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Conducting Polymer Implant for On-demand Ocular Drug Delivery

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ABSTRACT

Introduction: Implants have become an attractive treatment option for chronic posterior eye conditions. However, a patient’s disease state and any concurrent side-effects may require a dose adjustment which is not possible with currently marketed implants. Stimuli-responsive implants present an opportunity to tailor the release of the active. They may be composed of conducting polymers (CP) such as poly(3,4-ethylenedioxythiophene) (PEDOT), a robust CP with good biocompatibility and reproducible electroactivity. This thesis investigated the fabrication of a PEDOT-based system suitable for implantation to provide stimuli-responsive ocular drug delivery.

Methods: PEDOT films (non-porous and porous) were fabricated via vapour phase polymerisation (VPP) and characterised for their surface morphology, electrochemical behaviour and biocompatibility. Dexamethasone phosphate (dexP) was loaded after polymerisation as a dopant via active and passive ion-exchange. DexP release was determined in the absence and presence of an electrical stimulus. Dexamethasone base (dex) was also physically entrapped into the pores of porous PEDOT and a sealing layer was polymerized on top. PEDOT-coated cellulose membranes, replicating the PEDOT sealing layer, were also fabricated and characterised for their drug permeability using Franz-cells. Finally, a prototype implant for in-vitro evaluation was fabricated by 3D printing the casing and placing drug loaded VPP PEDOT films inside.

Results and Discussion: Porous PEDOT prepared by VPP exhibited a highly porous morphology and a three-fold higher electrochemically active surface area (as determined by cyclic voltammetry) compared to non-porous PEDOT prepared by VPP. Release medium extracts from non-porous and porous PEDOT films displayed no significant cytotoxicity. The amount of dexP loading achieved via active ion-exchange was almost three-fold higher compared to passive ion-exchange. The effect of redox state over dexP release was determined where a pulse stimulus released maximum amounts of drug. A faster rate of dexP release was observed for porous compared to non-porous films. Dex was physically entrapped into the pores to increase drug loading; however, it leaked through the PEDOT sealing layer within 1 h. PEDOT-coated cellulose membranes confirmed the high permeability of these sealing layers. Therefore, the assembled prototype implant contained only dexP loaded films and exhibited a burst in drug release during in-vitro stimulation aligning well with previous dexP release where a similar burst in drug release was observed upon the application of in-vitro stimulus.

Conclusion: A PEDOT-based system suitable for implantation was fabricated exhibiting a stimuli-responsive burst in drug release. However, drug loading and retention need to be further improved such as by physical entrapment and effective sealing to achieve long-term on-demand drug delivery.
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Contents of Chapter 1 (including Figure 1.1 and Figures 1.3 to 1.8) primarily consists of the published review article: M.N. Yasin, D. Svirskis, A. Seyfoddin, I.D. Rupenthal, Implants for drug delivery to the posterior segment of the eye: a focus on stimuli-responsive and tunable release systems. Journal of Controlled Release, 2014. 196: p. 208-21. It has been re-used with permission from Elsevier. The original article can be accessed at:


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RESEARCH OUTPUTS

Publications arising from this project


Published conference abstracts arising from this project


Conference presentations and seminars arising from this project

18th International Conference on Advanced Biomaterials and Nanomaterials (ICABN), *Poly(3,4-ethylenedioxythiophene) prepared by vapor phase polymerization for stimuli-responsive ion-exchange drug delivery*. Singapore on 8th-9th Sept, 2016. (E-poster presentation)

10th World Congress of Biomaterials (WBC 2016), *Highly porous poly(3,4-ethylenedioxythiophene) prepared by vapor phase polymerization for biomedical applications*. Montreal, Canada on 17th-22nd May, 2016. (Poster presentation)

Polymer Electronics Research Centre (PERC) 10th annual symposium, *Highly porous poly(3,4-ethylenedioxythiophene) prepared by vapor phase polymerization for biomedical applications*. Dept. of Chemistry, University of Auckland, Auckland, NZ on 16th Nov, 2015. (Oral presentation)
Research outputs

NZ National Eye Centre (NZ-NEC) seminar, Electro-responsive implant for on-demand drug delivery to back of the eye. Domain Lodge, Grafton, Auckland, NZ on 16th Oct, 2015. (Oral presentation)

HealtheX student conference, Porous poly(3,4-ethylenedioxythiophene) (PEDOT) structures for stimuli-responsive drug delivery. Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ on 11th Sept, 2015. (Oral presentation)


Polymer Electronics Research Centre (PERC) 9th annual symposium, Conducting polymer based scleral implant for efficient tunable drug delivery to posterior segment of the eye. Dept. of Chemistry, University of Auckland, Auckland, NZ on 23rd Nov, 2014. (Oral presentation)

PharmaTell (Departmental seminar at the School of Pharmacy), Conducting polymer based scleral implant for tunable drug delivery to the posterior eye. University of Auckland, Auckland, NZ on 29th Oct, 2014. (Oral presentation)

