advantage is that MSNs improve the solubility of water insoluble drugs, therefore providing an alternative to DDS of hydrophobic or poorly water soluble drugs. Furthermore, MSN shows good biocompatibility with no cytotoxicity to cells in vitro, and silica-based implants show no toxicity to liver, kidney or lymph nodes in animals. [35-37].

The aim of this study is to design a sustained drug delivery system based on the advantages of MSN and core-shell nanofibres. Levofloxacin (LVF) was adsorbed onto MSN and then
fabricated into nanofibre/MSN composites by core-shell electrospinning in order to reduce burst release behavior and obtain sustained release of LVF. The release profiles of core-shell electrospun composite mats were measured and compared with the control electrospun nanofibres and thin films. The polymer in this case was poly(ɛ-caprolactone). PCL was chosen due to its slow degradation rate (up to 24 months to complete degradation) [38, 39]. Finally, antimicrobial activity of the core-shell electrospun mats was assayed using E. coli [40].

3.3 Materials and Methods

3.3.1 Materials

Poly(ɛ-caprolactone) (PCL, MW 80000g/mol), MSN (200 nm particle size and 4 nm pore size), LVF (98.0%), dichloromethane (DCM) and N,N-dimethylformamide (DMF) were all purchased from Sigma-Aldrich. All other chemicals were of reagent grade and used without further purification.

3.3.2 Methods

3.3.2.1 Preparation of Drug-loaded MSN and Drug-loaded MSN/Electrospun Mats

LVF (75 mg) was fully dissolved in 30 mL deionised water, to which 150 mg of MSN was added and the solution was sonicated for 15 min and then stirred for a further 18 h. The MSN was collected after centrifuging (15 min at 25,000 g), dispersed in deionized water and sonicated for 15 min. This step was repeated twice to remove unbound LVF. The MSN/LVF were finally collected after centrifugation and dried at room temperature for 48 h.
The basic experimental setup for core-shell electrospinning process is shown in Figure 3-1. The setup contained an inner spinneret needle (25G, 0.52 mm outer diameter) placed inside in an outer spinneret needle (19G, 1.07 mm outer and 0.69 mm inner diameter) (Figure 3-1), syringe pumps (model NE-1000 New Era Pump Systems, Inc., Farmingdale, NY, USA), a high voltage supply (Spellman high voltage DC power supply, USA) and two syringes (3 mL for the core layer and 5 mL for the shell layer). The inner and outer spinneret needles produced the core and shell components in the resultant nanofibres, respectively. The electrospinning distance was varied between 0 and 300 mm (optimised distance of 150 mm) and applied voltage was varied between 0 and 40 (optimised value of 20 kV). Electrospun mats were collected for 10 h on a drum collector (100mm diameter).

The electrospun mats were prepared containing 1 wt% of LVF and 3 wt% of LVF-MSN (with respect to PCL). For core polymer solutions, 20 mg LVF and 60 mg LVF-MSN were added to two separate bottles containing 20 g of 10 wt% PCL in a 1:1 (by weight) mixture of DCM:DMF and stirred continuously until a homogeneous solution was obtained. A solution of 14 wt% PCL was also prepared separately to be used as the shell polymer solution. The control electrospun mat and drug/polymer film were made from 1% of LVF (with respect to PCL) added to a 14 wt% PCL solution and stirred until a clear solution was obtained.
Electrospun mats were collected over 4 h period, with core layer pump rates of 0.4 mLh^{-1} and shell layer pump rate of 0.7 mLh^{-1}. For a conventional electrospun nanofibrous mat a single pump was used at 0.7 mLh^{-1}. The drum collector was driven by a 12 V motor rotating at 60 mms^{-1} and moving horizontal oscillating speed 10 mms^{-1}.

### 3.3.3 Characterization

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses were carried out using a Philips XL30 field emission scanning electron microscope and a Tecnai12 transmission electron microscope. Fibre diameter was measured using Microstructure Measurement software. For each sample, 100 measurements were carried out and the mean fibre diameter determined. IR spectra in transmission mode were collected using
a Thermo Nicolet 6700 FTIR Spectrometer. TGA was performed using a Netzsch STA 449 F1 Jupiter TGA analyser at a heating rate of 10 °C/min under a nitrogen purge of 40 ml/min. Nitrogen adsorption-desorption isotherms were determined using a Micrometrics Tristar 3000 BET surface area analyser (Micrometrics, USA). Pore volumes and pore sizes of the MSN were determined from the desorption branches of isotherms by the Barrett-Joyner-Halenda (BJH) method. The specific surface area was calculated according to the Brunauer-Emmett-Teller (BET) method [34, 41, 42].

3.3.4 In Vitro Drug Release

The electrospun mats were cut into circular discs (14.3 mm in diameter). Four discs were weighted then placed in a glass vial containing 10 mL of phosphate buffer saline solution (PBS, 100 mM, pH 7.4) and were shaken continuously for 14 days at 150 rpm at 37°C. At various time intervals 1mL of release medium removed and replaced with an identical fresh buffer. The UV absorbance of LVF in buffer solutions was measured at 290 nm and converted to the LVF concentration according to the calibration curve. The amount of released LVF was calculated from the LVF concentration at each time points.

3.3.5 Antimicrobial Assay

Quantitative antimicrobial assays on the electrospun composite mats were carried out as per AATCC Test Method 100-1999 using E.coli (ATCC 4352) as the test species.
3.4 Results and Discussion

3.4.1 FTIR

MSN were first loaded with the antibiotic before they were incorporated in the core regions of nanofibres. FTIR was used to confirm the presence of the LVF in the MSN complex [34, 43]. The spectra of LVF, MSN and LVF-MSN were collected and shown in Figure 3-2. Characteristic FTIR transmission peaks of LVF were observed at 1294 cm\(^{-1}\) assigned to stretching of amines, 1620 cm\(^{-1}\) for aromatic C-C, 1724 cm\(^{-1}\) due to the presence of C=O and broad peak at 3265 cm\(^{-1}\) representing O-H of carboxylic acid group. No distinctive peaks were observed in the MSN spectra except for a strong peak at 1025 cm\(^{-1}\) due to the Si-O\(_2\) stretching. LVF-MSN exhibits a peak of carboxyl at 1717 cm\(^{-1}\). This peak was slightly shifted from 1724 cm\(^{-1}\) due to the hydrogen bonding of the carbonyl of the LVF to the silanol groups [34]. The broad peak at 3350 cm\(^{-1}\) is present in LVF-MSN due to the carboxylic group of LVF. It also shows another peak at 1619 cm\(^{-1}\) due to the aromatic C-C which is present in LVF (Figure 3-3 inset). The peaks observed in the spectra of LVF-MSN confirm that LVF was present in MSN mesoporous structure.
3.4.2 Nitrogen Adsorption Studies

The drug loaded MSN were further characterized by nitrogen adsorption-desorption studies. The surface area, the total pore volume and the average pore diameter size were calculated from the nitrogen adsorption-desorption isotherms using the BET and BJH method. Figure 3-3 shows typical IV isotherm curves for both MSN and LVF-MSN which confirm the existence of a uniform mesoporous structure [29]. The values for the BET specific surface area ($S_{BET}$), the total pore volume ($V_p$) and the BJH pore diameter ($D_p$) are given in Table 3-1.
The BET surface area and average pore size of the MSN before loading LVF are $690 \text{m}^2\text{g}^{-1}$ and 4.06nm. These parameters were decreased after loading LVF into MSN to $463 \text{m}^2\text{g}^{-1}$ and 3.5nm, respectively, in the case of LVF-MSN (Table 3-1). This indicates that the pores in MSN have been partially filled after loading with LVF thereby producing smaller values once the LVF is absorbed and occupying some of the volume and area that was previously available.

