ELECTROSPUN ETHYL CELLULOSE NANOFIBERS WITH PHASE CHANGE MATERIALS: DESIGNING TEMPERATURE-RESPONSIVE DRUG DELIVERY SYSTEMS

Michael Wildy
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ELECTROSPUN ETHYL CELLULOSE NANOFIBERS WITH PHASE CHANGE MATERIALS: DESIGNING TEMPERATURE-RESPONSIVE DRUG DELIVERY SYSTEMS

by

Michael J. Wildy

A Thesis

Submitted to the
Department of Chemistry and Biochemistry
College of Science and Mathematics
In partial fulfillment of the requirement
For the degree of
Master of Science in Pharmaceutical Sciences
at
Rowan University
October 13, 2023

Thesis Chair: Ping Lu, Ph.D., Assistant Professor, Department of Chemistry and Biochemistry

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Xiao Hu, Ph.D., Professor, Department of Physics and Astronomy
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Dedication

I would like to dedicate this work to my loving and encouraging mother, Andrea.
Acknowledgments

I would like to thank my advisor, Dr. Ping Lu, for his tireless work in providing insightful feedback and setting high standards for my work. I also want to thank my coworkers, John Schossig and Wanying Wei, for their assistance throughout my time spent completing the thesis research.
Abstract

Michael J. Wildy
ELECTROSPUN ETHYL CELLULOSE NANOFIBERS FOR TEMPERATURE-RESPONSIVE DRUG DELIVERY SYSTEMS
2023-2024
Ping Lu, Ph.D.
Master of Science in Pharmaceutical Sciences

In this study, ethyl cellulose (EC) nanofibers loaded with either Rhodamine B (RhB) or Doxorubicin HCl (DOX) and phase change materials (PCM) were fabricated by blend electrospinning. EC is a cellulose derivative widely used as an excipient in the pharmaceutical industry and an ideal polymer for controlled drug release. Lauric acid (LA) and stearic acid (SA) were used as a material with a melting point close to physiological body temperature. Good drug-polymer compatibility and an amorphous distribution of drugs were shown by Fourier transform infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction. The release rate of RhB was shown to be dependent on both drug and PCM loading at 37°C. Samples containing phase change material also showed an increased release rate of 8% RhB when the temperature was increased from 37 °C to 40 °C. A stimuli-controlled release of DOX was demonstrated by an increase of 27% and 41% release rate at pH 7.4 and pH 4, respectively, when the temperature was increased from 37 °C to 40 °C. The comparison of RhB and DOX showed the influence of the selected drug on release rate. The reported electrospun drug delivery system shows promise for the temperature-responsive release of DOX over an extended time-period. This approach may prove useful in the treatment of solid tumors while reducing side effects and improving patient compliance and outcomes.
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Chapter 1

Introduction

1.1 Electrospinning

Electrospinning is a straightforward method for the fabrication of nonwoven or aligned continuous polymer fibers on the scale of nanofiber to microfiber. Nanofibers possess a high specific surface area due to their extremely small diameters and high density of fibers. The process uses a high-voltage power supply to stretch fibers as a polymer solution in a syringe is forced through a needle. The DC power supply can be adjusted from roughly 10-30 kV and the solution feed rate is controlled by a programable syringe pump. The high-voltage power supply is connected to the needle, which causes a buildup of columbic forces in the charged polymer solution cause a droplet at the spinneret to elongate into a Taylor cone. The repulsive forces cause a polymer jet to eject and accelerate from the tip of the Taylor cone. Elongation and coiling of the polymer jet is caused by instability and pulling towards the conductive collector as a result of the repulsive Columbic forces. Rapid solvent evaporation and polymer solidification produces the membrane, which is deposited on the conductive metal collector positioned at a set distance from the needle tip.¹

Uniaxial electrospinning (Figure 1) uses a single polymer solution and is the simplest configuration. Coaxial electrospinning involves the use of multiple polymer solutions to form fibers with core-sheath composite structure. Electrospinnable polymers include polystyrene (PS),² poly(methyl methacrylate) (PMMA),³ poly(vinyl alcohol) (PVA),⁴ polylactic acid (PLA),⁵ polyvinylpyrrolidone (PVP),⁶ polycaprolactone (PCL),⁷
etc. Environmental and processing factors can influence the properties of electrospun nanofibers, such as relative humidity, temperature, polymer concentration and solvent system, solution feed rate, voltage, needle tip diameter, and tip-collector distance, etc.¹

Electrospun membranes have shown promise in a variety of applications including catalysis, sensors, filtration, energy storage, tissue engineering, and drug delivery, as well as others.⁸⁻¹³

**Figure 1**

*Diagram of Uniaxial Electrospinning Apparatus Setup*
1.2 Biopolymers and Cellulose Derivatives

Biopolymers are polymers that are naturally occurring or synthesized from precursors found in nature, and thus renewable resources. In addition, these abundant polymers have properties beneficial to pharmaceutical/biomedical applications such as biocompatibility, biodegradability, and antibacterial. The electrospinning of numerous types of biopolymers has been reported. A major class of electrospinnable biopolymers is polysaccharides. Polysaccharides include cellulose/cellulose derivatives, chitin, chitosan, and dextran. Another notable class of biopolymers are proteins such as silk, collagen, and gelatin.

Cellulose is the most plentiful natural resource and the main biopolymer component in lignocellulosic biomass, the most abundant type of biomass with an estimated 181.5 billion tons produced annually. Cellulose is very widely available since it is the main component of plant cell walls. The chemical structure, shown in Figure 2, contains a linear polysaccharide containing repeating glucose units which are connected by linkages at the β-(1→4) positions. Each glucose unit contains 3 hydroxyl groups, which can be reacted via substitution reactions to synthesize various cellulose derivatives. The insolubility of cellulose in most organic and inorganic solvents precludes the use of cellulose as a polymer for electrospinning. The material properties of cellulose can be improved by derivatization. Some common cellulose derivatives that can be electrospun include cellulose acetate (CA), methyl cellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), and ethyl cellulose (EC). Vueba et al. studied the effect of excipients by comparing dissolutions of tablets with formulations
with MC, HPC, or HPMC and ketoprofen (KET). The release of KET from HPMC polymer formulation tablets was shown to be zero order.21

**Figure 2**

*Cellulose Chemical Structure*

1.3 Pharmacology and Controlled Release

The goal of controlled drug delivery is to transfer the correct amount of active pharmaceutical ingredient (API) to the active site. This can be achieved by targeting specific windows for drug absorption to increase bioavailability, with most controlled release drug delivery systems (DDS) developed to produce a sustained or pulsated drug release to specific parts of the gastrointestinal (GI) tract. The function of the GI tract is to absorb nutrients but is also an obstacle to overcome in drug delivery as degradation and metabolism of API results in lower bioavailability. Gradual delivery to the upper GI tract is more important for drug bioavailability than direct gastric absorption in the stomach.22
1.4 Electrospun Drug Delivery Systems

Nanofibers possess unique properties including high surface to volume ratio, structure mimicking the extracellular matrix (ECM), and inter/intrafiber porosity. Electrospun (ES) DDS have emerged as promising drug delivery vehicles suitable for a variety of modes of administration to treat a range of conditions (Figure 1). Additional advantages of ES DDS are their high loading and encapsulation efficiency as well as scalability. The nanofiber composition and properties can be tuned using several methods to further adjust the release of drug payloads. One of the factors that affects the rate of drug absorption is the route of administration. The superior properties allow for unique drug release kinetics and biocompatibility of ES DDS. The drug release behavior and material properties of ES DDS can be tuned for various applications, making them attractive alternatives to traditional API formulations. The most efficient mode of administration varies depending on the drug/ailment. ES DDS for applications ranging from transdermal, subdermal, sublingual, vaginal, ocular, and oral have been reported.
Most pharmaceutical formulations are prepared for oral drug delivery since it is noninvasive, cost-effective, convenient, and patient-friendly. However, some problems associated with oral medication are fluctuating plasma concentrations and non-specificity of drug delivery. The need for frequent administration of medication can result in lack of patient compliance or contribute to overdose and systemic toxicity.\textsuperscript{22} ES DDS for oral administration have been reported such as water soluble polymers for immediate release of drug,\textsuperscript{43} and insoluble polymers for sustained release of API.\textsuperscript{44} The localized delivery of API is optimal for treatment of diseases that are concentrated in a particular organ or body area, such as for cancer treatments and skin conditions. The flexibility and biocompatibility of ES membranes make them great candidates for transdermal
applications as wound dressings and show promise for in improving healing rate.\textsuperscript{34, 35} Transdermal delivery is classified as parental as it does not involve absorption of drug through the GI tract and allows for localized drug delivery. Implantable DDS are marketed for the continuous release of API over long periods of time. Systemic toxicity is especially an issue with chemotherapy drugs since they traditionally exploit the rapid growth of cancer cells but the selectivity is not sufficient to completely avoid killing healthy cells.\textsuperscript{45, 46} The serious side effects of chemotherapy drugs warrant a cost benefit analysis prior to starting a treatment regimen.\textsuperscript{47} A more efficient delivery of therapeutic agents to the active site would be beneficial to reducing the unwanted side effects. ES membranes have been gaining attention as potential implantable scaffolds for the extended release of chemotherapy drugs at the site of action, post-surgical tumor removal.\textsuperscript{9, 36, 37}

There are several techniques employed with ES DDS to achieve controlled API delivery. The properties of the polymer can be altered by blending multiple polymers in a single solution, using multiple polymer solutions, or using various solvent systems. Another method to control the release of API is the use of additional materials that are capable bonding to the drug or encapsulation,\textsuperscript{48, 49} such as nanocarriers.\textsuperscript{50, 51} The choice of polymer, in addition to the processing parameters, plays a large role in the release mechanism. The polymer properties contributing to the release of API are swelling in water, polymer-drug affinity, and degradation rate. Several studies have reported the effect of polymer type on drug release kinetics. A comparison of typical rapid, biphasic, and sustained release profiles is shown in Figure 4.
Figure 4

Comparison of Different Release Profiles

Table 1

Uniaxial Electrospun DDS Fabricated from Blends of Polymers, Drugs, and Additional Materials

<table>
<thead>
<tr>
<th>Polymer/Materials</th>
<th>Drug</th>
<th>Release Profile</th>
<th>Ref</th>
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<tbody>
<tr>
<td>EC</td>
<td>IMC</td>
<td>Sustained</td>
<td>52</td>
</tr>
<tr>
<td>EC</td>
<td>Quercetin</td>
<td>Sustained</td>
<td>53</td>
</tr>
<tr>
<td>EC/CA</td>
<td>IMC</td>
<td>Sustained</td>
<td>54</td>
</tr>
<tr>
<td>EC/PVP</td>
<td>Naproxen (Nap)</td>
<td>Sustained/tunable</td>
<td>35</td>
</tr>
<tr>
<td>EC, PVP</td>
<td>CIF</td>
<td>Sustained</td>
<td>55</td>
</tr>
<tr>
<td>EC/HPMC/Tween 80</td>
<td>IMC</td>
<td>Rapid</td>
<td>19</td>
</tr>
<tr>
<td>CA</td>
<td>Thymol (THY)</td>
<td>Sustained</td>
<td>34</td>
</tr>
<tr>
<td>PLA</td>
<td>DOX</td>
<td>Sustained</td>
<td>36</td>
</tr>
<tr>
<td>PLA/MSN</td>
<td>DOX</td>
<td>Sustained</td>
<td>37</td>
</tr>
<tr>
<td>PLA/GO</td>
<td>RhB</td>
<td>Controlled</td>
<td>50</td>
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Researchers compared the release of ciprofloxacin HCl (CIF), a fluoroquinolone antibiotic, from polyvinyl acetate (PVAc) and PVA. The hydrophilic property of PVA produced a vastly different release profile, with a complete release in less than an hour for PVA compared to up to a week for PVAc. Table 1 summarizes DDS fabricated by uniaxial ES from blends of polymers, drugs, and/or nanocarriers/surfactants for various controlled drug release applications.

One of the most straightforward drug delivery applications using ES fibers is enhancing the dissolution rate of poorly soluble API (class II and IV). Some examples include by incorporating ibuprofen (IBU) and KET in hydrophilic polymers such as

<table>
<thead>
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<th>Polymer/Materials</th>
<th>Drug</th>
<th>Release Profile</th>
<th>Ref</th>
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<tr>
<td>PLGA</td>
<td>CIF</td>
<td>Biphasic</td>
<td>56</td>
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<tr>
<td>PLA/PLGA/Si</td>
<td>Gentamicin sulfate (GS)</td>
<td>Controlled</td>
<td>5</td>
</tr>
<tr>
<td>nanoparticles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA/PEO</td>
<td>DOX</td>
<td>Biphasic</td>
<td>9</td>
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<tr>
<td>PCL</td>
<td>IBU</td>
<td>Sustained</td>
<td>57</td>
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<tr>
<td>PCL</td>
<td>Carvedilol (CVD)</td>
<td>Controlled/tunable</td>
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<td>PCL/PEO/Si</td>
<td>DOX</td>
<td>Sustained</td>
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<tr>
<td>nanoparticles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVP</td>
<td>Cyclosporine A (CA)</td>
<td>Rapid</td>
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</tr>
<tr>
<td>PVP</td>
<td>Metronidazole (MET)</td>
<td>Rapid</td>
<td>40</td>
</tr>
<tr>
<td>PVP/Eudragit RS100</td>
<td>CBD, CBG</td>
<td>Rapid</td>
<td>13</td>
</tr>
<tr>
<td>PVA, PVAc</td>
<td>CIF</td>
<td>Rapid</td>
<td>4</td>
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Researchers compared the release of ciprofloxacin HCl (CIF), a fluoroquinolone antibiotic, from polyvinyl acetate (PVAc) and PVA. The hydrophilic property of PVA produced a vastly different release profile, with a complete release in less than an hour for PVA compared to up to a week for PVAc. Table 1 summarizes DDS fabricated by uniaxial ES from blends of polymers, drugs, and/or nanocarriers/surfactants for various controlled drug release applications.
PVP$^{13}$ and PVA$^{4}$ as well other types of polymers including CA.$^{27}$ This was achieved by fabricating fibers containing amorphous solid dispersion (ASD) of API as opposed to the more energetically favorable crystal structure of the bulk API material.$^{61}$ The lack of crystal lattice lowered the energy barrier for the dissolution of the poorly soluble API into the release medium. Accordingly, the release rate was vastly improved compared to the bulk API in pure crystalline form.$^{6,31,43,62}$ The enhanced dissolution of API can improve the bioavailability of poorly soluble drugs and provide immediate relief. Lopez et al. reported PVP fibers loaded with amorphous distributions of indomethacin (IMC) and griseofulvin (GSF) that showed significant immediate release of API and excellent stability with API retaining amorphous form after 8 months.$^{62}$ The authors reported amorphous distribution of API when loaded up to 33.33% w/w after optimizing electrospinning process parameters. Andriotis et al. reported the first ES DDS for the rapid delivery of cannabinoids cannabidiol (CBD) and cannabigerol (CBG) using PVP and Eudragit polymers.$^{13}$ Their work demonstrated a potential rapid release DDS for the poorly soluble cannabinoids to be administered orally.

