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Soft Template-Directed Polymerisation of Polypyrrole for Tuneable Dexamethasone Delivery

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BPharm, MS (Pharmacology & Toxicology)

Abstract

**Introduction:** Conducting polymers (CPs) are electroactive materials which can be loaded with drugs with release tuned by electrical stimulation. To enhance the versatility of CP platforms the levels of drugs delivered must be increased and responsiveness to stimulation enhanced. One approach to increase both the amount of drug delivered from CPs and their responsiveness is by synthesising CPs with micro- or nanostructures with an extended surface area. This thesis aimed to explore two types of soft templates, phytantriol liquid crystals and SDBS (sodium dodecyl benzene sulphonate) micelles, to direct the polymerisation of high surface area polypyrrole (PPy) platforms for tuneable delivery of dexamethasone. These systems have potential applications in the treatment of age-related macular degeneration (AMD) and diabetic macular edema (DME).

**Methods:** Phytantriol and water mixtures were prepared in different proportions and the resulting phase of liquid crystal determined. The liquid crystal phase was monitored during the addition of polymerisation reagents to the bicontinuous cubic phase, during polymerisation of PPy and under redox cycling of the polymer using small angle X-ray scattering. PPy films were prepared by using bicontinuous cubic phase as a template and were loaded with dexamethasone sodium phosphate (DexP) as a dopant. The formed films were characterised for their morphology and elemental make-up by scanning electron microscopy, and for electrochemical activity by cyclic voltammetry. Electrically tuneable release of DexP from the films was then studied. The cytotoxicity of the extracts from the films with and without stimulation was tested on a human retinal pigment epithelial cell line-19 (ARPE-19).

SDBS micelles were prepared and loaded with two drugs; the anionic and hydrophilic DexP and the non-ionic and hydrophobic dexamethasone (Dex). Electroactive PPy nanoparticles loaded with DexP and Dex were prepared by chemical polymerisation using drug loaded SDBS micelles as a template. Morphological, chemical and electrochemical characterisation of the formed nanoparticles was achieved. The particles were pressed into a pellet and adhered to an ITO coated glass slide using silver epoxy for electrochemical characterisation and drug release studies. Mechanisms driving the loading and release of hydrophilic DexP and hydrophobic Dex were investigated. The cytotoxicity of the extracts prepared from the pellets and silver epoxy
(with stimulation and without stimulation) and Dex and DexP at various concentrations were tested on ARPE-19 cells.

**Results and Discussion:** Different phases of phytantriol liquid crystals were studied and a phase diagram was constructed using the hot stage and cross-polarised light microscopy. Bicontinuous cubic phase was selected for polymerisation of PPy as it contains large, non-intersecting aqueous and lipid channels which helps in the movement of monomer and dopant to the working electrode. The addition of monomer and dopant, polymerisation and redox cycling had no effect on the bicontinuous cubic phase. DexP loaded PPy films were prepared using bicontinuous cubic phase liquid crystal as a template. SEM revealed a highly porous and interconnected nanorod like structures with a large increase in surface area (from 8.3 m$^2$.g$^{-1}$ to 224.2 m$^2$.g$^{-1}$) as determined by N$_2$ physisorption studies. The PPy film was found to be reversibly electroactive as evident from cyclic voltammetry. DexP release occurred in a more responsive fashion from the templated films compared to the conventional films. This was attributed to the increased surface area of the templated films which enhances the polymer/media interface. In cytotoxicity studies, no statistically significant difference was found between the films and the control indicating that DexP, unreacted monomer or unwashed liquid crystal that might leach from the films were not toxic.

PPy nanoparticles loaded with anionic, hydrophilic DexP and non-ionic, hydrophobic Dex were prepared by chemical polymerisation using SDBS micelles as a template. The formed particles were characterised under SEM and TEM, and the particles were found to be c.a. 50 nm in size. Large amounts of drug loaded nanoparticles were prepared and particles were pressed into pellets. From CV, it was confirmed that the pressed pellet was electroactive and the surface area was found to be 2.59 cm$^2$. The conductivity of the pellet (22.89 ± 5.49 S.cm$^{-1}$) was determined by four-probe conductivity meter, which shows that the pellet was conductive and charge can pass between particles in the pressed pellet. The entrapment efficiency of Dex was found to be 80.5 ± 1.19% and DexP was 58.3 ± 2.50%. This data suggests that more of the Dex was associated with the forming PPy nanoparticles as it is positioned in the hydrophobic core of the micelles, whereas DexP was associated near the head groups of the micelles and surrounding media. DexP release was faster on reduction and highest release was observed when a pulse stimulus was applied which indicates that the release of DexP is driven by electrostatic interaction. For Dex, the release was similar on oxidation, reduction or without stimulation, but showed higher release on applying pulse stimulus. As Dex is not a charged
molecule, electrostatic forces would not affect the release. Volume changes on applying pulse stimulus would have caused the release of Dex from the particles. Electrically tuneable release of both DexP and Dex was achieved on applying pulse stimulation. No statistically significant difference was observed among the groups, indicating a lack of cytotoxicity.

**Conclusion:** The soft templates, liquid crystals and micelles, were studied as a template to direct the polymerisation of polypyrrole. Tuneable release of DexP from porous PPy films prepared through liquid crystal was achieved with increased responsiveness compared to conventional films. DexP and Dex were loaded into PPy nanoparticles prepared by chemical polymerisation on micelle template and release of both the drugs was achieved on electrical stimulation. PPy particles could be compressed into different shapes and sizes, enabling customizability of these systems. These films and particles could form the basis of an implantable drug delivery system in the treatment of AMD and DME.
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Research Outputs

Publications arising from this thesis


Uppalapati D, Boyd B, Travas-Sejdic J, Svirskis D. Phytantriol liquid crystal templated high surface area polypyrrole films for stimuli-responsive drug delivery (Manuscript in preparation for submission to Drug Delivery and Translational Research).

Refereed Full Conference Papers arising from this thesis


Conference Presentations arising from this thesis


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<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>AAO</td>
<td>Anodised aluminium oxide</td>
</tr>
<tr>
<td>a.c.</td>
<td>Alternating current</td>
</tr>
<tr>
<td>Ag/AgCl</td>
<td>Silver/silver chloride</td>
</tr>
<tr>
<td>AMD</td>
<td>Age-related Macular Degeneration</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AOT</td>
<td>Sodium bis (2-ethylhexyl) sulphosuccinate</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulphate</td>
</tr>
<tr>
<td>ARPE-19</td>
<td>A human retinal pigment epithelial cell line</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated Total Reflection</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer-Emmett-Teller</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical Micelle Concentration</td>
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<tr>
<td>CP</td>
<td>Conducting polymer</td>
</tr>
<tr>
<td>CPLM</td>
<td>Cross-Polarised Light Microscopy</td>
</tr>
<tr>
<td>CPP</td>
<td>Critical Packing Parameter</td>
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<tr>
<td>CP/LC</td>
<td>Conducting polymer/ liquid crystal</td>
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<td>CV</td>
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<tr>
<td>DBSA</td>
<td>Dodecylbenzene sulphonic acid</td>
</tr>
<tr>
<td>DDAB</td>
<td>Didodecyldimethylammonium bromide</td>
</tr>
<tr>
<td>Dex</td>
<td>Dexamethasone</td>
</tr>
<tr>
<td>DexP</td>
<td>Dexamethasone sodium phosphate</td>
</tr>
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<td>DLS</td>
<td>Dynamic Light Scattering</td>
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<td>Diabetic Macular Edema</td>
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<td>Dodecyl trimethyl ammonium bromide</td>
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<td>---------</td>
<td>-------------</td>
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<tr>
<td>FITC-BSA</td>
<td>Fluorescein isothiocyanate labelled bovine serum albumin</td>
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<tr>
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<td>Fourier Transmission Infrared Spectroscopy</td>
</tr>
<tr>
<td>GO</td>
<td>Graphene oxide</td>
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<tr>
<td>HPLC</td>
<td>High-Performance Liquid Chromatography</td>
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<tr>
<td>ITO</td>
<td>Indium tin-oxide</td>
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<tr>
<td>MTT</td>
<td>3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide</td>
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<tr>
<td>MTX</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>MWNT-Ti</td>
<td>Multiwalled carbon nanotubes grown on titanium</td>
</tr>
<tr>
<td>NaDBS</td>
<td>Sodium dodecyl benzene sulphonate</td>
</tr>
<tr>
<td>NEXAFS</td>
<td>Near Edge X-ray Absorption Fine Structure</td>
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<tr>
<td>Pal</td>
<td>Palygorskite</td>
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<tr>
<td>PANI</td>
<td>Polyaniline</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PC</td>
<td>Polycarbonate</td>
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<tr>
<td>PEDOT</td>
<td>Poly(3,4-ethylenedioxythiophene)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactide-co-glycolic acid)</td>
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<td>PMMA</td>
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<td>PSS</td>
<td>Poly(styrenesulphonate)</td>
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<tr>
<td>P/S</td>
<td>Penicillin/streptomycin</td>
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<tr>
<td>pTS</td>
<td>Para-toluene sulphonic acid monohydrate</td>
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<td>XPS</td>
<td>X-ray Photoelectron Spectroscopy</td>
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<tr>
<td>Prof Sanjay Garg</td>
<td>Help with initial manuscript outline, manuscript review and editing</td>
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Chapter 3 (Polymerisation of Phytantriol in a Liquid Crystal Template) comprises of work published in this peer-reviewed conference proceeding.

Sections 4.5.9 and 4.6.6 of chapter 4 (Polypyrrole Films Polymerised in a Host Liquid Crystal Template for DexP delivery) comprises of work published in this peer-reviewed conference proceeding.


| Nature of contribution by PhD candidate | Planning of experiments, conducting experiments, analysis of the data, preparation of first draft, continuous improvement based on co-author feedback |
| Extent of contribution by PhD candidate (%) | 70 |

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<tr>
<td>Prof Ben J Boyd</td>
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Chapter 1: Introduction
Conducting polymers (CPs) are organic materials which possess metal-like electrical properties alongside the mechanical properties and processability of conventional polymers (1). These remarkable materials were discovered in 1977 by Shirakawa et al. (2). Since their discovery, CPs have been used for various applications including electrochromics, light emitting diodes, field effect transistors, energy storage devices, biosensing, neural interfaces, and drug delivery (3-13). They are prepared either by electrochemical or chemical polymerisation of monomer units. CPs are biocompatible, and through electrical stimulation control over drug release can be achieved. Charge, volume, molecular permeability, and hydrophobic/hydrophilic balance are modified on alteration of the redox state of CPs, which can be exploited to tune the release rate of drugs (14, 15). Anionic drugs like glutamate, salicylate, and dexamethasone sodium phosphate (DexP) (16-18), cationic drugs like dopamine, chlorpromazine, and risperidone (19-21), and neutral drugs like N-methyl phenothiazine, and progesterone (22, 23), have been reported to be loaded and released from CP based electrically tuneable delivery systems. The levels of drugs that can be delivered from CPs currently are suitable only for local delivery applications (24). To enhance the versatility of CP platforms to a wider range of applications and to extend the lifetime of these delivery systems, the levels of drugs delivered must be increased. In addition, while the influence of electrical stimulation on drug release has been demonstrated, approaches to achieve greater responsiveness over release are required for many applications.

One approach to increase both the amount of drug delivered from CPs and their responsiveness is by synthesising polymers with an extended surface area (25). CPs with defined micro- or nanostructures are exciting materials for biomedical applications due to their increased surface area offering enhanced responsiveness, reduced impedance, and high charge transfer capacity. CPs with defined micro- or nanostructures are commonly synthesised chemically or electrochemically, using a hard template (25) or a soft template (26) to direct polymerisation, or without a template by selecting appropriate polymerisation conditions (27, 28).

In drug delivery, CPs with defined micro- or nanostructures offer advantages due to their enhanced surface area with associated increases in drug loading capacity and responsiveness to stimulation. Increased drug loading is achieved if the template is retained with higher amounts of drugs loaded into the template or entrapped in the voids if the template is removed. Increase in the surface area increases the polymer/media interface, thereby increasing the
responsiveness. CPs with micro- or nanostructures have increased drug loading and can facilitate tighter control over the release of their payload.

Different mechanisms can support the loading of drugs into CPs. CP drug delivery systems often rely on electrostatic interactions between the CP and a charged drug. During synthesis of CP films, dopant anions are incorporated into the polymer to counterbalance the positive charge associated with the oxidative polymerisation. For polypyrrole (PPy), a dopant anion is incorporated every 3-5 pyrrole units (20, 29). With the increased surface area offered by CPs with defined micro- or nanostructures, ion exchange can be expected to be more efficient as distances between polymer bulk and polymer/media interface are reduced (18, 30). However, loading levels remain limited to 1 drug molecule per 3-5 monomer units if electrostatic forces alone govern loading (14). Meanwhile, where drugs can be physically entrapped within CPs, the use of templates to direct polymerisation creates many exciting possibilities. A template can be used which itself contains drug (25) or, if the template is removed after polymerisation, the empty space can act as a reservoir to contain drug (31).

Drug release from CPs is typically controlled by the redox state of the polymer which influences electrostatic interactions between drug and polymer (32), alongside volume changes (21, 33). Electrostatic interactions between CP and drug will result in anionic drugs being released on reduction, such as ferrocyanide (16) and DexP (34). Meanwhile, cationic drugs, dopamine (19), and chlorpromazine (20) are expelled from the polymer when oxidised.

The release of drugs from CPs with defined micro- or nanostructures utilises not only electrostatic interactions between drug and polymer but also relies on volume changes that occur upon switching of redox states. The movement of hydrated ions into and out of the polymer results in polymer expansion and contraction (35, 36). Volume changes rather than electrostatic interactions have been attributed to the triggered release of risperidone (37) and progesterone (23). Typically, CPs with defined micro- or nanostructures have increased effective electrochemical surface area. This higher apparent surface area promotes the speed and extent of ion movement into and out of the polymer; hence more precise and potentially greater magnitude of responses to both electrostatic switching and volume changes occur.

In this thesis, soft templates were explored to prepare CPs with defined micro- or nanostructures due to the advantages offered over hard templates. Post-polymerisation removal of the template can be challenging in the hard templated method, it may complicate the process
and destroy the fabricated nanostructures. In addition, the overall dimensions of the hard template restrict the amount of CP that can be produced by this method and may limit the ability to scale up processes, whereas large quantities of nanostructured CPs can be produced using soft template-directed polymerisation.

Two types of soft templates, liquid crystals and micelles, were explored in this thesis. The amphiphilic lipid phytantriol forms different liquid crystalline phases when added to water. The bicontinuous cubic phase has continuous aqueous channels separated by lipid channels which are hypothesised to support the electrochemical polymerisation (38). The other soft template investigated in this thesis was sodium dodecyl benzene sulphonate (SDBS) based micelles. SDBS is an anionic surfactant which forms micelles in water (39). These micelles can serve as a template for chemical polymerisation of CP to form nanoparticles.

Dexamethasone sodium phosphate (DexP) is a disodium salt of dexamethasone (Dex), a synthetic glucocorticosteroid used in the treatment of posterior eye conditions like age-related macular degeneration (AMD), diabetic macular oedema (DME), and uveitis (40). This thesis seeks to advance the current knowledge to increase the amount of bioactive that can be delivered from CP based drug delivery systems and to enhance the electrical responsiveness, with the goal to enhance the versatility of CP based drug delivery systems.

Different phases of phytantriol liquid crystals were studied and the effect of polymerisation reagents on the bicontinuous cubic phase was tested using small angle X-ray scattering. Porous PPy films were prepared by using bicontinuous cubic phase as a template. DexP was studied as a dopant to be loaded into the porous conducting films. The formed films were characterised for their morphology by scanning electron microscopy, elemental analysis, electrochemical activity by cyclic voltammetry and conductivity. The electrochemical release of DexP from the films was then studied.

Electroactive PPy nanoparticles were prepared by chemical polymerisation using SDBS micelles as a template. Morphological and electrochemical characterisation of the formed nanoparticles was achieved. In micelle-templated nanoparticles, lipophilic Dex and the more hydrophilic DexP were loaded, and the effect of stimulation on the release of both drugs was studied. Loading and release mechanism of hydrophilic DexP and hydrophobic Dex was investigated.
1.1 Thesis aims

The major aim of the thesis was to prepare nano-structured conducting polymers using soft templates for the controlled release of dexamethasone. The specific aims of this thesis were to:

- Prepare liquid crystal template suitable to direct PPy polymerisation and determine the effect of the presence of monomer and dopant alongside the polymerisation process on the liquid crystal phase.
- Electrochemically polymerise PPy through a liquid crystal template and characterise the formed porous PPy films.
- Investigate tuneable drug release from PPy films formed through a liquid crystal template compared to conventional PPy films.
- Prepare DexP and Dex loaded micelles and to synthesise electroactive PPy nanoparticles by chemical polymerisation using drug loaded micelles as a template.
- Investigate drug loading and release of anionic, hydrophilic DexP and non-ionic, lipophilic Dex from PPy nanoparticles.
- Determine the cytotoxicity of the extracts from the PPy films and PPy particles on ARPE-19 cells (a human retinal pigment epithelial cell line).

1.2 Thesis structure

A literature review is presented in Chapter 2 focussing in-depth on conducting polymers with defined micro- or nano structures and their application in drug delivery. The drugs dexamethasone and dexamethasone phosphate are then considered.

The majority of the literature review has been published as: Uppalapati D, Boyd BJ, Garg S, Travas-Sejdic J, Svirskis D. Conducting polymers with defined micro- or nanostructures for drug delivery. Biomaterials. 2016;111:149-62.

Chapter 3, presents the investigation of phytantriol liquid crystal as a template to direct the polymerisation of PPy/pTS (para-toluene sulphonic acid monohydrate). Tuneable DexP delivery from porous PPy prepared through liquid crystal template is presented in Chapter 4.

Chapter 5 investigates soft template-directed chemical polymerisation of electroactive polypyrrole particles loaded with dexamethasone for electrically responsive release.

Chapter 6 provides a general discussion, future directions arising from this work, and concluding remarks.
Chapter 2: Literature Review
2.1 Declaration for Chapter 2

The majority of this literature review has been published as: Uppalapati D, Boyd BJ, Garg S, Travas-Sejdic J, Svirskis D. Conducting polymers with defined micro- or nanostructures for drug delivery. Biomaterials. 2016;111:149-62.

2.2 Introduction

While many polymeric controlled release formulations have been developed, most release drug at predetermined rates that cannot be altered or stopped once administered. Meanwhile, delivery systems based on conducting polymers (CPs) offer the potential of tuneable drug release which can match the dose to the clinical need, even as the need might change. Only low levels of bioactives can be released from current CP delivery systems. We hypothesise that templating techniques can be used to prepare CPs with defined micro- or nanostructures capable of the tuneable delivery of increased levels of bioactives with improved responsiveness.

This literature review first introduces CPs with defined micro- or nanostructures and their synthesis using different fabrication approaches. The soft templates we have investigated in this thesis, phytantriol based liquid crystals and sodium dodecyl benzene sulphonate (SDBS) based micelles, are described in the soft template-directed polymerisation section (Section-2.5.2). Then a detailed insight into the current understanding of drug loading and drug release from CPs with defined micro- or nanostructures is provided. At the end of this chapter, dexamethasone and its disodium phosphate salt are introduced. We have studied dexamethasone in its base form and salt form in this thesis, which allows investigation of different mechanisms of loading and release from the CP systems. Dexamethasone base is uncharged and lipophilic, while the salt form is anionic and more hydrophilic. Local delivery of dexamethasone to target sites for conditions like age-related macular degeneration (AMD) and diabetic macular edema (DME) would be beneficial to reduce systemic toxicity and unwanted side effects.
2.3 Conducting polymers

Conducting polymers are organic materials which possess metal-like electrical properties while retaining the mechanical properties and processability of conventional polymers (1). These remarkable materials were discovered in 1977, which led to the Nobel Prize in chemistry being awarded to Shirakawa, McDiarmid, and Heeger in 2000 (2). The conductivity and reversible redox nature of CPs is attributed to the alternating double and single bonds with carbons in $Sp^2$ hybridisation. The delocalised $\pi$-electrons form a conjugated backbone which renders the materials conductivity through charge mobilisation (41). Polypyrrole (PPy) (42), polythiophene (43) and its derivative poly(3,4-ethylenedioxythiophene) (PEDOT) (25), polyaniline (PANI) (44), alongside poly(p-phenylene vinylene) (45) and its derivatives are the most widely explored CPs. Since their discovery, CPs have been used for various applications including electrochromics, light emitting diodes, field effect transistors, energy storage devices, in biomedical applications for bio sensing, at neural interfaces, and for drug delivery (3-13).

On alteration of the redox state of CPs, there are accompanied changes to the charge, volume, molecular permeability, and hydrophobic/hydrophilic balance. These changes can be exploited to tune the release rate of drugs (14, 15). Electrically tuneable delivery systems based on CPs have been reported for anionic drugs (glutamate, salicylate, dexamethasone sodium phosphate (DexP)) (16-18), cationic drugs (dopamine, chlorpromazine, and risperidone) (19-21), and neutral drugs (N-methyl phenothiazine and progesterone) (22, 23). The amounts of drugs currently delivered from CPs are suitable for local delivery applications (24). However, the levels of drugs delivered must be increased to enhance the versatility of these delivery platforms to a wide range of chronic disease states, for conditions requiring systemic delivery and to extend the lifetime of these delivery systems. In addition, while the influence of electrical stimulation on drug release has been demonstrated, approaches to achieve tight control over release are required for many applications.

One approach to increase both the amount of drug delivered from CPs and their responsiveness is by synthesising highly porous polymers with defined micro- or nanostructures (25).
2.4 Conducting polymer with defined micro- or nanostructures

CPs with defined micro- or nanostructures are exciting materials for biomedical applications due to their increased surface area offering enhanced responsiveness, reduced impedance, and high charge transfer capacity. CPs with defined micro- or nanostructures are commonly synthesised chemically or electrochemically using a hard template (25) or soft template (26) to direct polymerisation, or without a template by selecting appropriate polymerisation conditions (27, 28).

In addition, CPs with defined micro- or nanostructured systems have the potential to be loaded with a high level of a drug loading and are able to achieve more controlled and efficient drug delivery compared to the unstructured CP systems (46, 47).

2.5 Synthesis of CPs with defined micro- or nanostructures

CPs are oxidatively polymerised (Figure 2-1) from their respective monomers, polypyrrole has been selected as a representative example. Pyrrole monomers are oxidised to form radical cations, followed by a coupling reaction to form oligomers (48). These oligomers, in turn, couple with a monomer cation or another oligomer and the chain length extends to form a polymer. Oxidation can be driven either by a chemical oxidant (chemical synthesis) or by an oxidising potential applied through an electrode (electrochemical synthesis) (49). Frequently electrochemical methods are favoured in drug delivery as they offer finer control over the quantity and electrical properties of the final polymer by controlling the rate and amount of charge that is passed through.
Chapter 2 - Literature Review

1. Oxidation of monomer

\[
\text{H}_2\text{N} \xrightarrow{-e^-} \text{H}_2\text{N}^+.
\]

2. Radical coupling

\[
\text{H}_2\text{N}^+ + \text{H}_2\text{N} \xrightarrow{} \text{H}_2\text{N}^+ \text{H}_2\text{N}^+ -2\text{H}^+.
\]

3. Chain propagation

\[
\text{H}_2\text{N} \xrightarrow{-e^-} \text{H}_2\text{N}^+.
\]

\[
\text{H}_2\text{N}^+ + n \text{H}_2\text{N}^+ \xrightarrow{-2n\text{H}^+} \text{H}_2\text{N} \text{H}_2\text{N} \ldots \text{H}_2\text{N}.
\]

Figure 2-1: Oxidative polymerisation of polypyrrole (PPy). Adapted from (48).

Different fabrication approaches have been reported to synthesise CPs with defined micro- or nanostructures using either chemical or electrochemical polymerisation approaches (Figure 2-2). Template-directed polymerisation relies on existing structures to guide polymer growth. This includes hard template-directed polymerisation in which a hard micro- or nanostructured material is used as a template, whereas in soft template-directed polymerisation, a self-assembled molecular template is used. In template-free polymerisation, precise polymerisation conditions are employed to manipulate the initial nucleation reactions which influence the morphology and properties of the final polymer formed (48, 50, 51).
Figure 2-2: Template-directed and template-free fabrication approaches to synthesise CPs with defined micro- or nanostructures. Adapted with permission from (52).

2.5.1 Hard template-directed polymerisation

Micro- or nanostructured solid materials have been used as templates to direct the polymerisation of CPs. Synthesis of CP using a hard template was first explored by Martin et al. to synthesise CP nanofibres and tubules inside the pores of polycarbonate membranes (53). The resulting micro- or nanostructures of CPs match the shape and diameter of the template utilised in their synthesis.

The use of hard templates is the most common approach to prepare uniform micro- or nanostructures due to convenience, simplicity, and controllability. The size and thickness of the resultant micro- or nanostructures can be controlled by the size of the template features and polymerisation time. However, a drawback of hard template methods is when removal of the template is required. Post-polymerisation removal of the template can be challenging and may complicate the process and destroy the fabricated nanostructures. In addition, the overall dimensions of the template restrict the amount of CP that can be produced by this method and may limit the ability to scale up processes.