Australia-New Zealand CRS joint Student Workshop, Conducting polymer based implant for on-demand ocular drug delivery. Adelaide, South Australia on 23rd Oct, 2014. (Oral presentation)

HealtheX student conference, Developing an HPLC method for simultaneous quantification of a hydrophobic drug and its hydrophilic salt. Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ on 12th Sept, 2014. (Oral presentation)

Other publications


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Chapter 1 (Introduction) primarily comprises of this published review article:


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**Certification by Co-Authors**

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

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CHAPTER 1: INTRODUCTION

1.1 Background

Effective and sustained drug delivery to the posterior segment of the eye is challenging as there are structural and physiological barriers that limit the penetration of topically or systemically administered actives into the ocular tissues [1]. Thus, to treat ocular conditions of the posterior segment of the eye, invasive local therapy such as intravitreal injection of drug-containing solutions is generally required. Since these injections have to be performed relatively frequently by a specialist they increase the burden on health care professionals. Moreover, they only provide modest relief and generally result in low patient adherence to the therapy [2]. Finally, they may result in unwanted ocular complications such as cataract formation or retinal detachment [3]. The posterior segment of the eye is a confined space with slow clearance mechanisms when compared to the systemic circulation. Therefore, it offers great potential for local drug delivery systems such as implants [4].

While a number of drugs are available to treat posterior eye conditions, only a few implantable devices exist to deliver these drugs efficiently and over extended periods of time. Ocular implants provide a platform for the sustained release of drugs from either biodegradable or non-biodegradable polymeric systems over several months to years [5]. Currently, there are four ocular implants, Vitrasert®, Retisert®, Ozurdex® and Iluvien® approved by the FDA, with the first two being non-biodegradable systems anchored to the sclera, while Ozurdex® is a biodegradable rod injected into the vitreous. Iluvien® is a non-biodegradable rod-shaped implant injected into the vitreous, delivering the drug for up to 36 months [6-11]. However, while these systems can deliver the drug over long periods of time, the release rate cannot be altered. With the advances in polymer science and nanomaterial development for biomedical applications over recent years, there has been a paradigm shift from conventional to stimuli-responsive or tuneable devices [12-17]. Such systems also have great potential in the area of ocular drug delivery [18-20]. Thus, stimuli-responsive drug delivery systems (DDS) suitable for ocular implantation are of great interest as they have the potential to provide tuneable drug delivery to the affected areas [21, 22].

Stimuli-responsive systems are considered to be ‘intelligent’ as they respond to a stimulus, leading to initiation, termination, or an increase or decrease in drug release. This is of particular interest for drug delivery to the posterior segment of the eye as the currently available implants release drug at a constant rate, while different drug concentrations may be required at various time-points depending on the individual requirements and the disease state [23]. Such systems offer fine control over drug release, helping to optimise therapeutic outcomes in individual patients [24]. Conducting polymers (CP) are organic materials that possess both polymer- and metal-like properties [25]. CP offer exciting opportunities for use in stimuli-responsive implantable DDS to treat posterior eye conditions. These polymers are biocompatible, non-toxic and offer fine control over drug release through electrochemical stimulation [26]. A CP-based device presents an interesting opportunity for stimuli-responsive drug
delivery to the posterior segment of the eye. Figure 1.1 provides an overview of the approximate location used by selected implantable drug delivery systems for drug delivery to the posterior segment of the eye.

![Figure 1.1: Selected implants and their locations in the eye, adapted with permission from [27].](image)

1.2 Ocular conditions and current treatment options

Posterior segment diseases are a major health concern as these conditions directly impact on the patient’s vision and therefore their quality of life. Around 285 million people are estimated to be visually impaired or blind with this number increasing by at least seven million per year [28]. The main vision-threatening diseases affecting the posterior segment include age-related macular degeneration (AMD), diabetic retinopathy, proliferative vitreoretinopathy (PVR) and uveitis. While a number of drugs are available to treat these conditions, only a few implantable devices exist to deliver these drugs efficiently and over extended periods of time.