**Table 3-1** Surface area, pore size and pore volumes of MSN and LVF-MSN

<table>
<thead>
<tr>
<th></th>
<th>Surface area $S_{\text{BET}} \text{ (m}^2\text{g}^{-1})$</th>
<th>Pore size $D_p \text{ (nm)}$</th>
<th>Pore volume $V_p \text{ (cm}^3\text{g}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSN</td>
<td>690</td>
<td>4.06</td>
<td>0.74</td>
</tr>
<tr>
<td>LVF-MSN</td>
<td>463</td>
<td>3.50</td>
<td>0.53</td>
</tr>
</tbody>
</table>
3.4.3 TGA

The overall amount of loaded LVF was estimated by TGA. In the TGA measurement, the drug desorbs after decomposing, which is detected as a temperature-dependent weight reduction. Together with BET and BJK measurements, TGA provides further evidence that the LVF is adsorbed into the MSN structure [44, 45]. As shown in Figure 3-4, the loading fractions were estimated from the mass loss ratio between 0 and 700°C compared to the total initial weight. No weight loss was observed in TGA of MSN as expected due to the high melting point of the MSN (>1600°C) [34]. Conversely, the weight loss due to drug uptake was around 20% for the weight of the LVF-MSN. These results, together with that of FTIR, BET and BJK measurements confirm that LVF had been successfully adsorbed into the MSN porous structure.

![Figure 3-4 TGA of LVF, MSN and LVF-MSN](image)

Figure 3-4 TGA of LVF, MSN and LVF-MSN
3.4.4 Architecture of Electrospun PCL/LVF and PCL/LVF-MSN Mats

The architecture and nanofibre diameters of the electrospun mats with average diameter sizes of 600-700nm are shown in Figure 3-5 (A-H). Fibre size had a normal type distribution, with an average diameter of 600-900 nm (24%) for PCL/LVF-MSN and 500-800 nm (23%) for PCL/LVF. The results showed that the addition of MSN marginally increased fibre diameter. This is probably due to the increase in core solution viscosity as has been reported in different studies [29, 46]. Figure 3-5(E-F) show the TEM images of the structure of core-shell PCL/LVF and PCL/LVF-MSN composite nanofibres, respectively. The core-shell structure of the LVF/PCL nanofibres can be easily observed; the red arrows indicate the boundaries of the shell and core layers. In the case of PCL/MSN-LVF composite mats as it can be observed from the Figure 3-5(B&D), there are no LVF-MSN complexes observed on the surfaces of nanofibres which means the confinement of the LVF-MSN complexes in the core region (Figure 3-5 F).
Figure 3-5 SEM images of electrospun core-shell PCL/LVF mat (A-B) and PCL/LVF-MSN mat (C-D), TEM images of core-shell PCL/LVF (E) and PCL/LVF-MSN mats (F), Fibre diameter distribution of PCL/LVF (G) and PCL/LVF-MSN (H)
3.4.5 EDX Analysis

The EDX analysis of the composite nanofibres was carried out in order to investigate the distribution of MSN (Si element) in the electrospun fibres. The elemental analysis reveals the presence of Si distributed through the mats. The elemental ratio of C, O and Si are 83, 16.3 and 0.7% in the composite PCL/MSN composite nanofibres (Figure 3-6a). The layered SEM image (Figure 3-6b) shows even distribution of Si throughout the composite nanofibres.

![Figure 3-6 EDX spectrum (a) and MSN (Si) distribution (b) of electrospun core-shell PCL/MSN composite nanofibres](image)

3.4.6 Cumulative Release of LVF

Firstly, the release of LVF from drug-loaded MSN was measured separately to discover how long can MSN retain the drug. LVF release was measured by shaking the LVF-MSN in PBS solution at 37°C. Figure 3-7 shows that approx 70% of LVF in the nanoparticles was released in the first 20 min. The release plateaued by 60 min and there was little further release in the next 60 min. The MSN showed the ability to retain the antibiotic in the matrix for a short period of time with a burst release fashion. Similar release profiles have been previously reported. The
burst release of resveratrol was observed by Tsai et al, with 80% of the drug released in the first 10 min from resveratrol-MSN complexes. This indicates the drug was physically adsorbed into MSN [30].

![Graph showing cumulative release over time](image)

**Figure 3-7** The cumulative LVF release from LVF-MSN

In this study, the release of LVF from PCL/LVF, core-shell PCL/LVF, core-shell PCL/LVF-MSN composite nanofibres and PCL/LVF films were measured by shaking in PBS solution at 37°C. It was envisaged that the combination of MSN particles and the core-shell structure of the nanofibres would further modify burst release to provide drug release over a longer term. Figure 3-8 shows the release profiles of the LVF from uniform and core-shell nanofibres, and from the core-shell nanofibres where the drug had been adsorbed on MSN. The control PCL/LVF film was also included for comparison. The control PCL/LVF electrospun mat showed a strong burst nature, releasing 60% of the antibiotic in the first 24 h. Core-shell structure of the nanofibres slowed the initial release to 45% in the first 24 h. This observation is presumably due to the confinement of the drug in the core section of the nanofibres and the outer layer serving as a diffusion barrier [47]. In this study, although the initial release rates were quite different between control and core-shell nanofibres, both types of nanofibres released the same amount of the drug by 14 days (Figure 3-8). On the other hand, the release
profile of core-shell PCL/LVF-MSN was different to the nanofibres without MSN. It has been reported that presence of loaded nanoparticles in uniform nanofibres influences the release kinetics and could reduce the burst release [29, 48]. Experimental evidence shows a 39% release after 24 hrs and gradually increased to 69% after 14 days. This suggests that the LVF releasing behaviour was considerably influenced by the MSN incorporated within the core of the core-shell nanofibres. On the other hand, the control PCL/LVF film showed only a 15% release of LVF in first 6 hr, and then remains largely unchanged. Distinctive differences between the release rates of control film and electrospun mats clearly demonstrates the advantages of nanofibres over flat film such as the high surface area of nanofibres that plays a significant role in the release behaviour [49, 50].

![Cumulative LVF release from: PCL/LVF, Core-shell PCL/LVF, PCL/LVF-MSN electrospun mats and PCL/LVF film.](image)

**Figure 3-8** The cumulative LVF release from: PCL/LVF, Core-shell PCL/LVF, PCL/LVF-MSN electrospun mats and PCL/LVF film.

### 3.4.7 Antibacterial Activity Assay

The antibacterial properties of the various controls and core-shell electrospun mats were investigated quantitatively using *E. coli* reduction assays. *E. coli* is a Gram-negative bacterium that is commonly found in the lower intestine of warm-blooded organisms [40]. The assay was
done on samples after each time point in the cumulative release experiments. As expected, the control PCL electrospun mats containing no LVF showed no bacterial reduction. Both core-shell electrospun mats showed antibacterial ability with greater than 99% bacterial reduction after 7 days (Figure 3-9). This antibacterial behaviour of two core-shell electrospun mats can be attributed to the burst release of the drug in first few days of immersion in PBS where the majority of the drug content was released [49, 50].

Core-shell PCL/LVF nanofibres displayed significantly lower efficacies in bacterial inhibition compared to core-shell PCL/LVF-MSN nanofibres after day 10 where they have lost most of its LVF content (see Figure 3-8); bacterial reduction was decreased after that to 72% after 14 days. Conversely for the core-shell PCL/LVF-MSN nanofibres remained active and still showed bacterial inhibition after 7 days and roughly the same antibacterial ability after 14 days which is due to the reduced release rate.

![Antimicrobial assays of core-shell PCL/LVF, core-shell PCL/LVF-MSN and untreated PCL electrospun mats](image)

**Figure 3-9** Antimicrobial assays of core-shell PCL/LVF, core-shell PCL/LVF-MSN and untreated PCL electrospun mats
3.4.8 Conclusion

This chapter describes the fabrication and characterization of composite nanofibres for sustained drug release. The material combines loading the drug, LVF, into mesoporous silica nanoparticles and confining them to the core of bilayer nanofibres using core-shell electrospinning. Such an approach significantly reduced the release rate from burst release to a sustained release behaviour over two weeks in a phosphate buffer. As a result, the composite nanofibres exhibited strong antimicrobial activity even after they had been soaked in a phosphate buffer for 14 days. The findings show that physical adsorption of a drug on a nanostructure and constraining them in the shell of bilayer nanofibres provides an effective means of achieving sustained drug release.