Chemical or electrochemical polymerisation of CPs can be guided by hard templates. In chemical polymerisation, the hard template is immersed in a solution composed of monomers, dopant, and an oxidant. When employing electrochemical polymerisation, the hard template should be conductive or in contact with a conductive surface from which polymer growth begins. CPs with defined micro- or nanostructures result from polymerisation within the well-
defined spaces of the hard template or over existing discrete nanostructures, resulting in the structures as presented in Figure 2-3. Depending on the intended application of these structured CPs, the template can be removed leaving a negative copy of the starting material (51).

![Diagram of CP polymerisation](image)

**Figure 2-3:** Schematic diagram of CP polymerisation on or through a hard template: a) polymerisation in the pores or channels of a template and removal of the template resulting in nanofibres or nanotubes, b) polymerisation through the voids of a colloidal template and subsequent removal of the template produces a nanoporous film, c) polymerisation over discrete or individual nanostructures, d) nanocomposites of CPs polymerised with other nanomaterials. Adapted with permission from (52).

### 2.5.1.1 Membranes

CPs with a range of micro- or nanostructures including tubes, wires, rods, and fibres can be polymerised in the pores or channels of membranes (Figure 2-3a). The morphology of these formed structures depends on the initial template used. By controlling the polymerisation time, thin-walled tubules (short polymerisation times), thick-walled tubules (intermediate polymerisation times) and even solid fibres (long polymerisation times) can be produced within the pores of membranes. Track-etched membranes and anodised alumina are commonly used hard templates for this purpose. Track-etched membranes with diameters as low as 10 nm and pore densities of $10^9$ pores.cm$^{-2}$ can be produced by irradiating high energy heavy ions onto a polycarbonate (PC) or polyethylene terephthalate membrane (54). Polypyrrole (PPy) and polyaniline (PANI) polymerised on a PC membrane produced micro- or nanotubules...
preferentially due to the growth of polymer from the surface of pore walls (55-57). The production of solid fibres can be difficult due to limited diffusion of reagents into the formed nanotubules. To synthesise solid nanofibres, Duchet et al. proposed a modified method by the successive polymerisation of PPy in a PC membrane by immersing the membrane in fresh monomer and dopant solution after each polymerisation step (Figure 2-4) (58). The conductivity of PANI nanotubules produced either chemically or electrochemically on a PC membrane was greater than the bulk polymer, with higher conductivities at lower tubule diameters attributed to the presence of a higher proportion of oriented polymer chains within narrow PANI tubules (>60 S.cm\(^{-1}\) for tubules with diameters < 50 nm whereas conductivity fell to < 10 S.cm\(^{-1}\) for tubules >200 nm in diameter) (59).

![Figure 2-4: PPy nanotubules polymerised in a PC membrane after the dissolution of the PC template. Successive polymerisation steps increase the wall thickness of PPy nanotubules to form nanofibres a) after first synthesis, b) after second synthesis, c) after the third synthesis. Reprinted with Permission from (58).](image)

Alumina (anodised aluminium oxide-AAO) is formed on electrochemical anodic oxidation of aluminium with pore densities as high as \(10^{11}\) pores.cm\(^{-2}\) (60). Han et al. reported that the morphology of PEDOT chemically polymerised in alumina membranes could be controlled by changing the concentration of the oxidant and polymerisation temperature. They reported that at low FeCl\(_3\) concentration and low temperatures, thin-walled nanotubes can be obtained. As the concentration and temperature increased the wall thickness of nanotubes increased until solid nanorods were produced (61). In addition, the effect of monomer concentration and applied potential on PEDOT nanostructures in alumina has been demonstrated by Xiao et al. At sufficient monomer concentration and slower reaction rates, nanowires are formed as monomer can diffuse slowly and fill the pores of the alumina, whereas at low monomer concentrations and fast reaction rate nanotubes are formed (62).
An anodised aluminium oxide membrane with a pore diameter of 410 nm was used as a template for PPy polymerisation. The diameter was decreased to 380 nm when a layer of gold was coated on AAO membrane to make it conductive. PPy was polymerised for 60 s on a gold coated AAO membrane when the pore size further decreased to 200 nm as measured from scanning electron microscopy (SEM) images. Atomic force microscopy revealed the average pore size to be 190 and 140 nm in oxidised and reduced states, respectively. This actuation was used to control the release of the model drug fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA) and is discussed in detail in subsequent sections (35).

2.5.1.2 Colloidal arrangements

Arrangements of colloids can be used to guide the polymerisation of macroporous films. Self-assembled 3D colloidal templates comprising polystyrene (PS) and poly (methyl methacrylate) (PMMA) nanobeads deposited on conductive substrates have been used for this purpose. CPs are grown electrochemically in the voids of the colloidal template which can then be removed by dissolving in a suitable organic solvent, such as toluene, which leaves a macroporous interconnecting CP film (Figure 2-3b and Figure 2-5). The pore size of the resulting film depends on the diameter of the colloids used, typically in the range of few hundred nanometers. These colloids can be deposited on the substrate by gravitational sedimentation (63), vertical deposition (31) or electrophoretic deposition (64). Bartlett et al. prepared PPy, PANI and poly(bithiophene) macroporous films using PS latex beads. Interestingly, the pore diameter in the PPy and PANI films was less than that of the template diameter, however, this was not the case in poly(bithiophene) films. This is due to the shrinkage of PPy and PANI films on drying and may be attributed to the difference in polymer cross-linkings, type of dopant, and solvents used. The authors also reported ‘skin effect’ phenomena where concave triangular gaps were seen between the pores due to preferential polymerisation at the surface of the colloid before the void space is filled. This pattern is similar to the hollow nanotubule formation with the PC membrane template preceding the formation of solid nanorods (65).
Figure 2-5: SEM micrographs of a) PMMA colloidal crystal template on the stainless-steel substrate, b) inverse opal PPy film obtained by electropolymerisation of PPy and sodium dodecyl benzene sulphonate (NaDBS) and subsequent PMMA template removal. Adapted with permission from (31).

Macroporous CP films prepared over colloidal templates were reported to have highly efficient drug loading capacity while retaining their conductivity and mechanical stability. For instance, macroporous PPy films were synthesised using PS nanobeads with an approximate diameter of 46 nm (66, 67) and an inverse opal PPy film was synthesised using PMMA bead (approximately 430 nm) template (31) which are discussed in detail in later sections.

2.5.1.3 Discrete nanostructures

Various nanostructures can serve as hard templates over which CPs are polymerised (68, 69). The templates can then be selectively removed, leaving behind hollow CP nanostructures (Figure 2-3c). Polymerisation can be driven chemically, where monomer and oxidant are adsorbed onto nanostructures and the adsorbed monomer is polymerised. Alternatively, electrochemical polymerisation can be used to form CP onto nanostructures, which have been deposited onto a conductive substrate in the presence of a monomer and a dopant. Electrospinning of various materials produces nanostructures which serve as a template for the polymerisation of CP (70, 71). In this way, poly(lactide-co-glycolic acid) (PLGA) nanofibres electrospun onto electrodes have served as a template to direct the electropolymerisation of PEDOT nanotubes (25). Similarly, PPy and PEDOT nanofibres have been deposited by vapour phase polymerisation onto electrospun polyacrylonitrile (PAN) nanofibres (72). Electrospun polyvinylpyrrolidone nanofibres containing an oxidant have served as a sacrificial template to produce PEDOT nanofibres by vapour phase polymerisation (73). In an alternate approach, the chemical oxidant FeCl₃ was dropped during electrospinning onto spun fibres comprising 3,4-ethylenedioxythiophene (EDOT) monomer and PLGA (74).
2.5.1.4 Hard reactive templates

Reactive or seed templates can be used where the template is consumed during the polymerisation reaction thereby eliminating the need for an additional removal process. The reactive template can serve as both a template and an oxidant. CP nanostructures can be synthesised from existing oxidative inorganic templates such as V$_2$O$_5$ (75), MnO$_2$ (76) or Cu$_2$O (77). Nanofibrils of PPy can be obtained when small amounts of V$_2$O$_5$ nanofibres are introduced into a pyrrole monomeric solution. The monomer reacts with nanofibrillar V$_2$O$_5$ prior to the addition of oxidant, aiding in the fibrillar morphology of the end product (78). When the concentration of V$_2$O$_5$ nanofibres is increased in the reaction medium, PPy nanotubes are formed upon addition of the oxidant (FeCl$_3$). Here V$_2$O$_5$ acts as a polymerisation template which can be dissolved in dilute acids after polymerisation (75). Recently, a green method for synthesising PPy nanoparticles and nanofibres using V$_2$O$_5$ as a template has been reported. Here they replaced FeCl$_3$ with a green oxidant, H$_2$O$_2$ (79). Pan et al. reported the synthesis of PANI nanotubes by using MnO$_2$ as a reactive template. The morphology of the resulting PANI was dependent on the diameter and length of MnO$_2$ fibres. After the reaction, MnO$_2$ was reduced to soluble Mn$^{+2}$ ions (76). PPy nanostructures such as nanotubes, urchin-shape and flower-like microspheres have been synthesised by controlling the morphology of MnO$_2$ (Figure 2-6) (80, 81). PANI nanostructures (spheres and octahedrons) were synthesised using Cu$_2$O as a template in the presence of H$_3$PO$_4$ as a dopant. Cu$_2$O reacts with ammonium persulphate (APS) during the reaction to form the soluble Cu$^{+2}$ salt (77).

![Figure 2-6](image_url): SEM images of a) the cryptomelane- phase manganese oxide template and b) the resultant PANI nanotubes. The inset of b) is the TEM image of the PANI nanotubes. The scale bar is 1 µm. Adapted with permission from (76).
2.5.1.5 CP containing composite materials

Composites of CPs with other nanostructured materials combine the properties of both the materials. CP nanocomposites can be formed with metals and metal oxides (82), alongside carbon materials such as graphene oxide and carbon nanotubes (Figure 2-3d) (83, 84). The various properties of these materials such as high surface area enable customisability of the nanocomposites. Further, nanocomposites allow a wide range of drugs (both hydrophilic and hydrophobic) to be loaded due to the combined properties of these materials. Weaver et al. prepared nanocomposites of PPy with graphene oxide nanosheets. By changing the size and thickness of the nanosheets, significant differences were observed in the morphology, drug loading, and drug release from the nanocomposites (18). Nanocomposites formed from CPs with carbon nanotubes showed a significant increase in drug loading and controlled drug release compared to straight CP materials (83).

2.5.2 Soft template-directed polymerisation

Soft templates typically consist of self-directed assemblies of surfactants or amphiphilic lipids and can be used as a template to influence the growth of CPs and impart micro- or nanostructures. Self-assembly of the template is driven by non-covalent interactions such as Van der Waals forces, hydrogen bonds, and π-π interactions. Chemical polymerisation is typically used to form CPs with micro- or nanostructures using soft templates. CP micro- or nanoparticles, nanotubes, and nanofibres can be formed depending on the nature of monomer and dopant ions as shown in Figure 2-7.

Among the synthesis methods of CP with defined micro- or nanostructures, soft template-directed polymerisation is beneficial as the removal of the template after polymerisation is simple and straightforward, and large quantities of the final product can be synthesised using soft template-directed polymerisation thus rendering this method suitable for scale-up. In this thesis, two types of soft templates were explored: liquid crystals based on phytantriol and micelles based on SDBS.
2.5.2.1 Surfactants

Surfactants are amphiphilic molecules that form thermodynamically stable micelles above their critical micellar concentration (CMC) in a solvent (often water). Some amphiphilic molecules exhibit anisotropy and have multiple CMCs where transitions to rod-like cylindrical micelles or bilayers can occur (85). The size and shape of the micelles depend on the shape and geometric packing of the surfactant molecule and can be understood from the critical packing parameter (CPP), Equation 2-1 (86).

\[
CPP = \frac{v}{al}
\]

Equation 2-1
The CPP is calculated from Equation 2-1, where ‘v’ is the volume of the hydrocarbon chain, ‘a’ is the optimal area of the hydrophilic head group, and ‘l’ is the length of the fully extended hydrocarbon chain. There are a number of reports on the synthesis of CP nanoparticles, nanofibres or nanotubes using surfactant self-assembled structures as a soft template (87-89). CP nanoclips have been produced by Liu et al. using the cationic surfactant, cetyltrimethylammonium bromide (CTAB). CTAB forms (CTA)₂S₂O₈⁻ ions in the presence of an oxidant, APS. These ions act as a template for CPs to form nano-clips (90). In some cases, the surfactant can function as the dopant. PANI nanoparticles have been synthesised using micelles formed from an anionic surfactant, dodecyl benzene sulphonic acid (DBSA) as a template (91). Meanwhile, Wan et al. synthesised PANI nanotubes using β-naphthalene sulphuric acid as a dopant (92).

Reverse micelles are aggregates of surfactants with the polar head directed inwards and the non-polar part facing outside (93). These reverse micelles can solubilise various substances and have been used as templates to direct the growth of CPs (94). AOT (sodium bis (2-ethylhexyl) sulphosuccinate) is an anionic surfactant, which preferentially forms reverse micelles in an oil phase because of the two bulky hydrophobic tails compared to the hydrophilic head group. Nanotubes of PPy were prepared by reverse micelles formed from the surfactant AOT (95). Ferric ions from FeCl₃ were adsorbed to an anionic AOT head group region which explains the mechanism of PPy nanotube formation on the addition of pyrrole (96). Various PEDOT nanostructures were formed from AOT reverse micelles depending on the reaction conditions (97). At low concentrations of oxidant, PEDOT nanofibres were produced, whereas nanotubes were formed at higher oxidant concentration (98-100). Formation of PANI nanoparticles from DBSA reverse micelles has also been reported (101, 102).

**Sodium Dodecyl Benzene Sulphonate (SDBS)**

Micelles based on SDBS were used as a template to prepare PPy nanoparticles via chemical polymerisation. SDBS is an anionic surfactant with chemical formula C₁₈H₂₉NaO₃S (Figure 2-8). It appears as a white to yellow powder with a molecular weight of 348.48 g.mol⁻¹. The solubility of SDBS in water is 200 mg.ml⁻¹ (103). SDBS is a surface-active agent, which modifies the interfacial tension of water. SDBS has a hydrophilic group and a hydrophobic group and molecules will self-assemble in the presence of water to form micelles above the CMC. CMC of SDBS determine by different methods is shown in Table 2-1. These micelles
have a hydrophobic core and hydrophilic surface. In this thesis, SDBS has been studied as a template to prepare PPy nanoparticles loaded with two drugs and their tuneable release has been described in chapter 5.

**Figure 2-8:** Structure of SDBS.

**Table 2-1:** CMC of SDBS determined by different methods.

<table>
<thead>
<tr>
<th>Method/property used for determination</th>
<th>CMC of SDBS</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometer</td>
<td>1.5 ± 0.4 mM</td>
<td>(104)</td>
</tr>
<tr>
<td>Surface tension</td>
<td>0.86 ± 0.03 mM</td>
<td>(104)</td>
</tr>
<tr>
<td>Conductivity</td>
<td>0.87 mM</td>
<td>(104)</td>
</tr>
<tr>
<td>Surface tension</td>
<td>1.2 mM</td>
<td>(105)</td>
</tr>
<tr>
<td>Surface tension</td>
<td>1.4 ± 0.05 mM</td>
<td>(106)</td>
</tr>
<tr>
<td>Constant wavelength synchronous</td>
<td>1.48 ± 0.05 mM</td>
<td>(106)</td>
</tr>
<tr>
<td>Fluorescence spectrometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular absorption spectroscopy</td>
<td>1.58 ± 0.05 mM</td>
<td>(106)</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>1.63 ± 0.05 mM</td>
<td>(106)</td>
</tr>
</tbody>
</table>
2.5.2.2 Liquid crystals

Some amphiphilic polar lipids self-assemble in the presence of water to form liquid crystals. They form thermodynamically stable structures by non-covalent interactions (107). Different liquid crystalline phases such as lamellar, hexagonal, and bicontinuous cubic phases are formed. The phase of the liquid crystal formed depends on the lipid structure and the relationship is generally given by dimensionless critical packing parameter (Equation 2-1). Depending on the value of the dimensionless packing number (CPP), these self-assembled aggregates will form one-dimensional lamellar phase or two-dimensional hexagonal or three-dimensional bicontinuous cubic phase (6). The lamellar phase (L\textsubscript{α}) consists of stacked bilayer sheets arranged in a 1D array, while the bicontinuous cubic phase (Q\textsubscript{2} or V\textsubscript{2}) is composed of continuous, non-intersecting aqueous channels separated by a lipid layer arranged in a 3D network. The bicontinuous cubic phase has three further phase structures: Pn3m, Im3m, and Ia3d. The reverse hexagonal phase (H\textsubscript{2}) consists of cylindrical micelles packed in a hexagonal pattern and L2 consists of inverse micelles (Figure 2-9) (108). These liquid crystal nanostructures have been widely reported in the literature for their use in drug delivery (109, 110).
Figure 2-9: Depending on the V/al ratio, reverse micelles, reverse hexagonal, bicontinuous cubic, lamellar, hexagonal or micelles are formed. Adapted with permission from (108).

Hexagonal and reverse hexagonal liquid crystalline phases have been explored as templates for the polymerisation of CPs by both chemical and electrochemical synthesis. Polypyrrole was synthesised from a nematic liquid crystal and it was reported that as the orientation of liquid crystal changes a change in voltammetric response and conductivity was observed in PPy (111). Poly(p-phenylenevinylene) nanocomposites were prepared from hexagonal lyotropic liquid crystal (45). Polyaniline nanowires have been prepared from reverse hexagonal liquid crystals. Since aniline is hydrophilic, it gets polymerised in the hydrophilic core of the reverse hexagonal phase (112), whereas PEDOT nanowires have been synthesised by Hulvat et al. from hexagonal liquid crystals. EDOT being hydrophobic gets concentrated in the hydrophobic core of the hexagonal phase (113). The formed ordered nanowires demonstrated anisotropic properties (114). Similarly, PEDOT nanostructures have been prepared from chitosan based liquid crystals (115). Liquid crystals which are stable in an aqueous environment can be formed from the self-assembly of amphiphilic lipids such as phytantriol and glyceryl mono oleate.
Chapter 2- Literature Review

**Phytantriol**

Liquid crystal formed from phytantriol (3,7,11,15-tetramethylhexadecane-1,2,3-triol, Figure 2-11A) were explored in this thesis as a template to direct CP polymerisation. Phytantriol exists in different phases depending on the lipid and water compositions and external conditions like temperature and pressure (116).

The lamellar phase ($L_{\alpha}$) consists of bilayers of amphiphiles stacked on each other with tail groups facing inside, separated by an aqueous layer. When the lamellar phase is dispersed in water it forms liposomes (117). The bicontinuous cubic phase ($Q_2$ or $V_2$) consists of a single bilayer that is twisted and warped to form a complex 3D structure with continuous and non-intersecting water channels. The bicontinuous cubic phase can exist in different space groups: gyroid ($Ia3d$), diamond ($Pn3m$), and primitive ($Im3m$). With increasing water concentration, the bicontinuous cubic phases will appear in the order of gyroid, diamond, and primitive. The three space groups have the same surface area per unit cell; however, they differ in the way they are packed. When the bicontinuous cubic phase is dispersed in water cubosomes form. Meanwhile, the reverse hexagonal phase ($H_2$) consists of cylindrical micelles arranged in a hexagonal arrangement (Figure 2-10) (118, 119).

![Diagram of Phases](image)

**Figure 2-10:** Schematic diagram of lamellar ($L_{\alpha}$) phase, bicontinuous cubic phases: gyroid ($Ia3d$), diamond ($Pn3m$), primitive ($Im3m$) and reverse hexagonal phase ($H_2$).
The phase diagram of phytantriol was first proposed by Barauskas et al. (Figure 2-11B) (120). A detailed phase diagram was given by Dong et al, which shows that impurities influence the phase diagram when phytantriol was obtained from different suppliers (Figure 2-11C) (38).

Figure 2-11: A) Structure of phytantriol, B) Phase diagram of phytantriol by Barauskas et al, reprinted with permission (120). C) Phase diagram of phytantriol by Dong et al, adapted with permission (121).

While hexagonal and reverse hexagonal liquid crystals have been reported as a template for polymerisation of conducting polymers, to date the bicontinuous cubic phase has not been used as a template to polymerise PPy. Though there are stimuli responsive / on-demand liquid crystal drug delivery systems using other triggers, there are no electrical triggers reported. The bicontinuous cubic phase has large open aqueous channels. We hypothesised that these
channels can serve as a template for CP growth and can aid in the movement of monomer and dopant to the substrate where polymerisation occurs. In this thesis, the bicontinuous cubic phase of phytantriol liquid crystals was investigated as a soft template to polymerise porous PPy for drug delivery application and the effect of electrical stimuli on liquid crystal structure by tuning the CP. This is described in detail in chapters 3 and 4.

**Small-angle X-ray scattering (SAXS) to determine liquid crystal nanostructure**

Small-angle X-ray scattering is one of the most common methods to characterise and determine the nanostructure of liquid crystals. When an X-ray beam of a certain wavelength ($\lambda$) enters the sample, the X-rays are scattered by regions of electron density in the sample. The scattered X-rays are recorded which details the structure of the sample (Figure 2-12).

**Figure 2-12:** SAXS instrument setup with an incident beam ($\lambda$) and scattering angle of 20.

The scattering is converted to a plot of intensity versus the magnitude of scattering vector, $q$, by the following equation, Equation 2-2

$$ q = \frac{4\pi}{\lambda} \sin \theta / 2 $$

**Equation 2-2**

The scattering arises from ordered arrangements of eg. molecules in a crystal lattice or domains in a liquid crystalline material. The relative location of the peaks indicates the symmetry of the structure (Figure 2-13, Table 2-2). The interplanar distance between two reflecting planes of the material can be calculated from the scattering vector at which the peak occurs and is described by Bragg’s Law (Equation 2-3).
\[ 2d \sin \theta = n\lambda \]

Equation 2-3

Where \( d \) is the interplanar distance between two reflecting planes, \( \lambda \) is the wavelength.

**Figure 2-13:** A schematic diagram of phase structures (left) with their characteristic 2D SAXS pattern (middle) and scattering profiles (right), \( L_\alpha \)-lamellar, \( Q_2 \) or \( V_2 \)-bicontinuous cubic and \( H_2 \)-reverse hexagonal phases. Adapted with permission (122, 123).

**Table 2-2:** Characteristic SAXS peak ratios of different liquid crystal phases (124).

<table>
<thead>
<tr>
<th>Phases</th>
<th>Characteristic SAXS peak ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamellar (( L_\alpha ))</td>
<td>1:2:3:4…</td>
</tr>
<tr>
<td>Bicontinuous cubic (( Q_2 ) or ( V_2 ))</td>
<td>( \sqrt{2} : \sqrt{3} : \sqrt{4} : \sqrt{6} : \sqrt{8} : \sqrt{9} : \sqrt{10} : \sqrt{11} : \ldots )</td>
</tr>
<tr>
<td>Diamond-Pn3m (( V^{224} ))</td>
<td>( \sqrt{2} : \sqrt{3} : \sqrt{4} : \sqrt{6} : \sqrt{8} : \sqrt{9} : \sqrt{10} : \sqrt{11} : \ldots )</td>
</tr>
<tr>
<td>Gyroid-Ia3d (( V^{230} ))</td>
<td>( \sqrt{6} : \sqrt{8} : \sqrt{14} : \sqrt{16} : \sqrt{20} : \sqrt{22} : \sqrt{24} : \ldots )</td>
</tr>
<tr>
<td>Primitive-Im3m (( V^{229} ))</td>
<td>( \sqrt{2} : \sqrt{4} : \sqrt{6} : \sqrt{8} : \sqrt{10} : \sqrt{12} : \sqrt{14} : \ldots )</td>
</tr>
<tr>
<td>Reverse hexagonal (( H_2 ))</td>
<td>( \sqrt{1} : \sqrt{3} : \sqrt{4} : \sqrt{7} : \sqrt{9} : \sqrt{12} : \sqrt{13} : \ldots )</td>
</tr>
</tbody>
</table>
2.5.3 Template-free polymerisation

Template-free methods have focussed on manipulating polymerisation conditions to form CPs with desired structures. A template-free approach to prepare PANI nanostructures has been reported by Liang et al. by using current densities greater than 0.08 mA.cm$^{-2}$. At these higher than typical current densities nanoparticles rather than continuous films were formed. High current densities for polymerisation can damage CP materials by causing over oxidation, however, these nanoparticles were used as nucleation sites to guide the polymerisation of the second layer of nanostructures at low current density (27).

Gas bubbles, such as O$_2$ or H$_2$, can be formed by the electrolytic decomposition of water and have been used to guide the growth of CPs. The bubbles may be stabilised with an anionic surfactant, such as camphor sulphonic acid, naphthalene sulphonic acid, or polyelectrolytes like poly(styrene sulphonic acid) (28, 125). The resulting soap bubbles can act as a template for electropolymerisation when located on the surface of an electrode. PPy was electropolymerised over soap bubbles to form bowls, cups, or spherical structures depending on the size and shape of the gas bubbles released and the length of polymerisation (126).