Sustained release of API can be achieved by controlling the rate of matrix hydration and/or the rate of drug diffusion from the polymer matrix. Hydrophobic polymers are excellent candidates for delayed and sustained release DDS owing to their low wettability.$^{4}$ Some hydrophobic polymers used in ES DDS designed for sustained release include EC,$^{44,53}$ PLA,$^{63}$ poly-lactic-co-glycolic acid (PLGA),$^{56,64,65}$ polyurethane (PU),$^{66}$ and PCL.$^{57}$ Factors to be considered for designing sustained release DDS include polymer choice, drug and its physicochemical properties, and formulation properties. Wu et al. studied the release kinetics of CIF from PLGA.$^{56}$ The authors described 3 phases of
drug release kinetics. The first phase, lasting for only a few hours, followed first-order and was controlled by polymer swelling. The second phase followed zero-order release kinetics and was controlled by the diffusion of drug to the fiber surface, lasting several days. The final stage of CIF release was facilitated by the enzymatic degradation of PLGA. Polymer-drug interactions in both solution and solid form affect the dispersion of API and release rate. Therefore, the physicochemical properties of drugs are important to consider since small differences can lead to disparities between the release of various drugs. Accordingly, the insight gained from release profiles of model drug from a DDS may offer limited relevance with respect to the use of other drugs.  

ES solutions containing a blend of polymers, typically hydrophilic/hydrophobic, in varying ratios is a technique used to control the release rate of the drug to enhance therapeutic efficiency.  

By changing the ratio of different polymers/materials, the properties of the composite fibers can be tuned to control the release of API. The release can be fine-tuned to better suit the application of the specific drug being delivered. An initial burst release is undesirable in most drug delivery applications and can be reduced by the addition of hydrophobic polymers to achieve biphasic or sustained release of API. EC is commonly blended with other polymers to reduce the initial burst release and improve the sustained release of API. However, in cases such as treating a bacterial infection, a burst release is beneficial for patient outcome. 

A biphasic release consisting of an initial burst followed by a sustained, steady release of the loaded drug can be obtained by finding an ideal polymer ratio. A blend of CA and EC polymers were reported to be used to produce ES DDS with tunable burst and
sustained release rates. Alternatively, the release rate can be increased with the incorporation of a water-soluble polymer, such as gelatin.

The morphology of the ES DDS can affect the interaction between the polymer matrix and release medium. Fibers with bead-on-string structure have generally been regarded as low quality; however, they may prove useful in drug delivery applications. The morphology of bead-on-string fibers creates varying polymer thickness throughout the mat and thus simultaneous dual passive diffusion rates of API from the polymeric matrix. Porosity can also be used to control the diffusion rate of API from the DDS. Porosity of ES fiber can be controlled using various phase separation methods. Solvent choice plays the largest role in the formation of pores on the interior of surface of ES fibers. The addition of a nonsolvent can cause de-mixing of polymer in the solution and the formation of pores. ES of PU in DMF was reported to produced porous fibers that demonstrated a biphasic release of itraconazole. Binary solvent systems composed of solvents with contrasting properties can be used to tuning of porosity and control the release of API from the ES DDS. This can increase the surface area can improve diffusion of API from the hydrophobic polymer matrix by allowing greater penetration of release medium.

The method used for the loading of API into ES DDS can determine drug distribution and affect drug release kinetics. The most straightforward method for drug loading is by mixing in a blend ES polymer solution. Another method is by emulsion ES with the addition of surfactants to form micelles in the polymer solution. Micelles have been studied for their usefulness as drug carriers, with compatibility with both hydrophilic and hydrophobic model drugs. Emulsion ES can encapsulate drug
containing micelles in the polymer for controlled release.\textsuperscript{64, 81} Drugs can also be loaded into ES fibers after fabrication via absorption of API in solution.\textsuperscript{82}

**Figure 5**

*Diagram of Coaxial Electrospinning and Core-Sheath Fiber Structure*

Another method is core loading in multi-layered fibers. Composite ES fibers made of a mixture of polymers can also be fabricated by feeding multiple syringes to a coaxial ES spinneret, producing core-sheath fibers, as shown in Figure 5. The ratio of polymers can be easily adjusted in coaxial needles by varying the solution feed rate ratios, resulting in varying core and sheath layer thickness. Hydrophobic polymers such as EC as the sheath polymer and hydrophilic polymers such as PVP or other polymers, as the core polymer, have been reported.\textsuperscript{83, 84} Core loading of API also enables control of burst release with the additional outer layer barrier and distance for the diffusion of API.
The coaxial method of fabricating ES DDS with blends of multiple polymers has been shown biphasic,\(^{83,85}\) sustained,\(^{86,87}\) rapid,\(^{88}\) or stimuli-responsive\(^{86,89}\) release of API. Coaxial ES has been reported to be used to produce fibers that demonstrated controlled release of 2 model drugs by loading each in separate layers.\(^{72,90,91}\) Modified triaxial ES using 3 solutions, an core of KET, an inner of EC and KET, and an outer of ethanol showed a reduction in burst release compared to blend of EC and KET.\(^{92}\) Recent coaxial ES fibers for controlled release drug delivery applications are summarized in Table 2 below.

Advanced materials have also been incorporated into ES DDS to enhance selectivity of drug delivery and achieve greater therapeutic efficacy. Nanocarriers have gained attention for their high drug encapsulation efficiency and biocompatibility.\(^{31}\) Some examples of nanocarriers include metal organic frameworks (MOF),\(^{48}\) and 2-D materials like graphene oxide (GO),\(^{51}\) and MXene.\(^{93}\) Interactions like \(\pi - \pi\) stacking (hydrophobic interaction) and hydrogen bonding can be used to bond the drug/model to a nanocarrier, such as GO.\(^{51}\) Some nanocarriers are sensitive to external stimuli, such as pH and temperature, allowing for a trigger for the release of API. The stability of the metal organic framework can be readily adjusted using different metals. Zeolitic imidazolate framework (ZIF-8),\(^{48,94}\) is a MOF that has gained attention for its usefulness in targeted drug delivery owing to its nontoxicity and instability at low pH conditions. Magnetism can also be used to targeted release of API from magnetic nanoparticles\(^{95}\) and composite nanofibers.\(^{96}\)
Table 2

*Coaxial Electrospun Controlled Release DDS Consisting of Various Core-Sheath Polymer Combinations and/or Nanocarriers*

<table>
<thead>
<tr>
<th>Core</th>
<th>Sheath</th>
<th>Drug</th>
<th>Release Profile</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP</td>
<td>EC</td>
<td>Maraviroc</td>
<td>Controlled/tunable</td>
<td>83</td>
</tr>
<tr>
<td>EC</td>
<td>PVP</td>
<td>Quercetin</td>
<td>Biphasic</td>
<td>85</td>
</tr>
<tr>
<td>Zein</td>
<td>EC</td>
<td>Quercetin</td>
<td>Sustained</td>
<td>84</td>
</tr>
<tr>
<td>EC</td>
<td>Ethanol</td>
<td>KET</td>
<td>Sustained</td>
<td>97</td>
</tr>
<tr>
<td>Chitosan</td>
<td>EC/nanosheets</td>
<td>DOX/FA</td>
<td>Sustained</td>
<td>91</td>
</tr>
<tr>
<td>PCL, PVA</td>
<td>PVP</td>
<td>Quercetin,</td>
<td>Rapid</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tamoxifen citrate (TC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEO</td>
<td>PCL</td>
<td>DOX</td>
<td>Sustained</td>
<td>98</td>
</tr>
<tr>
<td>Carboxymethyl</td>
<td>PCL</td>
<td>DOX</td>
<td>Controlled/Sustained</td>
<td>86</td>
</tr>
<tr>
<td>Chitosan (CMC)</td>
<td>PCL</td>
<td>DOX</td>
<td>Sustained</td>
<td></td>
</tr>
<tr>
<td>PLGA/PCL</td>
<td>Gelaton/Genipin</td>
<td>DOX</td>
<td>Controlled/Sustained</td>
<td>87</td>
</tr>
<tr>
<td>DOX</td>
<td>PLCL/Gelatin</td>
<td>DOX</td>
<td>Sustained</td>
<td>99</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>PLA</td>
<td>Salicylic acid</td>
<td>Sustained</td>
<td>77</td>
</tr>
<tr>
<td>Glycol (PEG)</td>
<td>PLA</td>
<td>(SA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVA</td>
<td>PMMA</td>
<td>CIP</td>
<td>Sustained</td>
<td>28</td>
</tr>
</tbody>
</table>
Phase change materials (PCMs) utilize latent heat and possess higher energy density than sensible heat while being more cost efficient than chemical heat. Latent heat of fusion is absorbed or released by PCMs during the freezing or melting processes. The high enthalpy of fusion of PCMs as well as their relatively small change in volume makes solid-liquid PCMs particularly attractive as thermal energy storage materials. PCMs also have narrow ranges of upper and lower phase transition temperatures which can meet the requirements of the working range for desired applications.

PCMs can be classified as either organic or inorganic. Inorganic PCMs include salt hydrates and metallics. Metals and metal alloys with low melting temperatures are usually not considered practical PCMs due to their weight limitations. Organic PCMs include paraffin waxes, poly(ethylene glycol)s (PEGs), alcohols, fatty acids (FA), and their derivatives and eutectics. Paraffin waxes are one of the most commonly used PCMs due to their high latent heat, good stability, and low corrosivity and cost. Octadecane is a paraffin based PCM with a melting temperature close to that of the human body and has potential as a PCM to be used in the textile industry. Some advantages of organic PCMs are nontoxicity and no phase separation upon heating/cooling cycles. Fatty acids commonly used as PCMs include stearic, lauric, myristic, capric, palmitic, and caprylic acids. Biobased PCMs are a newer type of organic PCMs and are derived from sources like plant oils and beef tallow. Biobased PCMs have high latent heat, low flammability, and good thermal stability as a result of being fully hydrogenated. Many PCMs have been incorporated into ES nanofibers,
including paraffin waxes, biobased PCMs, PEGs, fatty acids and eutectics as well as others.

The proper containment of PCMs inside a thermal energy storage system is a major challenge. A typical thermal energy storage (TES) system is composed of a PCM and a framework/supporting material, in which the PCM is either encapsulated or incorporated into a form-stable material. Both organic and inorganic frameworks/supporting materials have been used for TES systems. Creating a TES system with the smallest possible size is important to allow for almost instantaneous freezing and melting of PCMs, making ES TES systems very attractive. Various TES systems made of composite nanofibers have been developed by researchers. Uniaxial electrospinning of a blend of PCM and polymer is a simple way to fabricate nanofiber based TES systems, which has been used to fabricate TES systems containing PS, PLA, Nylon 6 (PA6), polyacrylonitrile (PAN) and polyethylene terephthalate (PET) as the support materials. Coaxial electrospinning of a PCM as the core and a polymer as the sheath has also been reported, in which TiO₂-polyvinylpyrrolidone (PVP), polyurethane (PU), polyvinylidene fluoride (PVDF), polyvinyl butyral (PVB), and cellulose acetate (CA) and other polymers were used as sheath materials.

Recently, researchers have aimed at utilizing PCMs to create composite nanofibers possessing melting points slightly higher than the physiological body temperature to allow for controlled release of various types of drugs. Various types of DDS containing PCM have been reported. Earlier PCM-DDS used hydrogels to encapsulate PCM and drugs. Additionally, thermally sensitive polymers, such as poly
(N-isopropylacrylamide), have been used to produce hydrogels as stimuli-sensitive DDS and incorporated into blend ES DDS. However, some drawbacks of hydrogels are their lack of control of drug release. Micro- and nanoparticle DDS containing PCM have also been reported. Gelatin particles containing fluorescein isothiocyanate-dextran (FITC-dextran) were produced by emulsion and encapsulated in microbeads of 1-tetradecanol, a PCM with a melting point of 38-39 °C. Drug release from the microbeads was shown to be negligible at 37 °C over 24 h, yet quickly increased to near 100% after increasing temperature to 39 °C. The effect of temperature response on drug delivery was also shown to depend on the polymer chosen, with the highest release rate being gelatin microbeads, followed by chitosan, and poly lactic-co-glycolic acid (PLGA), respectively. Vitamin E was reported to be encapsulated in a eutectic mixture of lauric acid (LA) and stearic acid (SA). The resulting nanocapsules containing LA/SA in a ratio of 3:1 and a particle size distribution (PSD) of 70-550 nm demonstrated an immediate release of vitamin E upon heating to 37 °C. Pan et al reported the use of ES DDS consisting of a blend of Eudragit® RS 100 and poly(methyl methacrylate) for the temperature responsive delivery of antimicrobial octenidine. However, the thermal switch “on” temperature of 37 °C, while the “off” temperature was 25 °C was observed. Hu et al. reported the incorporation of thermally sensitive polymer Poly(N-isopropylacrylamide) (PNIPAAm) electrospun in a blend with EC. The ES DDS’ properties displayed a switch from hydrophilic to hydrophobic when the temperature was increased above the critical temperature of 32 °C.