3.4.9 Acknowledgment

I would like to thank Chi Huynh for her help in characterization studies with TEM.
3.5 References


Chapter 4

SLOW RELEASE OF LEVOFLOXACIN CONJUGATED ON MSN FROM PCL NANOFIBRES

The paper submitted for publication in the International Journal of Polymeric Materials in based on the work described in this chapter. The slow release of LVF was achieved by combining two approaches. First is covalently binding LVF to MSN (as a biocompatible nanocarrier) via cleavable bond. Conjugated LVF-MSN was then confined into biodegradable PCL nanofibres in second approach by electrospinning.
4.1 Abstract

A number of drug-nanofibrous composite mats have been reported as drug delivery systems. Their main disadvantage is a burst release of the drug. This section of the thesis presents experimental results which demonstrate new approaches to obtain more appropriate release of Levofloxacin (LVF) from composite nanofibres. Slow release was achieved through two approaches. The first approach is covalently binding LVF to the mesoporous silica nanoparticles (MSN) via a cleavable thioester bond and second approach is mixing the LVF conjugated MSN into poly(ε-caprolactone) (PCL) nanofibres. PCL/LVF nanofibrous mats were also fabricated as controls for comparison as in the previous chapter. The conjugated LVF-MSN was characterized by IR, DSC and solid-state $^{13}$C-NMR. After electrospinning, scanning electron microscopy (SEM) revealed the presence of MSN confined in the PCL nanofibres. Drug release profiles showed that release behaviour was decreased from 59% for the control PCL/LVF electrospun mats to 20% for the PCL/conjugated LVF-MSN mats after 1 day in phosphate buffer at 37°C, and continued gradual release was observed over the next 13 days in the latter. This slow release of the LVF is due to the cleavable bond between LVF and MSN which can be hydrolysed over a time. The results indicate that conjugation of the drug to MSN and confining them into nanofibres is a very effective way of minimizing burst release and achieving sustained release of the drug.
4.2 Introduction

Sustained release systems of drugs have been widely explored due to their advantages in improving therapeutic efficiency and reducing toxicity by delivering the drug at a controlled release rate consequently overcoming adverse side effects [1-3]. The requirements for drug delivery carriers are: biodegradable, protect the drug from undergoing decomposition while providing a controlled release of the drug. In the past decade several drug carriers have been investigated such as cast films[4], micro- and nanoparticles [5, 6] and nanofibres [7]. Among these forms, nanofibres show the most promise [8]. Nanofibres are fabricated by electrospinning and used in various applications such as filtration [9, 10], nanocomposites [11, 12] and medical textiles [13-15]. Electrospun nanofibres have been considered as drug delivery systems due to their advantages especially high surface area to mass ratio [14, 16, 17]. However, a disadvantage of drug loaded nanofibres has been the burst release behaviour of the drug that occurs where a large portion of the loaded drug is released in the first several h [18-21]. The drug release profile of electrospun nanofibres is dependent upon the drug concentration and its distribution throughout the nanofibre volume as well as surface area. The reasons for the burst release behaviour may include the presence of the drug on the surface of nanofibres, instead of throughout the fibre volume and the fast degradation rate of the polymer [14, 22, 23].

For drug-nanofibre systems to become useful it is necessary to slow drug release over a certain time period. One approach reported in the literature to reduce the burst release and prolong drug release is to employ drug (physically) loaded nanoparticles incorporated within the nanofibres [24-26]. In the past decade, a variety of biocompatible nanoparticles, including polymeric nanoparticles and silica nanoparticles, have been adopted as drug delivery applications [27-30]. Mesoporous silica nanoparticles have found widespread application in
drug delivery systems due to their high specific surface area, adjustable pore size, high pore volume and the presence of reactive groups. The reactive groups provide the opportunity to further functionalise the material (i.e. covalent conjugation to a drug) to allow better control of drug release [31, 32]. Numerous reports have been published on slow release of drugs from mesoporous silica nanoparticles [33-36]. For example, slow release of telmisartan from amine functionalised silica nanoparticles which is due to the strong interaction between the amine groups of MSN and carboxylic groups of drug has been reported [37]. Another approach is to covalently bind a drug to the nanocarriers via a cleavable bond. For example, doxorubicin (DOX) was chemically conjugated to poly(lactic-co-glycolic acid) (PLGA) by a cleavable linkage and then converted into nanoparticles [38]. The conjugated DOX-nanoparticles exhibited sustained DOX release profiles over 30 days whereas nanoparticles containing free DOX (unconjugated) showed a much faster release over 5 days as a result of simple drug diffusion through the matrix. This improved release profile was due to the slow and controlled hydrolysis of the ester linkage between DOX and PLGA over time.

The aim of this study was to design one sustained drug delivery system with a combination of different drug release mechanisms. The new system is based on the covalent binding of the drug to the nanocarriers and then incorporating the conjugated nanocarrier into a biodegradable polymer nanofibres ie poly(ε-caprolactone) (PCL). PCL was chosen as the carrier polymer because of its biocompatibility, electrospinnability and slow degradation rate (up to 24 months) [39]. Levofloxacin (LVF) was conjugated to propylthiol functionalised mesoporous silica nanoparticles (MSN), fabricated into a nanofibre/MSN composite and the release profile from the electrospun composites measured and compared to the composite nanofibres/control LVF-MSN (non-conjugated) and control drug blended electrospun nanofibres.
4.3 Materials and Methods

4.3.1 Materials

Poly(ɛ-caprolactone) (PCL, MW 80000 g/mol), propylthiol functionalised mesoporous silica nanoparticles (200 nm particle size and 4 nm pore size), levofloxacin (98.0%), dichloromethane (DCM) and N,N-dimethylformamide (DMF) were sourced from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Pierce. Dimethylsulfoxide (DMSO) was supplied by Fisher Chemical and 2-(N-morpholino)ethanesulfonic acid (MES) from Fluka. All other chemicals were of reagent grade and used without further purification.

4.3.2 Methods

4.3.2.1 Preparation of Conjugated LVF-MSN and PCL/LVF-MSN Nanofibres

LVF (100 mg) and EDC (56 mg) (1:1 molar ratio) were dissolved in 1 mL DMSO and mixed for 30 min to activate the LVF [40, 41]. The activated LVF solution was then slowly added to 5 mL of 100 mM PBS solution containing 100 mg of MSN and stirred continuously for 8 h. The conjugated nanoparticles were collected after centrifugation (15 min, 16000 g), dispersed in 5 mL of deionised water and sonicated for a further 5 min to remove unbound LVF. The MSNs were finally collected after centrifugation and dried at room temperature for 48 h. The control LVF-MSN without conjugation was prepared by mixing LVF and MSN for 8 h in deionised water and then collected by centrifugation.

Electrospun mats were prepared containing 1 wt% of LVF, 4 wt% of LVF-MSN (with respect to PCL). For polymer solutions, 30 mg LVF, 120 mg conjugated LVF-MSN and 120 mg control LVF-MSN were added to three separate bottles containing 20 g of 15 wt% PCL in a
1:1 (by weight) mixture of DCM:DMF and stirred continuously until a homogeneous solution was obtained.

The basic experimental setup for the electrospinning process is shown in Figure 4-1. The setup contained a spinneret needle (23 G Terumo), syringe pumps (model NE-1000 New Era Pump Systems, Inc., Farmingdale, NY, USA), a high voltage supply (Spellman high voltage DC power supply, USA) and a 5 mL syringes (Terumo). The electrospinning distance was varied between 0 and 300 mm (optimised distance of 150 mm) and applied voltage was varied between 0 and 40 (optimised value of 15 kV). Electrospun mats were collected for 10h on a drum collector of 100 mm diameter.