1.2.1 Age-related macular degeneration (AMD)

AMD is the leading cause of irreversible blindness in the population aged over 50 years with its prevalence projected to increase up to 50% in this age group by 2020 [29-31]. Early-stage AMD is characterised by the presence of a few medium-sized drusen in the retina as well as retinal pigment abnormalities. The advanced stage of AMD can be either dry (non-neovascular) or wet (neovascular). Wet AMD accounts for 10-15% of the overall prevalence of AMD but is responsible for over 80% of cases of legal blindness [32]. It is characterised by choroidal neovascularisation and can lead to
blindness in days to weeks as a result of haemorrhage or fluid accumulation. Anti-vascular endothelial growth factor (anti-VEGF) antibodies are the primary pharmacological course of treatment for wet AMD and are administered via the intravitreal route. These include the FDA-approved ranibizumab (Lucentis®) and off-label use of bevacizumab (Avastin®) [33, 34]. A fully recombinant human fusion protein called aflibercept (Eylea®), which acts as a soluble receptor for VEGF, is the most recent FDA-approved intravitreal drug for the treatment of this condition. It has shown higher affinity for VEGF-A compared to ranibizumab and bevacizumab and exhibits a longer half-life [35]. Corticosteroids such as triamcinolone acetonide and dexamethasone (dex) have also been proven effective against the progression of this disease when given intravitreally [36, 37]. Currently, three implantable DDS are in clinical trials for the treatment of wet AMD. Retisert (containing fluocinolone acetonide, which has FDA approval for uveitis) is in phase two clinical trials, while the ranibizumab-containing port delivery system is in phase one clinical trials [9, 38]. Iluvien (containing fluocinolone acetonide) is also in phase two clinical trials for use in this condition to lower the intravitreal dosing of ranibizumab [39]. Although bevacizumab has not been approved by the FDA for ocular use, it is widely used as an off-label intravitreal injection due to its cost-effectiveness [40, 41]. Interestingly, intravenous bevacizumab is also under investigation in phase two clinical trials for the treatment of wet AMD [42]. Currently, there is no implant in clinical trials containing a combination of an anti-VEGF and a corticosteroid drug although combination therapy has proven to be much more sustained and effective [43]. For dry AMD, a biodegradable implant of brimonidine is currently in phase two clinical trials. Brimonidine works by stimulating the release of neurotrophins, which protect retinal pigment epithelial cells [44-46].

1.2.2 Diabetic retinopathy (DR)

DR is also a major health issue in people aged between 30 and 64 years due to the worldwide diabetes epidemic and accounts for 12% of all new cases of blindness in the United States [47]. A landmark study, the Wisconsin Epidemiological Study of Diabetic Retinopathy, demonstrated that almost every individual suffering from diabetes has a 100% risk of developing some form of DR over the course of 30 years. DR can either lead to ischaemia or macular oedema, or both. Macular oedema arising from DR can be focal or diffuse. Focal macular oedema is associated with leakage of fluid from capillaries while diffuse macular oedema indicates an area of capillaries with no or poor perfusion [47, 48]. Intravitreal corticosteroids (dex, triamcinolone and fluocinolone) are used to treat this condition while bevacizumab has proven to be effective in macular oedema secondary to choroidal neovascularisation [49, 50]. Currently, only two implants have been approved by the FDA for use in this condition. Ozurdex is a biodegradable implant based on poly(lactic-co-glycolic acid) (PLGA) which is injected through a 22-gauge needle into the vitreous, releasing dex for up to six months [51]. Iluvien, on the other hand, is a non-biodegradable implant injected through a 25-gauge needle into the vitreous which releases fluocinolone acetonide for up to 36 months [9, 11]. However, dose requirements may change during the course of intravitreal corticosteroid therapy depending upon the disease state. Thus an increase or decrease in the drug release may be required [52].
1.2.3 Proliferative vitreoretinopathy (PVR)

PVR is a posterior eye condition characterised by the proliferation of ectopic cell sheets into the vitreous and peri-retinal area. This disease is a common problem after retinal detachment surgery and can lead to blindness [53]. Corticosteroids (triamcinolone) and anti-proliferative drugs (5-fluorouracil (5-FU), doxorubicin and daunomycin) are the main courses of treatment for this condition. Several implants containing a single or multiple drugs have been suggested and investigated to date [54-59]. The Codrug™ implant technology has been utilised for a combination implant containing 5-FU with either corticosteroids or NSAIDs. These implants showed positive outcomes in experimental disease-induced animals but have not entered into human clinical trials to date [60, 61]. The Codrug technology incorporates two drugs conjugated with a biodegradable spacer, capable of releasing both drugs at a pre-determined rate. Growth factor pathway inhibitors (hypericin, suramin and alkylphosphocholine) and antioxidants (N-acetylcysteine) have also been explored in disease-induced animals to halt the proliferation process and showed promising results without significant toxicity [62]. Anti-proliferative agents such as taxanes (docetaxel and paclitaxel) have also been used for the treatment of PVR and a magnetic stimuli-responsive implant, currently investigated for tunable delivery of docetaxel, will be discussed in more detail in Section 1.5.2 [63].