Interfacial polymerisation can be used to guide the growth of CPs when two reactive substances (monomer and oxidant) are each dissolved in different immiscible liquids (127). Uniform PANI nanofibres were formed in interfacial polymerisation, whereas irregularly shaped nanoparticles are formed by conventional chemical polymerisation. During conventional chemical polymerisation of PANI, regular PANI nanofibres are formed initially but due to the presence of excess reactants, secondary growth occurs on these nanofibres resulting in irregularly shaped particles. In contrast, during interfacial polymerisation, polymer forms at the interface only (128-130), and once the nanofibres are formed, they immediately diffuse into the aqueous layer with no secondary growth, resulting in a more uniform product (131).
2.6 Drug delivery

Effective utilisation of an electrically tuneable drug delivery system is determined by its stability of performance, drug loading capacity, and compatibility with surrounding tissues. Low amplitude electrical stimuli, typically less than 1 V, can be used to stimulate drug release from CP systems making them highly attractive (14, 132, 133). In drug delivery, CPs with defined micro- or nanostructures offer advantages due to their enhanced surface area with associated increases in drug loading capacity and responsiveness to stimulation (Table 1). However, the direct comparison of studies is difficult due to variations in the experimental setup and reporting of drug loading and release. Ideally, drug delivery would be standardised to the amount or volume of CP material. Nonetheless, the available data demonstrates how CPs with micro- or nanostructures have increased drug loading and can facilitate tighter control over the release of their payload.
### Table 2-3: Template-directed polymerisation of CPs with defined micro- or nanostructures for drug delivery.

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Template</th>
<th>Dopant</th>
<th>Polymerisation conditions</th>
<th>Drug loaded</th>
<th>Release conditions</th>
<th>Comments on Drug release</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 M pyrrole</td>
<td>AAO membrane</td>
<td>0.1 M NaDBS</td>
<td>0.6 V for 60 s</td>
<td>FITC-BSA</td>
<td>-1.1 V for 10 s and 0.1 V for 10 s</td>
<td>Cumulative release of approximately 0.9 µg.ml&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(35)</td>
</tr>
<tr>
<td>0.2 M pyrrole</td>
<td>1.0% w/v PS nanobead suspension by vertical deposition</td>
<td>0.01 M fluorescein</td>
<td>+0.9 V for 200 s</td>
<td>Fluorescein</td>
<td>-2.0 V for 10 s for 6 consecutive trials</td>
<td>&gt;10-fold increase in release from nanoporous films compared to non-porous films</td>
<td>(66)</td>
</tr>
<tr>
<td>0.2 M pyrrole</td>
<td>1.0% w/v PS nanobead suspension by vertical deposition</td>
<td>0.01 M fluorescein</td>
<td>+0.9 V for 200 s</td>
<td>Fluorescein as dopant and dexamethasone into nanopores sealed with second PPy layer on top</td>
<td>-2.0 V or -0.5 V for 5 s and 0 V for 5 s</td>
<td>Second sealing layer resulted in more fluorescein retained for release</td>
<td>(67)</td>
</tr>
<tr>
<td>0.1 M pyrrole</td>
<td>5.0% w/v PS nanobead suspension by vertical deposition</td>
<td>0.009 M biotin and 0.01 M NaDBS</td>
<td>+0.7 V</td>
<td>Streptavidin coated gold nanoparticles</td>
<td>-2.0 V to +2.0 V for 60 s</td>
<td>More release on reduction at -2.0 V compared to oxidation at +2.0 V</td>
<td>(134)</td>
</tr>
<tr>
<td>0.1 M pyrrole</td>
<td>PS nanobeads deposited by colloidal lithography</td>
<td>Rhodamine B</td>
<td>30 mC at 1 mA.cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Rhodamine B</td>
<td>0.25-1.0 mA.cm&lt;sup&gt;2&lt;/sup&gt; for 20-30 s</td>
<td>3 times more release compared to the non-porous films</td>
<td>(64)</td>
</tr>
<tr>
<td>0.1 M pyrrole</td>
<td>PMMA colloids by vertical deposition</td>
<td>0.1 M NaDBS</td>
<td>2 mA.cm(^{-2}) for 4 min</td>
<td>Risperidone</td>
<td>Alternate pulsed potential (±0.6 V, 0.5 Hz)</td>
<td>Drug release 162.69 ± 3.6 µg from inverse opal films and 42.5 ± 7.37 µg from unstructured films</td>
<td>(31)</td>
</tr>
<tr>
<td>0.2 M pyrrole</td>
<td>PMMA colloids by vertical deposition</td>
<td>0.05 M DexP</td>
<td>2 mA.cm(^{-2}) for 4 min</td>
<td>DexP</td>
<td>Alternate pulsed potential (±0.6 V, 0.5 Hz)</td>
<td>Inverse opal films released 3 times more than unstructured films</td>
<td>(36)</td>
</tr>
<tr>
<td>0.2 M pyrrole</td>
<td>PEDOT: PSS-Chitosan nanofibres</td>
<td>Ciprofloxacin hydrochloride</td>
<td>0.5 and 2.0 mA.cm(^{-2}) for 10 or 20 min</td>
<td>Ciprofloxacin hydrochloride</td>
<td>+0.3 V or -0.26 V for 72 h</td>
<td>Nearly 90% of loaded drug was released on reduction while only 30% was released by oxidation</td>
<td>(135)</td>
</tr>
<tr>
<td>0.02 M EDOT</td>
<td>Dexamethasone loaded PLGA nanofibres</td>
<td>PBS</td>
<td>0.9 mA.cm(^{-2}) for 30 min</td>
<td>Dexamethasone</td>
<td>+1 V with a scan rate of 0.1 V.s(^{-1}) for 10 s</td>
<td>Approximately 1.5 mg on stimulation compared to 0.25 mg in unstimulated films</td>
<td>(25)</td>
</tr>
<tr>
<td>0.2 M pyrrole</td>
<td>Graphene oxide nanocomposite</td>
<td>DexP and co-dopant graphene oxide</td>
<td>+0.8 V to reach charge density 400 mC.cm(^{-1})</td>
<td>DexP</td>
<td>Biphasic pulse of -2.0 V for 5 s and 0 V for 5 s</td>
<td>2.3 times more drug release than control films</td>
<td>(18)</td>
</tr>
<tr>
<td>0.34 ml pyrrole</td>
<td>Palygorskite (pal) nanocomposite</td>
<td>Aspirin and palygorskite</td>
<td>+0.8 V for 500 s</td>
<td>Aspirin</td>
<td>External stimulus of -0.6 V</td>
<td>Pal-PPy nanocomposite films were approximately 60 µg.ml(^{-1}) compared with 35 µg.ml(^{-1}) from conventional PPy films</td>
<td>(47)</td>
</tr>
<tr>
<td>Pyrrole Concentration</td>
<td>Nanomaterials Type</td>
<td>Drug Loading/Condition</td>
<td>Current/Scan Conditions</td>
<td>Drug Release Conditions</td>
<td>Highest Drug Release Remarks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
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<td>-------------------------</td>
<td>------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.4 M pyrrole</td>
<td>Carbon nanotubes</td>
<td>20 mg.mL⁻¹ DexP and 1 mg.mL⁻¹ carbon nanotubes containing DexP</td>
<td>+70 μA for 400 s</td>
<td>Dexamethasone (DexP) -2.0 V for 5 s followed by 0.0 V for 5 s for 20 h</td>
<td>Highest drug release with carbon nanotubes was 78.7 ± 2.1 μg compared to 38.8 ± 3.2 μg without nanotubes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 M pyrrole</td>
<td>Multi walled carbon nanotubes grown on titanium</td>
<td>Penicillin/streptomycin - 6.82 mM or Dexamethasone (DexP) 0.125 mM</td>
<td>CV from 0 to 1.1 V at a scan rate of 100 mV.s⁻¹ for 10 cycles</td>
<td>Penicillin/streptomycin or Dexamethasone (DexP) From -1 V to +1 V at scan rate of 100 mV.s⁻¹</td>
<td>Cumulative release of penicillin/streptomycin after 25 cycles was more than 80% and Dexamethasone was almost 100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 M pyrrole</td>
<td>Cetyl pyridinium, a cationic surfactant as modifier</td>
<td>Cetyl pyridinium and methotrexate</td>
<td>+0.7 V for 2400 s against Ag/AgCl</td>
<td>Methotrexate -0.7 V for prolonged time until no more methotrexate was released</td>
<td>Up to 100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15 M pyrrole</td>
<td>ATP as morphology directing agent</td>
<td>0.07 M lithium perchlorate and 0.20 M ATP</td>
<td>1.0 mA.cm² for 600 s</td>
<td>ATP -0.8 V at different</td>
<td>90% of ATP released from nanofibres after 45 h compared to 53% from unstructured film</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.145 M pyrrole</td>
<td>PPy nanofibres</td>
<td>0.085 M p-toluene sulphinic acid and 0.2 M PBS</td>
<td>0.477 mA.cm² for 1600 s</td>
<td>ATP and dexamethasone CV from -0.9 to +0.6 V at 100 mV.s⁻¹</td>
<td>More than 90% of the loaded ATP and dexamethasone was released</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.09 M pyrrole</td>
<td>DTAB/decyl alcohol micelles</td>
<td>NA</td>
<td>Chemical oxidation using FeCl₃</td>
<td>Fluorescein or daunorubicin -1.0 V for 10 s with 5 min interval for fluorescein and 0.5 V for 20 s each day repeated for 5 days</td>
<td>Electric field release using needle electrodes achieves electrically controlled release of fluorescein and daunorubicin</td>
<td></td>
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</table>
2.6.1 Drug loading

Different mechanisms can drive the loading of drugs into CPs. CP drug delivery systems often rely on electrostatic interactions between the CP and a charged drug. During synthesis of CP films, dopant anions are incorporated into the polymer to counterbalance the positive charge associated with the oxidative polymerisation. For PPy, a dopant anion is incorporated for every 3-5 pyrrole units. Either a small mobile anion (\(A_S^-\), Equation 2-4) or a large immobile anion (\(A_L^-\), Equation 2-5) can be loaded.

\[
PPy^0 + A_S^- \xrightarrow{\text{oxidation}} PPpy^+ + A_S^-
\]

Equation 2-4

\[
PPpy^+ + A_L^- + B^+ \xrightarrow{\text{reduction}} PPpy^0 + A_L^- + B^+
\]

Equation 2-5

Zinger and Miller first reported the loading of ferrocyanide as a dopant into PPy during polymerisation in 1984 (16). The anionic bioactives such as salicylate, naproxen, nicoside, ATP, and DexP have been loaded directly into CPs as dopants (17, 34, 137). However, only certain anions can be incorporated directly as dopants to form a polymer. If a drug molecule does not produce a satisfactory polymer product when used as a dopant the drug can be loaded after polymerisation through ion exchange by redox cycling the CP in appropriate media. For example, glutamate can be loaded into CP doped with perchlorate by reducing the polymer in 0.1 M sodium glutamate to promote the efflux of chlorate ions followed by oxidation to force glutamate ions into the film (16).

Alternatively, the reduction can be used to load cationic drugs, such as dopamine and chlorpromazine, into CPs through electrostatic attraction to large anionic dopants (\(A_L^-\)) which are immobilised in the polymer, Equation 2-5 (19, 20). However, electrostatic interactions are not the only mechanism for drug loading; the incorporation of cationic neurotrophin-3 during PPy synthesis has been attributed to hydrophobic interactions along with physical entrapment (138).
An advantage of using CPs with defined micro- or nanostructures is the ability to increase the amount of drug the material can carry. Increased drug delivery capacity will increase the utility of CP based drug delivery systems to manage systemic conditions and extend their operational lifetime. When a drug is incorporated directly as a dopant into the CP, loading is limited to 1 drug molecule per 3-5 monomer units (14). For drugs loaded by ion exchange, this theoretical loading level is unlikely to be reached, as it requires complete mobility of the polymerisation dopant out of the polymer bulk and of the drug into the polymer. With the increased surface area offered by CPs with defined micro- or nanostructures, ion exchange can be expected to be more efficient as distances between polymer bulk and polymer/media interface are reduced (18, 30). However, loading levels remain limited to exceed 1 drug molecule per 3-5 monomer units if electrostatic forces alone govern loading. Meanwhile, where drugs can be physically entrapped within CPs, the use of templates to direct polymerisation creates many exciting possibilities. A template can be used which itself contains drug (25) or if the template is removed after polymerisation the empty space can act as a reservoir to contain drug (31).

2.6.1.1 Drug loading into CPs with the template retained

Templates containing drug can be used to direct CP growth (Figure 2-14). Abidian et al. demonstrated synthesis of electrospun biodegradable PLGA fibres loaded with dexamethasone (Figure 2-14A). Electrodeposition of PEDOT on PLGA fibres produced PEDOT-coated PLGA fibres loaded with dexamethasone (25). Jeon et al. used a different approach by loading a model drug FITC-BSA into the pores of an AAO membrane template. After loading with FITC-BSA, the pores in the template were restricted to a certain diameter by polymerising PPY on the upper part of the template (Figure 2-14C). The AAO template with uniform pore size served as a depot system holding large quantities of drugs. By electroactuation of PPy, the pores were opened and closed controlling drug flux (35).

Nanocomposite materials containing a CP have been used in drug delivery (139-141). Graphene oxide (GO) nanosheets along with DexP were incorporated into PPy films as codopants, to form nanocomposites. During oxidative polymerisation, carboxylic groups were formed at the ends of GO, making them negatively charged allowing GO to be taken up as codopants into the CP along with DexP (Figure 2-14B). The high surface-to-volume ratio of GO allows large quantities of DexP to be taken up between the layers of GO nanosheets. Hence, DexP can be loaded into PPy not only as a dopant but also incorporated into GO resulting in
higher drug loading (18). Similarly, aspirin has been loaded into palygorskite-PPy (Pal-PPy) nanocomposites, which combine the electrical properties of PPy with the high surface area of Pal. The effective electrochemical surface area of the Pal-PPy nanocomposite was found to be approximately five times more than the unstructured PPy. The loading capacity of aspirin in the Pal-PPy nanocomposite was found to be 2.5%. The large ion-dipole attractions between anionic aspirin and cations in Pal resulted in higher drug loading than in PPy alone (47).

Carbon nanotubes are another class of materials, which have attracted considerable interest for biomedical applications, including drug delivery. This can be attributed to their biocompatibility and high drug loading capacity (142). Composites of CPs with carbon nanotubes possess improved electrical and mechanical properties (143). Luo et al. determined the drug loading capacity of the inner cavity of different sized carbon nanotubes. Carbon nanotubes, when sonicated in acid to open their ends, served as nano-reservoirs, which could be loaded with DexP. However, considerable leakage was observed from the ends of the carbon nanotubes. By sealing the carbon nanotubes with PPy, DexP loading significantly increased, the passive release was lower and release could be triggered electrically (Figure 2-14D). Carbon nanotubes with a thinner outer diameter and wider inner diameter displayed a drug loading level of 78.7 ± 2.1 µg compared to 38.8 ± 3.2 µg in unstructured PPy films (83). Sirivisoot et al. demonstrated the incorporation of the anionic drugs DexP and penicillin/streptomycin (P/S) into the polymer backbone during electropolymerisation of PPy on multiwalled carbon nanotubes grown on titanium (MWNT-Ti) (84).

A novel method of producing coaxial CP fibres loaded with ciprofloxacin has been accomplished by Esrafilzadeh et al. (135). They successfully polymerised PPy containing ciprofloxacin hydrochloride on PEDOT: PSS-chitosan hybrid fibres. PEDOT: PSS-chitosan fibres were formed by wet-spinning with chitosan used as a coagulant. Ciprofloxacin (isoelectric point-7.4) was maintained in a positive state at the polymerisation pH and was physically entrapped into the polymer during polymerisation while chloride ions acted as a dopant. The amount of ciprofloxacin-loaded into of fibre (diameter 65 ± 7 µm, weight 254 ± 13 µg) was found to be 42 ± 4 µg.cm⁻¹.

Alizadeh and Shamaeli used cetyl pyridinium to guide the growth of PPy films and to promote methotrexate (MTX) loading into nanostructured PPy films (136). MTX usually has very low doping efficiency into CPs. PPy with entrapped cetyl pyridinium improved MTX loading due to the electrostatic and aromatic interactions between the cationic cetyl pyridinium and anionic
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MTX. Ru et al. synthesised PPy nanofibre network using ATP as a dopant (30). Here ATP served as both dopant and morphology directing agent. These nanofibre networks contain micro- or nanogaps among the individual nanofibres, which can be loaded with a second drug. DexP, dopamine, hydroxy camptothecin, and paclitaxel were all loaded as a second drug using this approach (46).

Ge et al. reported the use of a soft template loaded with a drug to prepare CP nanoparticles using an emulsion as a soft template to direct polymerisation (26). They encapsulated fluorescein/daunorubicin in an emulsion formed from the cationic surfactant dodecyl trimethylammonium bromide (DTAB) and co-surfactant decyl alcohol. The addition of pyrrole monomer and ferric chloride to the drug encapsulated micelles resulted in the formation of drug loaded PPy nanoparticles with an average diameter of 60 nm. Entrapment efficiencies of 37.5% and 33.3% were reported for fluorescein and daunorubicin, respectively.

2.6.1.2 Drug loading into CPs after the template is removed

After the removal of a hard template, drugs can be loaded into the void spaces of CPs with defined micro- or nanostructures (Figure 2-14E). Entrapment of drugs in the voids of porous CP films produced from colloidal templates following the selective removal of the colloids has been demonstrated. Luo et al. were able to entrap dexamethasone into the pores of nanoporous PPy films polymerised using a PS nanobeads template. They produced nanoporous PPy films using a model drug, fluorescein as a dopant (66). As an extension of this work, they loaded a second drug, dexamethasone, into the pores of the nanoporous PPy films after the removal of the PS bead template. This allowed loading and simultaneous release of two drugs; fluorescein and dexamethasone (Figure 2-14E) (67). Similar work reported by Sharma et al. showed the entrapment of the hydrophobic drug risperidone in the macroporous inverse opal PPy films produced from a PMMA template. The surface area increased by almost four times and entrapment efficiency improved two-fold in inverse opal films compared to non-inverse opal films (31). Similarly, Pokki et al. fabricated nanoporous PPy film using PS beads deposited by colloidal lithography and loaded the resulting pores with rhodamine B (64). A related concept was reported by Cho and Borgens, in which nanoporous PPy/ biotin films were prepared using a sacrificial PS nanobead template. Streptavidin coated gold nanoparticles were then attracted to the macroporous PPy films through biotin-streptavidin interactions (134).
2.6.2 Drug release from CPs

Drug release from CPs is typically controlled by the redox state of the polymer which influences electrostatic interactions between drug and polymer (32), alongside volume changes (21, 33). Electrostatic interactions between CP and drug will result in anionic drugs being released on reduction, Equation 2-4. Ferrocyanide (16), salicylate and naproxen (17), ATP (137), and DexP (34) have all been released from CP films through this mechanism. Meanwhile, cationic drugs are expelled from the polymer when oxidised, Equation 2-5. The cationic drugs dopamine (19) and chlorpromazine (20) have been controllably released using this mechanism. Volume changes rather than electrostatic interactions have been attributed to the triggered release of risperidone (37) and progesterone (23).

**Figure 2-14**: Schematic diagram showing drug loading and release from different micro- or nanostructures. A) Polymerisation of PEDOT on DexP loaded PLGA fibres and actuation driven release on stimulation. Adapted with permission from (25), B) Electropolymerisation of PPy with GO and DexP as co-dopants and electrostatic release (18), C) Electrodeposition of PPy on the upper surface of the gold coated AAO membrane and the reversible change of pore size on oxidation and reduction with drug release. Reprinted with permission from (35), D) Polymerisation of PPy over DexP loaded carbon nanotubes and its release on stimulation. Adapted with permission from (83), E) Preparation of macroporous inverse opal films by polymerising on the colloidal template, loading drugs into pores (DexP) and as a dopant (fluorescein) and their subsequent release on stimulation. Adapted with permission from (67).
The release of drugs from CPs with defined micro- or nanostructures utilises not only electrostatic interactions between drug and polymer but is also able to take greater advantage of the volume changes that occur upon switching of redox states (Table 2-3). The movement of hydrated ions into and out of the polymer, described in Equation 2-4 and Equation 2-5, results in polymer expansion and contraction (35, 36). Typically, CPs with defined micro- or nanostructures have increased surface area. This higher effective electrochemical surface area promotes the speed and extent of ion movement into and out of the polymer; hence more precise and potentially greater magnitude of responses to both electrostatic switching and volume changes occur.

2.6.2.1 Electrostatic driven release

Electrostatic interactions were demonstrated to drive the release of anionic ciprofloxacin from coaxial PPy and PEDOT: PSS-chitosan fibres with a two-fold increase in release from the reduced state compared to the passive release from the as-prepared state. When electrochemically oxidised, a 20% reduction in ciprofloxacin release was demonstrated compared to passive release (135). Meanwhile, Sirivisoot et al. demonstrated the release of DexP and penicillin/streptomycin (P/S) from nanostructured PPy film polymerised on MWNT-Ti (84).

PPy nanocomposites have been co-doped with GO nanosheets to enhance DexP loading. This enabled more than twice the amount of DexP to be released from the nanocomposites compared to conventional PPy-DexP films upon application of a biphasic electrical pulse. The GO nanosheets remained within the polymer structure due to their large size (Figure 2-14B). The linear release of DexP was observed for 400 cycles from the films co-doped with DexP and GO (18). Similarly, DexP loaded into the inner cavity of carbon nanotubes and incorporated into PPy films, demonstrated 2 times more drug release compared to drug loaded directly as a dopant into PPy films (Figure 2-14D) (83). The higher amounts of drug release can be related to the increased drug loading with the GO nanosheets and carbon nanotubes acting as nano-reservoirs.

The electrostatically controlled release of aspirin from Pal-PPy nanocomposite films was approximately 1.7 times more than conventional PPy films in both stimulated and unstimulated samples. The difference was attributed to the increase in effective electrochemical surface area and electrostatic attraction between anionic aspirin and the cationic groups in Pal (47). ATP
release from PPy nanofibres polymerised using ATP as a dopant as well as structure guiding agent was found to be 90% compared to 53% from the unstructured cauliflower shaped PPy film on stimulation for 45 h (30).

A recent paper reported the release of anionic DexP loaded into macroporous inverse opal PPy films. DexP was loaded into both macroporous and conventional films as a dopant, yet stimulation could release more than 3 times the amount of drug from the same theoretical amount of PPy. This can be explained as the inverse opal film was more electroresponsive due to the high surface area resulting in improved ion exchange between the polymer and surrounding media (36).

Opening up exciting possibilities, CP films can function as self-powered galvanic cells when coated with a thin layer of active metal, such as magnesium, upon exposure to an electrolyte medium. In a recent report, magnesium acted as an anode and CP film as a cathode, thus together acting as a self-powered cell without the need for external stimulation. When the cell was placed in electrolyte medium such as NaCl, magnesium is oxidised to Mg$^{+2}$ in solution and the CP reduced resulting in the release of the drug (144). This galvanic mechanism has been used to release ATP from a PPy nanofibre network made using ATP as both dopant and morphology directing agent. The authors compared the release of ATP from the nanofibre network coated with magnesium to a conventional PPy film without coating. The nanofibre network showed 22% release compared to 13% released from the conventional PPy film (30).

### 2.6.2.2 Actuation driven release

CP systems can use redox-driven volume changes to drive drug release. The speed and extent of actuation rely on the influx and efflux of hydrated ions (21). Ion exchange between polymer and media is facilitated by the high surface area in CPs with defined micro- or nanostructures (25, 35). Abidian et al. reported that bursts of dexamethasone release could be achieved from PEDOT coated PLGA fibres by electrical stimulation which caused the PEDOT coating to actuate resulting in cracks which facilitated drug release (Figure 2-14A) (25).

Pulsatile release of FITC-BSA from a system comprising a PPy/NaDBS film electrodeposited on the upper part of the pores of an AAO membrane was demonstrated by Jeon et al. (Figure 2-14C). On reduction, hydrated cations move into the CP causing it to swell thereby decreasing the membrane pore size while on oxidation cations are expelled and the membrane pores open.
Chapter 2 - Literature Review

as the polymer contracts resulting in higher rates of drug release (35). Pore diameters were observed to decrease from 190 nm (60 L.m⁻² flux) to 140 nm (no flux) when the film is shifted from the oxidised to reduced states, with a switching time of less than 10 s.

Sharma et al. reported that the rapid contraction and expansion of the inverse opal films on electrostimulation resulted in increased risperidone release from inverse opal films. A 10% increase in interplanar spacing was seen in PPy inverse opal films on reduction. The release was found to be 162.69 ± 3.6 μg on stimulation compared to 119.8 ± 2.5 μg in unstimulated inverse opal films, whereas in conventional non-inverse opal films it was only 42.5 ± 737 μg on stimulation compared to 31.3 ± 0.4 μg from unstimulated films. The difference in release levels from inverse opal and conventional films can be attributed to higher drug loading in porous inverse opal films (31). Similarly, rhodamine B release from PPy film formed from PS nanobead template deposited by colloidal lithography was 3 times higher than non-porous films (64).

The simultaneous delivery of two drugs (fluorescein and dexamethasone) from nanoporous PPy films on electrical stimulation was demonstrated by Luo et al. The release of the anionic fluorescein can be attributed to a de-doping process from the PPy backbone, whereas volume changes of the porous structures on actuation squeezed dexamethasone out from the pores (Figure 2-14E) (67). Drug delivery from CPs with defined micro- or nanostructures is more efficient than conventional CPs, which can be attributed to both increased drug loading and higher responsiveness to electrical stimulation.