![Schematic diagram of preparation of LVF-MSN and PCL/LVF-MSN nanofibres.](image-url)

**Figure 4-1** Schematic diagram of preparation of LVF-MSN and PCL/LVF-MSN nanofibres.
4.3.3 Characterization

Scanning electron microscopy (SEM) analyses were carried out using a Philips XL30 field microscope. Fibre diameters were measured using the microstructure measurement software. For each sample, 100 measurements were taken to obtain an average fibre diameter. IR spectra were collected in the transmission mode using a Thermo Nicolet 6700 FTIR Spectrometer. The specific surface area and pore characteristics of the MSN were determined according to the Barrett-Joyner-Halenda (BJH) and Brunauer-Emmett-Teller (BET) method [42, 43]. DSC analysis of samples was carried out at a heating rate of 10°C/min under a nitrogen purge of 40ml/min using a Netzsch STA 449 F1 Jupiter DSC Thermal Analyser. TGA was performed using a Netzsch STA 449 F1 Jupiter TGA Thermal Analyser at a heating rate of 10°C/min under a nitrogen purge of 40ml/min. Solid-state $^{13}$C cross-polarization (CP-MAS) NMR spectra were recorded on a Bruker AV500 MAS spectrometer. A Varian UV-Vis spectrophotometer was used to measure the LVF concentration at 290 nm.

4.3.4 In Vitro Drug Release

The electrospun mats were cut into squares of 50x50 mm. Each square was weighed and then placed in 5 mL of phosphate buffer saline solution (PBS, 100 mM, pH 7.4) and shaken continuously for 14 days at 150 rpm at 37°C. 2mg of control and conjugated LVF-MSN placed in 5 mL of phosphate buffer saline solution (PBS, 100 mM, pH 7.4 and pH 9.0) and shaken continuously for 2 h at 150 rpm at 37°C. At each time points 1mL of release medium removed and replenished with an identical fresh release buffer. The UV absorbance of LVF in buffer solutions was measured at 290 nm and converted to the LVF concentration according to the calibration curve. LVF release profiles were calculated from the LVF concentration at each time point.
4.4 Results and Discussion

A novel drug delivery composite was formed by conjugating LVF to MSN then incorporating them into PCL nanofibres fabricated by electrospinning. LVF was used as a model drug because it is a common antibiotic with a carboxylic acid group that can be used for further chemical modification (i.e. conjugation) and has a chromophore for easy detection at $\lambda_{\text{max}}$ of 290 nm. The functionalised LVF/MSN and control were characterised and the LVF release profiles were measured.

4.4.1 FT-IR

FT-IR spectra of LVF, MSN, control LVF-MSN and conjugated LVF-MSN are shown in Figure 4-2. Characteristic IR transmission peaks of LVF can be observed at 1294 cm$^{-1}$ from amines, 1620 cm$^{-1}$ due to aromatic C-C bonds, 1720 cm$^{-1}$ due to carboxyl group C=O and the broad peak at 3265 cm$^{-1}$ from the O-H of the carboxylic acid. Spectra of MSN show a strong peak at 1025 cm$^{-1}$ due to Si-O$_2$ stretching. In the control spectra of LVF-MSN a peak at 1712 cm$^{-1}$ was observed due to the hydrogen bonding of the silanol groups and carboxylic groups of LVF (Figure 4-2c) [37]. There are also absorption band(s) at 3350 cm$^{-1}$ due to the presence of hydroxy groups of unbound LVF in the control LVF-MSN. This was not observed in the spectrum of the conjugated LVF-MSN; indicating a reduction of hydroxy group stretching after conjugation. The absorption at 1703 cm$^{-1}$ in the conjugated LVF-MSN is due to the formation of the thioester bond between LVF and thiol moieties [44, 45].
DSC analysis was used to determine the absence of the crystalline drug in the conjugated LVF-MSN form which is indicative of bound LVF instead of free LVF, resulting in no melting point depression for the LVF in the non-crystalline state [46-48]. As shown in Figure 4-3a the DSC curve of LVF exhibited one endothermic peak at 100°C due to the dehydration of LVF [49] and one peak at 244°C representing its melting point. No peak was detected in the MSN thermogram due to its high melting point (1600-1725°C) [37, 47]. The melting peak of LVF was observed to shift slightly from 244°C to 229°C in the control LVF-MSN. The absence of phase transition point(s) of LVF in the conjugated LVF-MSN thermogram is due to the

Figure 4-2 FT-IR Spectra of LVF (a), MSN (b), Control LVF-MSN (c) and conjugated LVF-MSN (d).
presence of LVF in a non-crystalline, amorphous state or disordered-crystalline state after LVF and MSN are conjugation [50, 51].

![DSC thermograms FT-IR Spectra of LVF (a), MSN (b), Control LVF-MSN (c) and conjugated LVF-MSN (d).](image)

**Figure 4-3** DSC thermograms FT-IR Spectra of LVF (a), MSN (b), Control LVF-MSN (c) and conjugated LVF-MSN (d).

### 4.4.3 TGA

TGA is an important method to quantify the amount of loaded drug [37, 52]. In the TGA measurement, the drug desorbs after decomposing, which is detected as a temperature dependent weight reduction. As shown in Figure 4-4, the loading fractions in all cases were estimated from the ratio of the weight loss between 0 and 700°C to the total initial weight. The weight loss of MSN is 13.1% due to the loss of functional groups of MSN, respectively. The weight loss due to drug uptake was 29.4% for the control LVF-MSN and 26.3% for conjugated LVF-MSN. The drug uptake values obtained by TGA were in agreement with those obtained by nitrogen adsorption studies in next section, as shown in Table 4-1.
4.4.4 Nitrogen Adsorption Studies

The mesoporous structure of the various version of the MSNs were further characterized by N\textsubscript{2} adsorption-desorption isotherms. Figure 4-5 shows typical IV isotherm curves for MSN, the control LVF-MSN and conjugated LVF-MSN. The isotherm confirm the existence of uniform mesoporous [28]. The BET surface area and the average pore size of the thiol derived MSN decreased from 640 m\textsuperscript{2}g\textsuperscript{-1} and 4.8 nm to 541 m\textsuperscript{2}g\textsuperscript{-1} and 4.17nm, respectively, in conjugated LVF-MSN and 534 m\textsuperscript{2}g\textsuperscript{-1} and 4.20 nm in control LVF-MSN. These indicate that LVF was loaded into the pores in both samples [37].

Figure 4-4 TGA of LVF, MSN, Control LVF-MSN and conjugated LVF-MSN.
Nitrogen isotherms of MSN and control LVF-MSN and conjugated LVF-MSN

Table 4-1 Structural parameters of MSN and control LVF-MSN and conjugated LVF-MSN

<table>
<thead>
<tr>
<th></th>
<th>$S_{BET}$ (m$^2$g$^{-1}$)</th>
<th>Dp(nm)</th>
<th>Vp(cm$^3$g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSN</td>
<td>640</td>
<td>4.8</td>
<td>0.77</td>
</tr>
<tr>
<td>Conj LVF-MSN</td>
<td>541</td>
<td>4.17</td>
<td>0.60</td>
</tr>
<tr>
<td>Ctrl LVF-MSN</td>
<td>534</td>
<td>4.20</td>
<td>0.56</td>
</tr>
</tbody>
</table>

4.4.5 NMR

Solid state $^{13}$C NMR was also used to confirm the conjugation of LVF to the MSN (Figure 4-6). The spectrum of MSN, Figure 4-6a, showed chemical shifts at 15.5, 23.5 and 26.8 ppm corresponding to the propyl carbons ($C_i$, $C_{ii}$ and $C_{iii}$) [53]. The shifts with asterisk (*) at 10.8 ppm and at 59.5 ppm were assigned to the presence of ethanol which was used as a stabilising agent in the supplied MSN (MSN was used as received). In the spectrum of the conjugated LVF-MSN, Figure 4(d) the shifts of carbonyl carbons ($C_{15}$ and $C_{16}$) are different from those of control LVF-MSN; $C_{16}$ changed to 160.3 ppm and $C_{15}$ to 171.6 ppm as a result of thioester
bond in the conjugated LVF-MSN. Similar changes in shifts have been reported in the literature whereby the shift of thioester carbonyl carbon had a higher value than other carbonyl carbons [54, 55]. Also, in the spectrum of the conjugated LVF-MSN, Figure 4-6d, the propyl C adjacent to the S changed from 23.5 ppm to 35.3 ppm. Similar shifts have been observed reactions with propyl thiols after conjugation due to the different cross-polarization dynamics of propylthiol carbons [56].