1.2.4 Uveitis

Uveitis is inflammation of the uvea, which comprises the iris, ciliary body and choroid. This condition can be divided into three types: inflammation of the iris is called anterior uveitis, inflammation of the ciliary body is intermediate uveitis and inflammation of the choroid is termed posterior uveitis [7]. The inflammation of all of these parts is termed panuveitis [64]. Immunosuppressants (cyclosporine A, tacrolimus and sirolimus), biological agents (infliximab, etanercept and interferons) and corticosteroids (dex and fluocinolone acetonide) are currently used to treat this condition, with corticosteroids being the mainstay of therapy for non-infectious uveitis [65, 66]. Drug delivery for the treatment of intermediate and posterior uveitis is challenging, as it demands sustained release over prolonged periods. Currently, three sustained-release intraocular implants (Ozurdex, Iluvien and Retisert) have been investigated for use in posterior uveitis. Biodegradable Ozurdex is approved by the FDA for use in this indication, while pilot studies have also been performed for the use of Iluvien and Retisert, with positive outcomes [67]. Implants containing a combination of a corticosteroid and an immunosuppressant have also been investigated for posterior uveitis treatment and showed sustained release of these drugs over several months [57]. A biodegradable scleral implant of betamethasone has recently been investigated that released effective amounts of the drug over a period of one month [68]. However, all these implants lack control over drug release after being implanted into the eye, limiting their use in the case of concurrent infections and exhibiting elevated side effects.
1.3 Dexamethasone sodium phosphate (dexP) and dexamethasone base (dex)

Dex and its phosphate salt dexP have both been widely used to treat chronic back-of-the-eye conditions as briefly discussed in Section 1.2. During this PhD, both of these drugs are explored for use in a conducting polymer-based implant; therefore, the relevant properties of these drugs and their potential use in ocular implants are discussed here. Figure 1.2 shows the chemical structure of dex and dexP.

![Chemical structure of a) dex and b) dexP.](image)

**Figure 1.2: Chemical structure of a) dex and b) dexP.**

### 1.3.1 Physicochemical properties and mechanism of action

Dex is a synthetic fluorinated corticosteroid drug, with the chemical formula C_{22}H_{29}FO_5 (Figure 1.2a) and the IUPAC name 9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one. It is only poorly soluble in water (solubility: 0.05 mg/mL at 25°C) but exhibits better solubility in organic solvents such as ethanol and chloroform. It is a very potent anti-inflammatory drug that exerts its action as an agonist at corticosteroid hormone receptors [69]. Corticosteroids like dex exhibit the increased expression of anti-inflammatory genes as well as suppressing the expression of activated inflammatory genes [70, 71]. They also abolish the expression of VEGF genes, leading to reduced endothelial cell proliferation and a slowing down of the formation of leaky blood vessels, thus ultimately improving vision in affected patients [71, 72].

DexP is the sodium phosphate salt of dex with the chemical formula C_{22}H_{28}FNa_2O_8P (Figure 1.2b) and the IUPAC name disodium:2-[(8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta- [a]phenanthren-17-yl]-2-oxoethyl]
Introduction

phosphate [73]. It is freely soluble in water and shows good solubility in organic solvents such as ethanol and methanol [74]. The therapeutic effect and mechanism of action for dexP are exactly the same as for dex, but the pharmacokinetic profiles differ due to the difference in solubility and polarity of both molecules. Therefore, the method of administration, the mechanism of elimination and/or half-lives may differ as well [75].

Table 1.1 elaborates on the physicochemical properties of dex and dexP; these properties dictate the formulation parameters of the drugs, such as the loading and release. Furthermore, they also play a very important role with respect to the permeability of the drugs across different biological barriers, as well as for other pharmacokinetic parameters.

Table 1.1: Physicochemical properties of dex and dexP [69, 73, 76, 77].

<table>
<thead>
<tr>
<th>Property</th>
<th>Dex</th>
<th>DexP</th>
</tr>
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<td><strong>Solubility</strong></td>
<td>Methanol: 25 mg/mL</td>
<td>Methanol: 20 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Water: 0.05 mg/mL</td>
<td>Water: 50 mg/mL</td>
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<tr>
<td><strong>pKa</strong></td>
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<td><strong>Molecular weight</strong></td>
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<td><strong>Log P</strong></td>
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<tr>
<td><strong>Polar surface area</strong></td>
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### 1.3.2 Intravitreal indications and administration

Intravitreal dex is widely used in the treatment of various posterior-segment eye conditions, including diabetic retinopathy (DR), diabetic macular oedema (DME), non-diabetic macular oedema (NDME), proliferative vitreoretinopathy (PVR), uveitis and age-related macular degeneration (AMD) [78, 79]. Unlike other corticosteroids, dex does not cause salt retention in the eye, making it the preferable candidate to be used in case of elevated adverse effects, such as increased intraocular pressure or glaucoma [80]. The average physiological concentration of natural corticosteroid hormone is reported to be 5 ng/mL in the vitreous humour and this hormone possesses the same anti-inflammatory potency as cortisone. Dex, on the other hand, is 30 times more potent than cortisone. Therefore, a minimum concentration of 0.2 ng/mL is required to mimic the physiological effect of natural corticosteroid hormone, whereas higher concentrations are needed to exert any anti-inflammatory effect in disease [81]. The average half-life of dex in the vitreous humour is 5.5 h with toxicity observed at or above 24 mg/mL [82, 83].
1.3.3 Potential use in ocular implants