2.7 Biological considerations

The majority of CPs are non-biodegradable. For many currently explored applications, this is entirely appropriate, with CP-based systems designed for removal from the body at the end of the device lifetime. To broaden future applications of these materials biodegradable CPs have been reported, this is achieved through enzymatic or hydrolytic degradation by certain modifications (145). Depending on the modification employed, biodegradability can be associated with a decrease in conductivity. Rivers et al. prepared pyrrole-thiophene oligomer chains containing degradable ester linkages. The ester bonds are cleaved by enzymes in-vivo and the resulting oligomer fragments can be engulfed by macrophages (146). Another approach is to modify the CP chain through the addition of ionisable or hydrolysable side groups. For
example, β-substituted pyrrole monomers with acid or ester groups attached to β-position have been reported as biodegradable without any loss in conductivity (147).

Although many CPs are regarded as biocompatible (10, 146), templates used to synthesise CP with defined micro- or nanostructures must also be considered for compatibility. This is important in cases where the drug is loaded into the CP delivery systems while the template is retained, and also if any traces of the templates are left behind after template removal. In addition, the bioactivity of the incorporated drug should not be affected by the template used or the electrical stimulation applied during synthesis or drug release.

A number of reports have presented data confirming the biocompatibility of CPs with cell cultures such as neurons (148, 149), osteoblasts, and fibroblasts (84). Drug release samples from PPy films doped with GO nanosheets, with or without DexP loading on biphasic stimulation, were added to the primary neuronal cultures with no changes observed in neuronal cell density indicating no toxicity of GO nanosheets (18). A recent study shows that the aqueous extracts obtained from DexP loaded PPy films evaluated on adult retinal pigment epithelium cells demonstrated negligible toxicity (150). Ge et al. demonstrated the biocompatibility of a temperature sensitive hydrogel (PLGA-PEG-PLGA) containing 1% weight of PPy nanoparticles the following injection subcutaneously at the dorsal site of FVB adult mice. Histological observation following H&E staining showed no infiltration by neutrophilic granulocytes and lymphocytes at 14 days with any fibrous tissue observed. However, hydrogels containing over 5% by weight of PPy nanoparticles caused fibrous tissue formation 2 weeks after injection (26).

The bioactivity of DexP released on electrical stimulation (-2 V for 5 s followed by 0 V for 5 s for 1000 cycles) was assessed using primary astrocyte cultures. The released DexP interrupted the primary astrocyte proliferation by down-regulating G-coupled receptors. The authors found a similar reduction in cell density and no difference in the bioactivity (p< 0.05) of the DexP released from nanocomposites films when compared to the control DexP solution (1 µM) (18). In another study, the bioactivity of DexP released from carbon nanotube nano-reservoirs in PPy was assessed using HAPI cells. No significant difference was found between the test group and control DexP solution of 10 μM concentration (p<0.05), indicating that DexP retained its activity during loading and stimulated release (83). Similarly, Esrafilzadeh et al. reported that the antibacterial activity of ciprofloxacin was retained following release from loaded coaxial CP fibres against both gram-positive and gram-negative bacteria (135). A clear zone of
inhibition was observed around the ciprofloxacin-loaded coaxial CP fibres compared to no zone of inhibition surrounding control fibres without drug.

Recording and stimulating bio-electrodes transduces bioelectric signals from cells to electronic signals (151). Due to the small size, neural electrodes typically have a high impedance. CP coatings are attractive at electrode neural interfaces as they lower impedance and can achieve controlled release of bioactives. CP coated electrodes have been used to deliver anti-inflammatory drugs to prevent pain, inflammation, neuronal loss, and scar formation (83); antibiotics to preclude infection (135); and neurotrophins such as nerve growth factor to promote neuronal growth at the implant site (152).

CPs with defined micro- or nanostructures have advantages over unstructured CPs at the bio-electrode interface due to their enhanced effective electrochemical surface area which lowers impedance and increases charge transfer capacity. Upon deposition of CP nanotubes on gold electrodes, the impedance decreased by 2 orders of magnitude at 1 kHz, the characteristic frequency of neuronal action potentials (25). The impedance of the glassy carbon electrode coated with PPy/carbon nanotube composite showed decreased impedance compared to PPy coated glassy carbon at frequencies between 0.5-100 kHz (83). Similarly, a drop in impedance has been seen when PPy is co-doped with GO and DexP at all frequencies (1Hz to 100 kHz) compared to the PPy doped with DexP alone (18).

### 2.8 Dexamethasone sodium phosphate (DexP) and Dexamethasone (Dex)

DexP and Dex have been investigated in this thesis for tuneable delivery from CP systems. They have been loaded into the conducting polymer systems prepared by soft template-directed polymerisation. DexP has been loaded into PPy films as a dopant prepared by electrochemical polymerisation using phytantriol bicontinuous cubic phase as a template. Both DexP and Dex have been loaded into PPy nanoparticles prepared by chemical polymerisation using SDBS micelles as a soft template. These drugs have differing logP and charge values. By examining the loading and release of each drug, this thesis seeks to understand the mechanism and release of drug with different properties from conducting polymers.
DexP (Figure 2-15A) is the disodium salt of the synthetic adrenal corticosteroid Dex (Figure 2-15B). DexP is a prodrug of Dex which is a potent anti-inflammatory agent (153). It acts by inhibiting the inflammatory pathways such as NF-κB activation and apoptotic pathways by binding to specific nuclear steroid receptors (40, 154). The physicochemical properties of DexP and Dex are elaborated in Table 2-4. These properties dictate the formulation parameters of the drug delivery system. These properties also determine the permeability across biological membranes as well as pharmacokinetic properties such as absorption and distribution.

Dex has been widely used in the treatment of ophthalmic conditions like age-related macular degeneration (AMD), diabetic and non-diabetic macular oedema, and posterior uveitis in the form of eye drops and eye ointments (155-157). Dex acts on glucocorticoids receptor similar to natural corticosteroid, cortisone. Unlike other corticosteroids, Dex lacks the salt retention in the eye making it an ideal candidate for cases with co-existing elevated intraocular pressure (158).
Table 2-4: IUPAC name, chemical formula and physicochemical properties of DexP and Dex (159, 160).

<table>
<thead>
<tr>
<th></th>
<th>DexP</th>
<th>Dex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C$<em>{22}$H$</em>{28}$FNa$_2$O$_8$P</td>
<td>C$<em>{22}$H$</em>{29}$FO$_5$</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>516.4 g.mol$^{-1}$</td>
<td>392.5 g.mol$^{-1}$</td>
</tr>
<tr>
<td>Physical state</td>
<td>White to beige powder</td>
<td>White powder</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>50 mg.mL$^{-1}$</td>
<td>0.05 mg.mL$^{-1}$</td>
</tr>
<tr>
<td>LogP</td>
<td>0.54</td>
<td>2.03</td>
</tr>
<tr>
<td>pKa</td>
<td>6.8</td>
<td>12.14</td>
</tr>
</tbody>
</table>

Recently, new systems are being investigated to provide site-specific delivery of Dex in order to minimise side effects. Ozurdex® is an intravitreal dexamethasone (0.7 mg) implant approved by the FDA, which releases Dex slowly over 6 months. Despite its usefulness, Ozurdex has shown side effects like the formation of cataract, anterior chamber inflammation, and retinal tearing. In the case of serious side effects, Ozurdex cannot be removed (161, 162). Hence, there is a need for novel drug delivery systems with stimuli responsive drug release for the treatment of ophthalmic conditions. Tuneable release of Dex from conducting polymer based drug delivery system has been investigated in this thesis.
Chapter 3: Polymerisation of Polypyrrole in a Phytantriol Liquid Crystal Template
3.1 Declaration for Chapter 3

Some of the work presented in this chapter was presented at the Advanced Materials and Nanotechnology-7 conference and has been published as peer-reviewed conference proceedings: Uppalapati D, Boyd B, Travas-Sejdic J, Svirskis D. Porous conducting polymer prepared through liquid crystal template for drug delivery. International Journal of Nanotechnology. 2017;14(1-6):422-31.

3.2 Introduction

Amphiphilic lipids can self-assemble into liquid crystal structures in an aqueous environment (163). These self-assembled structures vary in size and shape depending on the lipid concentration, solvent composition, pH, temperature, and pressure (164, 165). The forces responsible for the self-assembly of these structures are weak van der Waal’s forces, hydrophobic attraction, hydrogen bonding, and screened electrostatic interactions. The strength of these interaction forces determines the equilibrium structure formed (166). These liquid crystals are well established in controlled drug delivery (167, 168). Liquid crystals provide sustained release of drugs; different phases can have different drug release rates (169). The release of bioactives from lipid-based liquid crystal systems is diffusion controlled; generally, the larger bicontinuous channels of the bicontinuous cubic phase result in faster release rates of bioactive compared to lamellar and reverse hexagonal phases (170-172). Both hydrophobic and hydrophilic drugs can be loaded and released from liquid crystals (173).

Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) is an amphiphilic lipid and a well-known ingredient in hair and skin care cosmetics (6). When mixed with water, phytantriol forms different phases (Figure 3-6) depending on the lipid to water concentration and the temperature of the system (120). These phases can reversibly change from one form to another with alterations in external conditions.

Phytantriol forms the lamellar phase at low water compositions, typically less than 10% w/w water. At high water compositions, it forms a bicontinuous cubic phase (Q₂ or V₂) and when the temperature is increased, this bicontinuous cubic phase is converted to a hexagonal phase (H₂). This phase transition temperature of phytantriol is usually above 40 °C (120, 121). Impurities such as vitamin E acetate, when added to phytantriol at sufficient concentrations,
induce the formation of hexagonal phase instead of bicontinuous cubic phase at lower temperatures. It was proposed that voids at the intersection of the hydrophobic tails in phytantriol are filled by vitamin E acetate resulting in the formation of hexagonal phase at a lower temperature (168).

These phases can reversibly change from one form to another with alterations in external conditions, thus resulting in altered drug release rates. Temperature (167), salt (174), and pH (175) responsive liquid crystal systems have been applied to tuneable bioactive delivery. Boyd et al. have demonstrated a salt responsive system by studying the effect of ionic surfactant content in the lipid and ionic strength in the system on the phase behaviour of phytantriol dispersions (Figure 3-1). Two important trends were reported, firstly, an increase in the ionic surfactant content increased the curvature of the self-assembled system toward the hydrophobic region, resulting in the phase transition from bicontinuous cubic phase to lamellar phase. Secondly, an increase in ionic strength decreased repulsion between the head groups of the ionic surfactant, resulting in a phase transition from lamellar phase to bicontinuous cubic phase (174).

While various triggers have been reported to alter the liquid crystal phase, there are currently no electrical switches reported in the literature. We hypothesise that a hybrid system comprising both conducting polymer and liquid crystal components could be used to electrically trigger a phase transition in the liquid crystal via two possible mechanisms. CPs are semi-conductive, the resistance to the flow of current will result in heat evolution when a current is passed. This change in the temperature could be used to alter the liquid crystal phase. The second mechanism relies on the movement of ions into and out of the CP depending on redox state. The associated local changes in ion concentration could be used to alter the liquid crystal phase. Depending on the mobility of the anion into and out of the polymer, ion concentrations in surrounding solution will alter, which may result in phase transition in liquid crystal.
While a small number of conducting polymer/liquid crystal (CP/LC) hybrid systems are reported in the literature, none have been applied to drug delivery. Liquid crystals offer a highly ordered, nanoscale template for the growth of conducting polymers. Conducting polymer nanowires or nanotubes have been obtained by polymerising the polymer inside a liquid crystal template depending on the hydrophilic and hydrophobic nature of monomer used. Conducting polymers like polyaniline, PEDOT, polypyrrole nanostructures have been obtained either chemically or electrochemically from different hexagonal liquid crystals (112-114). Bicontinuous cubic phase was selected as a template to polymerise polypyrrole as this phase has continuous lipid and aqueous channels which helps in the movement of monomer and dopant through bicontinuous cubic phase to the substrate (116). Polymerisation occurs on the surface of the substrate, hence monomer and dopant should be in the vicinity of the substrate for the polymerisation to occur. Currently, there are no reports of conducting polymers polymerised within the bicontinuous cubic phase of a liquid crystal.

**Figure 3-1:** Schematic diagram of phase transition of ionic surfactant-phytantriol dispersions driven by composition and strength in the system. Adapted with permission (174).
Chapter 3- Polymerisation of Polypyrrole in a Phytantriol Liquid Crystal Template

In addition to being interested in the potential of an electrical stimulus to control bioactive release from the liquid crystalline phase, the liquid crystal templated growth of CP is expected to enhance the levels of bioactive that can be delivered from the CP/LC hybrid. By growing the CP inside a liquid crystal template micro- and nanostructure of the polymer is influenced. The increase in porosity of the polymer is expected to enhance responsiveness to the stimulation.

3.3 Aims and objectives

The aim of this chapter was to polymerise PPy through liquid crystal template and to determine the effect of additives, the polymerisation of polypyrrole and the redox cycling of PPy on the phase of liquid crystal template. The specific objectives are to:

- Construct a phase diagram of phytantriol and water to determine the required composition to achieve the bicontinuous cubic phase.
- Determine the effect of addition of pyrrole and pTS on the liquid crystalline phase.
- Explore approaches to fabricate conducting polymer through the liquid crystal template, and to perform morphological, chemical and electrochemical characterisation of the formed polymer.
- Explore the effect of potentiostatic polymerisation potential, temperature, and CV cycling of the conducting polymer on the liquid crystal phase.

3.4 Materials

Pyrrole was purchased from Aldrich (Australia), vacuum distilled, and stored under nitrogen at -20 °C until use. pTS was obtained from Aldrich, Australia. Phytantriol was obtained from DSM Nutritional Products Ltd (Singapore). Vitamin E acetate was purchased from Sigma MilliQ water was obtained from Millipore/Millipak system with filter size 0.22 µm and ITO (indium tin-oxide) coated glass slides with a resistance of 70-100 Ω were purchased from Delta technologies.
3.5 Methods

3.5.1 Distillation of pyrrole

As pyrrole is prone to oxidation in the presence of air, it was distilled under vacuum to prepare pure unoxidised monomer. Pyrrole was heated to 50 °C in a round bottom flask connected to the distillation apparatus under vacuum. The resulting vapours were collected and condensed into a receiving flask (Figure 3-2). The purified pyrrole was collected and stored under nitrogen at -20 °C in an airtight flask until further use.

![Distillation setup](image)

Figure 3-2: Vacuum distillation of pyrrole.

3.5.2 Determination of phytantriol/water phase diagram

Cross-polarised light microscopy (CPLM) is one of the most simple and convenient methods for the determination of liquid crystalline phases. Samples are heated on a heating stage and viewed under cross-polarised light. The samples are identified based on the birefringence and viscosity (176). Lamellar phase shows birefringence and is less viscous, bicontinuous phase is highly viscous and non-birefringent, whereas hexagonal phase is highly viscous and birefringent (177). The temperature of the sample can be controlled (increased or decreased) through a hot stage to determine the phase transition temperature. However, CPLM does not provide any information on dimensions and compositions.
Phytantriol and water at different ratios (from 5% w/w to 50% w/w water composition) were weighed accurately and heated to 75 °C in Eppendorf tubes. Phytantriol was injected using a syringe into the water at 75 °C and subjected to three repeat cycles of vortexing for 2 min and centrifugation for 5 min at 2800 g. The samples were then equilibrated at 37 °C for 48 h (5). To determine the composition of different phases of phytantriol liquid crystals and in turn to determine the phase diagram of phytantriol/water, the equilibrated samples were heated to different temperatures from 20 °C to 80 °C at 10 °C per minute using a hot stage and viewed under CPLM.

### 3.5.3 Effect of monomer and dopant on the phytantriol liquid crystal phase

Vitamin E Acetate at certain concentrations in phytantriol alters the phase transition temperature with bicontinuous cubic phase being converted to hexagonal phase at lower temperatures (121, 168). To determine the effect of the addition of the monomer (pyrrole) and the dopant (pTS) on the phytantriol liquid crystalline phase, equilibrium temperature ramps were performed using small angle X-ray scattering (SAXS).

SAXS was performed at the SAXS/WAXS beamline at the Australian Synchrotron. The transition temperature from bicontinuous cubic to hexagonal phase encountered on heating these systems is a highly sensitive parameter to the presence of composition variables, allowing an understanding of their influence on phase behaviour. Bicontinuous cubic phase liquid crystal samples with 50% w/w water composition were prepared with different concentrations of pyrrole (0.1 M, 0.5 M and 1 M) and pTS (0.1 M, 0.2 M and 0.5 M). The effects of different concentrations of monomer and dopant were studied. Bulk gel samples were placed in the multiwall gel holder, sealed with Kapton tape attached to the temperature controlled capillary holder and the structural attributes (liquid crystal phase dimensions) were determined with increasing temperature. The ramps were run from 25-85 °C with 2 °C intervals. The wavelength was 0.1127 nm (11 keV). The sample to detector distance was fixed to 1600 mm with an exposure time of 2 s. Diffraction patterns were collected on a Pilatus 1 M detector (Dectris) and integrated using the in-house software package scatterBrain.
3.5.4 Electrochemical polymerisation of PPy through liquid crystal template

For the electrochemical polymerisation of PPy through the liquid crystal template, an ITO coated glass slide was used as a working electrode, a steel mesh was used as a counter electrode, and Ag/AgCl as a reference electrode. One molar pyrrole monomer and 0.2 M pTS as a dopant was utilised for the experiments detailed below. All the samples were polymerised using potentiostatic mode at a constant voltage of +0.7 V for 1 h. Electrochemical polymerisation was carried out in a custom made horizontal setup shown in Figure 3-3A. For the fabrication of conducting polymer through the liquid crystal template, the following three approaches were explored.

**Approach 1:** In this approach bicontinuous cubic phase liquid crystal was prepared using a 50% w/w water composition, which forms bicontinuous cubic phase in the presence of excess water. Both phytantriol and water were heated to 75 °C, and water was injected into the lipid, alternatively vortexed for 2 min and centrifuged for 5 min for 3 times, and the formed liquid crystal was equilibrated for 48 h. After equilibration, 1 g of the liquid crystal was applied evenly on an ITO coated glass slide which works as a working electrode. Pyrrole and pTS were dissolved in MilliQ water and used as an electrolyte media to electropolymerise PPy in liquid crystal template. It was equilibrated for 1 h and then polymerised for 1 h in the horizontal setup.

**Approach 2:** In this approach, pyrrole was added to phytantriol. A thin layer of phytantriol containing pyrrole was applied onto the ITO coated glass slide using a spin coater. pTS was dissolved in MilliQ water, which acts as an electrolyte media. Phytantriol coated ITO glass slide was fixed in the horizontal set up and the electrolyte media was added to it. This allows in situ formation of a thin layer of liquid crystal on the ITO coated substrate. The samples were then electropolymerised potentiostatically for 1 h.

**Approach 3:** In this approach, liquid crystal templated PPy growth was achieved by first dissolving 2 M pyrrole in 1 g of phytantriol (lipid phase) and 0.4 M pTS in 1 g of water (aqueous phase). The phytantriol liquid crystal was then formed by heating both lipid and aqueous phases to 75 °C, injecting the lipid phase into the aqueous phase providing the final concentrations of 1 M pyrrole and 0.2 M pTS. Since pyrrole is prone to unwanted oxidation, samples were immediately transferred evenly onto an ITO coated glass slide which acted as the working electrode. Polypyrrole was grown electrochemically in potentiostatic mode at +0.7 V vs. an Ag/AgCl reference electrode for 1 h by keeping the working and counter electrodes (stainless
steel mesh) directly in contact with the liquid crystal, without additional electrolyte (Figure 3-3B).

![Diagram](image)

**Figure 3-3**: Schematic diagram of A) Custom build horizontal setup, B) synthesis of polypyrrole through liquid crystal template in a custom made horizontal setup.

To enable a comparison during characterisation studies, conventional untemplated PPy/pTS films were polymerised in the same horizontal setup by aqueous polymerisation of 1 M pyrrole and 0.2 M pTS in water at +0.7 V for 1 h.

### 3.5.5 Scanning Electron Microscopy (SEM)

SEM is a technique used to image the topography of a material at a higher resolution, around 2 to 5 nm (178). In this technique, the sample to be analysed is irradiated with a focussed electron beam. As the electron beam sweeps across the specimen, the signals from the secondary and backscattered electrons are collected. The signals collected vary according to the differences in surface topography. A large depth of focus of scanning electron microscope achieves a three-dimensional appearance of the material (179, 180).

SEM was used to determine the effect of the liquid crystal template on the morphology of the formed polypyrrole films. Samples were investigated using a Philips XL30S field emission microscope. Before visualisation under SEM, the liquid crystal was completely washed away using isopropanol, and the PPy samples were mounted on aluminium stubs using adhesive graphite tape to investigate surface morphology. Cross-sectional images were obtained in a similar manner by first cryo-fracturing the films under liquid nitrogen. The samples were lightly sputter coated with platinum using Quorum Q150RS sputter coater.
3.5.6 Cyclic Voltammetry (CV)

CV is a versatile analytical technique for characterisation of electroactive species to determine the redox behaviour of the material over a wide range of potential (181). In a typical CV, the potential is swept back and forth between two set points at a determined scan rate and current between working and counter electrodes is determined as a function of potential. The figure produced with current plotted against potential is a cyclic voltammogram. The area under the curve corresponds to the total amount of charge passed during the redox process. A higher area under the curve indicates a higher electrochemical surface area (182). The peak maxima represent the peak oxidation and reduction potentials. The CV of an electrochemical species has characteristic oxidation and reduction peaks and is usually used to identify and characterise the material. The reversibility of the electroactive material is represented by the reproducible oxidation and reduction peaks over several cycles (183).

Cyclic voltammetry is an electroanalytical technique to determine the electroactivity of a sample (10). A three-electrode setup was used to record CVs of the films. The films with liquid crystal were cycled between -0.7 V to +0.7 V (vs. Ag/AgCl) at a rate of 100 mV.s\(^{-1}\) without any additional electrolyte using an eDAQ potentiostat (model EA161) and E-corder 410 with E-Chem software (NSW, Australia).

3.5.7 X-ray photoelectron spectroscopy (XPS)

XPS is a semi-qualitative technique used to determine the chemical composition of the surface and the interactions among the atoms in the surface under various redox conditions (184, 185). XPS analyses on PPy films were carried out by our collaborator, A/Prof Geoff Waterhouse’s team on the Soft X-ray beamline at the Australian Synchrotron equipped with a SPECS Phoibos 150 hemispherical electron kinetic energy analyser. Spectra were excited at photon energies ranging from 1486.7 eV with a photon flux of approximately \(10^{11}-10^{12}\) photons.s\(^{-1}\) 200 m.A\(^{-1}\), pass energy of 5.0 eV and a sampling step of 0.01 eV. PPy films were carefully scraped off their conductive substrates and mounted onto drain current holders and then evacuated at UHV (\(2 \times 10^{-10}\) mBar) at room temperature before introduction into the analysis chamber.

NEXAFS measurements were also conducted on the Soft X-ray beamline at the Australian Synchrotron in the energy range between 90-2000 eV, with a photon flux of approximately \(10^{10}-10^{12}\) photons.s\(^{-1}\) 200 m.A\(^{-1}\) and at a high-resolution sampling step of 0.05 eV.
Measurements were carried out at the N K-edge (390-430 eV). Total electron yield (TEY), partial electron yield (PEY), and total fluorescence yields (TFY) were collected using drain current, a channeltron detector (CHN) and a multichannel plate (MCP) detector, respectively. All NEXAFS spectra were normalised against the $I_0$ current measured simultaneously on a gold mesh at each wavelength to remove any interference due to changes in the beam intensity during data acquisition.

3.5.8 Effect of electrochemical polymerisation and redox cycling of polypyrrole on the liquid crystal phase

To enable the study of the *in situ* effect of CP polymerisation through the liquid crystal template using SAXS, a 3D cell was designed with in-built working, counter, and reference electrodes with quartz windows. The setup (Figure 3-4) was suitable for connection to a potentiostat, enabling electrochemistry experiments to be conducted simultaneously with *in situ* scattering experiments. The cell was attached to a temperature-controlled capillary holder for *in situ* scattering experiments. SAXS measurements were recorded to govern the real-time growth kinetics of the conducting polymer in the bicontinuous cubic phase template, to determine whether the formation of conducting polymer has a transient or permanent influence on the nanostructure of the bicontinuous cubic phase. Acquisitions (0.05 s) were taken during polymerisation at 1-minute intervals for up to one hour. Equivalent acquisitions were undertaken without electrical stimulation to preclude the effects due to beam damage. The sample was positioned so that the polymerisation front moved vertically and the sample was moved between acquisitions perpendicular to the direction of polymerisation. Exposure time was limited to 50 ms to prevent beam damage.
Figure 3-4: Design of custom built cell with inbuilt working, counter, and reference electrodes used in the beam line to determine phase changes in bicontinuous cubic phase.