**Figure 4-6** Solid state NMR spectra of LVF (a), MSN (b), control LVF-MSN (c) and conjugated LVF-MSN (d).
4.4.6 Morphology of Electrospun Mats

The morphology and fibre diameter distributions of the electrospun mats are shown in Figure 4-7(a-f). Fibre diameter distribution followed a normal type distribution. The addition of MSN increased the average fibre diameter compared to the control PCL nanofibres from 0.6 µm to 0.8 µm in PCL/LVF-MSN composite mats, Figure 4-7 (c&f). This increase in average fibre diameter was probably caused by the increase in solution viscosity after the addition of MSN [57]. Figure 4-7(d-e) also shows the smooth surface of PCL/LVF electrospun nanofibres. However, after adding MSN, the composite electrospun nanofibres appeared to become rough with particles clearly noticeable on the surface of the nanofibres (Figure 4-7a&b). This phenomenon is due to the aggregation of MSNs particles in the PCL solution formed during electrospinning or the incomplete dispersion during the initial dispersion process into the PCL solution [26].
Figure 4-7 SEM images of electrospun PCL/LVF-MSN mat (a-b) and PCL/LVF (d-e); Fibre diameter distribution of PCL/LVF-MSN (c) and PCL/LVF (f) nanofibres.

4.4.7 EDX Analysis

EDX analysis was carried out in order to investigate the content and distribution of MSN (Si element) in PCL/LVF-MSN composite mats. As it’s shown in Figure 4-8 (left) EDX data from the area analyses of the nanofibres shows the presence of Si with ratio of 1 wt% which corresponds to the presence of MSN distributed in nanofibres. Figure 4-8 (right) shows even distribution of MSN (Si element) through nanofibres. As previously mentioned in SEM images
(Figure 4-7A&B) the aggregation of MSNs in nanofibres can be also observed in EDX layer image.

![Figure 4-7A&B](image)

**Figure 4-8** EDX spectrum (left) and MSN (Si) distribution (right) of electrospun PCL/LVF-MSN composite nanofibres

### 4.4.8 Cumulative Release of LVF

The release of LVF was measured in the control and conjugated LVF-MSN to measure if functionalized MSN could retain the LVF drug. The release of LVF from the nanoparticles was performed at different pHs (pH 7.4 and pH 9.0) in order to observe the influence of pH on the hydrolysis of thioester bonds in conjugated LVF-MSN. More release of LVF was expected to occur under conditions that promote the hydrolysis of thioester bond i.e. pH9.0 than compared tp pH7.4 [58]. As shown in Figure 4-9, LVF is released from conjugated LVF-MSN in basic medium to a higher extent, 90% of LVF released in 120 min than in at pH 7.4 with 74% of LVF released. In the control LVF-MSN 82% of LVF content was released (pH 7.4) in the first 20 min with the release plateauing by 60 min with no further release observed after 60 min. The LVF release profile of control LVF-MSN in pH 9.0 shows roughly the same release profile
of control LVF-MSN in pH 7.4 as a result of no stable chemical binding between LVF and MSN that can be affected by pH level.

![Graph showing cumulative LVF release](image)

**Figure 4-9** The cumulative LVF release from control LVF-MSN and conjugated LVF-MSN at pH 7.4 & 9.0.

The same release profile of LVF from MSN was observed in our previous study where LVF alone was blended with MSN [59]. This shows that MSN can retard LVF release in the matrix for a short period of time most likely due to the formation of weak hydrogen bonds between LVF and silanol groups on MSN [37, 60]. The slower release profile of the LVF from the conjugated LVF-MSN is attributed to the covalent binding (conjugation) of the LVF and MSN through the thioester bond followed by the hydrolysis of the thioester bond in alkaline medium [58, 61].

Figure 4-10 shows the release profiles of the LVF from the control and two composite nanofibres; one with drug adsorbed into the MSN (control LVF-MSN) and the other with drug covalently attached to the MSN. It was envisaged that the combination of conjugation and nanofibres would further slow burst release to provide drug release over a longer time period. The control PCL/LVF electrospun mat showed a strong burst nature, releasing 59% of the drug in the 24 h. This fast release of LVF was attributed to a simple diffusion process of the drug.
through the matrix of PCL nanofibres [38]. The adsorption of LVF on MSN (control LVF-MSN) in the nanofibres slowed the initial release of LVF to 42% in the first day. The mitigation of the burst release of a drug from drug-nanoparticles composite nanofibres has been reported in the literature [26, 62-64]. For example, Song et al fabricated polylactic-glycolic acid/MSN composite mats for prolonged release of fluorescein [65]. They found the burst release of fluorescein was reduced compared to uniform drug loaded nanofibres. On the other hand, the release profile of PCL/conjugated LVF–MSN composite nanofibres was different to the other two nanofibres. The release was 20% after 1 day and gradually increased to 51% after 14 days. This indicates that LVF releasing behaviour was considerably influenced by covalent binding between LVF and MSN that can be cleaved due to the selective hydrolysis of the thioester bond [58, 61]. Ibuprofen conjugated polyethylene glycol hydroxyethyl thioester (PEG-HET) was fabricated by Davaran et al and slow release behaviour of ibuprofen was reported due to the hydrolysis of thioester bond between ibuprofen and hydroxyl ethyl thiol [58].

![Figure 4-10 The cumulative LVF release from PCL/LVF, PCL/Control LVF-MSN and PCL/Conjugated LVF-MSN electrospun mats.](image)

120
4.5 Conclusion

This chapter follows on from the previous chapter and describes a composite nanofibrous material which exhibits sustained drug release behaviour. The new material is formed through covalently binding the carboxyl group of the LVF to thiol functionalised MSN through the formation of a thioester bond. Characterisation was confirmed prior to spinning electrospinning. Such an approach significantly reduced the burst release property of the drug and led to a sustained release over 2 weeks in phosphate buffer. The findings show that chemically binding of a drug to a nanocarrier and distribution of the nanocarrier in the form of nanofibres provides a highly effective means of achieving sustained drug release efficacy due to cleavable bond between drug and nanocarrier that can be broken by hydrolysis attack in buffer solution.
4.6 References


Chapter 5

SLOW RELEASE OF LVF FROM PVA/CONJUGATED CHITOSAN COMPOSITE NANOFIBRES

This Chapter has formed the basis of a paper submission to the Journal of Controlled Release in March 2016. LVF was conjugated to chitosan and then blended into PVA solution and then composite nanofibres were electrospun. The new drug-polymer composite nanofibres showed superior release behaviour of highly soluble PVA nanofibres.
5.1 Abstract

Various drug-nanofibrous composite mats have often been reported as drug delivery systems. Their main disadvantage is burst release of drug immediately after application. The aim of this chapter was to fabricate composite nanofibrous mats based on the incorporation of chitosan to which LVF had been covalently bound. The study showed that controlled release of levofloxacin (LVF) could be achieved by covalently binding LVF to low molecular chitosan (CS) via a cleavable amide bond and then blending the conjugated CS with polyvinyl alcohol (PVA) nanofibres prior to electrospinning. PVA/LVF and PVA-CS/LVF nanofibrous mats were also fabricated as controls. The conjugated CS-LVF was characterised by FTIR, DSC, TGA and 1H-NMR. Scanning electron microscopy (SEM) revealed that the blended CS-PVA nanofibres decreased the fibre diameter compared to control PVA nanofibres. Drug release profiles showed that burst release was decreased from 90% in the control PVA/LVF electrospun mats to 27% in the PVA/conjugated CS-LVF mats after 8 h in phosphate buffer at 37°C, and gradual release in the latter was observed over the next 72 h. This slow release is due to the cleavable bond between LVF and CS that slowly hydrolysed over time at neutral pH. The results indicate that conjugation of the drug to the polymer backbone is an effective way of minimizing burst release behaviour and achieving sustained release of the drug, LVF in this case.
5.2 Introduction