The use of an eutectic mixture of LA/SA (4:1) has been reported in several ES DDS. Inorganic nanomaterials including hollow SiO₂ nanoparticles with hole in the
wall,\textsuperscript{133} mesoporous SiO\textsubscript{2},\textsuperscript{134} and gold nanocages\textsuperscript{135} have been reported for the encapsulation of PCM for stimuli-sensitive DDS. Qiu et al reported the temperature responsive delivery of doxorubicin using LA/SA (4:1) encapsulated in silica-based nanocapsules with well-defined hole in the wall morphology.\textsuperscript{133} PCM can also be used to encapsulate API for stimuli-sensitive DDS. The temperature responsive delivery of nearly 100% Rhodamine B (RhB) in 10 minutes at 40 °C from electrosprayed microparticles composed of LA/SA shell and gelatin and RhB core was reported.\textsuperscript{49} Another PCM-DDS is nanofibrous hydrogels. Zhang et al reported temperature responsive release of aspirin from nanofibrous hydrogels containing LA/SA in a 4:1 ratio.\textsuperscript{136} Drug release after 48 h was found to be 87%, 56%, and 44% at 25 °C, 37 °C, and 40 °C, respectively. However, the sample preparation involved several post-ES steps. Gelatin methacryloyl (GelMA) nanofibrous hydrogels were ES and crosslinked under 365 nm ultraviolet lighting. LA/SA/aspirin was co-encapsulated in polydiacetylene (PDA) microspheres by oil-water emulsion, followed by Michael addition reaction between aminauric groups on GelMA and PDA at pH 8.5 to attach the microspheres to the nanofibrous hydrogels.

1.5 Materials

1.5.1 Ethyl Cellulose

EC was chosen as the polymer for ES of the DDS. EC is an ethyl ether cellulose derivative (Figure 6) synthesized via substitution of 44-51% of cellulose’s hydroxyl groups. The viscosities of EC solutions depend on the polymeric backbone chain length, with viscosity increasing with chain length. EC polymer can withstand the release of drugs and is included in tablet formulations as a binding agent and thickener. The
cellulose derivative is biocompatible, biodegradable, light and heat-resistant, nontoxic, colorless, and tasteless. EC is used in pharmaceutical, cosmetic, and food industries.\textsuperscript{137} The water-insoluble polymer commonly used for coatings of solid-dosage form and is also used for masking taste. EC is employed in modified release dosage form due to its hydrophobic properties and swelling capacity. Pharmaceutical effectiveness can be improved by controlled release of drugs at a steady rate to maintain consistent drug blood concentrations. EC coatings allow for extended modified release of pharmaceuticals due to the hydrophobic nature limiting the wetting of the polymer matrix. The release rate of drugs can also be modified with the use of different molecular weight EC polymers.\textsuperscript{138} The surface contact angle of EC films was reported to be 80.3-106.3°,\textsuperscript{139} depending on EC concentration and solvent used. The encapsulation of API in EC microspheres,\textsuperscript{140} microcapsules,\textsuperscript{141} and films\textsuperscript{137,142} for controlled release applications has been reported. EC nanofibers have the potential for modified release and targeted drug delivery due to the insoluble and biodegradable nature of the polymer. Accordingly, EC was the chosen polymer for the ES drug delivery system discussed herein.

\textbf{Figure 6}

\textit{Ethyl Cellulose Chemical Structure}

\[
\begin{array}{c}
\text{RO} \\
\text{OR} \\
\text{OR} \\
\text{OR} \\
n \\
\end{array}
\]

\[R = \text{H or CH}_2\text{CH}_3\]
EC is commonly used as a polymer in controlled release ES DDS. The hydrophobic and insoluble properties of EC limit the diffusion rate of API from the polymer matrix. The addition of varying ratios of EC to composite ES DDS can tune release kinetics of API for various drug delivery applications. Composite nanofibers DDS consisting of EC and hydrophilic polymers such as PVP and PEO, as well as zein, have been reported. Lipophilic drugs, such as KET, IBU, and IMC have good compatibility with EC, stabilizing the API in amorphous form. A benefit of using additional polymers with EC is the improved electrospinnability over EC alone. Additionally, numerous examples of coaxial ES of EC and other polymers, most commonly PVP, have been reported with various polymers. Ball et al. reported coaxially ES PVP core and EC sheath fibers loaded with antiviral maraviroc. The release of API was shown to be biphasic as well as adjustable by varying the thickness of EC outer layer. Nouri et al. reported the simultaneous release of chemotherapy agents folic acid (FA) and doxorubicin (DOX) from 2-D nanosheets encapsulated in coaxial ES fibers made of a chitosan core and EC sheath. The DDS showed continuous zero order release of API over 20 days. Another reported ES DDS produced from EC used bead-on-string morphology to achieve controlled release.

EC can be dissolved in various organic, polar solvents. While ethanol is the most commonly used solvent, it is not very ideal for ES. A binary solvent system of ethanol and water in an 80:20 ratio has been reported to produce optimal EC fibers. Other solvents such as dimethylformamide (DMF), dichloromethane (DCM), acetic acid, acetone, and tetrahydrofuran (THF)/dimethylacetamide (DMAc) have been used to produce EC nanofibers. The use of solvents with high vapor
pressure/volatility in mixtures with low vapor pressure/volatility solvents can produce pores on the surface of the fibers.\textsuperscript{76} Thus, the drug diffusion rate can be controlled by adjusting porosity. Coaxial and triaxial ES of EC with various solvents for the sheath fluid has been used as a technique to improve the electrospinning and quality of fibers.\textsuperscript{92, 97, 145}

1.5.2 Rhodamine B

RhB was used as a model of a hydrophilic drug. The chemical is a fluorescent xanthene dye (Figure 7) commonly used as for human-medial cell diagnosis and also found in paper, textiles, and cosmetic products.\textsuperscript{149} The dye was obtained from Sigma Aldrich in the chloride salt form. RhB is commonly used as a model hydrophilic drug in ES DDS.\textsuperscript{3, 7, 51, 64} The cationic chemical also contains a carboxylic group allowing for hydrogen bonding. This hydrophilic dye has a diameter of $\sim 1.5$ nm,\textsuperscript{150} similar to that of DOX,\textsuperscript{151} the target model drug of our research. The molecular weight of RhB and DOX are also very similar, 479 g/mol and 513 g/mol, respectively. The solubility of RhB in water, ethanol, and DMF allowed for the seamless integration of the dye into the blend polymer electrospinning solutions and subsequent nanofibers. Additionally, the resonance of the planar fused ring system can facilitate $\pi - \pi$ stacking for loading in 2-D materials, such as GO.\textsuperscript{51} These attributes allow for good compatibility with the polymer and increased stability. RhB produces a bright pink color with a wavelength of maximum absorbance at $\sim 554$ nm. This is far from the UV absorbance of EC, between $\sim 200-250$ nm, and therefore does not cause any interference when measuring RhB concentration of the medium. The strong intensity of the dye’s color also aided in its quantification in the release medium.
1.5.3 Doxorubicin

Doxorubicin HCl (DOX) was incorporated in the DDS as a model hydrophilic drug after the concept was validated with RhB. DOX is a common anthracycline chemotherapy drug (Figure 8) administered by IV to patients with various forms of cancer including sarcomas, carcinomas, and hematological.\textsuperscript{152} The mechanism of action is still unclear; however, DOX can inhibit macromolecular biosynthesis by interacting with DNA through intercalation and subsequently preventing DNA replication.\textsuperscript{153} DOX can also form free radicals that are capable of damaging both cell membranes and DNA. There is a need for improved selective delivery of DOX to the desired site to increase its efficiency and patient outcome. Serious side effects can lead to diminished quality of life as well as death for patients. This is due to injury of healthy tissues and vital organs, such as the heart, liver, kidney, and brain.\textsuperscript{152} An improved delivery system for DOX could alleviate unwanted side effects and increase the drug’s efficacy. Localized administration would be beneficial to avoiding systemic toxicity associated with intravenous drugs.
Treating tumors offers additional localized drug delivery potential due to the metabolic microenvironment in tumors. Some of these unique properties, such as acidosis and hypoxia, could be exploited as triggers for the targeted delivery of chemotherapy drugs. Therefore, stimuli-sensitive ES DDS present an emerging technique for localized chemotherapy delivery targeting tumor sites.

Figure 8

*Doxorubicin HCl Chemical Structure*

Several reported ES DDS containing DOX have been reported for the sustained release of DOX. The release of DOX from PLA ES DDS has been widely reported with or without nanocarriers, such as graphene oxide, mesoporous silica nanoparticles (MSNs). PLA or PLA polymer blends has been used to fabricate ES DDS containing DOX as implantable scaffolds. Gohary et al reported a coaxial ES DDS using a core solution containing PEO and DOX and PCL sheath. The release rate was shown to be controlled by varying the polymer solution feed ratios. A lower burst release over blend ES was observed as well as increased in vitro efficacy compared to pure DOX. However, a biphasic release of chemotherapy drugs may be more efficient
in suppressing tumor growth. An ES DDS made of a blend of PEO and PLA polymers showed biphasic release of DOX and good *in vivo* cancer treatment efficacy.\(^9\)

**1.5.4 Fatty Acids**

PCMs are commonly used for TES systems with application of storing latent energy;\(^{157}\) however, our research aimed at utilizing PCMs to create composite nanofibers with a melting point slightly higher than the physiological body temperature to allow for controlled release of various types of model drugs. Fatty acids (FA) are a class of PCMs suitable for DDS due to their biocompatibility, biodegradability, and stability. FA eutectics, mixtures of 2 or more fatty acids in specific ratios, have lower melting points than either FA alone. Lauric acid (LA) and stearic acid (SA), 12 and 18 carbon chain saturated fatty acids (Figure 9), respectively, were selected as the FA components in a binary eutectic mixture used as the PCM as the LA/SA eutectic mixture consisting of a 4:1 ratio has been reported to possess a sharp melting point around 39 °C,\(^{158,159}\) slightly above the normal human physiological temperature of 37 °C. The LA/SA in the composite material DDS can be melted at a temperature slightly above the eutectic FA mixture m.p. (i.e., 40 °C) to allow diffusion of API through the PCM and achieve targeted release of drug payload.\(^{49}\)
1.6 Methods

1.6.1 Experimental Objectives

EC was selected as the polymer for the fabrication of ES DDS. The sustained release and low burst release can be enhanced by the additional properties of external stimuli sensitive material. LA/SA and LA were selected as PCMs to impart temperature sensitivity to the composite nanofibers. The addition of PCM may improve the slow release of API from EC. Additionally, the thermal sensitivity may act as a switch for the increased release rate of API upon melting of PCM at elevated temperature. This DDS could be used for the targeted delivery of drugs. Temperature sensitive drug delivery can be used to increase the localized concentration of API, thereby possibly improving efficiency and patient outcome. RhB was used as a model drug in the first project to validate the concept of PCM increasing the release rate of API at elevated temperature and study the effect of PCM concentration to determine the optimal loading. A DDS made of EC nanofibers blended with PCM with model chemotherapy drug DOX to study the release kinetics and cytotoxicity.
1.6.2 Electrospinning

EC nanofibers were electrospun (ES) from a solution containing 20 wt% EC (rated at 9-11 mPa-s) in a binary solvent system of ethanol and water (80:20). Electrospinning parameters were optimized including polymer viscosity rating (9-11 mPa-s, 90-110 mPa-s), concentration (5-22%), solvents (DMF, ethanol), ethanol/water binary solvent ratios (70:30-82:18), solution feed rate (0.4-1.8 ml/h), needle tip to collector distance, voltage, needle size, coaxial ES with solvent, solvent vapor sheath, or solvent core (ethanol, water, acetone). ES solutions were prepared by the addition of solvent to the polymer and vigorous stirring for at least 24 hours prior to use. PCM and RhB or DOX were then added to the fully dissolved polymer solution and allowed to mix well overnight. Once optimized, all ES was conducted using a solution feed rate of 1 ml/h, voltage of 15 kV, and a needle tip to collector distance of 15cm.

1.6.3 Characterization

1.6.3.1 Optical Microscope. Electrospun fibers can be visibly seen by the naked eye when a sufficiently thick mat is deposited on the conductive collector. The chosen polymer and parameters employed dictate the rate and spread of the deposited mat, but it can typically be observed within a few minutes. A small amount of fibers collected on a glass microscope slide can be used to inspect the sample quality on an optical microscope before continuing to collect more of the sample. This technique can be used to obtain a rough assessment of the fiber quality by looking for beading and defects in the fibers as well as the production of particles.
1.6.3.2 Scanning Electron Microscope. The physical morphology of ES fibers is typically analyzed using scanning electron microscopy (SEM). SEM is type of microscope that uses a beam of electrons, excited by a high energy supply, to analyze the surface of a material by measuring the interaction of signal of the electron beam and sample. The insulating properties of polymers necessitate the use of gold plasma sputter coating to provide a conductive outer layer to the samples. The images obtained can provide an accurate representation of the surface and interior morphology (by examining the cross-section) of ES fibers. Publicly available computer software ImageJ allows for the determination of fiber diameter to further classify the sample. A Thermofischer Apreo FEI SEM was used to image all samples after gold sputter coating.