Dynamic activation experiments were conducted which allowed the detection of any phase changes when exposed to stimulus in real time using the custom-built cell described above. After polymerisation of the conducting polymer within the liquid crystal, the conducting polymer was subsequently stimulated through repeated oxidation and reduction cycles to determine the effects on liquid crystal nanostructure. Cyclic voltammetry was run from -0.6 V to +0.6 V for 20 cycles at a high scan rate (100 mV.s⁻¹) and a low scan rate (10 mV.s⁻¹). The resulting cyclic voltammetry was time-correlated with the observed liquid crystal nanostructures. Similarly, concentration and movement of dopant ions into and out of the polymer is determined by applying potential pulses (±0.6 V at 0.5 Hz) to the liquid crystal templated polymer in which 7% didodecyldimethylammonium bromide (DDAB) was added. DDAB was reported to cause a phase transition from lamellar phase to bicontinuous cubic phase with increasing concentration (174). DDAB above 7% showed birefringence indicating the presence of lamellar phase. We hypothesise that when the polymer is cycled between reduction and oxidation, the concentration of DDAB might change in liquid crystal, which may cause a phase change in the liquid crystal template.
3.6 Results and discussion

3.6.1 Phase diagram of phytantriol/water

Mixtures of the amphiphilic lipid phytantriol and water formed different liquid crystal phases depending on the lipid to water ratio and temperature as presented in Figure 3-5. Phytantriol forms lamellar, bicontinuous cubic, and reverse hexagonal phases depending on the water content and temperature, which could be identified by cross-polarised light microscopy. The lamellar phase was less viscous and displayed dispersed birefringence (Figure 3-5A), and the reverse hexagonal phase was highly viscous and revealed fan-textured birefringence (Figure 3-5C). Meanwhile, the bicontinuous cubic phase was highly viscous and did not show any birefringence due to the 3D arrangement (Figure 3-5B). Lamellar and reverse hexagonal phase showed birefringence due to the refraction of light by layered arrangement in these phases.

Figure 3-5: A) Lamellar (<10% w/w water concentration), B) Bicontinuous cubic (>35% w/w water concentration) and C) Reverse hexagonal phase (>35% w/w water concentration above 60 °C) under cross-polarised light microscopy.

To determine the phase transition from one phase to another, different concentrations of phytantriol and water were used. The sample temperature was controlled by hot stage and the samples were viewed under a cross-polarised light microscope. The phase change was recorded and plotted with water composition versus temperature. The resultant phase diagram is represented in Figure 3-6. Phytantriol formed lamellar phase (Lα) below 10% w/w water composition which was non-viscous and birefringent. When the water composition was increased, it formed bicontinuous cubic phase (Q2 or V2) which remained stable in the presence of excess water with increasing water composition. When the bicontinuous cubic phase was heated above 50 °C, bicontinuous cubic phase was transitioned to a viscous birefringent reverse hexagonal phase (H2). The black vertical line separates the bicontinuous cubic phase and hexagonal phases with their corresponding phases with excess water. When the lamellar phase
or hexagonal phase was heated, a reverse micellar phase ($L_2$) is formed. The determined phytantriol phase diagram agrees with the ones reported in the literature (38, 120).

Figure 3-6: Phase diagram of phytantriol and water, $L_2$-Inverse micellar, $L_\alpha$- Lamellar, $H_2$- Reverse hexagonal, $Q_2$-Inverse bicontinuous cubic phase, $H_2 + H_2O$-Reverse hexagonal phase in the presence of excess water, $Q_2 + H_2O$- Inverse bicontinuous cubic phase in the presence of excess water. The black vertical line represents the phase separation. The right side of the vertical line contains phases corresponding to the left side with the presence of excess water.

From the phase diagram, 50% w/w was selected for the electrochemical polymerisation of PPy. At this ratio, the liquid crystal was in the bicontinuous cubic phase with an excess of water present, which was confirmed by a lack of birefringence on cross-polarised light microscopy. The bicontinuous cubic phase has bicontinuous aqueous channels, which helps in the penetration of monomer and dopant to the working electrode and is hypothesised to aid in directing the polymerisation through the channels.
3.6.2 Investigating the effect of monomer and dopant on the phytantriol liquid crystal

SAXS was used to determine the phase of the liquid crystal and examine any changes, which may occur with the inclusion of pyrrole or pTS. The data was analysed using indexing of Bragg peaks for known liquid crystalline structures expected to arise from the materials and an in-depth comparison of relative peak intensities and background intensity changes. The presence of the Pn3m space grouping was revealed in the control bicontinuous cubic phase liquid crystal, with peak spacing ratios of $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, and $\sqrt{9}$ as shown in Figure 3-7.

![X-axis (Å$^{-1}$) vs. Intensity (A.U.)](image)

**Figure 3-7:** SAXS data of bicontinuous cubic phase in Pn3m space group with $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, and $\sqrt{9}$ group spacing.
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The phase transition temperature of phytantriol bicontinuous cubic phase to hexagonal phase was found to be around 50 °C (Figure 3-6). Additives such as vitamin E acetate, when added to the bicontinuous cubic phase, caused a phase transition to hexagonal phase at a lower temperature (38, 121). It was found that upon addition of 3-4% of vitamin E acetate the phase transition was decreased to body temperature (37 °C), and it was further decreased to room temperature (25 °C) by addition of 5-6% vitamin E acetate as determined by cross-polarised light microscopy when the sample was heated on hot stage.

The addition of monomer and dopant to the bicontinuous cubic phase is required for PPy polymerisation. Bicontinuous cubic phase liquid crystals were prepared using different concentrations of pyrrole (0.1, 0.5 and 1 M) and pTS (0.1, 0.2, 0.5 M) to determine if there is any phase change caused by these additives. Stock plots were plotted for samples to compare the effect of various concentrations of pyrrole and pTS (Figure 3-8).
Figure 3-8: Stock plots of bicontinuous cubic phase with different concentrations of pyrrole and \( pTS \). In all the samples, except with samples containing pyrrole 0.5 M, \( pTS \) 0.5 M, and pyrrole 1 M, \( pTS \) 0.5 M the liquid crystal existed in Pn3m space grouping. In those 2 samples, it is in Ia3d.

Although the phase was not changed from bicontinuous cubic, there is a change from Pn3m to Ia3d in samples containing pyrrole 0.5 M, \( pTS \) 0.5 M, and pyrrole 1 M, \( pTS \) 0.5 M. The Pn3m phase is expected to convert to the Ia3d phase at low water composition/activity, indicating that the addition of high concentrations of pyrrole and \( pTS \) occupy significant amounts of water in their hydration leading to a slight nett dehydration of the liquid crystal structure.

Equilibrium temperature ramps were performed on all the samples from 20 °C to 50 °C and, after cooling back to 20 °C, to determine the effect of the added monomer, dopant, and
temperature. Figure 3-9 displays 50% w/w water composition of bicontinuous cubic phase with and without 1 M pyrrole and 0.2 M \( \rho \)TS. This is the concentration of pyrrole and \( \rho \)TS used to prepare PPy films, hence the SAXS profile as stacked plots for all the temperatures are shown below. These stacked plots have peaks at peak spacing ratio which corresponds to Pn3m space grouping at all temperatures. The minor peak shifts are not significant as the peak ratios are not varied. This indicates that after heating bicontinuous cubic phase with and without 1 M pyrrole and 0.2 M \( \rho \)TS from 20 °C to 50 °C, there was no change in the phase structure.

**Figure 3-9:** Stacked SAXS plots of a) bicontinuous cubic control and b) bicontinuous cubic phase with pyrrole 1 M and \( \rho \)TS 0.2 M.
3.6.3 Electrochemical polymerisation of PPy through liquid crystal template

For the electrochemical polymerisation of PPy through the liquid crystal template, a 50% w/w water composition was selected which forms bicontinuous cubic phase with excess water. Bicontinuous cubic has large, non-intersecting, aqueous and lipid channels, which helps in the movement of pyrrole and \( \rho \)TS to the working electrode. Since the resistance offered by the liquid crystal template is higher than the aqueous polymerisation it might increase the voltage which would over-oxidise the PPy formed. Hence, potentiostatic polymerisation at 0.7 V was used for polymerisation through liquid crystal template instead of galvanostatic polymerisation.

Figure 3-10: A) Bicontinuous phase liquid crystal template on an ITO slide masked with Kapton tape, B) Polymerisation of PPy through approach 1 with polymer only present on the outside edges of the working electrode, C) Polymerisation of PPy through approach 2, D) Polymerisation of PPy through approach 3.

**Approach 1:** In the first approach, bicontinuous cubic phase liquid crystals were prepared, equilibrated, and then polypyrrole was polymerised in the presence of pyrrole and \( \rho \)TS dissolved in electrolyte media. Polypyrrole was formed on the outer edges of the liquid crystal. Liquid crystal was unevenly spread on the working electrode with a thinner layer on the edges and a thick layer in the centre. Polymerisation requires diffusion of the reactants through the liquid crystal to the working electrode. The growth pattern around the edges suggests that
diffusion of the reactants occurred more rapidly at the edge of the working electrode where liquid crystal was thinner (Figure 3-10 A and B).

**Approach 2:** Due to the improper penetration of reactants in the first approach through the liquid crystal bulk, in this approach, a thin layer of lipid containing pyrrole was spin coated onto the conductive substrate to which water containing pTS was added. This has helped not only in the formation of liquid crystals *in situ* but also acts as an electrolyte media for the polymerisation of polypyrrole. However, this approach resulted in the formation of very thin film. This might be because of the limited amounts of pyrrole present in the thin layer of phytantriol and exhaustion of pyrrole (Figure 3-10 C).

**Approach 3:** In this approach as the monomer was added to phytantriol, and pTS was added to water before the formation bicontinuous cubic phase liquid crystal. The working electrode and counter electrode were in direct contact with liquid crystal. When potential was applied, polypyrrole was polymerised through the liquid crystal. The resulting film was uniform with a rough surface. Since pyrrole and pTS were present in the liquid crystal, the formation of PPy indicates that PPy was formed through liquid crystal template (Figure 3-10).

### 3.6.4 Scanning Electron Microscopy

Scanning electron microscopy was used to investigate the surface morphology and cross-section of the template films after washing away the liquid crystal template with a porous structure evident. Typical polypyrrole demonstrates a cauliflower surface morphology (Figure 3-11A) with a non-porous cross-section (Figure 3-11C), however, the images in Figure 3-11B show a distinctive porous morphology on both the nodular surface (Figure 3-11B) and in cross-section (Figure 3-11 D and E) in imitation of the liquid crystal structure (21, 186). This demonstrates that PPy was formed through liquid crystal template.
Figure 3-11: SEM of A) Typical polypyrrole, B) porous polypyrrole prepared in a liquid crystal template through approach 3, C) cross-section of typical polypyrrole film, D) cross-section of porous polypyrrole prepared through approach 3, E) Cross-section of porous polypyrrole prepared through approach 3 at higher magnification.
3.6.5 Cyclic Voltammetry

CV was used to determine the electroresponsiveness of the PPy films produced through the liquid crystal template. Clear oxidation peaks and reduction peaks at were observed, indicating the reversible electroactivity of the liquid crystal templated PPy (Figure 3-12). Over the 20 cycles measured (1st, 5th, 10th, 15th, and 20th cycles are shown), a decrease in oxidation and reduction behaviour was observed. This indicates a loss of electroactivity of PPy on repeated cycling. The low amount of current was due to the limitation of the movement of ions to the polymer surface by bicontinuous cubic phase liquid crystal template. Also, on repeated cycling, there may have been changes to the liquid crystal nanostructure resulting in changes in ion movement across the polymer/liquid crystal interface and changes in the observed CV.

![Cyclic voltammetry of porous polypyrrole prepared through bicontinuous cubic phase liquid crystal template when cycled from -0.7 V to +0.7 V at a rate of 100 mV.s⁻¹ inside host liquid crystal template without any additional electrolyte media.](image)

**Figure 3-12:** Cyclic voltammetry of porous polypyrrole prepared through bicontinuous cubic phase liquid crystal template when cycled from -0.7 V to +0.7 V at a rate of 100 mV.s⁻¹ inside host liquid crystal template without any additional electrolyte media.
3.6.6 X-ray Photoelectron Spectroscopy (XPS)

XPS was performed to determine the atoms present in the sample and the redox state of the polymer. Spectra collected for conventional and templated PPy films were presented in Figure 3-13. Spectra showed characteristic peaks for oxygen, nitrogen, carbon, and sulphur. This was consistent with the elements present used for the synthesis of PPy, with the latter element arising from the dopant, suggesting that pTS was successfully incorporated into the PPy backbone as a dopant. Spectra from both conventional and templated films appeared similar, regardless of the oxidation state of PPy, thus higher resolution narrow scans were performed over the C 1s to ascertain subtle chemical differences.

![Figure 3-13: XPS spectra for conventional and templated PPy films in oxidised and reduced states.](image-url)
Figure 3-14: XPS C 1s narrow scan spectra for conventional and templated PPy.

C 1s spectra for PPy shown in Figure 3-14 shows a single peak located at 284.6 eV, which can be assigned to neutral carbon species. The intensity of the peak was primarily related to the neutral carbon species present in PPy with a small contribution arising from externally surface adsorbed adventitious hydrocarbons/carbonates from the atmosphere. No obvious chemical differences were observed for the C 1s signal, thus was not subject to peak fitting.
Figure 3-15: N K-edge total electron yield NEXAFS spectra for conventional and templated films.

N K-edge Near Edge X-ray Absorption Fine Structure (NEXAFS) measurements were performed to further evaluate the surface electronic properties of PPy in both oxidised and reduced states. Spectra for two sample redox pairs presented in Figure 3-15 revealed the presence of three main peaks as annotated in the figure. The main peak located at 400.7 eV arises from $\pi^*$ pyrrole ring resonances (i.e. this energy relates to the conduction band). Below this peak exists a weak feature located between 397.5-397.8 eV, which can be attributed to the formation of unoccupied mid-gap states upon formation of polarons and bipolarons. An intense and broad feature at higher photon energies of 405-407 eV is evident and relates to $\sigma^*$ resonances. Small variations in peak energies may be due to different extents of oxidation/reduction which directly affects N-C bonding. No differences were observed in the conventional and templated PPy films. Despite the electroactivity of the PPY films observed by CV, there were no obvious changes in redox state that could be detected by XPS.
3.6.7 Effect of electrochemical polymerisation and redox cycling of polypyrrole on the liquid crystal phase

To determine the effect of polymerisation potential of conducting polymer on the phase of the liquid crystal host template, liquid crystal in the bicontinuous cubic phase containing monomer (pyrrole) and dopant (pTS) was prepared by approach 3 described above. Bicontinuous cubic phase containing pyrrole and dopant was placed into the 3D cell and mounted in the sample holder. Polymerisation was carried out for 1 h and scattering experiments were performed by passing the X-ray beam through the sample. Acquisitions were taken every 5 min at 10 different positions of the sample.

The phase structure of the liquid crystal before and after PPy polymerisation was determined to investigate if PPy polymerisation induced any change in liquid crystal phase. SAXS data revealed the presence of the Pn3m space group bicontinuous cubic phase, with peak spacing ratios of $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, and $\sqrt{9}$. The bicontinuous cubic liquid crystal and the templated polypyrrole in liquid crystal show no differences indicating that the addition of monomer and dopant and polymerisation had no effect on the nanostructure of the bicontinuous cubic phase liquid crystal (Figure 3-16).

![SAXS profile of a) bicontinuous cubic phase, b) bicontinuous cubic phase with polypyrrole after polymerisation.](image)

**Figure 3-16:** SAXS profile of a) bicontinuous cubic phase, b) bicontinuous cubic phase with polypyrrole after polymerisation.
Chapter 3- Polymerisation of Polypyrrole in a Phytantriol Liquid Crystal Template

After polymerisation of polypyrrole through the bicontinuous cubic phase, polypyrrole was electrically stimulated to determine the effect of stimulation on the bicontinuous cubic phase structure. The resistance due to the current flow through the polypyrrole results in heat evolution, which can alter the liquid crystal structure. The kinetics of the transition was converted to equivalent temperature vs time, and this data was compared across the different systems and with changes in the experimental variables. This is directly analogous to the approach taken in near infrared radiation activated transitions in Fong et al. (187).

To electrically stimulate the formed polypyrrole, cyclic voltammetry (-0.6 V to +0.6 V at 20 mV.s\(^{-1}\)) and pulse (-0.6 V to +0.6 V, 0.5 Hz for 1 min) were applied. Subsequently, electroactive behaviour within the host liquid crystal matrix was determined. Similarly, the effect of salt concentration was determined by the movement of dopant into and out of the polymer depending on redox state, which may alter the liquid crystal phase. No change was observed in the bulk liquid crystal phase during stimulation, indicating that the movement of ions into and out of the polypyrrole has no influence on the bicontinuous cubic phase structure. One consideration is that the thickness of the polymer film is only approximately 20 μm whereas the X-ray beam is rather large at approximately 250 μm. Hence, any local change in the bicontinuous cubic phase close to the polymer may not be seen as the structure measurement is for the entire sample. The scattering from the cubic phase in Figure 3-16 was however very clean (eg. no split peaks or shoulders) indicating that a change to the state of polymer in this case probably did not induce a change in structure.

Although there was visible darkening seen in a few positions, it was concluded that it was caused by beam damage. This was verified by repeatedly exposing the control bicontinuous cubic liquid crystal without any polypyrrole and electrical stimulation. At certain positions, visible darkening and a phase change was seen due to the melting of bicontinuous cubic phase.

Some experiments were stopped prematurely due to the low current during polymerisation. The low current might be due to the exhaustion of pyrrole and pTS at the polymerisation site and at the polymer/media interface. After 45 min, the samples appeared visibly more liquid than at the start. This could be due to the hutch temperature being raised to 28 °C, (although at this temperature conversion to a low viscosity phase is not expected), or the raised temperature may have accelerated water evaporation from the sample, causing the formation of lamellar phase as seen from the less viscous nature of the liquid crystal.
Chapter 3- Polymerisation of Polypyrrole in a Phytantriol Liquid Crystal Template

Future studies would require a small beam diameter (at a different synchrotron with a micro source SAXS beamline) to test the hypothesis that redox activity of the polymer can change liquid crystalline phase structure by allowing profiling of the polymer-liquid crystal interface at high spatial resolution.

3.7 Conclusion

A phase diagram of the phytantriol liquid crystal was determined using the hot stage and cross-polarised light microscopy in agreement with previous reports. Upon addition of monomer and dopant to the bicontinuous cubic phase no effect was observed in the overall phase structure, but changes were evident in the packing arrangement within the phase. Different approaches were explored to fabricate porous conducting polymer through a bicontinuous cubic phase of liquid crystal template. Conducting polymers were polymerised through a liquid crystal host template. The formation of the conducting polymer did not alter the liquid crystal phase. Temperature, beam exposure, and water evaporation from the host liquid crystal had to be controlled or these variables would alter the liquid crystal phase.

Following polymerisation of the polymer within the liquid crystal host matrix, porous PPy films were evident on SEM. Cyclic voltammetry revealed the formation of a reversibly redox active polymer. XPS has revealed the presence of atoms present in pyrrole and pTS, but no obvious changes in redox state could be detected by XPS, despite the electroactivity observed by CV. The liquid crystal phase was monitored by SAXS during CV sweeps and pulsed potentials; no change was observed in the liquid crystal phase. In some samples changes in scattering were observed; however, following comparisons with appropriate controls, this was found to be the result of beam damage. Due to the beam size being relatively larger (250 μm) than the thin polymer layer (20 μm) and the local changes at the surface of the polymer-liquid crystal layer might not be detected. Hence, the hypothesis that a phase transition would be observed in the CP/LC hybrid system when an electrical trigger was applied was not able to be proven.
Chapter 4: Polypyrrole Films Polymerised in a Host Liquid Crystal Template for DexP Delivery
Chapter 4- Polypyrrole Films Polymerised in a Host Liquid Crystal Template for DexP Delivery

4.1 Declaration for Chapter 4

Some of the work presented in this chapter (Sections 4.5.9 and 4.6.6) was presented at Advanced Materials and Nanotechnology-7 conference and has been published in the conference proceedings as: Uppalapati D, Boyd B, Travas-Sejdic J, Svirskis D. Porous conducting polymer prepared through liquid crystal template for drug delivery. International Journal of Nanotechnology. 2017;14(1-6):422-31.

4.2 Introduction

Amphiphilic lipids such as phytantriol self-assemble in the presence of water to form liquid crystals by non-covalent interactions. Phytantriol can form liquid crystals in different phases depending on the composition of lipid and water. Hexagonal and reverse hexagonal liquid crystalline phases have been explored as templates for the polymerisation of CPs by both chemical and electrochemical synthesis. Hulvat et al. synthesised PEDOT nanofibres using poly(oxyethylene)n-oleyl ether (n=10) hexagonal liquid crystals as a template (113, 114). PANI (188), PPy (111), PEDOT (115) and poly(p-phenylenevinylene) (45) nanostructures such as nanofibres and nanocomposites have all been synthesised from liquid crystal templates.

Templated micro- or nanostructured films have been reported in the literature for the delivery of drugs, using the hard template. Luo et al. produced nanoporous films using polystyrene nanobead template and fluorescein as a dopant (66). They also added a second drug dexamethasone by entrapping it into the porous structure (67). Other studies which have explored the loading and release of drugs from templated structures after the removal of the hard template are described in detail in chapter 2 (31, 64, 134). Unlike a hard template, a soft template can be removed easily after synthesis of CP. Drug delivery from micro- or nanostructured CPs can be attributed to either increase in drug loading capacity or higher responsiveness to electrical stimulation or both.

A phytantriol based liquid crystal in the bicontinuous cubic phase was used as a soft template in this study to prepare PPy films loaded with DexP. As polymerisation occurs on the working electrode, pyrrole and DexP should be available. Bicontinuous cubic phase has continuous, non-intersecting aqueous and lipid channels which were hypothesised to enhance the movement of pyrrole monomer and DexP to the working electrode.
Chapter 4- Polypyrrole Films Polymerised in a Host Liquid Crystal Template for DexP Delivery

The PPy films produced using bicontinuous cubic liquid crystal as a template mentioned in chapter 3 were more porous than conventional PPy films based on SEM. Increasing the porosity of the CP provides a mechanism to increase the responsiveness of the system, due to an increase in surface area. This higher effective electrochemical surface area, as evidenced by CV, enhances the polymer/media interaction thereby promoting the speed and extent of ion movement into and out of the polymer; hence more precise and potentially greater magnitude of responses can be achieved on electrical stimulation.

Dexamethasone sodium phosphate (DexP) was loaded as a dopant into the PPy and used to test the releasing capabilities of the developed porous PPy films compared to the conventional films. DexP is a synthetic glucocorticoid that has anti-inflammatory and immunosuppressant effects. It is used in many inflammatory conditions including in certain types of cancers, arthritis, and other autoimmune disorders, to prevent transplant rejection and to prevent nausea and vomiting by some chemotherapy drugs (40, 153, 154). The delivery system being developed in this thesis has application in age-related macular degeneration (AMD) and diabetic macular edema (DME).

4.3 Aims and objectives

The main aim of the work presented in this chapter was to prepare DexP loaded porous PPy films using a liquid crystal template and to achieve electrically tuneable drug release. The specific objectives were to:

- Determine the effect of pyrrole and DexP on the bicontinuous cubic liquid crystalline phase.
- Polymerise PPy/DexP films through a bicontinuous cubic liquid crystal template by electrochemical polymerisation.
- Perform morphological, chemical and electrochemical characterisation of the PPy/DexP films prepared through bicontinuous cubic phase and make a comparison with conventional PPy/DexP films.
- Determine the electrically tuneable DexP release from conventional and templated PPy films.
- Determine the cytotoxicity of the extracts on ARPE-19 cells.
4.4 Materials

Pyrrole was purchased from Aldrich (Australia), vacuum distilled and stored under nitrogen at -20 °C until use; dexamethasone sodium phosphate (DexP) was obtained from Jai Radhe Sales, India. Phytantriol was obtained from DSM Nutritional Products Ltd (Singapore). MilliQ water was from Millipore/Millipak system with filter size 0.22 µm and resistivity of 18.2 MΩ.cm. ITO slides with a resistance of 70-100 Ω were purchased from Delta Technologies. PBS tablets were obtained from Sigma-Aldrich, Australia, and each tablet when dissolved in 200 ml of MilliQ water at 25 °C yield 0.137 M sodium chloride, 0.0027 M potassium chloride, 0.01 M Na₂HPO₄ and 0.0018 M KH₂PO₄ at pH 7.4. ARPE-19 cells (a human retinal pigment epithelial cell line) were obtained from American Type Culture Collection (USA) and were used within 20 passages from the time of purchase. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Molecular Probes, Thermo Fischer Scientific (USA). The DMEM/F12, GlutaMAX™ medium and TrypLE™ Express were purchased from Gibco, Thermo Fisher Scientific (USA).

4.5 Methods

4.5.1 Effect of pyrrole and dexamethasone on bicontinuous cubic phase

It was desirable to maintain the phytantriol liquid crystal in the bicontinuous cubic phase through polymerisation as this phase has large bicontinuous lipid and aqueous channels to facilitate the movement of pyrrole and dexamethasone to the vicinity of the working electrode. As mentioned in chapter 3, adding new components may induce a change in the bicontinuous phase of liquid crystal template. The phase structure of the bicontinuous cubic liquid crystal phase after the addition of pyrrole and DexP was determined by Small Angle X-ray Scattering (SAXS) at the Australian Synchrotron. Bicontinuous cubic phase liquid crystal samples with 50 % w/w water composition were prepared with different concentrations of pyrrole (0.1 M, 0.5 M and 1 M) and DexP (0.1 M, 0.2 M and 0.5 M). Bulk gel samples were placed in the multiwall gel holder, sealed with Kapton tape attached to the temperature controlled capillary holder, and the structural attributes (liquid crystal phase dimensions) were determined with increasing temperature. The wavelength was 0.1127 nm (11 keV). The sample to detector distance was fixed to 1600 mm with an exposure time of 2 s. Diffraction patterns were collected.
4.5.2 Preparation of drug loaded porous PPy prepared through liquid crystal template

To prepare bicontinuous cubic phase liquid crystal, a 50% w/w water to lipid composition was selected. Phytantriol (1 g) and water (1 g) were weighed accurately in glass vials. Pyrrole (2 M) was dissolved in lipid and dopant DexP (0.4 M) was dissolved in water and pH adjusted to 3 using 0.1 N HCl to facilitate polymerisation. Both aqueous and lipid phases were heated to 75 °C in a water bath. Aqueous phase was injected in lipid phase by a syringe, vortexed and centrifuged alternately to ensure proper mixing and formation of bicontinuous cubic phase with a final concentration of 1 M pyrrole and 0.2 M DexP. For polymerisation, the formed bicontinuous cubic phase with pyrrole and DexP was transferred onto an ITO coated glass slide which works as a working electrode. This ITO coated glass slide with bicontinuous cubic phase containing pyrrole and DexP was fixed in a custom build horizontal setup. Platinum mesh was used as a counter electrode which was kept directly in contact with the bicontinuous cubic phase without any additional electrolyte. Ag/AgCl was used as a reference electrode. Polymerisation was carried out at +0.7 V for 1 h in a potentiostatic mode.