Controlled slow release systems of drugs have been widely explored due to their advantages compared to other methods of drug administration [1, 2]. The advantages include reducing the side effects of general drug administration by delivering the drug at a controlled release rate, improved therapeutic efficiency, continuous sustained release of small amounts of drug instead of several large doses decreases side effects and improves of patient compliance [3]. In the past decade several drug delivery systems have been investigated for a controlled release of drugs from nanoparticles [4] and biodegradable nanofibres [5]. Also nanofibres have gained considerable attention as a drug delivery system in the last decade and have been proved to offer an efficient drug delivery system for providing sustained release of drugs [6, 7]. Various biodegradable nanofibres have been investigated for controlled release of drugs [8-10]. However, a major drawback of drug loaded nanofibres is the undesired burst release behaviour of drug from nanofibres [11-14].

For example, ciprofloxacin loaded nanofibres of poly(vinyl alcohol) (PVA) have been reported [14] with high initial burst release (80%) of total ciprofloxacin content in the first 40 h. The burst release of salicylic acid (SA) from poly(vinyl alcohol) nanofibres with different SA concentration was also reported [15]. The release profiles showed very high burst release within the first h with 40-65% of the total drug released and then increased rapidly after 10 h in all cases until reaching equilibrium with approximately 95% of total drug released. The burst release behaviour of the drugs may be due factors such as a large proportion of the drug on the fibre surface due to insufficient mixing and rapid drug diffusion out of the polymeric matrix because of fast polymer degradation [14, 16, 17].
In order to obtain the controlled slow release profile desired it is necessary to overcome the initial burst release. One approach reported in the literature for achieving a controlled release of drug is covalent conjugation of drugs to the polymer backbones [18-20]. This approach requires the presence of functional groups on both the drug and polymer on which chemistry can be done. For example, docetaxel (DTX) was covalently attached to low molecular weight CS via a cleavable amide linker. Prolonged slow release of DTX was obtained in simulated intestinal fluid (pH 7.5) which resulted in higher bioavailability along with prolonged circulation time in the blood stream than the intravenously injected DTX [21]. In another study, composite nanofibres of poly(lactide-co-glycolide) (PLGA) and poly(ethylene glycol)-g-chitosan-ibuprofen (PEG-g-CS-IBU) conjugates were fabricated by covalently linking IBU to the amine groups of chitosan via amide bond [22]. There it was observed that presence of conjugated CS-IBU in the matrix reduced the burst release due to the cleavable amide bond between chitosan and ibuprofen. Another report includes synthetising of doxorubicin conjugated stearic acid-g-chitosan polymeric micelles (DOX–CS–SA) [23]. The release profile showed that release behaviour of DOX is pH-dependent where the release rate of DOX increased with the reductions of the pH for release medium from 7.2 to 5.0.

PVA is widely used in biomedical applications due to its well-known characteristics such as biocompatibility, biodegradability, water solubility, nontoxicity, swelling capability in an aqueous medium, and appropriate mechanical properties [24-26]. It has been reported that by incorporating a second polymer component such as chitosan (CS) into the PVA solution, leads to an improvement in biocompatibility and mechanical properties (e.g. solubility) of the nanofibres. Chuang et al [27] fabricated PVA/CS blended membranes for cell culture. They observed that the PVA/CS blended membrane was more favourable for the cell culture with better spreading of cultured cells than the pure PVA membrane.
The aim of this chapter was to design a controlled drug delivery system by conjugating a LVF from the carboxylic groups to the amine groups of chitosan using carbodiimide chemistry [28, 29], followed by mixing the conjugated system with PVA prior to electrospinning. This method was used in order to reduce the burst release of drug from highly water soluble PVA nanofibres which has been reported [14, 15]. The release profiles of PVA/CS-LVF electrospun composite mats were measured and compared to the control PVA/LVF and PVA-CS/LVF (non-conjugated) electrospun composite mats.

5.3 Materials and Methods

5.3.1 Materials

PVA (MW 85,000-124,000, 98-99% hydrolysed), low molecular weight CS (deacetylation ≥ 75%), LVF (98.0%) and acetic acid were all purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) crosslinker was purchased from Pierce. Dimethyl sulfoxide (DMSO) was from Fisher Chemical and sodium hydroxide flake was from Chem Supply. All other chemicals were of reagent grade and used without further purification.

5.3.2 Methods

5.3.2.1 Synthesis of CS-LVF

The conjugation of LVF and CS was based on the mechanism of carbodiimide chemistry [28, 29]. CS (1g) was dissolved in 0.1M acetic acid solution (500mL). A mixture solution of LVF and EDC (1:1 molar ratio) was prepared by dissolving LVF and EDC in 10mL anhydrous dimethyl sulfoxide (DMSO). Then, the mixture solution was added dropwise to the CS solution under magnetic stirring continuously for 12 h. The conjugated CS was then coagulated by adding 1M NaOH to increase the pH level of the CS solutions. CS-LVF was collected by
centrifugation (15 min, 16000 g), washed by DI water and then dried at room temperature for 24 h.

![Diagram showing the preparation of CS-LVF](image)

**Figure 5-1** The preparation of CS-LVF

### 5.3.2.2 Preparation of Composite Electrospun Mats

Electrospun mats were prepared containing 1 wt% of LVF, and 5 wt% of CS-LVF (with respect to PVA). For polymer solutions 20mg LVF, 100mg CS-LVF were added to two separate vials containing 20 g of 10 wt% PVA (dissolved in water) and stirred continuously until a homogeneous solution was obtained. A solution of 10 wt% PVA-CS (9:1) was also prepared with 1 wt% LVF (with respect to PVA-CS). To obtain homogeneous solutions of PVA/CS, the pH of the PVA solution was adjusted to 6 by dropwise adding 1M HCL.

The basic experimental setup for the electrospinning process is shown in Figure 5-2. The setup contained a spinneret needle (23 G Terumo), syringe pumps (model NE-1000 New Era Pump Systems, Inc., Farmingdale, NY, USA), a high voltage supply (Spellman high voltage DC power supply, USA) and a 5 mL syringes (Terumo). The electrospinning distance was varied between 0 and 300 mm (optimised distance of 150 mm) and applied voltage was varied
between 0 and 40 (optimised value of 12 kV). Electrospun mats were collected for 10h at 0.6mL/h pump rate on a drum collector of 100 mm diameter.

**Figure 5-2** Schematic diagram of preparation of CS-LVF and PVA/CS-LVF nanofibres

### 5.3.3 Characterization

IR spectra were collected in the transmission mode using a Thermo Nicolet 6700 FTIR Spectrometer. DSC analysis of samples was carried out at a heating rate of 10°C/min under a nitrogen purge of 40ml/min using a Netzsch STA 449 F1 Jupiter DSC Thermal Analyser. TGA was performed using a Netzsch STA 449 F1 Jupiter TGA Thermal Analyser at a heating rate of 10°C/min under a nitrogen purge of 40ml/min. $^1$H NMR spectra of the samples were recorded on a Bruker Av400 NMR spectrometers. Scanning electron microscopy (SEM) analyses were carried out using a Philips XL30 field microscope. Fibre diameters were measured using the microstructure measurement software. For each sample, 100 measurements were taken to obtain an average fibre diameter. A Varian UV-Vis spectrophotometer was used to measure the LVF concentration at 290 nm.
5.3.4 In Vitro Drug Release

The electrospun mats were cut into squares of 50x50 mm. Each square was weighed and then placed in 5 mL of phosphate buffer saline solution (PBS, 100 mM, pH 7.4 and pH 5.0) and shaken continuously for 3 days at 150 rpm at 37°C. At each time points 1mL of release medium removed and replenished with an identical fresh release buffer. The amount of released LVF at each time point was determined by UV spectrophotometer. The UV absorbance of LVF in buffer solutions was measured at 290 nm and converted to the LVF concentration according to the calibration curve. LVF release profiles were calculated from the LVF concentration at each time points.