1.6.3.3 Fourier Transform-Infrared Spectroscopy. The integration of additional polymers, drugs, and PCMs can be confirmed by various instruments. Fourier Transform Infrared Spectroscopy (FTIR) is used to study the vibrational frequencies by measuring absorbance/transmittance of IR radiation and attributing them to the presence of different chemical bonds present in the sample. Attenuated total reflection (ATR) is a versatile, simple, and fast technique for the analysis of both solids and liquids. A crystal is used to allow the IR radiation to transfer back and forth between the sample before reaching the detector. The spectra are produced by converting the interferogram, a measurement of the attenuated IR beam after some of the energy has been absorbed by the sample. This characterization technique can provide insight into the physical form and distribution of API or other additives in a composite material. The major peak in API FTIR spectra is typically present in spectra of the DDS but shifted in wavenumber or disappear when the drug intermolecular interactions are minimized. This is the case when
API is distributed amorphously in a sample or weak intermolecular interactions, such as hydrogen bonding, between the API and polymer occur.\textsuperscript{32, 52, 54, 68, 97} A PE-Frontier FTIR spectrometer with ATR attachment was used to analyze all samples.

\textbf{1.6.3.4 Thermogravimetric Analysis.} Thermogravimetric analysis (TGA) was conducted using a TA SDT Q600 at a heating rate of 10.0\textdegree C/min under nitrogen gas. TGA is a thermal analysis technique that measures the weight of a sample as a function of temperature to determine the thermal stabilities, weight percents, and presence of various chemicals in a sample. Thermal degradation is typically performed under inert atmosphere (nitrogen, vacuum, or noble gases) for the simplest measurements. The simplest thermogram is a single sigmodal curve produced as the sample degrades over a temperature range. TGA can be combined with other instruments such as mass spectrometer and FTIR to further characterize the components in the sample and associate them to a specific temperature range of thermogram.\textsuperscript{161}

\textbf{1.6.3.5 Differential Scanning Calorimetry.} A common technique to measure melting temperature and heat of fusion is differential scanning calorimetry (DSC). DSC is a thermo-analytical technique that measures the difference in heat needed to change the temperature of sample and the reference material as a function of temperature. The recommended reference material for PCMs is alumina (Al\textsubscript{2}O\textsubscript{3}) since it has a well-defined heat capacity over the scanned temperature range. All DSC data was obtained using a TA Q2500 DSC at a temperature ramp of 5-10 \textdegree C/min in Tzero pans. During a phase change, the sample requires either more heat if it is exothermic (i.e., melting) or less heat if it is endothermic (i.e., exothermic) processes. DSC calculates the amount of heat absorbed or released by the sample during the temperature change and/or phase change by measuring
the difference in heat between the reference and sample. The DSC scans are overlaid and plotted as the DSC curve. The phase transition temperature range is between the phase transition temperature (i.e., melting or crystallization temperature), found as the onset of the line fitting of the rising portion of the peak, and the temperature corresponding to the peak. Latent heat of fusion is calculated as the area under the curve during the heating/melting process.\textsuperscript{162}

DSC can be useful in characterizing the distribution of drugs in polymeric DDS. The physical form of API can be investigated by analyzing the enthalpy of fusion of the sample in the temperature range corresponding to the melting point of the API. The melting of a crystalline material will produce an endothermic peak on a DSC thermogram as a result of energy released during breaking of bonds in the tightly packed crystal structure. The absence of an endothermic enthalpy peak in the range of the melting point of the API indicates the lack of highly crystalline structure, alluding to an amorphous distribution of API.\textsuperscript{19}

1.6.3.6 Goniometer. The static contact angles of the samples were measured using ultrapure water and a Rame Hart NRL C.A. goniometer with U5 series camera upgrade. Surface contact angles can provide insight into the material’s properties by measuring the interface of a solvent, most commonly water, air, and a solid material. Specifically, the contact angle between the liquid-vapor interface and surface of a solid material from the liquid side. The contact angle provides a measure of the propensity of a surface to be wetted.\textsuperscript{163} A contact angle of $\theta < 90^\circ$ is generally consider hydrophilic, while $\theta > 90^\circ$ is hydrophobic. Hydrophobic materials can be further classified as superhydrophobic if $\theta > 150^\circ$.\textsuperscript{164} A hydrophilic contact angle implies that the surface can
be wetted and allow for the solvent to spread across the surface. A hydrophobic contact angle alludes to the material being able to repel the droplet and not allow it to adhere to the surface. The permeation pressure of the release media can be quantified by contact angle/surface tension. The permeability of the matrix in the release media has been shown to be a limiting factor in the release of loaded drugs.\textsuperscript{165} The wettability, or degree of hydrophilicity, of a material is an important material property, especially on the nano-microscale, that can play a large roll on the diffusion of API from the polymer matrix.\textsuperscript{166} The contact angle of a material can be used to estimate the release behavior of drugs/biomolecules from a DDS.\textsuperscript{167} The addition of different surfactants has been used to lower the contact angle of hydrophobic polymer and illustrate the dependance of release rate of API on surface wettability.\textsuperscript{167, 168} Although additional mechanisms besides increased wettability were responsible for the increase in release rate of API from the hydrophobic polymer when surfactants were used. The wettability can also be adjusted by combining multiple polymers in specific ratios, a concept that can be easily employed by ES.\textsuperscript{169}

1.6.3.7 X-ray Diffraction. X-ray Diffraction (XRD) is a nondestructive analytical technique to study the crystallographic structure of materials by measuring the diffraction angle of incident x-rays scattered by the sample. The diffraction intensity is recorded over a range of angles ($\theta$). The diffraction peaks are shown in XRD patterns, a plot of diffraction intensity vs. diffraction angle. All XRD patterns were collected on a Bruker D8 Discover XRD using Cu Ka radiation at 40 kV and 40 mA with degrees/step and time/step at 0.02° and 0.5 s, respectively, at 20 from 5-90°. XRD is commonly used to analyze materials in solid-state and allows for determining the distance between atoms
and the composition and arrangement of atoms in the crystal lattice/unit cells. The incorporation of crystalline materials in a sample can be confirmed by XRD as diffraction patterns are unique to a material and the corresponding crystal structure. The crystal structure of the material provides information that can be used to further understand the release mechanism of the API from DDS. The physical form of API in DDS can be investigated using XRD and comparing the patterns of the composite DDS to that of the pure API.\textsuperscript{19,54} XRD data of pharmaceuticals can be used to gather insight into the structure of API but provides limited useful characterization information.\textsuperscript{170} Some pharmaceutical formulations require freeze-drying preparation and additives known as lyoprotectants to prevent crystallization of API. XRD has been used to study state of API and confirm the amorphous state of lyoprotectants.\textsuperscript{171}

\textbf{1.6.3.8 \textit{In vitro} Drug Release.} \textit{In vitro} drug release experiments generally follow or mirror the guidelines found in pharmacopeias. A common method for \textit{in vitro} drug release experiments is sample and separate (SS), where the dosage form is added to the release medium which is kept at a constant temperature and the drug concentration is measured by taking a sample of the release media.\textsuperscript{172} When available, the use of paddle method\textsuperscript{27,43,92,97,141} is optimal for reproducibility. Some drawbacks of the paddle method include expensive equipment and the use of 0.9 L of release media requiring a large amount of sample for each experiment. The temperature is typically kept constant at 37 °C by keeping the sample and release media in either an incubator shaker\textsuperscript{4,37,83} at 50-200 rpm or water bath.\textsuperscript{31,127} An alternative method involves the sample enclosed in dialysis tubing which is then immersed in the release medium.\textsuperscript{33} Concentration of drug released is measured by ultraviolet visible (UV-Vis) spectrometry or liquid chromatography (LC) to
determine the cumulative drug released and create a release profile. The release of RhB and DOX was analyzed by \textit{in vitro} drug release studies. The conditions used were temperatures of either 37 °C, 40 °C, or room temperature, 7.4 pH or 4 pH phosphate buffered saline (PBS) release media, and constant release medium volume. The release medium and samples were contained in 50 ml conical centrifuge tubes kept at a constant temperature in a water bath or incubator shaker. Drug concentrations were determined by measuring aliquots of release media using a Perkin Elmer Lamda 35 UV/Vis spectrometer and 10-point calibration curves, shown in Figure 10. This allowed for the calculation of total drug and percent drug released using equation 1 to produce drug release profiles. \textit{In vitro} drug release profiles are an essential tool for estimating the release mechanisms of drugs from the dosage form. These tests allow further optimization and development to improve release profiles.\textsuperscript{22} The experimental method was verified using a water-soluble polymer to prepare a RhB loaded fast-dissolving DDS. The release profile, shown in Figure 11 below, confirmed the method validity with a cumulative release of \~100\% RhB after 0.5 h.

\begin{equation}
\textit{Cumulative Drug Released} (\%) = \frac{V_r \sum_{i=1}^{n-1} C_r + V_r C_n}{M_d} \times 100\% \tag{1}
\end{equation}

Where $V_r$ is the volume removed for each aliquot, $C_r$ is the concentration of drug in the release medium, $V_t$ is the total volume of release medium, and $M_d$ is the total mass of drug encapsulated.\textsuperscript{173}
Figure 10

*Calibration Curves*

(A) y = 0.208x + 0.0189
   R² = 0.9992

(B) y = 0.0208x - 0.0028
   R² = 0.9977

*Note.* A) RhB and (B) DOX.

Figure 11

*Method Validation Release Profile*
Chapter 2

Exploring Temperature-Responsive Drug Delivery with Biocompatible Fatty Acids as Phase Change Materials in Ethyl Cellulose Nanofibers

2.1 Introduction

Cellulose, an abundant natural resource, forms the backbone of numerous applications due to its versatility.¹⁵ Cellulose derivatives, owing to their enhanced solubility, are commonly employed in electrospinning (ES) as opposed to pure cellulose. Ethyl cellulose (EC), a derivative synthesized via cellulose esterification, has emerged as a prevalent excipient because of its desirable attributes, such as biocompatibility, water insolubility, and its capability to modulate drug release. These qualities make EC an exemplary polymer for tablet coatings designed for continuous or extended release of active pharmaceutical ingredients (APIs) from orally administered formulations.¹³⁸ Additionally, the solubility of EC in ethanol, a non-toxic solvent, makes it ideal for electrospinning in pharmaceutical and biomedical contexts. Electrospun nanofibers laden with APIs have gained prominence due to their inherent ability to control drug release, thereby enabling versatility in treatment applications.⁴⁵,¹⁷⁴ The integration of materials sensitive to external stimuli such as pH or temperature can further augment the targeted efficiency of pharmaceutical delivery systems through electrospun nanofibers.

Conventional oral administration of medication has several limitations, including fluctuating plasma concentrations and a lack of specificity in drug delivery. These drawbacks often result in poor patient adherence due to the necessity of frequent drug administration, thereby escalating the risk of overdose and systemic toxicity. Systemic toxicity poses a particular challenge in the administration of chemotherapy drugs, which,
while designed to target rapidly growing cancer cells, often lack the selectivity needed to avoid harming healthy cells.\(^{45,46}\) The severe side effects necessitate a thorough cost-benefit analysis before initiating a treatment regimen.\(^ {47}\) Thus, an efficient delivery system that can accurately deliver therapeutic agents to the active site has the potential to reduce undesirable side effects significantly.

Drug delivery systems (DDS) developed using electrospun nanofibers loaded with APIs present a versatile platform for numerous administration methods and show potential for treating an array of medical conditions.\(^ {4,26-28}\) The unique characteristics of nanofibers, such as a high surface-to-volume ratio, structural resemblance to the extracellular matrix, and tunable inter/intra-fiber porosity, enable the design of innovative drug release behaviors appropriate for various pharmaceutical applications.\(^ {23-25}\) Additional benefits of electrospun DDS include high drug loading and encapsulation efficiency and scalability.\(^ {29,30}\) Moreover, the composition and properties of the nanofibers can be fine-tuned to control the release of drug payloads further. By adjusting the blend of polymers in electrospun solutions, typically involving hydrophilic and hydrophobic components in various ratios, the release rate of the drug can be controlled to optimize therapeutic efficacy.\(^ {3,33,68,69}\) EC, due to its hydrophobic properties that limit the diffusion rate of APIs, is a widely used polymer in controlled and sustained-release electrospun DDS.\(^ {33,44,54,69,72,83,85,129,143}\) Incorporating additional polymers or materials into EC during electrospinning can modify the release rate; helping to curb the burst release of the drug. Composite nanofiber DDSs, composed of EC and other polymers, have been documented in some studies.\(^ {19,33,52,70,72,83,85,143}\) An additional advantage of incorporating other polymers with EC is the enhancement of its electrospinnability compared to using EC alone. Polymer-drug
interaction can also influence the drug release rate. The encapsulation of drugs that exhibit low polymer compatibility can result in fewer drug-polymer intermolecular interactions, lower degree of amorphous distribution and higher chance of aggregation of drug molecules. Rapid burst release of the drug can occur due to accumulation of drugs on the surface. Alternatively, good drug-polymer compatibility can allow for improved control of drug release by maintaining an amorphous distribution of drugs.