For intravitreal administration, dex has been injected previously as an aqueous solution with an injection volume of 0.05-0.1 mL, containing 0.4-0.5 mg of dex per dose \[82, 84\]. Injected via the intravitreal route, this dose results in much higher concentrations than required to produce any anti-inflammatory effect, but is necessary to obtain the therapeutic outcomes due to the rapid clearance and less sustained effect of dex \[72\]. Ozurdex is the first FDA-approved implant to contain 0.7 mg of dex. Once injected into the vitreous, it releases the drug slowly and has proven therapeutic efficacy for up to 6 months. When compared to the amount of drug previously being injected via intravitreal injection of dex suspension to obtain the same therapeutic efficacy, Ozurdex offers many advantages with fewer complications \[85\]. These factors indicate the high potential for dex to be used in ocular implants. Despite all the successes, there are problems associated with Ozurdex, such as anterior chamber migration and the inability to remove the implant in cases of elevated adverse effects (discussed briefly in Section 1.4.2). Therefore developing a stimuli-responsive implant of dex for the treatment of chronic posterior-segment conditions is of great interest.

1.4 Current ocular implants and their limitations

The use of polymers for compression, spray and dip-coating, or encapsulation to modify the release characteristics of drugs has been in practice in the pharmaceutical industry for over 50 years \[86\]. Such polymers are also used to prepare ocular implants, as they can entrap the drug and release it over prolonged periods of time. For the purpose of this thesis introduction, ocular implants have been classified into non-biodegradable, biodegradable and stimuli-responsive polymeric systems, with the latter group comprising biodegradable or non-biodegradable systems depending on the polymer used.

1.4.1 Non-biodegradable polymeric implants

These implants can entrap drug either by dispersion throughout a polymer matrix or storage inside a reservoir surrounded by a release-controlling non-biodegradable polymer membrane. Drug release from non-biodegradable matrix systems is governed by diffusion and an initial burst is often observed. On the other hand, reservoir systems release the drug either through permeable non-biodegradable membranes or via a small orifice in an impermeable membrane. Both of these systems have shown near zero-order drug release of effective concentrations over extended periods of time \[86-88\]. Polyvinyl alcohol (PVA), ethylene vinyl acetate (EVA) and silicon are the most commonly used non-biodegradable polymers for ocular implants. Silicon and EVA are hydrophobic in nature and are mainly used as membranes with limited permeability in reservoir-based systems. PVA, on the other hand, is more hydrophilic and therefore permeable to a broader range of drugs. A combination of PVA with either EVA or silicon is often utilised to optimise drug release from the implant. Another polymer called polysulfone has also been investigated for ocular implants, with limited success until its recent use in the encapsulated cell technology implants from Neurotech \[87-90\].
Vitrasert, approved by the FDA in 1996, is a non-biodegradable implant containing 4.5 mg of ganciclovir for the treatment of CMV retinitis. It contains the drug in the form of a pellet coated with two layers of non-biodegradable polymers. The inner coating is PVA, with an impermeable outer coating of EVA on three sides and an additional PVA layer on the remaining side. The drug permeates via the PVA-coated side at a rate of approximately 24 µg/day and the implant has shown clinical suppression of disease symptoms over five to eight months [91, 92]. Vitrasert is sutured to the pars plana region of the sclera. Common complications with the use of this implant include endophthalmitis, cataract formation, intraocular pressure increase and risk of retinal detachment [93].

Retisert is the second FDA-approved non-biodegradable implant with a PVA and silicon coating and it contains 0.59 mg of fluocinolone acetonide for the treatment of uveitis. The drug is present in the form of a cylindrical pellet that is attached to a PVA suture tab and coated with additional PVA and silicon. The drug is released via a diffusion port in the PVA-silicone laminate through the PVA suture tab, as silicon is impermeable to fluocinolone acetonide [94]. Like Vitrasert, Retisert is sutured to the sclera and therefore needs to be surgically inserted and removed. Retisert has proven effective for up to 30 months, with an initial steady drug release rate of 0.6 µg/day during the first month decreasing to a 0.3-0.4 µg/day after 30 days for up to 30 months. Common complications associated with this implant include increased intraocular pressure as well as a higher incidence of cataract formation and retinal detachment [95].