5.4 Results and Discussion

In this study we designed a novel drug delivery system in order to improve the release profile of highly soluble PVA nanofibres. The system is based on conjugating LVF to CS, combining them with PVA and then fabricating composite nanofibres by electrospinning. Again LVF was used as the model drug for its functional carboxylic acid group and ease of analytical detection by absorption at its peak at 290 nm.

5.4.1 FTIR

FTIR spectra of LVF, CS and CS-LVF were obtained and shown in Figure 5.3. Characteristic FTIR transmission peaks of LVF can be observed at 1294cm⁻¹ (assigned to stretching of amines), 1620cm⁻¹ for aromatic C-C, 1720cm⁻¹ due to carboxyl C=O and a broad peak at 3265 cm⁻¹ representing O-H of carboxylic acid. The peak assignment of CS shows characteristic bands at 3351 cm⁻¹ assigned to combined stretching of NH₂ and OH groups, 1643 cm⁻¹ due to NH₂ scissoring vibration, 1590 cm⁻¹ for carbonyl asymmetric stretching, and at 1028 cm⁻¹ due
to the C-O of the pyranose ring [30, 31]. The conjugated CS-LVF spectrum shows a peak at 1652 cm\(^{-1}\) due to a secondary amide band (C–O stretch of acetyl group); and the peak at 1575 cm\(^{-1}\) most likely due to N–H stretch of amide bond, confirming the formation of amide bond [29, 31-33].

Figure 5-3 FTIR spectra of LVF, CS and CS-LVF.

5.4.2 \(^1\)H-NMR

The conjugated CS-LVF was characterized and the formation of amide linkage between CS and LVF was confirmed by \(^1\)H-NMR spectroscopy. As shown in Figure 5-4, CS-LVF gave rise to a series of peaks at 4.60, 3.11, 3.90 and 3.72 ppm corresponding to \(^1\)H linked to the carbon
atoms in sugar ring (H_a, H_b, H_c, H_d, respectively) [33, 34]. The new signal appeared at δ=2.5–2.7 ppm corresponding to H_a due to the formation of an amide bond in between LVF and chitosan chains [34-36].

![Figure 5-4 1H-NMR spectra of LVF, CS and CS-LVF.](image)

**5.4.3 DSC**

Thermograms of LVF, CS, and CS-LVF are presented in Figure 5-5. DSC analysis was conducted to determine the interaction of CS with LVF. The DSC thermogram of pure CS indicated one exothermic transition at 309.6°C along with one endothermic transition at 101.9°C. The exothermic transition is indicative of CS degradation at 309.6°C and the
occurrence of an endothermic transition at 101.9°C could be attributed to the moisture present in samples or the presence of acetic acid moieties [37-39]. The DSC thermogram of LVF showed endothermic transitions at 94.2°C and 237.2 °C due to the decomposition of LVF. The CS-LVF conjugate showed a new sharp endothermic transition at 250.5°C that was neither present in thermogram of CS nor of LVF. This endothermic transition at 250.5°C could be hypothesized to have arisen due to carboxylic linkage between -COO⁻ moieties of LVF and -NH₃⁺ moieties of CS and formation of amide bond between LVF and CS [38].

![DSC thermograms of LVF, CS and CS-LVF.](image)

**Figure 5-5** DSC thermograms of LVF, CS and CS-LVF.

### 5.4.4 TGA

The thermogram of CS exhibits two distinct stages of weight loss. One is in the range of 30–107 °C due to the loss of adsorbed and bound water and decomposition of low molecular weights polymers (Figure 5-6). The second stage starts at 240 °C and continues up to 370 °C due to the degradation of chitosan biopolymer [40]. This stage is ascribed to dehydration of the
sugar rings, depolymerization and decomposition of the acetylated and deacetylated units of the polymer [41]. TGA spectra of the conjugated CS-LVF demonstrated slow weight loss at about 90 °C due to evaporation of water and moisture content in the polysaccharide. The second decomposition stage starts at about 195 °C which is lower than that of CS. The fast process of weight loss appears from 195 to 310°C. This is attributed to the thermal destabilization of the chitosan chains due to the conjugation of LVF in a polymer chain and related dissociation of the strong intra-molecular bonding [40, 42].

The morphology and fibre diameters of the electrospun mats with diameter sizes distributions are shown in Figure 5-7 (A-C). PVA electrospun nanofibres have uniform structure and fibres diameters compare to the PVA/CS composite nanofibres. The addition of CS in the electrospinning solution greatly influences the morphology of the electrospun nanofibres [43]. The fibre diameter distribution of the PVA/LVF nanofibres followed a normal distribution.

5.4.5 Morphology of the Nanofibres

The morphology and fibre diameters of the electrospun mats with diameter sizes distributions are shown in Figure 5-7 (A-C). PVA electrospun nanofibres have uniform structure and fibres diameters compare to the PVA/CS composite nanofibres. The addition of CS in the electrospinning solution greatly influences the morphology of the electrospun nanofibres [43]. The fibre diameter distribution of the PVA/LVF nanofibres followed a normal distribution.
Incorporation of chitosan reduced the fibre diameter by approximately 50%. The average fibre diameters were marginally decreased from 200-300nm to 100-200nm. This reduction in diameter is due to the presence of CS a cationic polysaccharide with amino groups, which are ionizable under acidic conditions. It has been reported that the addition of cationic and anionic polyelectrolytes would affect the conductivity of polymer solution thus result in thinner fibre [44, 45].

![SEM images of electrospun PVA/LVF (A), PVA-CS/LVF (B) and PVA/CS-LVF (C); and their relative diametere distribution.](image)

Figure 5-7 SEM images of electrospun PVA/LVF (A), PVA-CS/LVF (B) and PVA/CS-LVF (C); and their relative diametere distribution.
5.4.6 Cumulative Release of LVF

Figure 5-8 shows the release profiles of the LVF from the controls and two composite nanofibres; one with CS/LVF in the composition and the other with drug covalently attached to CS (Conjugated CS-LVF). The uniform PVA/LVF electrospun mat showed an extreme burst nature, releasing 90% of the drug in the first 8 h. This very fast release behaviour of LVF was attributed to the swelling properties and dissolution of the PVA matrix within the medium. It has been reported that as soon as the PVA matrix began to swell, LVF molecules were leached out from the matrix very quickly [46, 47]. A similar phenomena has been reported by Kenawy et al [48]; they observed a fast burst release of ketoprofen from PVA with a release of about 85.24% of the drug content within the first 2h.