Conventional PPy films, without the use of a template, were polymerised by dissolving 0.2 M DexP in water and the pH was adjusted to 3. To this 1 M pyrrole was added and polymerisation was carried out in potentiostatic mode in a horizontal setup at +0.7 V for 1 h.

4.5.3 Scanning electron microscopy

The surface morphology of the PPy films was investigated using a Philips XL30S field emission gun scanning electron microscope at an accelerating voltage of 5 kV using an EDS detector. Before visualisation under SEM, the liquid crystal was completely washed away using isopropanol and the PPy samples were mounted on aluminium stubs using adhesive graphite tape to investigate surface morphology. Cross-sectional images were obtained in a similar manner by first cryo-fRACTURING the films under liquid nitrogen. The samples were lightly sputter coated with platinum using Quorum Q150RS sputter coater.
After imaging the surface morphology, elemental analysis was also performed on the same samples by energy dispersive X-ray spectroscopy (EDS). using SUTW-Sapphire detector at a resolution of 133.10. The X-ray signals from the sample are collected by the detector which converts their energy into electrical charge. The charge is then processed to identify the elemental source. EDAX ZAF quantification was used to determine the amounts of elements present in the final sample by applying corrections for atomic number (Z), absorption (A) and fluorescence (F).

4.5.4 N\textsubscript{2} physisorption measurements

N\textsubscript{2} physisorption measurements were performed based on the Brunauer-Emmett-Teller (BET) method which involves physical adsorption of gas (N\textsubscript{2}) molecules onto a solid surface to determine the specific surface area of the solid. N\textsubscript{2} physisorption isotherms were determined using a Micromeritics Tristar 3000 instrument at liquid nitrogen temperature (-195 °C). PPy films were carefully removed off the ITO glass slides and the analysis was performed on freestanding films. Specific surface area of the templated and conventional films was calculated by BET equation from the N\textsubscript{2} adsorption data using relative pressure P/P\textsubscript{0} values in the range of 0.05-0.2 (189).

4.5.5 Fourier Transmission Infrared Spectroscopy (FTIR)

Infrared spectroscopy is a useful technique to examine the stretching, bending and vibrations of the intramolecular bonds within a molecule (190). An infrared spectrum is obtained by transmitting an IR beam on sample and plotting the transmitted light versus wavelength. Molecules in the sample absorb IR frequencies characteristic to the structure which corresponds to its vibrational frequency. Changes to the dipole moment in the molecule causes atoms to move with respect to each other which results in change in bond length (stretching) and bond angle (bending) (190, 191).

FTIR spectra of conventional and templated PPy film and DexP were recorded in ATR mode using a germanium (Ge) crystal of Bruker Tensor 37 FTIR spectrometer, with OPUS spectroscopic software (OPUS 6.5, Germany). Measurements were recorded between 400 and 4000 cm\textsuperscript{-1} with a resolution at 4 cm\textsuperscript{-1}. 

4.5.6 Cyclic voltammetry

The electroactivity of the PPy films was characterised using CV. A three electrode, electrochemical set up was utilised for recording the CV of the films. After polymerisation, the liquid crystal was washed away using isopropanol. The templated or conventional PPy film on ITO covered glass slide was used as a working electrode. Platinum mesh was used as a counter electrode and Ag/AgCl as a reference electrode. The films were cycled between -0.7 V to +0.7 V at a rate of 100 mV.s⁻¹ in PBS for 100 cycles using a Bio-Logic potentiostat, USA, and data was analysed using the EC-Lab® software.

Cyclic voltammetry was also used to determine the surface area of the porous templated films and conventional films using the same setup. But the films were cycled from 0 V to +0.4 V in 5 mM ferri-ferrocyanide (5 mM ferricyanide and 5 mM ferrocyanide) at 100 mV.s⁻¹.

The charge passed is related to the surface area and the square root of time according to the Equation 4-1, (47, 192).

\[ Q = (2nFAD^2\pi^{-\frac{1}{2}}C)\frac{1}{t^2} \]

Equation 4-1

where Q is the charge passed, n is the number of electrons used in the reaction (n=1 for Fe(CN)₆³⁻/⁴⁻), F is the Faraday constant (9.65 x 10⁴ C.mol⁻¹), A is electrode surface area, D is the diffusion coefficient (6.2 x 10⁻⁶ cm².s⁻¹), C is a mediator concentration (5 x 10⁻⁶ mol.cm⁻³ Fe(CN)₆³⁻/⁴⁻ in PBS is used as a mediator).

When charge Q is plotted against the square root of time, the slope of the curve gives K, from which the surface area of the electrode is calculated by Equation 4-2.

\[ Q = K\frac{1}{t^2} \]

Equation 4-2

4.5.7 Electrochemical Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) is defined as a resistance including capacitance and inductance offered by the material to the alternating current (a.c.) when a voltage is applied.
Impedance is a measure of dielectric properties of the electroactive material as a function of frequency (193, 194).

EIS was performed using a three-electrode setup with a Bio-Logic electrochemical workstation. Either templated or conventional PPy films were used as working electrodes, with a platinum mesh counter electrode and Ag/AgCl reference electrode. Impedance was measured at open circuit potential in PBS electrolyte solution with a 10 mV a.c. component from 10 kHz to 1 Hz. Impedance data was analysed using the EC-Lab® software.

4.5.8 Conductivity

The conductivity and reversible redox nature of CPs are attributed to the alternating double and single bonds with carbons in $\text{SP}^2$ hybridisation. The delocalised $\pi$-electrons form a conjugated backbone which renders the materials conductive through charge mobilisation. Conductivity ($\sigma$) of a material is its ability to pass a current through it and is the inverse of resistivity ($\rho$). Resistivity is the ability of material to oppose the flow of current through it. Conductivity of PPy typically ranges between 1 and 60 S.cm$^{-1}$ (195, 196).

Resistivity is calculated by Equation 4-3.

$$\rho = \frac{4.532 \times V \times t}{I}$$

Equation 4-3

Where $V = $ voltage (V), $t = $ sample thickness (cm), $I = $ current (A) and $\rho = $ resistivity ($\Omega$.cm).

Electrical conductivities (S.cm$^{-1}$) of templated and conventional PPy films were measured using a Jandel (RM2 model) Multi Height Probe, Resistivity Test Unit with a four-point linear probe (1.0 mm tip spacing). The films were peeled off the substrate and placed on a glass slide and four replicate measurements were taken for each film. Measurements were taken by the application of constant current (0.5 to 300 $\mu$A) and the voltage measured. These values were used to calculate the resistivity ($\rho$ in $\Omega$.cm).
4.5.9 Drug release studies

Four freshly prepared films were used in each of the four investigated groups, templated stimulated, templated unstimulated, conventional stimulated, and conventional unstimulated. For stimulated drug release experiments, films were stimulated using a three-electrode setup in phosphate buffered saline (PBS), pH-7.4 with a pulse of ±0.6 V, 0.5 Hz at 24, 48, 72, and 144 h for 5, 10, 10 and 60 min, respectively. Sink conditions were maintained (concentration of the DexP in the solution is less than 10% of its saturated solubility) at all the times by adding fresh PBS each time when a sample was taken. For controls, unstimulated films were observed under the same experimental conditions without any electrical stimulation. The released drug was quantified by High-performance liquid chromatography (HPLC). An ANOVA test was applied to determine the statistically significant difference in the release rates of the templated stimulated films.

Briefly, HPLC analysis was conducted on an Agilent 1200 series HPLC (Agilent Technologies, USA) equipped with an autosampler (injection volume: 10 μL) a 100-well sample tray, a vacuum solvent degasser, a quaternary pump and an online diode array detector. The results were analysed using ChemStation® software Agilent Technologies, Germany. A reverse phase C18 Phenomenex column (250 x 4.6 mm, particle size 5 μm) and a mobile phase consisting of 50% phosphate buffer (24 mM NaH₂PO₄), 27.3% acetonitrile, 22.7% methanol at 40 °C and a flow rate of 1 ml.min⁻¹ were used. The signals were detected at a wavelength 254 nm. DexP was calibrated over a range of 5 μg.ml⁻¹ to 1000 μg.ml⁻¹ with satisfactory linear regression achieved (R²=0.999).

4.5.10 Cytotoxicity studies

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric assay is used to measure cytotoxicity and cell proliferation (197). The assay is dependent on the ability of viable cells to metabolise a water-soluble tetrazolium salt into a water-insoluble formazan product, which determines the mitochondrial activity (198, 199).

ARPE-19 cells were used to determine the cytotoxicity of the templated films by MTT assay. PBS extracts from the films were tested for cytotoxicity because in the final device PPy will not be exposed to the tissue, instead it will be enclosed in biocompatible, non-biodegradable polymer such as polyvinyl acetate. PBS extracts were obtained from the films with and without
electrical stimulation (±0.6 V, 0.5 Hz for 1 hour). Negative control cells were treated with DMEM/F-12, GLutaMAX (100% cell viability) whereas positive control cells were treated with Triton X-100 in DMEM/F-12, GlutaMAX to kill the cells (0% cell viability). ARPE-19 cells were seeded at a density of 2x10^5 cells/ml in 96 well plates and incubated at 37 °C in a 5% CO₂ humidified atmosphere overnight. PBS extracts from each sample (n=6) in DMEM/F-12, GlutaMAX (1:1) were added by replacing the cell culture medium. Plates were then incubated for 1 h at 37 °C and 5% CO₂. After the incubation, solutions from each well were removed and replaced with MTT solution (0.5 mg.ml⁻¹) and incubated at 37 °C for 4 h. MTT solution was removed from all the wells and replaced with 0.04 M HCl-isopropanol solution to dissolve the formed insoluble formazan. Formazan dissolves in HCl-isopropanol solution to give a purple colour. The intensity of the purple colour was quantified by measuring the absorbance at 570 nm with correction of any interference at 650 nm (Bio-Tek Synergy HT) and percentage cell viability was calculated.

4.5.11 Pilot study: Preparation of drug loaded porous PPy prepared through liquid crystal template by potentiostatic polymerisation to pass a current of 1000 mC

Another set of films were prepared using the same concentrations mentioned in section 4.5.2 except that the pH of the DexP was not adjusted to 3. When pH was adjusted with HCl, the chloride ion from the HCl competes with DexP to be loaded as a dopant in the PPy films. To verify the loading and release of DexP, films were prepared without adjusting the pH. Briefly, for templated films, pyrrole was added to lipid, DexP was dissolved in water and pH was not adjusted. Aqueous phase was injected into the lipid phase and polymerisation was carried out at +0.7 V for more than 4 h instead of 1 h to reach a total charge of 1000 mC. For conventional films, DexP was dissolved in water, pyrrole was added and polymerisation was carried out at +0.7 V to reach a total charge of 1000 mC.
4.6 Results and discussion

The films prepared through the liquid crystal template produced noticeably less polymer product compared to the conventional films when they were polymerised for same time at a constant potential. The reduced efficiency of polymerisation within the liquid crystal can be attributed to the limitation in the diffusion of monomer and dopant to the working electrode where they are consumed during polymerisation.

4.6.1 Effect of pyrrole and dexamethasone on bicontinuous cubic phase

The addition of monomer and dopant to the liquid crystal should not affect the bicontinuous cubic phase; this was important as we intended to polymerise in the bicontinuous cubic phase. Bicontinuous cubic phase liquid crystals were prepared using different concentrations of pyrrole (0.1, 0.5 and 1 M) and DexP (0.1, 0.2, 0.5 M) to determine if there is any phase change caused by these additives. Stacked plots were plotted for samples to compare the effect of various concentrations of pyrrole and pTS (Figure 4-1).

The addition of pyrrole and DexP has caused a change of phase structure from Pn3m (cubic phase with excess water) to Ia3d (√6:√8:√14:√16:√20:√22:√24…) in samples containing pyrrole 0.1 M and 0.5 M regardless of the concentration of DexP. DexP being hydrophilic will dissolve in the aqueous channels of bicontinuous cubic phase. The Pn3m phase is expected to convert to the Ia3d phase at low water composition/activity, indicating that the addition of DexP occupies significant amounts of water in their hydration leading to a slight nett dehydration of the liquid crystal structure. But when the pyrrole concentration is increased to 1 M, again Pn3m phase (√2:√3:√4:√6:√8:√9…) structure was observed. This indicates that pyrrole (logP = 0.7) may be occupying in the lipid channels.
Figure 4-1: SAXS stacked plots of phytantriol liquid crystals with different concentrations of pyrrole and DexP. Bicontinuous cubic phase samples with pyrrole 0.1 M and 0.5 M, at all DexP concentrations, exist in Ia3d ($\sqrt{6}:\sqrt{8}:\sqrt{14}:\sqrt{16}:\sqrt{20}:\sqrt{22}:\sqrt{24}...$) whereas samples with pyrrole 1 M exist in Pn3m ($\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}...$).
4.6.2 Morphological characterisation

The surface morphology of PPy is usually cauliflower like with smaller projections coming off larger projections (138). SEM of conventional PPy/DexP films demonstrated a highly nodular surface as shown in Figure 4-2 A and B (32). SEM of PPy polymerised in bicontinuous cubic phase liquid crystal template demonstrated an interconnected network of nanorod like growth of PPy/DexP as shown in Figure 4-2 C and D, which is unusual in conventional PPy/DexP film indicating that bicontinuous cubic phase template has an influence on the polymerisation of PPy. SEM images in Figure 4-2 E and F have nanorods of varying diameters, which indicates that the polymerisation of PPy was initiated in the channels of bicontinuous cubic phase and as the polymerisation continued, the polymer became thick. It is also interesting to observe that thin nanorods were polymerised from the thick nanorods, representing the vertical polymerisation of PPy through liquid crystal.

The growth of PPy through the liquid crystal had a considerable effect on the specific surface area of the templated films as evident from N₂ physisorption measurements. The specific surface area for the conventional film was found to be 8.311 m².g⁻¹, whereas the specific surface area of the templated porous film was found to be 224.2 m².g⁻¹.
Figure 4-2: SEM of A, B) Conventional PPy/DexP at different magnifications showing highly nodular surface, C-F) PPy/DexP polymerised through bicontinuous cubic liquid crystal template at different magnifications showing an interconnected nanorod like structures.
4.6.3 Drug loading

To determine the loading levels of DexP, elemental analysis was performed by EDS (Figure 4-3). The percentage weight of different elements present in conventional and templated films was determined and is shown in Table 4-1. Fluorine and phosphorous (in the phosphate group) are present in DexP. Determining the percentage weight of fluorine and phosphorus, determines the amount of DexP loaded into the films. Percentage weight of fluorine and phosphorous in the templated films was found to be much lower than the conventional films. Interestingly, it was also observed that in the templated films (chlorine-7.61%), a higher percentage of chlorine was present than that is present in conventional films (chlorine-4.28%). The movement of the large DexP to the working electrode was hindered by the presence of bicontinuous cubic phase in the templated films. HCl was added to the DexP solution to adjust pH to 3. Chloride from HCl, being a small highly mobile anion, can easily move through the bicontinuous cubic phase to the working electrode. Hence, a higher amount of chlorine was being loaded into the templated films compared to DexP as a dopant.

Table 4-1: Percentage weight of elements present in the conventional and liquid crystal templated films determined by scanning electron microscope.

<table>
<thead>
<tr>
<th>% weight of elements present</th>
<th>Fluorine (F)</th>
<th>Chlorine (Cl)</th>
<th>Phosphorous (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional film</td>
<td>1.57</td>
<td>4.28</td>
<td>3.15</td>
</tr>
<tr>
<td>Templated film</td>
<td>0.95</td>
<td>7.61</td>
<td>2.20</td>
</tr>
</tbody>
</table>
Figure 4-3: Elemental composition of A) conventional film, B) templated film determined by EDS.
4.6.4 FTIR

FTIR spectra of the conventional PPy film, templated PPy film, and DexP from 4000 cm\(^{-1}\) to 800 cm\(^{-1}\) are displayed in Figure 4-4a. The FTIR spectra of the samples from 1800 cm\(^{-1}\) to 800 cm\(^{-1}\) in Figure 4-4b to clearly observe the peaks in the region. The FTIR spectra of the PPy match with the peaks reported in the literature. In FTIR spectra of both the conventional films and templated films, the peak at 919 cm\(^{-1}\) is due to the C-H band out of plane deformation vibrations. The peak at 1218 cm\(^{-1}\) is due to the stretching vibration in doped PPy. The peaks between 1460 cm\(^{-1}\) and 1570 cm\(^{-1}\) are due to the antisymmetric ring stretching and symmetric stretching vibrations of C=C and C-N in pyrrole ring. Peaks at 1325 cm\(^{-1}\) and 1058 cm\(^{-1}\) are due to N-H and C-H deformation vibration (31, 200, 201). Both conventional and templated films have peak at similar positions indicating that bicontinuous cubic phase serves as a template and no chemical interactions were formed between phytantriol and PPy.

In the FTIR spectra of DexP, peak at 1251 cm\(^{-1}\) is due to the stretching vibration of the C-F bond, peaks at 1620 cm\(^{-1}\), 1664 cm\(^{-1}\) and 1708 cm\(^{-1}\) corresponds to the stretching vibration of C=O and double bonds conjugated to C=O. The presence of a peak between 2850 cm\(^{-1}\) and 3000 cm\(^{-1}\) in the spectra of both conventional film and templated films indicates the incorporation of DexP in the PPy which corresponds to the presence methyl group. Similarly, the presence of a peak between 1665 cm\(^{-1}\) and 1760 cm\(^{-1}\) corresponds to the carbonyl group of DexP in both conventional and templated PPy (32). The peak at 1666 cm\(^{-1}\) is more prominent in conventional films than templated suggesting the presence of higher amounts of DexP in conventional films.
Figure 4-4: FTIR spectra of conventional PPy film, templated PPy film, DexP a) from 3500 cm\(^{-1}\) to 800 cm\(^{-1}\), b) from 1800 cm\(^{-1}\) to 800 cm\(^{-1}\).
4.6.5 Electrochemical characterisation

The electroactivity of both conventional non-porous and templated porous films was established by CV and conductivity measurements. The films when cycled from -0.7 V to +0.7 V at 100 mV.s\(^{-1}\) for 100 cycles showed reversible nature of conventional (Figure 4-5a) and templated (Figure 4-5b) films. Over 100 cycles a slight decrease in the current passed could be seen in both conventional and templated films which shows loss of charge that is transported in and out of the membrane. This indicates a loss of electroactivity of PPy on repeated cycling. The area under the curve of a CV represents the charge passed during each cycle. The charges passed during cycle 5 and 100 of conventional and templated PPy are shown in Table 4-2. The charge passed for the conventional is higher than the templated films due to more polymer formed during conventional polymerisation. The percentage loss in electroactivity of the templated films (16.63\%) was more prominent than that of the conventional films (11.10\%), indicating large movement of ions into and out of the polymer due to the higher surface area of the templated films.

Figure 4-5: CV of PPy films run from -0.7 V to +0.7 V at 100 mV.s\(^{-1}\) in PBS for 100 cycles. a) conventional, b) templated. Arrows indicate the direction of flow of current.
Table 4-2: Charge passed during cycle 5 and 100 conventional and templated films. Over 100 cycles, charge passed decreased gradually indicating a loss of electroactivity.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Charge passed</th>
<th>Percentage loss in electroactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 100</td>
</tr>
<tr>
<td>Conventional film</td>
<td>312.42 mC</td>
<td>260.44 mC</td>
</tr>
<tr>
<td>Templated film</td>
<td>194.03 mC</td>
<td>172.48 mC</td>
</tr>
</tbody>
</table>

The electrode surface area of PPy films was calculated from a cyclic voltammogram run from 0 V to +0.4 V at 100 mV.s\(^{-1}\) in 5 mM ferri-ferrocyanide. The electrode surface area of porous templated film was found to be 17.79 cm\(^2\), whereas the electrode surface area for the conventional PPy film was found to be 5.49 cm\(^2\). Hence, the roughness factor was calculated to be 3.24. The increase in surface roughness increases the polymer/media interface, thereby decreasing the impedance and increasing the responsiveness of the system. BET measurements also revealed an increase in the surface area of the templated films measured by gas adsorption.

The conductivity of the conventional films was 18.36 ± 3.82 S.cm\(^{-1}\) and that of templated films was 14.89 ± 2.02 S.cm\(^{-1}\). The conductivity was consistent with PPy films reported in other studies, which typically ranged between 1 and 60 S.cm\(^{-1}\) (195, 196, 202). The slight decrease in the conductivity of the templated films is due to the porous nature which increases the voids in the film thereby increasing the resistance (152).

Impedance determines the resistance including capacitance and inductance of the polymer offered on application of current as a function of frequency (194, 203). Impedance modulus (|Z|) at 0.5 Hz (frequency used during stimulation of drug release) was found to be 115.95 Ω for conventional films and 92.44 Ω for templated films. The decrease in impedance may be attributed to an increase in roughness which results in a higher electrochemical surface area (also indicated by CV results). Decreasing impedance values are favourable for drug releasing systems. A lower impedance value suggests that less current or voltage is required to drive drug release from the polymer systems.
4.6.6 Drug release studies

Dexamethasone phosphate was loaded into the films as a dopant during electrochemical polymerisation [16]. The release was determined with and without electrical stimulus. While release was slow for the unstimulated films, the stimulated templated films demonstrated bursts of release in response to electrical stimuli (Figure 4-6B). The duration of stimulation was different at different trigger time points, and interestingly there was no significant difference in the release rate per minute (p > 0.8, one-way ANOVA) (Table 4-3). Conventional non-porous films released greater masses of drug (Figure 4-6A); this can be attributed to the more efficient polymerisation process producing more polymer. Also from the elemental analysis data, it was confirmed that less DexP (from the percentage weight of fluorine and phosphorus) was loaded as a dopant in the templated films compared to the conventional films. Interestingly, the percentage of chlorine was much higher in the templated films, indicating that bicontinuous cubic phase restricts the movement of large molecule DexP to the polymerisation site. Hence, chloride might have been loaded as a dopant in the templated films which explains the lower amounts of release. For the conventional films, electrical stimulation had only moderate effects on drug release. All the groups investigated showed a burst of release before the first stimulation point. This is typically seen from CP drug releasing systems and may be due to the diffusion driven release of drug close to the polymer/media interface. This initial burst was smaller from the template PPy systems. It is possible that a thin layer of liquid crystal remains at the PPy/media interface which reduces unstimulated release from the templated films.
Figure 4-6: Drug release profiles of A) conventional films, and B) templated films prepared when polymerised for the same time and when the pH of DexP solution was adjusted to 3 using HCl, with and without stimulation. Data points indicate mean, error bars represent standard deviation (n=4).
Table 4-3: Rate of drug release from the porous template films during periods of electrical stimulation.

<table>
<thead>
<tr>
<th>Stimulation time (h)</th>
<th>Duration of stimulation (min)</th>
<th>Rate of drug release (µg.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>5</td>
<td>2.43 ± 1.17</td>
</tr>
<tr>
<td>48</td>
<td>10</td>
<td>2.02 ± 0.75</td>
</tr>
<tr>
<td>72</td>
<td>10</td>
<td>2.35 ± 0.63</td>
</tr>
<tr>
<td>144</td>
<td>60</td>
<td>2.40 ± 0.62</td>
</tr>
</tbody>
</table>

4.6.7 Cytotoxicity studies

The cytotoxicity of extracts from the PPy films prepared with and without electrical stimulation was determined on ARPE-19 cells by MTT assay. Comparisons were made with cell culture media as a negative control and Triton-X-100 as a positive control. There were no significant differences in cell viability among stimulated or unstimulated groups compared with the control (Figure 4-7), indicating a lack of cytotoxicity of the films. The lack of cytotoxicity of the films also indicates that the substances that might be released from the films, such as unreacted pyrrole monomer, unwashed phytantriol, and the dopant DexP were not cytotoxic to the ARPE-19 cells.
Figure 4-7: Cytotoxicity of the extracts determined by MTT assay on APRE-19 cells indicating that the extracts from templated (stimulated and unstimulated) films are not cytotoxic.

4.6.8 Pilot study: Drug release from PPy films prepared by potentiostatic polymerisation to pass a current of 1000 mC

The amount of DexP released from conventional films was higher than from the templated films. The release was further tested from the films prepared by using PPy and DexP dissolved either in liquid crystal for templated films or in water for conventional films without adjusting the pH and until a similar charge (1000 mC) is passed. It can be explained that when HCl was not used to adjust the pH of DexP solution, only DexP was available to be loaded as a dopant into the PPy film.