Renexus (formerly known as NT-501) contains cells from the human retinal pigment epithelium (RPE) that are genetically modified to secrete recombinant human ciliary neurotrophic factor (CNTF). The outer shell of this device is fabricated from polyether sulfone and is 9 mm long with an inner diameter of 0.87 mm and an outer diameter of 1.07 mm. It is threaded with a non-biodegradable polyethylene terephthalate yarn and contains a titanium loop at one side and a filling tube on the other side composed of perfluoroalkoxy copolymer. After sterilisation, it is loaded with approximately 10 µL of a suspension containing CNTF-secreting cells (39,000 cells/µL) [96]. The device is placed into the vitreous through a small incision in the sclera, fixed with a suture, and has shown to release clinically effective amounts of CNTF over a period of 18 months. Renexus is currently in phase two clinical trials for the treatment of retinitis pigmentosa and dry AMD. Based on the same platform, NT-503 is another non-biodegradable polymer implant currently in phase one clinical trials that secretes a VEGF antagonist for the treatment of choroidal neovascularisation [90, 97-100].

An additional refillable functionality has been achieved in the port delivery system (PDS)—a scleral plug consisting of a porous container, a semi-permeable non-biodegradable membrane with a refill port and one or more exit ports to release the drug into the vitreous humour. This implant has been tested with ranibizumab and exhibited significant positive outcomes in phase one clinical trials, showing improved visual acuity, reduced macular thickness and lower leakage in choroidal neovascularisation when compared to monthly intravitreal injections of the same drug [101, 102].

Another non-biodegradable implant, Iluvien, which is currently FDA-approved for the treatment of diabetic macular oedema, contains 190 µg of fluocinolone acetonide in a PVA matrix inside a polyimide tube. This cylindrical-shaped implant has membrane caps on both ends to control the rate of drug release. It is injected into the vitreous through a 25-gauge needle to release drug at a rate of approximately 0.6 µg/day for up to 36 months. Common complications with this implant include
increased intraocular pressure, glaucoma, retinal detachment, cataract formation and an increased incidence of infection [103-105]. The fate of this implant is still unclear, as it is non-biodegradable and would therefore accumulate in the vitreous humour, with surgical removal of this implant from the vitreous not reported so far.

A non-biodegradable polymeric implant for the delivery of multiple drugs at independent rates has been fabricated by Nagai et al. [106]. This subconjunctival implant consists of a polymeric drug reservoir prepared by pouring tri(ethyleneglycol)dimethacrylate (TEGDM) onto a micro-fabricated PDMS mould, which is then loaded with active and sealed with a polymer mixture of poly (ethyleneglycol) dimethacrylate (PEGDM) and TEGDM. Low molecular weight compounds (330 to 480 g/mol) with fluorescent markers were used to study drug delivery, with this new device exhibiting controlled release of these substances across the sclera into the vitreous humour over a period of four weeks.

Another interesting non-biodegradable implant described by Molokhia et al. [107] is intended for implantation into the lens. This device contains bevacizumab, for the treatment of wet AMD, dispersed within a PVA matrix that is supported by an outer PMMA structure (Figure 1.3). Release is controlled through a semi-permeable membrane on one side, with silicon valves to refill the reservoir. Due to its intended placement into the lens, there is the possibility of delivering drug to both the anterior and posterior segments of the eye, depending on the device orientation. Bevacizumab was released at a rate of 60-80 µg/day, which is within the effective concentration range. However, this release rate was only achieved for a few days. Although a long-term release profile is required to confirm its use in chronic retinal conditions, this refillable implant system may be a promising technology for the simultaneous delivery of various drugs to either the anterior or posterior segments of the eye.

Figure 1.3: Capsule drug device showing the drug reservoir, semi-permeable membrane and location of the silicon valves, adapted with permission from [107].
In general, non-biodegradable polymers are preferred for implant fabrication to deliver therapeutics to the posterior segment as they release the drug in a more controlled manner over extended periods of time, while also offering easier removal in case of adverse reactions. They also give better control over the drug release rate. Fabrication of refillable, non-biodegradable implants might therefore be advantageous in terms of patient compliance and overall treatment costs.

1.4.2 Biodegradable polymeric implants

These implants are made from biodegradable polymers that entrap the drug, either throughout the polymer matrix or in the form of a reservoir with a biodegradable polymer coating. The drug is released from these matrix systems through a combination of diffusion and polymer degradation. In reservoir-based implants drug is mainly released through a pore in the otherwise impermeable membrane, with the biodegradable polymer coating degrading relatively slow compared to the drug release out of the system. With biodegradable matrix systems there is generally an initial burst and then a fairly constant release rate over time, with a final burst observed in reservoir systems. Drug release can vary depending on the surface area, rate of polymer degradation, polymer swelling, molecular weight and nature of the drug molecule [88, 108, 109]. Compared to biodegradable particles, which have also been widely used to treat ocular diseases [110], implants offer higher drug loading due to their larger dimensions as well as extended drug release due to their smaller surface-to-volume ratio [22].

Polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactones (PCL), polyanhydrides (PA) and poly(ortho esters) (POE) are biodegradable polymers commonly used for ophthalmic drug delivery, with PLGA being the most widely studied. These polymers are all aliphatic esters that are degraded in the body by water and/or enzymes with their degradation products being metabolised into CO₂ and water [109, 111]. PCL is a hydrophobic polyester and is degraded by slow hydrolysis, offering extended periods of drug release. It is often combined with PLGA to fabricate PLGC implants, which are less immunogenic than pure PLGA devices [87, 112, 113]. POE is a hydrophobic biodegradable polymer that degrades by surface erosion making the drug release dependent solely on the chemical degradation of the implant system. POE are divided into four subtypes (subtype 1, 2, 3 and 4), with subtypes 3 and 4 being the most suitable for ophthalmic drug delivery as they have been extensively studied for their biocompatibility and slow erosion properties [87, 88, 114]. PA are bio-erodible polymers suitable for the incorporation of hydrophobic drugs, exhibiting surface erosion and excellent biocompatibility. The most widely studied PA is 1,3-bis(carboxyphenoxypropane) with sebacic acid (SA), which exhibits increased hydrophilicity and faster degradation when the SA portion is increased. Therefore, water solubility and the extent of surface erosion by hydrolysis can be controlled by changing the polymer composition [115].

Ozurdex, the first biodegradable intravitreal implant approved by the FDA, is a cylindrical implant comprising dex dispersed in a PLGA matrix based on the NOVADUR® technology (Allergan). It contains 700 µg of dex and has proven to be clinically effective for up to six months in the treatment of diabetic macular oedema and non-infectious uveitis. It is injected into the vitreous via a 22-gauge
needle and exhibits fast drug release over the first two months due to diffusion of the drug and degradation of the polymer, after which polymer degradation is solely responsible for slower drug release over the following four months. The same technology has recently also been used to investigate the delivery of brimonidine and is currently in phase two clinical trials for the treatment of dry AMD and in a phase one clinical trial for the treatment of retinitis pigmentosa. Like Ozurdex, this implant is injected into the vitreous, releasing the drug for up to six months, with brimonidine aimed at preventing cell death in the retinal pigment epithelium [9, 44, 46, 116]. It has been shown that the low micro-pH of PLGA degradation products can cause inflammation of the ocular tissues, which may be masked if delivering a steroid (Ozurdex) but may become more apparent with the brimonidine implant. To eliminate the inflammation seen with conventional PLGA formulations, GrayBug has developed a proprietary PLGA-based technology proven to reduce this inflammation observed with the use of conventional PLGA particles, with their lead product for wet AMD treatment (GB102) targeted to last up to six months [117, 118].

The Verisome technology is another biodegradable drug delivery platform believed to contain drug within a lipid/oil layer that is injected via a 30-gauge needle into the vitreous. The drug-containing lipid gels in-situ, forming a spherule in the vitreous humour that releases therapeutic levels of drug for up to 12 months [9, 116, 119]. Verisome containing triamcinolone acetonide (IBI-20089) is currently in phase two clinical trials and has proven safe and effective in the treatment of macular oedema for up to one year. The formed sphere degrades slowly as the drug releases out of the in-situ forming implant. While this implant technology exhibits the same safety and efficacy profile as Ozurdex, it can achieve drug release over a longer duration depending upon the injection volume, rendering the Verisome technology superior.

The most recent developments in biodegradable implantable systems include the ENV705™ implant from Envisia Therapeutics and the nanoporous film device by Zordera. The ENV705 implant is based on the PRINT technology where a trehalose/bevacizumab combination is dispersed throughout a PGA matrix. This technology allows customised drug release by slight fabrication modifications; therefore, offering an advantage over other biodegradable implants. Like Ozurdex, this implant is placed into the vitreous and has shown drug release at effective concentrations over three to six months [120]. The nanoporous film device by Zodera is also injected into the vitreous and has shown zero-order sustained drug delivery to the retina. This device consists of a drug pellet sandwiched between two thin layers of impermeable biodegradable membrane (Figure 1.4). One side has nanopores of the same diameter as the active, permitting only one drug molecule to leave the reservoir from each pore at a time. This approach yields a very thin device with a diameter of only 40 µm and almost zero-order sustained drug release. The polymer layers degrade at a later time-point when most of the drug has been released, eliminating the need for device removal. This implant offers control over the release rate by adjusting the pore size and has been shown to deliver ranibizumab over four months in a sustained manner. It may therefore become a promising biodegradable implant technology for treating chronic retinal conditions [121].
Figure 1.4: Nanoporous film device showing the drug pellet sandwiched between two polymer layers with one side containing nanopores to allow sustained zero-order drug release, adapted with permission from [121].

Most biodegradable implants are fabricated as injectables, eliminating the need for complicated and specialised surgical procedures for insertion. However, their release kinetics are often not ideal as they exhibit an initial and final burst—except for the nanoporous film device described above which has shown sustained zero-order drug release [122]. As most of these implants are placed into the vitreous humour without any fixation, they may move away from the injection site. Problems have been reported with Ozurdex, which showed anterior chamber migration resulting in reduced drug concentrations at the required site and direct contact with the corneal endothelium resulting in corneal oedema leading to surgical removal of the implant [123]. Therefore, in conditions and situations where the drug is no longer required, it is quite difficult to recover biodegradable implants when compared to non-biodegradable systems anchored into the sclera.