The amount of released LVF increases with decreasing pH value. As shown in Figure 5.8 the addition of CS in to PVA matrix only slightly reduced the burst release to 82% after 8 h in pH 7.4. This indicated that addition of CS to the PVA matrix may have enhanced the swelling and dissolution properties of PVA [49]. The release profile of LVF from PVA-CS/LVF at pH 5.0 shows a faster release than LVF due to faster dissolution of CS in acidic medium. In other words, incorporation of hydrophobic CS into PVA nanofibres decreased the diffusion and therefore the release from the nanofibres. A recent report shows that composite nanofibres of PVA and polyvinyl acetate could reduce the burst release of ciprofloxacin to 55% after 24 h from 82% in control PVA nanofibres [14]. On the other hand, the release profile of the PVA/conjugated CS-LVF nanofibres at pH 7.4 was significantly different to the other two nanofibres. The release was 27% after 8 h and gradually increased to 59% after 72 h. At pH 5.0 it released 32% of LVF content after 8 h at pH 5 and reached to 85% after 72 h. This is due to the covalently grafted (via an amide bond) to CS. The release occurs first by CS dissociation and hydrolysis of amide bond from composite nanofibres and then by simple
diffusion. The cleavage of the amide bond depends on the pH value, the lower the pH value
the faster hydrolysis of amide bond and higher drug release from the composite nanofibres
containing conjugated chitosan [50]. These results indicate the notable stability of the amide
bond in buffered solution (at pH 7.4) which resulted in limiting the diffusion rate of LVF in the
matrix. A similar drug release profile has been reported by Jiang et al [22]. Composite
nanofibres of poly(lactide-co-glycolide) (PLGA) and poly(ethylene glycol)-g-chitosan (PEG-
g-CS) with ibuprofen (IBU) conjugated to CS were fabricated. They observed that the burst
release of IBU from PLGA membrane dropped from 85% to 20% in PLGA-PEG-g-CS (IBU
was covalently conjugated to the CS via amide bond) after 4 days. This slow release is due to
the amide bond between IBU and CS that can be cleaved in release medium. Another report
shows the slow release behaviour of IBU from polyethylene glycol hydroxyethyl amide (PEG-
HEA) due to the hydrolysis of amide bond between IBU and hydroxyl ethyl amide [51].

Figure 5-8 The cumulative release of LVF from: PVA/LVF, PVA-CS/LVF and PVA/Conjugated CS-LVF
electrospun mats (pH 7.4 and pH 9.0).
5.5 Conclusion

This Chapter describes a new composite nanofibrous material for achieving sustained drug release. The novel nanofibrous material combines covalently binding the carboxyl group of the LVF to the amine group of CS through the formation of a amide bond and confining the conjugated LVF into PVA nanofibres using electrospinning. Such an approach significantly reduced the burst release of the drug from uniform PVA nanofibres after 8 h and led to a sustained release over 72 h in phosphate buffer. The findings show that chemically binding of a drug to the polymer backbone of chitosan and distribution of the conjugated polymer in the nanofibres provides an effective means of achieving sustained drug release efficacy due to cleavable bond between drug and nanocarrier that can be cleaved by hydrolysis attack in buffer solution. Approximately 50% of the drug was delivered over 72 h.
5.6 References


41. Cai, H., Z.P. Zhang, P.C. Sun, B.L. He and X.X. Zhu, Synthesis and characterization of thermo-and pH-sensitive hydrogels based on Chitosan-grafted N-


Chapter 6

CONCLUSION AND SUGGESTIONS FOR FUTURE RESEARCH
6.1 Summary

The purpose of this thesis was to investigate mechanisms that can slow the release of a drug from nanofibres. Different composite nanofibrous mats were designed and fabricated and the release of the drug monitored. The research originated from the idea that the drug release profile of nanofibres could be improved by physical and chemical approaches. The objectives of the thesis have been successfully accomplished by slowing down the release profiles in order to reduce the burst release from conventional uniform biodegradable nanofibres and films.

The followings are the main conclusions obtained from this research:

- The first approach to achieve the slow release in this research has been carried out by physically adsorbing the drug into mesoporous silica nanoparticles and confining them in the core section of the core-shell nanofibres using core-shell electrospinning. The LVF-MSN complexes were characterized by different methods such as FTIR and BET analysis to prove the presence of the drug in the mesoporous structure of MSN. This combination method significantly reduced the burst release of the LVF from conventional biodegradable nanofibres. The slow release of drug from core-shell PCL/LVF-MSN composite nanofibres was achieved and the sustained release was followed over a two week period. As a result, the composite electrospun nanofibres exhibited strong antimicrobial activity even after they had been soaked in a phosphate buffer for 14 days. The findings show that physical adsorption of a drug on a nanostructure and distribution of the nanostructure in the core-shell nanofibres provides effective means to achieving sustained drug release efficacy.
• Besides physically adsorbing drug on to the nanoparticles, covalently binding the drug to nanoparticles was shown to have an important role in reducing the burst release of drug. This approach includes conjugating the drug to nanoparticles. The LVF-MSN conjugation were fabricated by covalently binding the carboxyl group of the LVF to a thiol functionalised MSN through the formation of a thioester bond. The conjugated complexes were characterized by IR and $^{13}$C CP MAS to confirm the successful covalent bond formation between the LVF and MSN. The release profile of LVF from the conjugated MSN showed slower release in pH 7.4 buffer solution than in pH 9.0 due to faster hydrolysis of thioester bond in alkaline solutions. The conjugated MSN then were fabricated into the nanofibres using electrospinning. This approach significantly reduced the burst release of the drug as compared to that from the control unconjugated electrospun nanofibres. Sustained release was achieved over 2 weeks in phosphate buffer. The results show that chemically binding of a drug to a nanocarrier and distributing drug conjugated nanocarriers in the nanofibres is an effective strategy to achieve sustained drug release via a cleavable thioester bond between a suitable drug and suitable nanocarrier that can be cleaved by hydrolysis attack at suitable pHS.

• The final approach in this thesis is about reducing the burst release of highly soluble PVA nanofibres. This approach including conjugating drug to another polymer as a carrier of drug and then blending conjugated polymer with PVA nanofibres via electrospinning. LVF was covalently bound to the amine group of CS via amide bond. The conjugation of CS-LVF was characterized and confirmed by FTIR, DSC, TGA and H-NMR. The conjugated CS-LVF was blended with PVA solution and the composite nanofibres were electrospun. The results showed that the burst release of LVF in PVA/LVF and PVA-CS/LVF considerably reduced in PVA/conjugated CS-
LVF. The slower release profile was achieved to the cleavable amide bond between LVF and CS that can be hydrolysed. The results showed that release of LVF is pH dependant; faster hydrolysis of amide bonds occurred in acidic buffered solution (pH 5.0) consequently resulting in a faster LVF release profile.

6.2 Further Work

Electrospinning is a promising method to fabricate a drug loaded composite membrane. However, there are still limitations in the electrospinning of nanofibres that requires improvement. Starting from electrospun composite mats investigated in this thesis, there are issues to be considered. Therefore, some suggestions are proposed for future work.

1. At the current stage, the biodegradable polymers studied in this thesis are all “bulk eroding” polymers, where the polymer degradation and erosion occurs through the bulk of the polymers. This issue leads to a fast release of drug molecules from the matrix. There is another class of biodegradable polymers which are called “surface eroding” polymers such as poly-anhydrides. The degradation and erosion in this class occurs via the layer by layer erosion of the surface of the polymer as new surface is exposed to an environment. This class of polymers is very expensive to synthesis so there are few studies reported on them. Employing polyanhydride as the main structure of the nanofibres matrix should be very promising in controlling the release of drug from the nanocarriers.

2. The electrospinning technique has room to improve specially co-axial electrospinning. There is a new “burn” electrospinning technique called tri-axial electrospinning. This technique can fabricate up to three layers in one nanofibre which can be designed as a
new nanofibrous drug delivery systems to achieve even longer drug release. This technique can contain the combination of different biodegradable polymers in each layer to achieve longer release profiles of one or more drugs simultaneously.
Publications and Conference Presentations

**J. Jalvandi**, Y. Gao, Y. B.Truong, M. White, R. Padhye, I. L.Kyratzis, Mesoporous silica nanoparticle and β-Cyclodextrin as biocompatible nanocarriers of levofloxacin, *Poster presentation at International Conference on Nanoscience and Nanotechnology (ICONN), Adelaide, Australia (February 2014).*

**J. Jalvandi**, Y. Gao, Y. B.Truong, M. White, R. Padhye, I. L.Kyratzis, Release profile of levofloxacin from poly(ε-caprolacton)/mesoporous silica nanoparticles co-axial electrospun composite mat, *Poster presentation at BIOENGINEERING CONFERENCES (BIOENG), Istanbul, Turkey (November 2015).*