As a proof of the concept, polymerisation of both templated and conventional films was carried out in a potentiostatic mode to reach a similar charge passed which should result in equal
amounts of polymer formed. Equal amounts of polymer have similar drug loading levels. As DexP is loaded as a dopant into PPy during polymerisation, a dopant molecule is consumed for every 3-5 molecules of pyrrole monomer. The drug release data from the conventional unstimulated (Figure 4-8A) and templated unstimulated (Figure 4-8B) was almost similar, indicating that when a similar charge is passed, drug loading is similar. Interestingly, the release data was more efficient from the porous templated films compared to the conventional films on stimulation. Increase in responsiveness in templated films on stimulation was due to high surface area of the templated films which allows more media to interact with the polymer and hence higher drug release. Future work is required which could explore drug loading levels and the morphology and porosity of the templated films compared to the conventional films when a similar charge is passed. Further characterisation was not performed on these films.
Figure 4-8: Drug release profiles from A) conventional films, and B) templated films prepared when a similar charge is passed and when pH of DexP is not adjusted, with and without stimulation. Data points indicate mean, error bars represent standard deviation (n=3).
4.7 Conclusion

A conducting polymer based stimuli responsive drug delivery system has been developed using a phytantriol bicontinuous cubic phase liquid crystal as a template. Conventional PPy films revealed a highly nodular surface whereas templated PPy films revealed interconnected nanorod like structures under SEM. From the elemental analysis, conventional films were loaded with higher amounts of DexP compared to the templated films. FTIR spectra confirms that both conventional and templated films have transmittance peaks corresponding to PPy indicating that bicontinuous cubic phase serves just as a template and no interaction was found between liquid crystal and PPy. The porous PPy films formed through liquid crystal templates were electroactive as evident from cyclic voltammetry and conductivity. Impedance was found to be lower for templated films, indicating their porous nature and increase in surface area. This porous system was capable of releasing bursts of dexamethasone phosphate in response to electrical stimulation. Templated films were more responsive to electrical stimulation compared to the conventional films. Extracts from both templated (stimulated and unstimulated) films showed no cytotoxicity. Such CP based stimuli responsive drug delivery systems offer promising advantages in conditions like AMD and DME where the required drug dosing changes with time.
Chapter 5: Micelle Directed Chemical Polymerisation of Electroactive Polypyrrole Particles for Triggered Release of Dexamethasone and Dexamethasone Phosphate
Chapter 5- Micelle Directed Chemical Polymerisation of Electroactive Polypyrrole Particles for the Triggered Release of Dexamethasone Base and Dexamethasone Phosphate

5.1 Introduction

PPy can be synthesised through the oxidation of pyrrole monomer units which can be initiated electrochemically using electrical stimulation (204, 205) or chemically using chemical oxidants such as iron (III) chloride (FeCl\(_3\)) (206, 207), iron (III) perchlorate (208), iron (III) sulphate (209, 210) and ammonium persulphate (APS) (211), in both aqueous and non-aqueous media. While electrochemical polymerisation is the most widely used approach, polymerisation is limited to the size of the conductive substrate on which it is polymerised with thin films, typically less than a few micron, produced (33). Chemical polymerisation overcomes these limitations as a conductive substrate is not required, with polymerisation resulting in insoluble polymer precipitates forming in solution. The large amounts of conducting polymer that can be rapidly produced makes chemical polymerisation suitable for scale-up (212).

A range of conducting polymer systems are reported in the literature for the release of anionic drugs (16, 17), cationic drugs (19) and neutral drugs (22). These systems can be used to modify drug delivery rates over time, depending on the patient’s needs. To improve the versatility of these systems for wider applications, the amount of drug delivered should be increased while the ability to electrically tune drug release rates should be maintained.

Template-directed growth of PPy can be utilised to increase the overall surface area of the delivery system, the drug loading and the release efficiency (31, 52). Large amounts of micro or nano-structured CPs can be synthesised using a soft template-directed chemical polymerisation and, unlike a hard template, this template can be removed easily after synthesis. Ge et al. have demonstrated the release of fluorescein or daunorubicin loaded polypyrrole nanoparticles prepared by polymerisation of micelles formed from DTAB and decyl alcohol. These particles were dispersed in a temperature sensitive hydrogel, which was injected directly into mice with the release of the loaded drugs triggered upon application of an electrical potential (26). The present work investigates the use of micelles as soft templates over which PPy is grown. Sodium dodecyl benzene sulphonate (SDBS) is an anionic surfactant which readily forms micelles in water with a hydrophobic core (39). These micelles act as a soft template with high drug solubilising capacity in their own right. It is hypothesised that following polymerisation of PPy over the micelles drug will be entrapped. In this chapter, we
investigated the loading and release of two drugs, the hydrophobic and neutral dexamethasone (Dex) and its more hydrophilic anionic salt form dexamethasone sodium phosphate (DexP). Dexamethasone is a synthetic steroid that has anti-inflammatory and immunosuppressant effects. DexP is a sodium salt of dexamethasone and a prodrug which gets converted into dexamethasone in the body (40, 153, 154).

Drug loaded PPy particles were prepared utilising micelles as soft templates capable of enhanced drug encapsulation and loading. Two forms of dexamethasone with different properties were incorporated into the particles. Electrochemical and morphological characterisations of the formed particles are described in detail. Release of both drugs was examined under different forms of electrical stimulation, while the toxicity of extracts prepared from electrical stimulation of the nanoparticles was tested on a human retinal pigment epithelium-19 (ARPE-19) cells. The formed particles could be compressed into desired shapes and sizes, to achieve drug loaded levels based on clinical need. The PPy particles described have the potential to be used as a platform material in triggered drug delivery systems for ocular conditions like age-related macular degeneration (AMD) and diabetic macular edema (DME).

5.2 Aims and objectives

The aim of this chapter was to prepare PPy nanoparticles loaded with DexP and Dex by chemical polymerisation using micelles as a template for tuneable DexP and Dex delivery. The specific objectives were to:

- Prepare SDBS micelles loaded with hydrophilic DexP and hydrophobic Dex.
- Synthesise PPy nanoparticles by chemical polymerisation using drug loaded micelles as a template.
- Perform morphological, chemical and electrochemical characterisation of the formed PPy nanoparticles.
- Determine drug loading and electroresponsive drug release from PPy nanoparticles.
- Determine the cytotoxicity of extracts on ARPE-19 cells.
5.3 Materials and methods

5.3.1 Materials

Pyrrole was purchased from Sigma-Aldrich (Australia) and was distilled and stored at -20 °C under nitrogen until use. Sodium dodecyl benzene sulphonate (SDBS) and ammonium persulphate (APS) were obtained from Aldrich (Australia) and used without further purification. Dexamethasone sodium phosphate (DexP) was purchased from Jai Radhe Sales (India), dexamethasone base (Dex) was purchased from Cfm Oskar Tropitzsch (Germany). ITO slides (resistance 70-100 Ω) were purchased from Delta Technologies (USA) and silver conductive epoxy (four hour working time) was obtained from MG chemicals (Canada). Milli-Q water was from a Millipore/Millipak system with filter size 0.22 µm and resistivity of 18.2 MΩ.cm. Phosphate Buffered Saline (PBS) tablets were obtained from Sigma-Aldrich (Australia). All other reagents were of analytical grade. ARPE-19 cells (a human retinal pigment epithelial cell line) were obtained from American Type Culture Collection (USA) and were used within 20 passages from the time of purchase. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyldiazotetrazolium bromide (MTT) was purchased from Molecular Probes, Thermo Fischer Scientific (USA). The DMEM/F12, GlutaMAX™ medium and TrypLE™ Express were purchased from Gibco, Thermo Fischer Scientific (USA).

5.3.2 Determination of the CMC of SDBS

A series of SDBS solutions at increasing concentrations of up to 25 mM were prepared in Milli-Q water. The surface tension of the prepared solutions was measured using a Du Nouy ring tensiometer, (White Electrical Instruments Co Ltd, England) in order to determine the CMC of SDBS. Briefly, the platinum ring of the tensiometer was immersed in each specific concentration of SDBS solution. The ring was slowly lifted from the liquid. Surface tension, $\gamma$, was determined by the force required to separate the ring from the surface of the liquid at 25 °C. Triplicate measurements of surface tension for each concentration were obtained.

To further confirm the formation of micelles and CMC of SDBS, dynamic light scattering measurements were made. A series of dilutions of SDBS were prepared from 25 mM SDBS solution. The average size of the micelles was determined using a Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK). All measurements were recorded in triplicates at 25 °C.
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The size was measured at a scattering angle 90°, wavelength 633 nm, and viscosity 0.8872 mPa.s.

5.3.3 The ability of SDBS to solubilise Dex

Dex is lipophilic (logP = 2.03) and sparingly soluble in water (0.035 mg.ml⁻¹; 0.089 mM). SDBS micelles have a hydrophobic core which can solubilise hydrophobic drugs such as Dex. A series of concentrations of SDBS were prepared into which excess Dex was added. The solutions were stirred continuously for 48 h until the solution was saturated with Dex. The solutions were filtered to remove undissolved Dex and analysed for drug content by High-performance liquid chromatography (HPLC).

5.3.4 Simultaneous detection of DexP and Dex

Both DexP and Dex concentrations were estimated by HPLC in this study. Briefly, HPLC analysis was conducted on an Agilent 1200 series HPLC (Agilent Technologies, USA) equipped with an autosampler (injection volume: 10 μL) with a 100-well sample tray, a vacuum solvent degasser, a quaternary pump, and an online diode array detector. The results were analysed using ChemStation® software Agilent Technologies, Germany. A reverse phase C18 Phenomenex column (250 x 4.6 mm, particle size 5 μm) and a mobile phase consisting of 50% phosphate buffer, 27.3% acetonitrile, 22.7% methanol at 40 °C, and a flow rate of 1 ml.min⁻¹ were used. The signals were detected at a wavelength 254 nm. A primary stock solution containing DexP (1 mg.ml⁻¹) and Dex (1 mg.ml⁻¹) was prepared in 50% v/v methanol. The stock solution was serially diluted with 50% v/v methanol to produce working solutions in the range of 5 μg.ml⁻¹ to 100 μg.ml⁻¹.

5.3.5 Fabrication of drug loaded PPy particles

SDBS (25 mM) was dissolved in Milli-Q water to prepare the micelles. Dex (1.25 mM) and DexP (1 mM) was then added to the formed micelles with continuous stirring until a clear solution was obtained. Pyrrole was added dropwise to the stirred drug loaded micellar solution to achieve a concentration of 0.05 M and allowed to equilibrate for 30 min. The solution was transferred into an ice bath (4 °C) and stirring was continued for another hour. The oxidant APS (0.15 M) was dissolved in 2 mL of Milli-Q water and added to the pyrrole/SDBS/drug
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solution to initiate polymerisation. The reaction was carried out for 4 h at 4 °C. The reaction was terminated by adding an excess amount of water and the precipitated PPy nanoparticles were washed with excess Milli-Q water to remove unreacted monomer, SDBS and APS. The resulting PPy particles were dried overnight at room temperature (Figure 5-1A).

5.3.6 Morphological characterisation:

5.3.6.1 Size and Zeta potential

The size and zeta potential of the formed particles were determined by dynamic light scattering (DLS) measurements. Measurements were taken for the nanoparticle dispersion at a concentration of 0.1 μg of particles per ml in Milli-Q water.

5.3.6.2 Scanning Electron Microscopy (SEM)

The surface morphology of the PPy particles was investigated using a Philips XL30S field emission gun scanning electron microscope at an accelerating voltage of 5kV using an EDS detector. The samples for SEM were mounted on aluminium studs using adhesive graphite tape and lightly sputter-coated with platinum using Quorum Q150RS Sputter Coater.

5.3.6.3 Transmission Electron Microscopy (TEM)

TEM characterisation was carried out on a Tecnai™ G² Spirit Twin transmission electron microscope, operated with Xplore 3D software. The fine PPy powder was mixed with Milli-Q water to form a dispersion and 5 μL was added to the coated grid. The coated grid was left under a hot lamp to evaporate water leaving a thin layer of particles on the grid.

5.3.7 FTIR

FTIR spectra of the particle pellet, DexP, Dex and SDBS were recorded in ATR mode using a germanium (Ge) crystal of Bruker Tensor 37 FTIR spectrometer, with OPUS spectroscopic software (OPUS 6.5, Germany). Measurements were recorded between 400 and 4000 cm⁻¹ with a resolution at 4 cm⁻¹.
5.3.8 Electrochemical characterisation

To support electrochemical characterisation the particles (Figure 5-1A) were compressed into a pellet (13 mm in diameter, around 0.2 mm in thickness) using a hydraulic pellet making machine which applied 8 tonnes of pressure. The pellet was firmly adhered to an ITO covered glass slide working electrode by means of silver epoxy. The exposed ITO was masked by Kapton tape leaving only the pellet in contact with the surrounding environment for all the samples (Figure 5-1B). The ITO substrate with attached pellet was fixed into the custom-built horizontal setup (Figure 5-1C).

Figure 5-1: A) Polypyrrole particles, B) Particles pressed into a pellet and adhered on an ITO covered glass slide using silver epoxy with exposed ITO masked by Kapton tape, C) Schematic diagram of electrochemical setup.
5.3.8.1 Cyclic Voltammetry (CV)

The electroactivity of the PPy particles was characterised using CV. A three electrode, electrochemical setup was utilised for recording the CV of the particles. The PPy particle pellet adhered to an ITO covered glass slide was used as a working electrode. Platinum mesh was used as a counter electrode and Ag/AgCl as a reference electrode (Figure 5-1C). The particles were cycled between -0.7 V to +0.7 V at a rate of 100 mV.s\(^{-1}\) in PBS for 100 cycles using a Bio-Logic potentiostat, USA and the data was analysed using the EC-lab\textsuperscript{®} software.

Cyclic voltammetry was also used to determine the surface area of the nanoparticle based electrode using the same setup. The particles were cycled between -0.2 V to +0.6 V at a rate of 100 mV.s\(^{-1}\) in PBS containing 5 mM ferri-ferrocyanide (5 mM ferricyanide and 5 mM ferrocyanide).

The charge passed is related to the surface area and the square root of time according to Equation 5-1 (47, 192).

\[
Q = (2nFA D^2 \pi^{1/2} C^{1/2}) t^{1/2}
\]

Equation 5-1

where \(Q\) is the charge passed, \(n\) is the number of electrons used in the reaction (\(n=1\) for \(\text{Fe(CN)}_{6}^{3/-4}\)), \(F\) is the Faraday constant \((9.65 \times 10^{4}\ \text{C.mol}^{-1})\), \(A\) is electrode surface area, \(D\) is the diffusion coefficient \((6.2 \times 10^{-6}\ \text{cm}^2\cdot\text{s}^{-1})\), \(C\) is a mediator concentration \((5 \times 10^{-6}\ \text{mol.cm}^{-3}\ \text{Fe(CN)}_{6}^{3/-4}\) in PBS is used as a mediator).

\[
Q = K t^{1/2}
\]

Equation 5-2

When charge \(Q\) is plotted against the square root of time, the slope of the curve gives \(K\), from which the surface area of the electrode is calculated.
5.3.8.2 Conductivity

Electrical conductivities (S.cm\(^{-1}\)) of the PPy particles pressed into pellets were measured using a Jandel (RM2 model) Multi Height Probe, Resistivity Test Unit with a four-point linear probe (1.0 mm tip spacing). The pellets were placed on a glass slide and four replicate measurements were taken for each pellet. The current was set at 500 µA and the voltage was measured. These values were used to calculate the resistivity (ρ in Ω.cm) according to the Equation 5-3.

\[
\rho = \frac{4.532 \times V \times t}{I}
\]

Equation 5-3

Where \(V\) = voltage (V), \(t\) = sample thickness (cm), \(I\) = current (A) and \(\rho\) = resistivity (Ω.cm). The reciprocal of resistivity gives conductivity (Equation 5-4).

\[
\sigma = \frac{1}{\rho}
\]

Equation 5-4

Where; \(\rho\) = resistivity (Ωcm) and \(\sigma\) = conductivity (S cm\(^{-1}\)).

5.3.8.3 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy was performed using a three-electrode setup with a Bio-Logic electrochemical workstation. Either PPy pellets, bare ITO, or silver epoxy coated ITO were used as working electrodes, with a platinum mesh counter electrode and Ag/AgCl reference electrode. Impedance was measured at open circuit potential in PBS with a 10 mV a.c. component from 10 kHz to 1 Hz. Impedance data was analysed using the EC-Lab® software.

5.3.9 Drug loading and electrically responsive release

The PPy particles were investigated for drug loading and encapsulation efficiency indirectly by determining the amount of drug remaining in the supernatant by HPLC. Drug loading determines the weight of drug in proportion to the weight of all components used to create the
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particles (Equation 5-5), while entrapment efficiency determines the effectiveness of the methodology in loading the drug into particles (Equation 5-6) (213-215).

\[
Drug Loading = \frac{Mass \ of \ drug \ added - mass \ of \ drug \ in \ the \ supernatant}{Total \ mass \ of \ drug \ loaded \ particles} \times 100
\]

Equation 5-5

\[
Encapsulation \ Efficiency = \frac{Mass \ of \ drug \ added - mass \ of \ drug \ in \ the \ supernatant}{Mass \ of \ drug \ added} \times 100
\]

Equation 5-6

The same setup of pressed pellets on ITO covered glass slides used for electrochemical characterisation was used for drug release studies with phosphate buffered saline (PBS) as the media (Figure 5-1C). To determine the effect of redox state of PPy on drug release, the release was determined under different conditions. +0.6 V was applied to oxidise PPy, -0.6 V was applied to reduce PPy, and ±0.6 V was applied at 2 Hz to alternately oxidise and reduce PPy pellet for 3 h. Release under these conditions was compared against passive release in the absence of electrical stimulation for 3 h. Sink conditions were maintained at all the times by adding fresh PBS each time when a sample was taken. A paired t-test was applied between the stimulated group and other groups (oxidation, reduction, and no stimulation) to determine the statistically significant difference in release after 3 h.

To further determine the ability to alter drug release from the nanoparticles, pulses of stimulation in the form of ±0.6 V at 2 Hz were applied for 5 hours after 24 h and 48 h. Sink conditions were maintained at all the times. Release samples were analysed by HPLC.

5.3.10 Cytotoxicity studies

ARPE-19 cells were used to determine the cytotoxicity of the particle pellets by MTT assay. PBS extracts were obtained from the pellets in the presence (±0.6 V, 0.5 Hz for 1 hour) and absence of electrical stimulation. As conductive silver epoxy was used to adhere PPy pellets to ITO coated glass slides, extracts were obtained from silver epoxy with and without stimulation to verify if it releases any toxic chemicals into the extracts. Negative control cells were treated
with DMEM/F-12, GLutaMAX (100% cell viability), whereas positive control cells were treated with Triton X-100 in DMEM/F-12, GlutaMAX to kill the cells (0% cell viability). ARPE-19 cells were seeded at a density of 2x10^5 cells/ml in 96 well plates and incubated at 37 °C in a 5% CO₂ humidified atmosphere overnight. PBS extracts from each sample (n=6) in DMEM/F-12, GlutaMAX (1:1) were added by replacing the cell culture medium. Plates were then incubated for 1 h at 37 °C and 5% CO₂. After the incubation, solutions from each well were removed and replaced with MTT solution (0.5 mg.ml⁻¹) and incubated at 37 °C for 4 h. MTT solution was removed from all the wells and replaced with 0.04 M HCl-isopropanol solution to dissolve the formed insoluble formazan. Formazan dissolves in HCl-isopropanol solution to give a purple colour. The intensity of the purple colour was quantified by measuring the absorbance at 570 nm with correction of any interference at 650 nm (Bio-Tek Synergy HT) and percentage cell viability was calculated.

5.4 Results and discussion

5.4.1 CMC of SDBS

In order to construct micellar templates used to direct the chemical polymerisation of PPy, the CMC of SDBS was determined from a change of surface tension using a Du Nouy ring tensiometer (216). The surface tension of water was 72.8 mN.m⁻¹ which decreased upon addition of SDBS until the CMC was reached, after which the surface tension plateaued (after 2 mM). Three linear portions were found in the graph (before 1 mM, between 1 mM to 2 mM and after 2 mM). This indicates that there might be a transition between different micelle structures. The point of intersection of first and last linear portions indicates critical micellar concentration which was determined to be 1.5 mM (Figure 5-2). CMC is not a constant, which can be affected by many variables such as nature and concentration of counterions in solution, buffer, temperature, and salt (106). As shown in Table 2-1, CMC varies slightly with the method of determination. Since we have used SDBS at a concentration of 25 mM, which is higher than CMC of SDBS, we have not determined and compared the CMC by other methods.
Chapter 5 - Micelle Directed Chemical Polymerisation of Electroactive Polypyrrole Particles for the Triggered Release of Dexamethasone Base and Dexamethasone Phosphate

Figure 5-2: Surface tension of SDBS determined by Du Nouy ring tensiometer. Data points represent mean values and error bars represent SD (n=3).

The size of the SDBS micelles was determined by dynamic light scattering measurements. The presence of SDBS micelles was confirmed by the presence of a peak between 3-4 nm. This agrees with previous reports of SDBS micelles (39). The intensity of this peak on DLS measurements corresponding to micelles decreased as the concentration of SDBS decreased with the peak disappeared when the concentration was diluted below 1.5 mM (Figure 5-3), corroborating the surface tension data.
5.4.2 Solubility of Dex in SDBS

As the concentration of SDBS reaches its CMC, a sharp increase in the solubility of the hydrophobic Dex was observed, indicating the ability of the SDBS micelles to solubilise this poorly water soluble drug in the hydrophobic core (Figure 5-4). Above the CMC, the solubility of Dex increased as the concentration of SDBS increased in a linear fashion. For subsequent drug loading and release experiments, 25 mM of SDBS was used which could solubilise 1.25 mM (0.5629 mg.ml⁻¹) of Dex.
Figure 5-4: Solubility of Dex with increasing SDBS concentration. SDBS micelles were formed above the CMC, which solubilise the poorly water soluble Dex. Data points represent mean values and error bars are SD (n=3).

5.4.3 Simultaneous detection of DexP and Dex

The concentration of DexP and Dex were analysed by HPLC. The signals were detected at a wavelength 254 nm. DexP was eluted with a retention time of 6.1 min and Dex with a retention time of 10.5 min (Figure 5-5). DexP and Dex were calibrated over a range of 5 μg.ml\(^{-1}\) to 100 μg.ml\(^{-1}\) with satisfactory linear regression achieved (\(R^2=0.999\)) for both the drugs (Figure 5-6).
Figure 5-5: Chromatogram displaying a peak corresponding to DexP with a retention time of 6.1 min and Dex with a retention time of 10.5 min. Insets a) show peak purity of DexP, b) overlapping spectra of different spectra of DexP, c) show peak purity of Dex, d) overlapping spectra of different spectra of Dex.
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Figure 5-6: Linearity range of DexP and Dex from 5 \( \mu \text{g.mL}^{-1} \) to 100 \( \mu \text{g.mL}^{-1} \).
5.4.4 Preparation of PPy particles

PPy particles, loaded with both Dex and DexP, were successfully prepared by chemical oxidative polymerisation of pyrrole using micelles as a soft template. APS was used as the chemical oxidant to initiate the polymerisation process, as iron salts such as FeCl₃ causes precipitation of SDBS micelles (217). Micelles were formed above the CMC of the anionic surfactant (Figure 5-7A). SDBS is proposed to solubilise Dex (logP = 2.03) in the hydrophobic core of the micelles while associating DexP (logP = 0.54) near the hydrophilic head groups of the micelles in close proximity to the surrounding media (Figure 5-7B).

![Figure 5-7: Schematic illustration of the synthesis of the PPy particles prepared by chemical oxidative polymerisation. A) assembly of SDBS into micelles, B) associating of Dex in the core and DexP in the outer shell of the micelles, C) association of pyrrole with the outer shell of the micelles and D) chemically driven polymerisation of PPy over the micellar template.](image-url)
These drug loaded micelles were then used as the template to carry out the further polymerisation process. Pyrrole (logP = 0.7) was then added to the micellar solution which tends to orient towards the hydrophilic heads inside micelles (Figure 5-7C) (218). Subsequently, pyrrole monomer was polymerised by adding the oxidant APS (Figure 5-7D). The black PPy particles that formed over 4 hours were collected by filtration and the unreacted oxidant and monomer washed away with excess water. The yield of particles was found to be around 80-90 mg for every 100 ml of the micelle solution.

5.4.5 Morphological characterisation

SEM demonstrated aggregates of particles around 50 nm in diameter (Figure 5-8), with individual particles able to be visualised under TEM (Figure 5-8 inset). DLS measurements confirmed this data with particles of 50 nm in size. The zeta potential of the PPy particles was -45.7 mV. The zeta potential remained stable after a week indicating stability of particles in solution (219, 220). A zeta potential value greater than +30 mV or less than -30 mV indicates a high degree of colloidal stability due the repulsion between particles. A zeta potential value between -30 mV to +30 mV will eventually cause aggregation of the particles due to Van der Waal attraction (221).