In the present study, we have introduced PCMs into EC nanofibers via an electrospinning technique, thereby creating a temperature-responsive DDS. The PCMs operate as thermal switches, controlling the drug release based on temperature variations. PCMs, through their solid-liquid phase transitions, can effectively modulate the nanochannel dynamics in nanofibers, facilitating or restricting drug release in response to temperature fluctuations. Biocompatible, biodegradable, and stable fatty acids (FAs) offer a promising class of PCMs that can be effectively integrated into DDSs. Interestingly, eutectic mixtures comprising two or more FAs at specific ratios exhibit only a single melting point. Notably, a eutectic mixture of lauric acid (LA) and stearic acid (SA) manifests a sharp melting point around 39 °C at an LA/SA ratio of 4:1, slightly above the standard human physiological temperature of 37 °C, making it a fitting candidate for our DDS. Rhodamine B (RhB), a frequently employed fluorescent dye molecule, was selected as our hydrophilic small-molecule model drug. To our knowledge, this is the first report on a nanofiber-based DDS incorporating PCMs via blend electrospinning. The DDS demonstrated sustained drug release, along with a burst release at high drug loadings when PCMs were absent. Further, the LA/SA loadings impacted the release rate at 37 °C, thus suggesting the
tunability of drug release. The drug release experiments conducted below and above the PCM melting point underscored the temperature-mediated release characteristics of RhB. In addition, this study investigated the material properties and molecular interactions, providing valuable insights into the underlying mechanisms governing drug release.

2.2 Experimental Section

2.2.1 Chemicals and Materials

Ethyl cellulose (EC), a derivative of cellulose, was acquired as a solution (9-11 mPa·s at 25 °C, 5% in an 80/20 toluene/ethanol mixture) from TCI America. Rhodamine B (RhB, ≥95% as verified by HPLC), utilized as a model drug dye, was procured from Sigma-Aldrich. We employed two naturally occurring fatty acids, Lauric acid (≥ 98%, Sigma-Aldrich) with a 12-carbon chain and Stearic acid (SA, 97%, AGROS Organics) with an 18-carbon chain, as temperature-responsive phase change materials (PCMs) to regulate drug release. Standard solvents, including ethanol (200 proof, ACS grade) and anhydrous N, N-dimethylformamide (≥ 99.9%), were sourced from VWR and were used to dissolve EC, LA, and SA. Water purification was performed using a Millipore Direct-Q 8 UV water purification system, achieving a resistivity of 18.2 MΩ cm at 25 °C. All chemicals were utilized as received without any further purification.

2.2.2 Drug Encapsulation in Nanofibers Using Electrospinning

The electrospinning solutions were prepared by dissolving EC in a binary solvent mixture of ethanol and water at a 4:1 ratio to reach a concentration of 20 wt%. Both lauric acid and stearic acid were incorporated into the solutions at a weight-to-weight ratio of 4:1 (LA: SA). The concentrations of RhB and LA/SA were calculated relative to
the EC weight. The constituents and concentrations of these solutions, as well as the resulting nanofiber sample names, are outlined in Table 3.

A uniaxial electrospinning setup was used to generate the nanofibers. The apparatus, shown in Figure 12, consists of a programmable syringe pump (Legato 110, KD Scientific), a 22-gauge blunt metal needle, a high voltage source (ES30P-5W,
Gamma High Voltage Research), and a grounded aluminum foil collector. The distance from the needle tip to the collector was maintained at 15 cm. Voltage was set to 15 kV, and the solution was fed at a rate of 1.0 mL/h.

Figure 12

Schematic Illustration Showing the Uniaxial Electrospinning Setup and Nanofiber Formation

2.2.3 Characterization

Fourier Transform Infrared (FTIR) spectra were recorded using a PerkinElmer Frontier FTIR Spectrometer equipped with an ATR attachment. The morphological evaluation of the samples was performed via a Thermo Fisher Apreo FEI SEM after the samples were subjected to gold sputter coating. SEM images were analyzed using ImageJ to measure the average fiber diameters, with a sample size of at least 50 randomly
selected fibers. Thermal properties were analyzed through Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). DSC curves were recorded on a TA Q2500 DSC, employing a temperature ramp of 5-10 °C/min in Tzero pans. TGA curves were generated on a TA SDT Q600 using a temperature ramp of 10.0 °C/min under a nitrogen gas atmosphere. The crystalline structures of the samples were analyzed via X-ray Diffraction (XRD), employing a Bruker D8 Discover machine and Cu Kα radiation set at 40 kV and 40 mA. The scan parameters were set at 0.02° per step and 0.5 s per step at 2θ ranging from 5-90°. Surface wettability was evaluated by measuring the water contact angles using a ramé-hart A-100 NRL contact angle goniometer equipped with a U5 series camera.

2.2.4 In Vitro Drug Release

Experiments for in-vitro drug release were performed at different temperatures: 37 °C and 40 °C in a controlled water bath, as well as at ambient room temperature. For a typical procedure, nanofiber samples precisely weighing 10±0.4 mg were immersed in 30 ml of pH 7.4 Phosphate Buffered Saline (PBS) solution in 50 ml conical centrifuge tubes. At pre-set time intervals, 3 ml aliquots were carefully withdrawn from the solution and promptly replaced with an equivalent volume of fresh PBS. The concentration of Rhodamine B (RhB) in the release medium was determined with a PerkinElmer Lamda 35 UV/Vis spectrometer using a 10-point calibration curve.
2.3 Results and Discussion

2.3.1 Chemical Structure and Intermolecular Interactions

FTIR provides a reliable method to analyze intermolecular interactions between the polymer matrix and incorporated molecules, such as the model drug and PCMs, by identifying characteristic peak shifts and assessing molecular compatibility.\(^{28,54}\) Figures 13 and 14 depict the IR spectra of samples with the incorporation of RhB and LA/SA molecules. Major IR peaks for pure EC nanofibers were observed within the ranges of 900-1500 cm\(^{-1}\) and 2800-3000 cm\(^{-1}\) (Figure 13A). The identical IR spectra of pristine EC and EC nanofibers affirmed the unaltered polymer structure post-electrospinning. The alkane C-H bonds and C-O-C ether bonds in EC were represented by peaks within 2800-3000 cm\(^{-1}\) and at 1055 cm\(^{-1}\), respectively. The pristine RhB’s IR spectra revealed a strong peak at 1583 cm\(^{-1}\). The presence of a peak at 1591 cm\(^{-1}\) across all EC-RhB samples, containing varying RhB amounts (Figure 13B), corresponds to the carboxylic acid carbonyl (-COO) peak in RhB, further validating the model drug’s incorporation into the nanofibers. The increase in RhB content was accompanied by rising intensity at 1591 cm\(^{-1}\). The observed peak shift from 1582 cm\(^{-1}\) in pristine RhB to 1591 cm\(^{-1}\) in EC-RhB nanofibers suggests a weak intermolecular interaction between EC and RhB, most likely H-bonding. Figure 4 diagrams these possible H-bonding interactions, which could occur between EC’s hydroxyl group (as a proton donor) and RhB’s carboxylic acid carbonyl group (as a proton acceptor). Generally, weak interactions between drug and polymer in electrospun DDSs indicate their compatibility, as reflected in our findings.

In EC-RhB5-LASA nanofiber samples containing different amounts of LA/SA, several peaks emerged and intensified with increasing LA/SA content (Figure 14). The
peak at 1697 cm\(^{-1}\) in pure LA/SA (Figure 14A) corresponds to the carbonyl group of LA/SA, whereas in EC-RhB5-LASA5, EC-RhB5-LASA10, EC-RhB5-LASA20, and EC-RhB5-LASA40, it appeared at 1732, 1732, 1733, and 1734 cm\(^{-1}\), respectively (Figure 14B). A similar peak shift was also observed in the spectra of EC-LASA5, EC-LASA10, EC-LASA20, and EC-LASA40. This shift from 1697 cm\(^{-1}\) in LA/SA to 1732-1734 cm\(^{-1}\) likely results from H-bonding between the carbonyl group of LA/SA and the hydroxyl group of EC. It has been reported that additives, such as polyethylene glycol (PEG) and fatty acid methyl ester (FAME) derivatives, to the polymer solution of electrospun nanofibers act as plasticizers by reducing polymer crystallinity and modifying mechanical properties.\(^{179-181}\) Pure EC nanofibers exhibited a good degree of flexibility yet were difficult to peel from the collector as intact membranes without fracturing. We also observed that the mechanical properties of EC nanofibers improved after LA/SA addition, which could be a consequence of these intermolecular interactions.

**Figure 13**

*FTIR Spectra of EC Nanofibers with Different RhB Content*

![FTIR Spectra](image)

*Note.* (A) full range and (B) selected range.
Figure 14

*FTIR Spectra of EC-RhB5 Nanofibers with Different LASA Content*

![FTIR Spectra](image)

*Note.* (A) full range and (B) selected range.

Figure 15

*Intermolecular Hydrogen Bonding Between RhB/LASA and EC Molecules*

![Hydrogen Bonding](image)

Rhb or LA/SA

EC  
(R = CH₂CH₃)

Rhb or LA/SA
2.3.2 Thermal Stability and Physical State

Figure 16 presents the TGA thermograms, which illustrate our samples’ thermal stability and decomposition patterns. A single-step decomposition occurred between 260-400 °C for pure EC nanofibers, resulting in a 90.41% weight loss. The thermograms for EC-RhB1, EC-RhB5, and EC-RhB10 nanofibers displayed an initiation of decomposition at 185 °C, a temperature lower than that for EC, indicating RhB’s reduced thermal stability. The weight losses for these samples at 400 °C were 97.11%, 92.42%, and 84.49%, respectively, showing a decrease in weight loss with increased RhB loading—the lowest observed with a 10% RhB load. The TGA thermograms for LA/SA-loaded nanofibers exhibited two-step thermal decomposition curves. The first step, ranging between 110-280 °C, can be mainly attributed to LA/SA content, while the second curve, within 280-400 °C, corresponds to RhB and EC. The percentage weight losses for LA/SA, noted as 5.20%, 9.64%, 18.85%, and 36.62% for EC-RhB5-LASA5, EC-RhB5-LASA10, EC-RhB5-LASA20, and EC-RhB5-LASA40 nanofibers, respectively, increased with rising LA/SA concentrations. This progression in weight loss correlates with the concentration of LA/SA in solutions, thereby validating the encapsulation of greater amounts of LA/SA with higher PCM concentrations in electrospinning solutions.
The DSC thermograms, displayed in Figure 17, facilitated the examination of the melting point and enthalpy of both LA/SA and RhB present in the nanofibers. The thermogram illustrating a heating-cooling cycle of a 4/1 LA/SA mixture shows a sharp melting point at 39.06 °C (Figure 17A). To further investigate the physical state of RhB within the polymer matrix, we analyzed the melting point of EC nanofibers loaded with varying amounts of RhB. RhB’s melting point, observed at 203 °C, is immediately succeeded by thermal degradation—a finding corroborated by our TGA analysis. Interestingly, this melting point is not discernible in EC nanofibers containing 1%, 5%, and 10% RhB, indicative of RhB’s homogeneous and amorphous distribution within the blend electrospun nanofibers. This occurrence is attributed to the stabilizing effect of the polymer matrix on the energetically unstable amorphous state of RhB, as opposed to the relaxed crystal structure characteristic of pure RhB.⁶¹
The crystalline nature of RhB and LA/SA within EC nanofibers was further examined through XRD analysis, with patterns presented in Figure 18. The XRD patterns of pure EC nanofibers exhibited a broad halo, indicative of an amorphous structure (Figure 18A). The XRD of pure RhB, on the other hand, presented a highly crystalline structure evidenced by intense peaks. The two most prominent peaks of RhB were observed at 22.2° and 25.3°. However, the XRD patterns of EC nanofibers loaded with 1%, 5%, and 10% RhB did not reveal any discernible peaks, supporting the conclusion from the DSC analysis about the homogenous and amorphous distribution of RhB in the EC nanofibers. Similarly, the XRD of pure LA/SA showed a highly crystalline structure, as indicated by a significant peak at 21.4° and a smaller one at 25.0°. However, the diffractograms of EC-RhB5 nanofibers containing 5%, 10%, 20%, and 40% LA/SA revealed only a faint band at 21.6°, suggesting a homogeneous and predominantly amorphous distribution of LA/SA within the EC-RhB nanofibers. Additionally, a small
peak appears alongside the broad halo at 7.0° in the EC-RhB5-LASA nanofibers, indicating a weak interaction between the fatty acids LA/SA molecules and EC-RhB molecules (Figure 18).

**Figure 18**

*XRD Patterns of (A) EC Nanofibers with Different Amount of RhB and (B) EC-RhB5 Nanofibers with Varying LASA Content*

2.3.3 Surface Characteristics

The surface wettability of EC nanofibers with varying RhB and LA/SA loadings was evaluated through water contact angle measurements, presented in Figure 19. The water contact angle of pure EC was 144.2°, falling within the hydrophobic range. The incorporation of up to 10% hydrophilic RhB exerted negligible impact on the surface wettability of the nanofibers, signifying that the majority of RhB remained encapsulated within the EC nanofibers (Figure 19A). To further verify the changes in surface wettability resulting from the LA/SA presence on nanofibers, we tested EC nanofibers incorporating 5%, 10%, 20%, and 40% LA/SA without RhB. The contact angles for EC-
LASA5, EC-LASA10, EC-LASA20, and EC-LASA40 remained comparable to those of EC nanofibers, with a slight difference of just 3.6° (Figure 19B). This consistent trend underlined that the LA/SA did not influence the surface wettability of the EC nanofibers. Considering the chemical structures of fatty acids LA and SA, they feature lengthy lipophilic aliphatic chain tails (12 and 18 carbons respectively) along with a hydrophilic carboxylic acid head. The long carbon chains are anticipated to govern the interaction with water, given their known lipophilic nature in acidic form.\(^{182}\)

Intriguingly, the introduction of both LA/SA and RhB to the electrospinning solution yielded nanofibers that exhibited reduced hydrophobicity, as suggested by lower water contact angles compared to pure EC nanofibers (Figure 19C). This augmented wettability became evident during in vitro release experiments, where membranes containing higher LA/SA concentrations rapidly dispersed in the release medium rather than sticking to themselves upon submersion. Moreover, the EC-RhB5-LASA40 nanofibers were observed to sink to the bottom of the release container over several days, indicative of their enhanced water absorption. This decrease in the water contact angle could be attributed to a heightened presence of RhB on the surface, facilitated by LA/SA. The RhB presence on the nanofibers was visually corroborated by the intensifying pink hue with increasing LA/SA content, where EC-RhB5-LASA40 nanofibers exhibited the deepest color among these four samples despite the consistent RhB content (5%) in all nanofibers. This observation was ascribed to the exterior mix of RhB with LA/SA on the nanofibers.
2.3.4 Nanofiber Morphology

Figure 20 depicts the SEM images of EC nanofibers containing varying concentrations of RhB or LA/SA. The pure EC nanofibers, displayed in Figure 20A, are characterized by rough surfaces with wrinkles and irregular shapes, such as elongated beads. Among the samples, EC-RhB1 nanofibers demonstrate the highest surface
porosity. In contrast, EC-RhB5 nanofibers present lower porosity and varied fiber sizes, indicating reduced uniformity. EC-RhB10 samples exhibit a mostly smooth surface with visible pores and fewer small fibers, revealing an improvement in quality over EC-RhB5. The inclusion of RhB led to an increase in average fiber diameters, measured at 0.354 µm for pure EC and 0.477, 0.513, 0.524 µm for RhB1, RhB5, and RhB10, respectively (Figure 20A). The introduction of LA/SA affected the fiber quality, especially at higher concentrations. EC-RhB5-LASA5 samples show more significant fiber irregularities and nonporous surface morphology. EC-RhB5-LASA10 and EC-RhB5-LASA20 samples, however, display improved fiber uniformity. The EC-RhB5-LASA40 samples are notable for their larger, interconnected fibers with interfiber junctions, suggesting a significant presence of LA/SA on the nanofiber surface. The average diameters of EC-RhB5-LASA with 5%, 10%, 20%, and 40% LA/SA loadings correspondingly increased with LA/SA concentrations (Figure 20B).
Figure 20

SEM Images of As-Spun Nanofibers

Note. (A) EC, (B) EC-RhB1, (C) EC-RhB5, (D) EC-RhB10, (E) EC-RhB5-LASA5, (F) EC-RhB5-LASA10, (G) EC-RhB5-LASA20, and (H) EC-RhB5-LASA40. The scale bar in (A) applies to all images.

Figure 21

Nanofiber Diameters of (A) EC-RhB and (B) EC-RhB5-LASA Samples with Varying RhB or LA/SA Loadings Before and After Release Experiments at 37 °C
2.3.5 Model Drug Release Kinetics

The *in vitro* release profiles of model drug RhB from nanofibers are depicted in Figure 22, where release kinetics were investigated under various conditions. In the case of EC nanofibers with differing RhB loadings (Figure 22A), EC-RhB1 and EC-RhB5 both exhibited slow initial release rates. However, with EC-RhB10, a minor burst release was apparent, most likely due to a significant amount of RhB being located near the surface. EC-RhB1 demonstrated a steady release pattern over the first week, which then intensified, an outcome attributable to the nanofibers’ high porosity and low mechanical strength. Contrasting this, the release rate for EC-RhB5 declined after one week, an observation that could be explained by the combination of increased mechanical strength and lower surface porosity. EC-RhB10 nanofibers showed a burst release of about 12% within the first 4 hours. After 4 weeks, cumulative RhB releases for EC-RhB1, EC-RhB5, and EC-RhB10 were found to be 21%, 7%, and 42% respectively. SEM images (Figure 23) confirmed that the EC nanofibers were preserved, supporting the assumption of a diffusion-controlled mechanism of drug release. Water contact angles on all samples after RhB and/or LA/SA encapsulation (Figure 19) confirmed the hydrophobicity of nanofibers, facilitating a sustained RhB release from EC-RhB1, EC-RhB5, and EC-RhB10 nanofibers.

Examining the influence of LA/SA loading with a constant RhB loading of 5% (Figure 22B) led to some intriguing findings. With increased LA/SA loading, the release rate escalated, culminating in a substantial burst release at 37 °C for the EC-RhB5-LASA20 and EC-RhB5-LASA40 samples. EC-RhB5-LASA5 exhibited a slow and steady release, with approximately 8% of RhB released after 168 hours, an increment of
2.5% over the release from EC-RhB5 nanofibers. Meanwhile, EC-RhB5-LASA10 showed release profiles with a minor initial burst release of 19%. The pronounced burst releases observed for EC-RhB5-LASA20 and EC-RhB5-LASA40, 77% and 83% respectively within the initial 4 hours, indicate that LA/SA loading significantly impacts release kinetics. The increase in release is probably due to the majority of RhB molecules migrating towards the surface and the resultant increase in wettability due to the increased LA/SA loading (Figure 8). This suggests that biocompatible fatty acid PCMs could serve as alternative materials to modulate the API’s release rate, facilitating controlled release.

The RhB release from EC-RhB5-LASA10 nanofibers was measured at room temperature, 37 °C, and 40 °C (Figure 22C). The release rates displayed marked differences during the initial 4 hours. The samples kept at room temperature had the smallest burst release of 7% RhB, while those kept at 37 °C and 40 °C had burst releases of 20% and 28% RhB, respectively. The increase in fiber diameter post-release at 40 °C compared to 37°C points to a higher degree of penetration of the release medium and polymer swelling, validating the temperature-responsive behavior of this drug delivery system.

Moreover, the substantial increase in the release rate of 32% after 240 hours at 37 °C when compared to room temperature, underscored the potential of these nanofiber-based DDSs for controlled release under normal physiological conditions, and additionally, for temperature-responsive release at elevated temperatures. The DDS’s ability to release the API under physiological conditions without any additional stimuli can potentially reduce the widespread overuse of antibiotics. After the initial burst
release, a steady release was observed, indicating a biphasic controlled release. While an initial burst of API is typically undesirable, the succession of an initial weak burst release followed by a sustained release could potentially improve patient outcomes.\textsuperscript{71}
Figure 22

*RhB Release Profiles*

Note. (A) EC-RhB nanofibers with 1%, 5% and 10% RhB loadings at 37 °C, (B) EC-RhB5-LASA with 5%, 10%, 20%, and 40% LA/SA loadings at 37 °C, and (C) EC-RhB5-LASA10 at ambient temperature, 37 °C, and 40 °C.
Figure 23

SEM Images of Nanofibers Post In Vitro Release at 37 °C

Note. (A) EC-RhB1, (B) EC-RhB5, (C) EC-RhB10, (D) EC-RhB5-LASA5, (E) EC-RhB5-LASA10, (F) EC-RhB5-LASA20, and (G) EC-RhB5-LASA40 nanofibers. (G) SEM Image of EC-RhB5-LASA10 nanofibers post in vitro Release at 40 °C. The scale bar in (A) applies to all images.

SEM images of nanofibers following in vitro release have shed light on the diffusion-controlled mechanism underlying drug release. Figure 12 illustrates the SEM images of EC-RhB1, EC-RhB5, and EC-RhB10 (Figure 23, A-C) after in vitro release at 37 °C. Interestingly, the morphology of all the nanofibers post-release closely resembled that of the as-spun samples (Figure 21). This consistency can be attributed to the stability of EC in aqueous solution, owing to its water-insoluble nature and slow biodegradability. Nonetheless, an increase in fiber diameters was observed across all samples post-release, signifying the penetration of the release medium and swelling of the nanofibers (Figure
21). The SEM images reaffirm that the majority of the polymer nanofiber structure was retained following in vitro release.

The SEM images of EC-RhB5-LASA5, EC-RhB5-LASA10, EC-RhB5-LASA20, and EC-RhB5-LASA40, also displayed in Figure 23D-G, revealed no visible surface pores in any samples containing LA/SA, after in vitro release at 37 °C. A distinct observation, however, was made in the post-release SEM image of EC-RhB5-LASA10 at 40 °C (Figure 23H), where a highly porous surface morphology was apparent. This change is attributed to the melting of LA/SA on the nanofibers. The fiber diameter increased from 0.544(±0.198) μm before release to 0.926(±0.531) μm after release at 40 °C, a result that verifies the significant swelling of nanofibers due to the solid-to-liquid phase transition of LA/SA at this temperature. Notably, this marked an increase in comparison to the fiber size following release at 37 °C, which measured 0.615 ± 0.323 μm. Our results confirmed the underlying release mechanism and emphasized the complex interplay of temperature and material properties in determining the release profiles.

2.4 Conclusions

In this study, temperature-responsive EC nanofibers encapsulating PCMs (i.e., LA/SA) and RhB, a model dye drug, were meticulously investigated. EC nanofibers were successfully fabricated for the encapsulation of RhB and LA/SA using a straightforward blend electrospinning technique. FTIR spectra demonstrated favorable drug-polymer compatibility, and XRD and DSC analyses confirmed the amorphous physical state of RhB within the nanofibers. RhB release kinetics were found to be dependent on drug loading, with a slow release at 1% and 5% RhB loadings and a burst release at 10% RhB
loading. By manipulating the LA/SA loading, the RhB release rate was made tunable, with nanofibers containing 5% and 10% LA/SA demonstrating steady release at 37 °C. The PCM-integrated nanofibers exhibited distinct temperature-mediated release patterns, including a 32% increase in the release of API from room temperature to 37 °C and an 8% release rate increase when the temperature was raised to 40 °C. These findings underscore the potential of EC nanofibers for the controlled release of chemotherapy drugs, with the ability to fine-tune drug release through variations in LA/SA concentrations and drug loadings. The successful integration of PCMs into EC nanofibers has unveiled a novel approach to creating stimuli-responsive DDSs, paving the way for further exploration and optimization and holding significant promise for advancing personalized medical treatments.
Chapter 3

Temperature-Responsive Delivery of Chemotherapy Drugs from Ethyl Cellulose Nanofibers Incorporating a Biocompatible Fatty Acid Phase Change Material

3.1 Introduction

According to the CDC, cancer was the 2nd leading cause of mortality in the US in 2022.\textsuperscript{183} Cancer treatment regimens using traditional chemotherapy drugs requires careful planning due to the narrow therapeutic window of earlier chemotherapy drugs with non-specific mechanism of action.\textsuperscript{184} Doxorubicin (DOX) is one of the most commonly used Anthracycline, a class of traditional chemotherapeutic drugs that exploits the rapid growth of cancer cells. DOX has several mechanisms of action, including free radical formation and topoisomerase II inhibition.\textsuperscript{58} Unfortunately, the selectivity of DOX is insufficient to avoid affecting healthy cells as well.\textsuperscript{45, 46} The high dosage required for effective treatment with DOX delivered by IV infusion leads to a significant risk of exposure to toxic levels by patients, which has been linked to multi-organ toxicity.\textsuperscript{185, 186}

Another challenge in cancer treatment is the recurrence of tumor growth. The most effective treatment for solid tumors is surgical removal. Yet, the persistence of immunosuppressive cells known as tumor-associated macrophages (TAMs) allows for tumor recurrence and patient mortality over the long term despite immunotherapy administration following surgical tumor removal.\textsuperscript{187} A more efficient delivery of therapeutic agents to the active site is beneficial since it allows for a lower dosage of active pharmaceutical ingredient (API) to be administered and lowers the risk of toxicity and side effects.
Electrospinning is an efficient technique for encapsulating stimuli-sensitive materials or drug carriers within fibers to control the release of API. The nonwoven mats of electrospun fibers are excellent materials for biomedical applications, owing to their high surface area-to-volume ratio and biocompatibility. Controlled drug delivery offers advantages such as targeted and sustained release that can achieve steady plasma drug concentrations and improved bioavailability. Temperature-sensitive drug delivery systems (DDS) containing phase change materials (PCMs) hold the potential for targeted drug release using elevated body temperature or other forms of targeted heating such as near-infrared light (NIR) or magnetic nanoparticles to trigger drug release.

Cellulose-derived biopolymers are sustainable, renewable, and biocompatible, making them excellent polymer candidates for drug delivery systems. Ethyl cellulose (EC) is a hydrophobic and water-insoluble cellulose derivative widely used in the pharmaceutical industry for controlled-release tablet coatings and more recently for electrospun nanofibers with controlled-release applications. Electrospun membranes loaded with chemotherapy drugs show potential for use as implantable scaffolds. The release and cytotoxic efficacy of DOX-loaded polylactic acid (PLA) nanofibers with potential applications as biomedical scaffolds have been reported. Additional encapsulation of DOX in nanocarriers such as graphene oxide (GO) and mesoporous silica nanoparticles (MSNs) using PLA nanofibers has been shown to offer additional control of drug release.

Nouri et al. reported the sustained release of chemotherapy agents folic acid (FA) and DOX over 20 days from 2-D nanosheets encapsulated in chitosan-EC core-sheath fibers fabricated by coaxial electrospinning. Li et al.
demonstrated sustained release and inhabitation of bacterial growth using PVP/EC blend nanofibers containing antibiotic ciprofloxacin (CIF). In a previous study, we demonstrated the temperature-responsive drug delivery of Rhodamine B (RhB) from ethyl cellulose nanofibers containing a eutectic mixture of fatty acid PCMs - lauric acid (LA) and stearic acid (SA) - in a 4:1 ratio, which were fabricated using a simple blend electrospinning technique. This work encapsulated DOX, RhB, and LA in EC nanofibers by electrospinning. LA is a fatty acid, a class of biocompatible and biodegradable PCMs suitable for biomedical applications. The incorporation of PCMs in DDS can be used to achieve temperature-mediated control of API release. The melting of PCM allows for enhanced diffusion of API from the DDS. A lowered melting point of the composite nanofibers to a temperature range suitable for drug delivery applications allowed for the use of LA instead of LA/SA as PCM in the current DDS. Additional comparison studies using RhB showed decreased electrospinnability and fiber quality with LA/SA, while no difference in temperature response of drug release was observed when LA was used. DOX was chosen as a model hydrophilic chemotherapy drug, while RhB, a dye with similar properties, was studied for comparison. The drug release was measured at various temperatures and pH to examine the effect of different stimuli on the drug release behavior. The reported DDS demonstrated temperature and pH-dependent release profiles. Drug properties were also found to influence release kinetics. In vitro cell viability assay showed sustained inhibition of HEK-293 cells treated with EC-DOX-LA nanofibers.
3.2 Experimental Section

3.2.1 Chemicals and Materials

Ethyl cellulose (EC) was obtained from TCI America (9-11 mPa·s in 80:20 toluene/ethanol at 25 °C). Rhodamine B (RhB, ≥95% verified by HPLC), obtained from Sigma-Aldrich, and Doxorubicin HCl (DOX, ≥95% verified by HPLC), obtained from TCI America were used as model hydrophilic drugs. Lauric acid (LA) (98%) was obtained from Sigma Aldrich. Ethanol (200 proof, ACS grade), obtained from VWR, was used to dissolve EC, LA, and RhB. Pure water with a resistivity of 18.2 MΩ·cm at 25 °C, obtained by filtration using a Millipore Direct-Q 8 UV water purification system, was used for dissolving DOX and preparing electrospinning solutions.