Figure 5-8: SEM of polypyrrole nanoparticles. The inset shows TEM of an individual particle. SEM and TEM reveal a rounded particle morphology with diameter around 50 nm.
5.4.6 FTIR

FTIR spectra of particle pellet, DexP, Dex base and SDBS from 4000 cm\(^{-1}\) to 800 cm\(^{-1}\) are displayed in Figure 5-9a. The FTIR spectra of the samples from 1800 cm\(^{-1}\) to 800 cm\(^{-1}\) in Figure 5-9b to clearly observe the peaks in the region. FTIR spectra of PPy pellet shows a peak at 1033 cm\(^{-1}\) which corresponds to C-H and C-N in-plane deformation vibration. The peak at 1166 cm\(^{-1}\) is due to vibration of pyrrole ring. The peak at 1300 cm\(^{-1}\) shoulder band due to C-C in-ring stretching and C-N deformation mode. The peaks at 1548 cm\(^{-1}\) and 1456 cm\(^{-1}\) are due to asymmetric stretching vibration of C=C and C-N (200, 201). The FTIR spectra of DexP and Dex were similar with the presence of peak between 2850 cm\(^{-1}\) to 3000 cm\(^{-1}\) due to the presence of methyl group, and the presence of this peak in PPy particles indicate the presence of Dex in the particles. Similarly, the peak between 1665 cm\(^{-1}\) to 1760 cm\(^{-1}\) corresponds to the presence of carbonyl group in DexP and Dex and incorporation of these in the PPy the particles (32). The absence of characteristic peaks corresponding to the SDBS (222) at 2840 cm\(^{-1}\) and 2920 cm\(^{-1}\) due to the presence of CH\(_2\) and CH\(_3\) and peak at 1650 cm\(^{-1}\) due to the SO\(_2\) stretching in PPy particles indicate that SDBS served as a template and has been washed away completely.
Figure 5-9: FTIR spectra of particle pellet, DexP, Dex base and SDBS a) from 3500 cm\(^{-1}\) to 800 cm\(^{-1}\), b) from 1800 cm\(^{-1}\) to 800 cm\(^{-1}\).
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5.4.7 Electrochemical characterisation

The electroactive nature of the prepared PPy particles was established by CV and conductivity measurements. The PPy particles were cycled at a constant rate of 100 mV.s\(^{-1}\) between two set of points (-0.7 V and +0.7 V vs Ag/AgCl) in PBS Cyclic voltammetry over 100 cycles confirmed the reversible electroactive nature of the prepared polypyrrole particles (182). Over 100 cycles, a slight shift in the peak position was observed which could be attributed to the de-doping of DexP and persulphate ions and doping of ions from the electrolyte solution. The area under curve determines the total amount of charge passed during the redox process, which was found to be 7.85 mC for 1\(^{st}\) cycle which decreased slightly to 6.25 mC by the 100\(^{th}\) cycle. The oxidation peak was found at +0.42 V and reduction peak was found at -0.18 V. (Figure 5-10).

![Cyclic Voltammetry](image)

**Figure 5-10:** Cyclic Voltammetry run between -0.7 V to +0.7 V at 100 mV.s\(^{-1}\) for 100 cycles in PBS.

The electrode surface area of the PPy pellet was calculated from CV cycled between -0.2 V and +0.6 V at 100 mV.s\(^{-1}\) in ferriferrocyanide, which was found to be 2.59 cm\(^2\), whereas electrode surface area for silver epoxy on ITO was found to be 1.22 cm\(^2\) (Figure 5-11). Hence, the roughness factor was calculated to be 2.12. The increase in surface roughness increases the polymer/media interface, thereby decreasing the impedance and increasing the responsiveness of the system.
Figure 5-11: Cyclic Voltammetry run between -0.2 V to +0.6 V at 100 mV.s⁻¹ for 100 cycles in 5 mM ferri-ferrocyanide.

The conductivity of the PPpy particles pressed into a pellet was 22.89 ± 5.49 S.cm⁻¹. The conductivity of the PPpy particle pellet was consistent with PPpy films reported in other studies, which typically ranged between 1 and 60 S.cm⁻¹ (195, 196, 202). This verifies that the PPpy particles contain polymerised polymer chains that are conductive and that charge can pass between the particles in the pressed pellet (33).

Impedance determines the resistance including capacitance and inductance of the polymer offered on the application of current as a function of frequency (194, 203). The PPpy particle pellet had the lowest impedance modulus (|Z|) over all frequencies. A comparison can be made between samples by taking (|Z|) at 2 Hz (frequency used during stimulation of drug release). ITO had an impedance of 2337.01 Ω, silver epoxy on ITO had an impedance of 1240.22 Ω, while particle pellets stuck on ITO using silver epoxy had an impedance of 205.47 Ω (Figure 5-12).
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Figure 5-12: Impedance of silver epoxy on ITO, ITO coated glass and PPy particles over a range of frequencies.

The decrease in impedance may be attributed to an increase in roughness which results in a higher electrochemical surface area (also indicated by CV results). Decreasing impedance values are favourable for drug releasing systems. A lower impedance value suggests that less current or voltage is required to drive drug release from the polymer systems.

5.4.8 Drug loading and electrically responsive release

Drug loading and entrapment efficiency data for DexP and Dex in the PPy nanoparticles are shown in Table 5-1. Entrapment efficiency of Dex is higher than DexP, indicating that Dex, being a poorly water-soluble drug, could have been entrapped in the hydrophobic core of the micelle, whereas DexP could have been solubilised between micelles and the surrounding medium. This suggests that PPy was indeed formed over the micelles.
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This drug loading is comparable to that achieved in conducting polymer films (33). The entrapment efficiency of this process is very high (80.5 ± 1.19 %) for Dex, (31) compared to DexP and the drug loading was limited by the amount of drug solubilised in the micelles. Since these particles can be compressed into different shapes and sizes, the overall drug loaded into the final system can be increased depending on the requirement.

Table 5-1: Entrapment efficiency and drug loading in the particles.

<table>
<thead>
<tr>
<th></th>
<th>Entrapment efficiency (%) (Mean ± SD) (n=3)</th>
<th>Drug loading (%) (Mean ± SD) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DexP</td>
<td>58.3 ± 2.50</td>
<td>4.50 ± 0.26</td>
</tr>
<tr>
<td>Dex</td>
<td>80.5 ± 1.19</td>
<td>6.24 ± 0.04</td>
</tr>
</tbody>
</table>

Drug release from conducting polymers depends on the redox state of the polymer which determines both the electrostatic interactions between polymer and the loaded drug and drives volume changes in the polymer (52). The slowest rates of release of the anionic DexP were observed when the PPy particles were maintained in the oxidised state and when they were not stimulated (Figure 5-13a). Without stimulation, PPy would be expected to remain close to the oxidised state as the result of polymerisation. In the oxidised state, the positive charges on the polymer backbone will attract the anionic DexP. DexP was released faster on reduction to maintain charge neutrality as more negative charge enters the polymer, thus forcing the anionic DexP out of the polymer. DexP release was highest when a pulsed stimulus of ±0.6 V at 2 Hz was applied. This pulsing caused de-doping of DexP from the polymer in reduced state and doping of anions from the PBS on oxidation. The non-ionic drug Dex showed a similar release pattern to DexP (Figure 5-13b). The main point of difference was that reduction did not increase drug release rates beyond no stimulation. As Dex is not a charged molecule, electrostatic charges would not be expected to directly cause release. Interestingly, alternating the redox state of the polymer resulted in increased release rates. This could be attributed to volume changes in the polymer in different redox states altering the rates of drug diffusion from the PPy particles into the surrounding solution (23). The release of both DexP and Dex were
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statistically significant (p<0.001) on pulsed stimulation compared to oxidation, reduction, or no stimulation after 3 h.
Figure 5-13: Release of a) DexP and b) Dex on oxidation (+0.6 V), reduction (-0.6V), pulse (±0.6 V) and passive (n=6).
The release of both the anionic drug DexP and the non-ionic drug Dex was highest on the application of an alternating potential stimulus. To further demonstrate the tuneability of the PPy nanoparticles, the release of DexP and Dex was determined by intermittent bursts of an alternating potential stimulus (Figure 5-14). After an initial 24 h of passive diffusion mediated release, application of a 5 h period of ±0.6 V stimulation at 2 Hz caused a surge in release. A similar trend was observed when this stimulation was applied again at 48 h. The experiment was terminated at 72 h as the pellets started delaminating from the substrate.

Interestingly, for both Dex and DexP, more drug was released during the second period of stimulation than the first. The pellets could be seen visibly swelling during stimulation, eventually resulting in delamination from the underlying substrate. This swelling may be due to electroactuation of the polymer (21) and may have increased the rate of drug release. Future work is required to ensure the stability of function of the PPy particle-based delivery system. During these release experiments, a higher percentage of the DexP was released than Dex. This indicates electrically driven release of the anionic DexP is far more efficient than the release of the uncharged base form of the drug.
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Figure 5-14: Release of a) DexP and b) Dex over 72 hours with periods of stimulation indicated by the black bars. Data indicates mean ± SD (n=6).
Chapter 5- Micelle Directed Chemical Polymerisation of Electroactive Polypyrrole Particles for the Triggered Release of Dexamethasone Base and Dexamethasone Phosphate

5.4.9 Cytotoxicity studies

The cytotoxicity of extracts prepared with and without electrical stimulation of PPy particles was determined on ARPE-19 cells by MTT assay. Comparisons were made with cell culture media, as a negative control and extracts prepared from the silver epoxy alone. Three different concentrations of Dex and DexP were also tested. There were no significant differences in cell viability among any of the groups compared with the control (Figure 5-15) indicating a lack of cytotoxicity. The slight increase in cell viability in the group containing DexP (0.5 mg.ml⁻¹) and Dex (0.05 mg.ml⁻¹) indicates an increase in metabolic activity of ARPE-19 caused by the corticosteroid dexamethasone as previously reported (223).

![Figure 5-15: Cytotoxicity of the extracts determined by MTT assay on ARPE-19 cells.](image-url)
5.5 Conclusion

SDBS micelles loaded with the anionic and hydrophilic drug DexP and the non-ionic and hydrophobic Dex were prepared. PPy nanoparticles were polymerised chemically using drug loaded SDBS micelles as a soft template. Chemical polymerisation can prepare large amounts of polymer and is suitable for scale-up. The formed nanoparticles were around 50 nm in size and negatively charged. The PPy particles were conductive and reversibly electroactive. The polymerisation process was more effective at encapsulating the lipophilic Dex than the more hydrophilic DexP. Pressing the particles into the desired form can be achieved while retaining the electrically responsive drug release of CPs. An increase in the release of DexP was observed on application of a reducing potential suggesting electrostatic forces influence the release of the anionic drug. Meanwhile, a reducing potential had no effect on the release of the uncharged Dex. An alternating potential was the most efficient stimulation to trigger the release of both Dex and DexP. Overall, the electrically driven release of the anionic DexP was more efficient than the electrically driven release of the uncharged Dex. Periods of alternating potential resulted in bursts of release for both drugs. Extracts obtained from the PPy particles indicated no cytotoxicity in ARPE-19 cells. These systems have potential application in ocular conditions like age-related macular degeneration and diabetic macular edema. These particles could be compressed into different shapes and sizes, enabling customizability of these systems. In addition, the particles could be loaded with drugs of different properties widening the scope of application of these smart nanoparticles to a range of disease states.
Chapter 6: General Discussion, Future Outlook and Conclusion
6.1 General discussion

Conducting polymers (CPs) can be electrically stimulated to tune the release of loaded drugs. Strategies are required to increase the amount of drug loaded and to enhance the responsiveness of these systems to stimulation. Previously reported CPs with defined micro- or nanostructures were discussed in detail in Chapter 2. CPs with a variety of micro- or nanostructures can be reliably synthesised through hard template-directed, soft template-directed or template-free polymerisation methods. Drugs can be loaded into these CP structures along with the template or loaded subsequent to the removal of the template. While hard template-directed polymerisation has been the most widely explored, particularly for drug delivery applications, it can be problematic to remove the template without damaging the micro- or nanostructures. Hard template-directed polymerisation approaches are also more likely to be complicated during scale-up. Soft template-directed or template-free approaches offer alternate fabrication avenues and are beginning to be explored more widely. By imparting micro- or nanostructures into CPs, the drug delivering capacity can be increased with enhanced responsive drug release to electrochemical stimulation.

Phytantriol liquid crystals were explored as a template for the synthesis of porous polypyrrole (PPy) films in Chapter 3. Different phases of liquid crystals were determined using a hot stage in combination with cross-polarised light microscopy and a phase diagram was constructed. Bicontinuous cubic phase was selected as a template to synthesise PPy as it contains continuous, non-intersecting, aqueous and lipid channels which allow the movement of monomer and dopant to the working electrode. The effect of the addition of monomer, pyrrole and dopant, pTS at different concentrations was studied by small angle X-ray scattering (SAXS) at the Australian synchrotron. Bicontinuous cubic was maintained after the addition of pyrrole and pTS. Different approaches were explored to polymerise PPy through the bicontinuous cubic phase template. PPy was polymerised by adding pyrrole to the lipid and pTS to the water before injecting aqueous phase into the lipid phase to form liquid crystals. Electropolymerisation was carried out at +0.7 V for 1 h in a three-electrode setup by keeping working and counter electrodes directly in contact with the liquid crystal. For comparison, conventional films were prepared without any template using the same concentrations of pyrrole and pTS and under similar polymerisation conditions. Aqueous electrochemical polymerisation (by dissolving pyrrole and pTS in water) was used for preparing conventional
films. The morphology of the conventional and templated films was characterised by SEM which revealed a porous morphology of the templated films, indicating that PPy has been polymerised in the bicontinuous cubic template. The electroactivity of the formed PPy film in the template was tested by CV which shows reversible redox activity. Electrochemical polymerisation and redox potential of PPy had no effect on the liquid crystal phase structure.

Bicontinuous cubic phase was further explored as a template to prepare drug loaded porous PPy films in Chapter 4. DexP was selected as a model drug which is used to treat posterior eye conditions like age-related macular degeneration (AMD) and diabetic macular edema (DME). The effect of the addition of pyrrole and DexP to the liquid crystal template was studied by SAXS which showed that the phase was maintained in the bicontinuous cubic phase. The morphology of the templated PPy demonstrated a highly interconnected network of nanorod like structures compared to the conventional films which showed a typical nodular morphology under SEM. Under similar polymerisation conditions (potential and time), more polymer formed without a template than when a bicontinuous cubic phase template was used to direct polymerisation. This is attributed to the resistance caused by bicontinuous cubic phase to the movement of pyrrole and DexP to the working electrode from which polymerisation occurs. The amount of DexP loading was determined by determining the percentage weight of elements such as fluorine and phosphorous present in DexP and not in the polymer itself by EDS. The percentage weight of fluorine and phosphorus in conventional films was found to be higher than templated films. Interestingly, the percentage weight of chlorine was found to be higher in the templated films than in the conventional films, indicating that chlorine has been included as a dopant instead of DexP. HCl was used to adjust pH of the DexP solution. Chloride derived from HCl, being a small highly mobile anion, is able to diffuse better in the bicontinuous cubic phase than the large DexP molecule to the working electrode. CV shows that both conventional and templated films are electroactive but the charge passed during each cycle in the conventional PPy films was much higher than the templated films, as higher amounts of the polymer was formed in the conventional method. The effective electrochemical surface area was found to be higher for templated films compared to the conventional films as expected from the morphology. Conductivity was found to be similar for templated and conventional films. The impedance modulus of the templated films at 0.5 Hz (frequency used during stimulation of drug release) was found to be slightly lower than conventional films which results from increased surface area in templated films. The amount of DexP released from the conventional films with and without stimulation was found to be much higher than the
templated film. This was due to the more efficient polymerisation producing more polymer and also due to higher drug loading levels as determined by elemental analysis on EDS. Templated films demonstrated a highly responsive DexP release on stimulation while the stimulation has only moderate effect on release from the templated films. To further prove the effect of stimulation, films loaded with DexP alone as a dopant (chloride not present as pH was not adjusted) and polymerisation was carried out until the same amount of charge was passed in both templated and conventional films so that similar amounts of polymer were formed. Higher levels of DexP release occurred in a more responsive fashion from the templated films compared to the conventional films. This was due to the increased surface area of the templated films which enhances the polymer/media interface. Cytotoxicity of the release extracts from the templated films with and without stimulation was tested on ARPE-19 cell lines which demonstrated that films or any substances that might be leached from the polymer, such as unreacted pyrrole monomer or unwashed lipid or DexP, did not result in any toxicity on cells.

A different soft template (micelle) was explored to verify if increased amount of drug loaded polymer could be produced by chemical polymerisation in Chapter 5. SDBS, an anionic surfactant, was used as a template and two drugs, non-ionic, hydrophobic Dex and its anionic salt, hydrophilic DexP, were studied. This allowed us to study the mechanism of loading and release of drugs with different properties from PPy. CMC of the SDBS was determined to be 1.5 mM. Dex (logP = 2.03) is hydrophobic and poorly soluble in water; the solubility of Dex was enhanced in the presence of micelles. The solubility of Dex was found to be 0.5629 mg.ml$^{-1}$ at 25 mM of SDBS. Meanwhile, DexP (logP = 0.54) was associated near the head groups of the micelles. Monomer (pyrrole) and oxidant (APS) were added to the drug loaded micelles. As pyrrole (logP = 0.7) tends to orient towards the hydrophilic heads inside the micelles and APS is highly water soluble, polymerisation occurs at the micelle-water interface to form drug loaded PPy nanoparticles. The formed particles were characterised under SEM and TEM, and the particles were found to be c.a. 50 nm in size. The particles were pressed into pellets and adhered to an ITO coated glass slide using silver epoxy. From CV, it was confirmed that the pressed pellet was electroactive and the surface area was found to be 2.59 cm$^2$. The conductivity of the pellet (22.89 ± 5.49 S.cm$^{-1}$) was determined by four-probe conductivity meter, which shows that the pellet was conductive and charge can pass between particles in the pressed pellet. The impedance of the pellet was less than that of the bare electrode, indicating an increase in surface area. The drug loading and entrapment efficiency were determined indirectly by estimating the amount of unloaded drug in the supernatant after the formation of
particles. Entrapment efficiency of Dex (80.5 ± 1.19%) was found to be higher than DexP (58.3 ± 2.50 %), indicating that due to Dex being a hydrophobic molecule it was entrapped in the hydrophobic core of the micelle, whereas DexP was solubilised between micelles and the surrounding media. DexP being an anionic molecule must have been loaded by electrostatic attraction. It is also interesting that Dex loading was found to be higher despite DexP being anionic and attracted electrochemically to the forming PPy. To understand the mechanism of release of DexP and Dex, different stimulation conditions were verified. DexP release was slowest when the particles were oxidised or when they were not stimulated. DexP release was faster on reduction and highest release was observed when a pulse stimulus was applied which indicates that the release of DexP is driven by electrostatic interaction. For Dex, the release was similar on oxidation, reduction or without stimulation, but showed higher release on applying pulse stimulus. As Dex is not a charged molecule, electrostatic forces would not affect the release. Volume changes on applying pulse stimulus would have caused the release of Dex from the particles. To further determine the release of drugs on stimulation, pulse potential was applied intermittently after an initial period without any stimulation. An increase in the release was observed for both DexP and Dex when a stimulation was applied after 24 h. The second stimulation after 48 h demonstrated a higher rate of release due to the swelling of the pellets and due to electroactuation. The cytotoxicity of the extracts from the pellets and silver epoxy (with stimulation and without stimulation) and Dex and DexP at various concentrations were tested on ARPE-19 cell line. No statistically significant difference was observed among the groups, indicating a lack of cytotoxicity.

This thesis explored two different soft templates, liquid crystal based template to prepare films by electrochemical polymerisation and micelle based template to prepare particles by chemical polymerisation. PPy films showed similar drug loading levels to that reported in the literature but an increase in responsiveness was achieved in the porous templated films. Loading in the particles was also similar to the previous reports but large amounts of drug loaded particles could be synthesised by micelle based chemical polymerisation. In addition, the particles could be loaded with drugs of different properties widening the scope of application of these smart nanoparticles to a range of disease states. The particles could be compressed into different shapes and sizes, enabling customisability of these systems. These systems have the potential to be used in conditions such as AMD and DME.
6.2 Limitations and future outlook

This project describes the soft templated directed polymerisation of PPy based drug delivery systems. Porous PPy films with increased responsiveness were prepared by bicontinuous cubic phase of phytantriol liquid crystal as a template. The levels of drug loading were similar between templated and conventional films. Loading levels need to be increased for these systems to be applicable to a wide range of drugs. This can be achieved by increasing the polymerisation efficiency in a liquid crystal host template and by incorporating smaller dopants than DexP which improves the movement in liquid crystal template. When polymerisation efficiency is increased, higher amounts of polymer will be formed. This results in higher drug loading since DexP was loaded as a dopant.

The current operational lifetime of the films was less than a week and particle pellet was 3 days. A loss of reversible redox activity and conductivity inevitably occurred due to over-oxidation during repeated cycles. In addition, repeatedly forcing the polymer through redox cycling resulted in structural damage to the polymer caused by swelling and de-swelling as ions move into and out of the polymer. This was evident as cracks in the polymer film and particle pellet, and delamination from the substrate. Hence, the stability of the system in the final product needs to be enhanced. It was reported in the literature that PPy and PEDOT nanotubes remained firmly attached to the substrate compared to the conventional PPy and PEDOT, indicating that CP with defined micro- or nanostructures are more stable on repeated cycles due to their porous structure withstanding strain (224). However, new approaches are required to promote performance stability in conducting polymers to ensure the translation of CP-based delivery systems to the patient bedside.

In this thesis, release was tuned effectively using electrical stimulation. But for many clinical applications, the ability to completely stop release is required, therefore strategies (such as combining CPs with other materials which further control the release; such as diffusion limited release by hydrogels) need to be designed in the future to inhibit unstimulated release from CP platforms. CPs have exciting potential which is gradually being realised to achieve delivery systems where drug release rates are tuneable to provide individualised patient treatment.

The developed PPy films and particles are non-biodegradable and if they are intended to be used in the treatment of ophthalmic conditions like AMD and DME, a procedure is required to implant and a second procedure is required to remove after the drug is exhausted. If in the
future CP-based systems could be rendered biodegradable (146, 225), this would extend and promote the exploration of new applications where systems would degrade at the end of their usable in-vivo lifetime without the need for a removal procedure.

CPs with defined micro- or nanostructures are highly promising platforms for drug delivery applications, however, there are no marketed products using these materials in drug delivery. Responsive materials which can be triggered by heat, light, and electrical stimulation offer advantages for drug delivery whereby the bioactive payload is delivered only when required. An example is ThermoDox® (226), a doxorubicin-based thermosensitive liposomal formulation for liver cancer. Following targeting to tumour tissue, these liposomes change structure when heated to a certain temperature releasing doxorubicin to the target tissues and reducing off-target effects (227, 228). To follow other materials and move further along the development pipeline, fabrication pathways are required to enable scale-up of delivery platforms. In addition, standardised protocols need to be developed for drug loading, release, analysis and reporting of data as there is a large variation to how studies are designed and reported (229). CP based biosensors are well described and could be used to sense changes in the body. For instance, a recent study reported a bio-switch chip, in which they loaded PPy with glucose oxidase. On reduction, they found that glucose from the external solution entered PPy to cause oxidation of glucose (230). In the future electrically triggered drug release from CPs will be combined with sensing properties (biomolecular species (7), pressure (231) or electricity (152)) of these materials to make a closed-loop, self-regulating drug delivery system. This concept was realised by Zhou et al. with an enzyme based logic-controlled anode to sense lactic acid and lactate dehydrogenase in the body produced during tissue trauma which triggered the release of acetaminophen from the cathode (232). Similarly, pressure sensing CP materials are being reported which could find use in glaucoma monitoring and personalised treatment (233). In the future, these materials will be used as drug delivering implants where drugs release can be tuned either in response to an external stimulation or as a self-regulating device.
6.3 Conclusion

Liquid crystal templated porous PPy films loaded with DexP and micelle templated PPy nanoparticles loaded with Dex and DexP were prepared for tuneable drug delivery. Phytantriol liquid crystals were studied, different phases were identified and a phase diagram was constructed. Bicontinuous cubic phase liquid crystal was explored as a template for the first time for the polymerisation of PPy. The effect of added additives (monomer and dopants), polymerisation and electrical stimulation of PPy on bicontinuous cubic phase demonstrated no change in the phase structure. Bicontinuous cubic phase liquid crystal was further explored as a template to prepare DexP loaded PPy film. The PPy film was found to be porous and electroactive. The electrically tuneable release of DexP with higher responsiveness was achieved compared to conventional films.

SDBS micelles loaded with the anionic and hydrophilic DexP and the non-ionic and hydrophobic Dex were prepared. PPy nanoparticles were synthesised by chemical polymerisation using SDBS micelles as a template. Large amounts of drug loaded nanoparticles were prepared; the formed particles were pressed into a pellet and adhered to ITO coated glass slides. The entrapment efficiency and drug loading capacity of Dex were higher than DexP. Dex was entrapped into the hydrophobic core of the micelle, whereas DexP was loaded by electrostatic interactions. The release of DexP was more efficient than Dex indicating that DexP was released due to electrostatic interactions whereas Dex was released by volume changes and actuation of the polymer. These particles could be compressed into different shapes and sizes, enabling customizability of these systems.

Extracts prepared from both films and particles were found to be non-toxic in ARPE-19 cells. These films and particles could form the basis of an implantable drug delivery system in the treatment of AMD and DME.
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