3.2.2 Encapsulation of Drug/PCM in Nanofibers

Electrospinning solutions were prepared by dissolving EC in a binary mixture solvent system of ethanol: water (4:1). Polymer solutions were vigorously stirred overnight following the addition of the drug to ensure even distribution. Electrospinning solutions containing 20 wt% EC (EC) and 10% LA (EC-LA), 1% DOX (EC-DOX-LA), and 1% RhB (EC-RhB-LA) were prepared. PCM and drug concentrations were calculated relative to polymer weight. Electrospinning was conducted using a uniaxial setup (Figure 1). The uniaxial ES setup used consisted of a programmable syringe pump (Legato 110, KD Scientific), a 22-gauge blunt metal needle, a high voltage source (ES30P-5W, Gamma High Voltage Research), and a grounded flat aluminum foil collector. The distance to the conductive collector was kept at 15 cm, a constant 15 kV voltage was applied, and the solution feed rate was set at 1.0 mL/h to prepare all samples.
3.2.3 Characterization

Fourier transform infrared (FTIR) spectra were collected using the ATR attachment of a PerkinElmer Frontier FTIR. The surface morphology of gold sputter-coated samples was analyzed on a Thermo Fisher Apreo FEI scanning electron microscope (SEM). SEM images were used to determine the average diameter of samples by measuring at least 50 representative fibers on ImageJ. Differential scanning calorimetry curves (DSC) were collected on a TA Q2500 DSC with $T_{\text{zero}}$ pans and a temperature ramp of 5 °C/min under nitrogen gas. Thermogravimetric analysis (TGA) curves were collected on a TA SDT Q600 using a temperature ramp of 10.0 °C/min under nitrogen gas. X-ray Diffraction (XRD) patterns were collected on a Bruker D8
Discover XRD using Cu K\(\alpha\) radiation at 40 kV and 40 mA with 0.02° per step and 0.5 s per step at 20 over the range of 5-90°. Static contact angles were measured using ultrapure water and a ramé-hart A-100 NRL C.A. goniometer connected to a U5 series camera. Fluorescence microscopy images were recorded on a Thermo Fisher Invitrogen EVOS M5000 fluorescence microscope in GFP mode.

### 3.2.4 In Vitro Release

*In vitro* drug release experiments were conducted at 37 °C and 40 °C in an incubator shaker and at ambient temperature on an orbital shaker. Nanofiber samples weighing 20 +/- 0.4 mg were submerged in 20 ml of pH 7.4 phosphate-buffered saline (PBS) in 50 ml conical centrifuge tubes. Aliquots of 3 ml were retrieved at predetermined intervals and immediately replaced with an equal volume of fresh PBS. PerkinElmer Lambda 35 UV/Vis spectrometer was used to measure the concentrations of RhB and DOX in the release medium using 10-point calibration curves. Degradation of DOX in pH 7.4 PBS was measured, and cumulative drug concentrations were adjusted accordingly.

### 3.2.5 Cytotoxicity Assay of Nanofibers

HEK-293 cells were cultured in a DMEM supplement medium containing 10% FBS, 1% L-glutamine, 1% penicillin, and 1% streptomycin inside an incubator with 5% CO\(_2\). Cells were collected via trypsinization and resuspended in fresh DMEM. Cells were seeded (60,000 per well and 1 mL final volume) in a 24-well plate and incubated for 24 h at 37 °C. Samples were sterilized under UV light with a wavelength of 254 nm for three hours. Nanofibers were suspended in 1 mL of fresh medium for exchange at 24 h. Positive control (free DOX), negative control (fresh medium), and 25 µg/ml of total drug
concentrations were used. The morphology of cells was monitored on a Thermo Fisher Invitrogen EVOS M5000 fluorescence microscope at 24, 48, and 72 h.

3.3 Results and Discussion

3.3.1 Chemical Structure and Intermolecular Interactions

FTIR spectra allowed for analysis of the intermolecular interactions of polymer and drug molecules and drug-polymer compatibility. Figure 2 shows the IR spectra of LA, RhB, DOX, EC, and EC fibers with drugs. EC nanofibers spectrum displayed major peaks in the 3000-2800 cm$^{-1}$ and 1500-900 cm$^{-1}$ ranges (Figure 2A).

Figure 25

*FTIR Spectra of EC, RhB, DOX, LA, EC-DOX-LA, EC-RhB-LA, and EC-LA*

![FTIR Spectra of EC, RhB, DOX, LA, EC-DOX-LA, EC-RhB-LA, and EC-LA](image)

*Note.* (A) full and (B) select-range spectra.

The alkane C-H bond vibrations attributed peaks in the 3000-2800 cm$^{-1}$ range. The characteristic peak at 1055 cm$^{-1}$ was attributed to the C-O-C ether bonds in EC. The major peaks observed in the pristine LA spectrum were in the regions of 3000-2800 cm$^{-1}$,
also corresponding to alkane C-H bonds, and a major peak at 1697 cm\(^{-1}\) attributed to a carbonyl group (-COO). The IR spectrum of pristine RhB showed a characteristic peak at 1583 cm\(^{-1}\), also attributed to a carbonyl group. However, this peak was not observed in the spectrum of EC-RhB-LA. Similarly, the spectra of EC-DOX-LA did not show any peaks associated with DOX. The absence of these representative peaks of DOX/RhB in EC-DOX-LA/EC-RhB-LA suggests that the drug molecules were efficiently embedded in the EC nanofibers and, therefore, not on the surface. However, the signal intensity was likely not strong enough due to the low DOX/RhB concentrations (1%). Select-range spectra (Figure 2B) highlight the characteristic peak corresponding to LA. The carbonyl group peak, seen at 1697 cm\(^{-1}\) in the spectrum of pristine LA, was observed at 1737, 1737, and 1732 cm\(^{-1}\) in the spectra of EC-LA, EC-RhB-LA, and EC-DOX-LA, respectively. The slight shift observed in peaks was attributed to H-bonding between the carbonyl of LA (proton donor) and the oxygen of ether in EC (proton acceptor).

Furthermore, we observed improved mechanical strength of EC nanofibers after adding LA to the blend solution, possibly resulting from compatibility and intermolecular interactions arising between EC and LA.

### 3.3.2 PCM/Drug Encapsulation

The incorporation of DOX and RhB was confirmed through visual inspection. EC and EC-LA membranes were white, while EC-DOX-LA and EC-RhB-LA membranes displayed orange and pink colors, respectively (Figure 26). Fluorescence microscopy was used to verify the presence of API in the composite nanofibers. Fluorescent images of DOX and RhB under 20X magnification (Figure 27) displayed strong fluorescence, confirming the presence of both DOX and RhB in nanofibers. Furthermore, the
distribution of DOX and RhB appears homogenous, as seen by the continuous fluorescence throughout the nanofibers.

**Figure 26**

Photos of (A) EC, (B) EC-LA, (C) EC-DOX-LA, and (D) EC-RhB-LA Nanofiber Membranes

**Figure 27**

Fluorescence Microscope Images of (A) EC-DOX-LA and (B) EC-RhB-LA Nanofibers

*Note.* The scale bar in (A) applies to both images.

DSC thermograms were used to analyze the melting point of PCM and composite nanofibers as well as to provide insight into the distribution of drug molecules. The DSC thermograms of pristine LA and EC nanofibers containing 40% LA (Figure 28A)
demonstrated a decrease from a melting point of 44.18 °C in bulk PCM material to 37.38 °C after encapsulation in the composite nanofibers. The additional broadening of the fusion temperature range and lowering of PCM melting point indicated a predominately amorphous and homogeneous distribution of LA. While the PCM concentration in EC-DOX-LA was kept at 10%, the LA concentration in EC nanofibers was increased to 40% for DSC measurements to ensure an adequate PCM phase transition heat flow signal. Ethyl cellulose-lauric acid interactions allowed for the lowering of the solid-liquid phase transition temperature, achieving melting slightly above the normal physiological body temperature. Phase transitions were not observed in the thermograms of EC-DOX-LA or EC-RhB-LA nanofibers (Figure 28B) upon heating above the melting point of pure RhB or DOX, 229 °C and 203 °C, respectively. The absence of heat flow corresponding to the heat of fusion of DOX or RhB in the nanofibers suggests amorphous solid dispersion of API. DDS containing amorphous solid dispersions (ASDs) of API have been electrospun from EC and other polymers. The EC polymer matrix stabilized RhB or DOX molecules in amorphous form.
TGA thermograms (Figure 29) were used to confirm PCM loading in nanofiber samples. The TGA thermograms of pure EC nanofibers displayed a single thermal decomposition of 93.39% weight loss at 600 °C. Pristine LA also showed a single thermal decomposition in the 150-240 °C range, corresponding to a weight loss of approximately 100%. As a result, EC-DOX-LA and EC-RhB-LA nanofibers displayed two-step thermal decomposition, with the first step occurring in the temperature range of 240-300 °C, attributed to LA content, and a second curve in the range of 300-400 °C, attributed to EC and DOX or RhB. Percentage weight losses at 310 °C, used to estimate LA loading, were found to be 9.79% and 10.14% for EC-DOX-LA and EC-RhB-LA nanofibers, respectively.
The crystallinity of drug-loaded composite nanofibers was analyzed using X-ray diffraction data for composite nanofibers, pristine drugs, and PCM powders (Figure 30). XRD patterns of EC, EC-LA, EC-DOX-LA, and EC-RhB-LA nanofibers showed a broad halo, indicative of amorphous material. In contrast, pristine LA, RhB, and DOX showed highly crystalline structures with intense signals in their XRD patterns. Major peaks in pristine RhB were present at 22.2° and 25.3°, while DOX displayed several crystalline peaks, with the most prominent peaks at 16.7°, 22.6°, and 25.1°. Sharp peaks were observed in the XRD of pristine LA at 6.7°, 9.7°, and 24.2°. The characteristic humps of amorphous forms of EC, LA, DOX, and RhB in composite nanofibers suggest homogeneous distribution and amorphous dispersion of drug and PCM molecules, agreeing with DSC data. These results further prove the good compatibility among EC, DOX/RhB, and LA.
Figure 30

$XRD$ Patterns of $EC$, $EC$-$LA$, $EC$-$DOX$-$LA$, $EC$-$RhB$-$LA$, $DOX$, $RhB$, and $LA$
3.3.3 Surface Morphology and Properties

Figure 31 shows SEM images of pure EC, EC-DOX-LA, and EC-Rhb-LA (Figure 31, A-F) nanofibers with irregular diameter and smooth surface morphology. These SEM images provided further evidence of efficient LA and DOX/Rhb encapsulation in EC nanofibers. The addition of the drug and PCM led to the formation...
of slightly enlarged fibers. The average diameter of nanofibers increased from 418 nm for pure EC to 520 nm and 526 nm for EC-DOX-LA and EC-RhB-LA nanofibers, respectively. All nanofiber samples displayed nonuniform fibers and fiber defects, resulting in a wide distribution of fiber diameters (Figure 31, G-I). Yet, the distribution range of diameters of nanofibers was observed to increase after the addition of DOX/RhB and LA. The retention of smooth surface morphology and absence of excess material on the surface of EC-DOX-LA and EC-RhB-LA nanofibers confirmed the successful encapsulation of both DOX/RhB and LA within the EC nanofibers.

The surface wettability of nanofiber membranes was analyzed by water contact angle measurement. EC, EC-LA, EC-DOX-LA, and EC-RhB-LA membranes displayed hydrophobicity with contact angles of 143.6°-144.2° (Figure 32). Altering the wettability of ES DDS can affect polymer dissolution and drug release rates. Lauric acid, a saturated fatty acid ester containing a nonpolar 12-carbon long acyl chain, is predominantly hydrophobic in nature. Adding fatty acid LA and hydrophilic model drugs DOX and RhB did not alter the surface wettability of samples. The retained hydrophobicity of samples further verified the encapsulation of most DOX and RhB within nanofibers.
3.3.4 In Vitro Drug Release

In vitro drug release profiles of DOX and RhB under different conditions are shown in Figure 33. Retention of EC’s hydrophobicity in EC-DOX-LA and EC-RhB-LA allowed for sustained and controlled stimuli-responsive drug release. Release profiles of DOX at ~25°C, 37 °C, and 40 °C in pH 7.4 release media (Figure 33A) were examined to probe the temperature effect on drug release under normal physiological acidity. Burst release of 30%, 37%, and 61% at 4 h were observed for samples at ~25°C, 37 °C, and 40 °C, respectively. At 96 h, the cumulative release of DOX was found to be 56%, 73%, and 100% at ~25°C, 37 °C, and 40 °C, respectively. A temperature-responsive release of DOX was observed when the temperature was increased from ~25°C to 37 °C, resulting in an increase of 17% DOX released. Furthermore, the temperature rise from 37°C to 40 °C allowed for an additional boost of 27% in release. The ethyl cellulose-lauric acid interactions and resultant amorphous state of LA caused a shift in the solid-liquid phase transition temperature, allowing for melting slightly above the normal physiological body
temperature. The diffusion of DOX molecules trapped within water-insoluble EC fibers facilitated the melting of LA at the elevated temperature, thereby creating a thermal switch that can be activated by raising the temperature to 40 °C.

The pH effect on the release rate of DOX was analyzed under acidic conditions of pH 4 (Figure 33B). Notably, the response of DOX release to temperature increase was found to be greatest under acidic in vitro conditions. The cumulative DOX released at 96 h in pH 4 PBS was approximately 56% and 98% at 37 °C and 40 °C, respectively, representing a substantial increase of approximately 41% upon increasing the temperature. The solubility and stability of DOX are much higher in pH 4 PBS than in pH 7.4 PBS. DOX is known to precipitate in pH 7.4 PBS due to dimerization.200 Interestingly, the initial release rate of DOX in pH 4 was lower, with approximately 27% release after 4 h at 40 °C, compared to approximately 61% released at pH 7.4. The reduction in burst release and delayed release in acidic pH conditions was unexpected, considering the higher solubility of DOX at lower pH. Furthermore, at 96 h, the cumulative release of DOX at 40 °C and pH 4 was only approximately 2% less than the respective release at pH 7.4.

The cationic form of DOX resulting from the protonation of amine at low pH has been reported to cause unfavorable interactions between DOX and electrospun hydrophobic polymers Polycaprolactone (PCL) and Polyglycolic lactic acid (PLGA).201 Faster release of DOX at pH 5 compared to pH 7.4 was due to unfavorable interactions, and increased hydrophilicity was reported.201 The pKₐ of the primary amine of DOX is reported to be 8.3.202 Accordingly, the cationic protonated form of DOX is present in higher concentrations at pH 4 than 7.4. The effect of pH on release rate is likely caused
by changes in the compatibility of DOX and EC, resulting from higher cationic DOX concentrations present at low pH. However, the rate of penetration of the release medium into water-insoluble ethyl cellulose was retarded under acidic pH conditions, and the protonation of DOX was not immediate. This was observed in the reduction of burst release of DOX in pH 4 release media at both 37 °C and 40 °C. The pH-responsive release of DOX has profound implications considering the application of the DDS as a scaffold for implantation directly at tumor sites. Notably, the pH in the microenvironment surrounding tumor cells is known to be acidic and slightly elevated in temperature.\textsuperscript{154} Additional inflammation could raise the temperature further. Environmental stimuli provided by the microenvironment of tumor pH may be exploited by the reported stimuli-responsive DDS to increase the localized delivery and efficacy of DOX. Moreover, the DOX release kinetics at pH 4 and 7.4 showed promise for treating recurring tumor growth. An initial burst release is beneficial to preventing tumor cell growth, while a sustained release allows for an extended inhibition of remaining tumor cell growth.\textsuperscript{203, 204}

Release profiles comparing the release of DOX and RhB from EC nanofibers containing 10% LA (Figure 33C) demonstrated the dependence of release kinetics on drug properties. While the molecular size and weight of DOX and RhB are very close\textsuperscript{150, 151}, the solubility of RhB in pH 7.4 PBS is higher than DOX. After 4 h, significant burst releases of 57% and 73% were observed at 37 °C and 40 °C, respectively. Increased solubility of RhB allowed for a greater extent of release at the physiological body temperature of 37 °C compared to DOX. An increase of 28% release of RhB was noted when the temperature was raised from ~25 °C to 37 °C. Due to the substantial burst release at 37 °C, a less pronounced temperature-responsive effect of drug release was
observed when the temperature was elevated to 40 °C. Nonetheless, a temperature response was noted by an increase of 10% in the release of RhB observed when the temperature was increased from 37 °C to 40 °C. The discrepancies observed between the release profiles of RhB and DOX demonstrate the importance of drug-release medium interactions on release kinetics. Thus, careful consideration should be taken when choosing model drugs, and caution should be used when interpreting release kinetics.
Figure 33

DOX/RhB Release Profiles

Note. (A) Temperature-responsive release of DOX, (B) pH effect on release kinetics of DOX, and (C) comparison of release rates of DOX and RhB.
Figure 3

*SEM Images of EC-DOX-LA and EC-RhB-LA Nanofibers Post In Vitro Release*

Note. EC-DOX-LA nanofibers after *in vitro* release at (A) 37 °C, (B-C) 40 °C, (D) pH 4 and 37 °C, (E-F) pH 4 and 40 °C and EC-RhB-LA nanofibers after *in vitro* release at (G) 37 °C, and (H-I) 40 °C.
SEM images of nanofibers post _in vitro_ release (Figure 34) were analyzed to better understand the mechanisms governing drug release. Ethyl cellulose is known to facilitate sustained release resulting from the polymer’s low degree of wettability. Diffusion-controlled release of RhB from EC nanofibers containing LA/SA was confirmed from SEM images in our previous study. SEM images of EC-DOX-LA post _in vitro_ release at 37 °C and 40 °C, 37 °C and pH 4, and 40 °C and pH 4 (Figure 34, A-F) revealed largely intact morphology of nanofibers. The diameters of EC-DOX-LA nanofibers post _in vitro_ release were 622 nm and 762 nm at 37 °C and 40 °C, respectively. A similar trend of increased fiber size was observed in diameters of EC-DOX-LA nanofibers post _in vitro_ release at 37 °C and 40°C in pH 4 release media. The increased diameter size can be attributed to release media penetration and water-insoluble ethyl cellulose swelling. SEM images of EC-DOX-LA and EC-RhB-LA membranes post _in vitro_ release at 40 °C in acidic or neutral conditions (Figure 34) suggested increased LA leakage at the elevated temperature. Significant accumulations of particles on the surface and excess material forming interconnected fibers observed were likely due to the increased amount of LA migrating out from EC fibers. However, the solubility of lipophilic LA in pH 7.4 PBS is very low\(^\text{205}\), and thus, the majority remained in close contact and adhered to the nanofiber membrane. While average diameters of as-spun EC-DOX-LA and EC-RhB-LA nanofibers differ by only 6 nm, SEM images of EC-RhB-LA nanofibers post _in vitro_ release at 37 °C and 40 °C displayed larger diameter sizes of 672 nm and 888 nm, respectively (Figure 35). The rapid burst release of RhB is most likely caused by the increased leakage of LA and interfiber connections from PCM aggregations. The increased average diameter notes in EC-RhB-LA nanofibers post _in
vitro release at 40 °C may be due to greater penetration of release media to fill the void of additional leaked LA or LA present on the surface of fibers.

Figure 35

*Fiber Diameters of EC-DOX-LA and EC-RhB-LA Samples After In Vitro Release Under Different Conditions*

3.3.5 In Vitro Cytotoxicity Assay of Nanofibers

Morphological cytotoxicity assays were performed with EC-DOX-LA against HEK-293 cells to evaluate the efficacy of DOX-loaded nanofibers (Figure 36). Cells treated with EC and EC-LA nanofibers displayed cell morphology and density consistent with negative controls over 72 h, confirming the nontoxicity of biocompatible ethyl cellulose and lauric acid. Rounding of cells was observed in HEK-293 cells treated with free DOX at 25 µg/mL after 24 h. The positive control assay indicated the prevention of cell-cell adhesion and cell apoptosis. Treatment of HEK-293 cells with EC-DOX-LA nanofibers also led to the rounding up of cells. While round-shaped cell morphology was
noted in free DOX and EC-DOX-LA treated cells after 48 h, significant rounding of cells observed after 72 h of treatment with the DOX-loaded nanofiber signaled increased cell death. Both free DOX and EC-DOX-LA nanofibers displayed effective growth inhibition of HEK-293 cells. Notably, EC-DOX-LA treatment of cells showed sustained drug efficacy after 72 h. Cytotoxicity assay results confirmed sustained drug release observed in release profiles. The sustained and temperature-sensitive drug release signal potential \textit{in vivo} efficacy when employed as an implantable DOX-loaded scaffold to inhibit the recurrence of tumors.

**Figure 36**

\textit{Micrographs of HEK-293 Cells Treated with EC, EC-LA, and EC-DOX-LA Nanofibers and Free DOX After Incubation for 0 h, 24 h, 48 h, and 72 h}
3.4 Conclusions

The stimuli-responsive release of hydrophilic model drugs DOX and RhB from the reported electrospun DDS was demonstrated at various temperatures and acidity of release media. Model drugs and PCM (LA) were encapsulated in EC nanofibers by blend electrospinning, a straightforward and scalable technique for fabricating continuous nanofiber membranes. FTIR, DSC, and XRD analysis showed good compatibility and amorphous drug distribution. The release rate of API was shown to be dependent on temperature, pH, and drug properties. A temperature-responsive release of DOX was demonstrated by an increase of 41% in the release when the temperature was elevated to 40 °C at pH 4, an improvement over the temperature-responsive increase in the pH 7.4 release medium. Additionally, the pH 4 release medium allowed for a reduced burst release of DOX. A significant burst release of 73% RhB was observed at 40 °C. In vitro cytotoxicity assays revealed sustained inhibition of the growth of HEK-293 cells treated with DOX-loaded nanofibers. Accordingly, the reported DDS shows promise as an implantable scaffold for post-surgical cancer tumor treatment. The higher temperature and acidic conditions at tumor sites may provide a suitable target for the reported temperature-responsive DDS to deliver water-soluble chemotherapy drugs, such as DOX. This approach to solid tumor therapy could prove useful by lowering the risk of systemic toxicity and reducing the likelihood of tumor recurrence.
Chapter 4

Future Outlook

Electrospinning of other polymers was also tested due to the difficulty of working with EC. In addition, the mechanical properties EC could be improved with blend ES or coaxial ES using other polymers to meet the requirements for applications such as implantable scaffolds. PLA is a widely used biodegradability, biocompatibility, and bioresorptivity biopolymer that is a highly attractive material for use in developing drug delivery systems. The material has even been recently approved by the FDA for biomedical applications such as scaffolds. The biopolymer can be synthesized sustainably by fermenting sugar cane or corn starch to produce the lactic acid monomer. PLA is a thermoplastic with excellent polymer properties such as mechanical strength and high modulus. PLA’s ease of electrospinning and exceptional properties have led to its extensive use in ES nanofibers.

A goal of future research is to use coaxial ES to encapsulate DOX within the PCM in the core and surrounded by a polymer sheath outer layer, limiting the release of the drug until a sufficient temperature is reached. However, coaxial ES adds an extra layer of complexity to the ES system. EC coaxial ES system became problematic due to the difficulty of ES with the cellulose derivative polymer. PLA proved to be much easier to ES and provide more consistent and uniform samples. Multiple additional factors can influence the coaxial ES process. The interaction between multiple solutions can greatly affect the ease of ES. The interfacial tension between solutions should be minimized, which can be easily achieved with miscible core and sheath solutions. A benefit of
coaxial ES over uniaxial or blend ES is the increased encapsulation efficiency. The solvent is typically immediately volatilized/evaporated during ES,213 leaving the drug/PCM in the center of the composite nanofibers. If the ES process is running ideally the defects resulting from core/sheath blending is minimized and 2 layers are produced. The concentration of the majority of the drug towards the center of the nanofibers results in an increased distance between the drug and the surface of the material. This allows for slower diffusion of API and increased control of the release rate by employing additional mechanisms. The increased complexity of coaxial ES permits combining polymers and additives with incompatible (different) solubilities and further tunability of properties. However, the intricacies of multiple solutions complicates the optimization of processing parameters such as solution feed rate and necessitates attention to additional interactions such as miscibilities.212 Coaxial ES can also be used to modify the morphology of ES fibers through phase separation. The incompatibility of solvents and water vapor can be exploited to control the porosity and drug diffusion rate.23, 78, 214, 215 Nevertheless, the coaxial ES DDS fabricated from EC or PLA did not show vast improvement over blend ES. Coaxial ES of consisted of polymer sheaths with/without PCM and core solutions containing model drugs in various solvents were tested. The results proved more inconsistent than blend ES due to the complexity. The drug release generally increased with increasing PCM content; however, a temperature responsive increase in release between 37 °C and 40 °C was rarely observed or difficult to reproduce. The lack of reproducibility was likely caused by fiber defects during ES as well as mixing of core-sheath solutions. Further optimization of coaxial ES and other parameters affecting drug release are necessary to achieve publishable results.
The API could also be changed to study the effect on release rate. Lipophilic drugs like IBU and KET have good compatibility with EC and display relatively higher release rate.\(^{216, 217}\) The addition of PCM to these DDS could result in a greater thermal switch effect on the release rate of API. Additionally, alternative modes of heating can also be used to control the release of API from DDS containing PCM. The combination of PCM and magnetic particles has been shown to control the release of API. Thermal energy is created when a magnetic field is applied, thus generating sufficient heat to melt the PCM and increase the release rate. Heat generated from magnetic nanoparticles has been reported.\(^{218, 219}\) High intensity focused ultrasound can also be used as a method of providing heat and control the release of API from PCM-DDS.\(^{220}\)

The ES DDS presented in this work demonstrated the potential of electrospinning as a means for fabricating nanofibers capable of temperature-responsive drug release. Future work can build on the blend electrospinning by using additional nanocarriers or multiple polymer/solvents in coaxial electrospinning. Improved control of drug release using external stimuli can be achieved by optimizing the coaxial electrospinning process is also possible. The incorporation of different types of drug molecules can result in different drug release kinetics that can also be further investigated. Additionally, release profiles can be fine-tuned for the treatment of different conditions and improved pharmaceutical efficacy. The versatility of electrospinning allows for infinite modifications and new approaches to explore in designing novel controlled drug delivery systems.
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