1.5 Stimuli-responsive implants

Implants made from stimuli-responsive polymers exhibit abrupt changes in structure, solubility, charge, volume and hydrophobic-hydrophilic balance in response to physical or chemical changes in the environment and can be utilised to tune drug release rates [124]. This may be of particular interest for drug delivery to the posterior segment of the eye as currently available implants release drug at a constant rate while different drug concentrations may be required at various timepoints depending on the individual requirements and the disease state [23].

1.5.1 Light-activated implantable systems

The transparent nature of the cornea and the lens makes the eye an optimal organ for light-activated DDS [125], with a number of interesting nanomaterials already investigated for biomedical release applications [16]. In the eye, the use of photo-activated drug delivery started with photodynamic therapy (PDT) to treat choroidal neovascularisation using the photosensitiser verteporfin (Visudyne®), which is activated by non-thermal red laser light to produce single oxygen molecules that induce
coagulation [19]. A number of photo-responsive polymers have already been investigated over recent years with the major hurdles remaining the toxicity of some chromophores as well as potential tissue damage by the wavelength of light used for activation. Generally, photo-responsive polymer technologies can be divided into two groups: (i) irreversible polymers that can result in photo-polymerised (methacrylates), photo-degradable (nitrobenzoxycarbonyl) or photo-triggered (PDT) systems and (ii) photo-reversible systems that are based on photo-isomers (azobenzene and spirobenzopyran) or photo-dimers (coumarin, nitrocinnamate and anthracene) [19].

Generally, light-activated DDS have been prepared in the form of either polymeric micelles or gels by incorporating light-sensitive materials into the formulation that either respond to a specific wavelength or convert light into heat, as is the case for gold nanoparticles. These systems are comparatively new, with limited application for drug delivery to the posterior segment of the eye due to the wavelength of light required. Electromagnetic radiations (EMR) ranging from 250-380 nm (UV: ultraviolet) and 700-900 nm (NIR: near infrared) are typically utilised to activate such systems, while wavelengths above 900 nm cannot penetrate ocular tissues and are therefore unsuitable for drug delivery to the back of the eye [126, 127]. UV-sensitive DDS generally include leuco-derivatives in the polymer structure. These compounds are usually unionised and become ionised under the influence of UV radiation, creating an osmotic pressure in the polymer/gel system that results in an influx of solvent causing increased drug release [128]. Visible or NIR light-sensitive micelles are generally prepared by chemically binding a chromophore onto the hydrophobic part of the polymer [129], which is cleaved by application of a specific wavelength of light—resulting in drug release. Disruption of the chromophore can be reversible or irreversible, allowing an increase in drug release for short or extended periods of time. Chromophores that exhibit reversible disruption in response to a specific wavelength of EMR include azobenzene, cinnamoyl, spirobenzopyran and triphenyl methane-derivatives, with these compounds converted to a less stable state in the presence of suitable EMR and returning back to their stable state when the stimulus is removed. Pyrene-derivatives, on the other hand, show irreversible disruption of the chromophore when activated by light, thus releasing the entire drug amount or leading to a permanent increase in drug release [130]. Chromophores are also under investigation for incorporation into other polymeric systems. Peng et al. [131] developed a hydrogel consisting of biocompatible biodegradable dextran and azobenzene, incorporating green fluorescent protein (GFP) to observe photo-responsive protein release. They showed that 70% of the loaded GFP was released in response to an EMR stimulus within 90 min, compared to only 5% in the absence of light. Such a drug delivery technology might be ideal where a reversible increase in drug release can be achieved by application of a light stimulus.

Nanoparticles comprising an impermeable metal shell such as gold have also been explored for light-activated drug delivery. These metal shells are capable of absorbing EMR and converting it into thermal energy, which leads to disruption of the micelle structure [132]. Similarly, implantable DDS composed of an impermeable metal core have been explored for the purpose of drug delivery, with the metal portion of the implant capable of producing heat upon exposure to EMR, leading to degradation of specific areas in the implant. This resulted in the formation of an orifice in the implant, releasing drug upon application of light. It is important to focus a specific wavelength of light on a very small surface
area of this metal shell in order to create a suitable orifice for drug release. For ocular drug delivery, one such system has been developed by On Demand Therapeutics (ODTx), which contains several drug reservoirs that can be activated individually by laser light (Figure 1). While the patent does not give any detailed information on the constituents, it states that the light-responsive core of the implant may consist of a highly impermeable silicone polymer or thin walled titanium tube. The release from each individual reservoir, with the potential of carrying different drugs, can hereby be initiated when required by use of non-invasive laser technology already available in ophthalmic practice to treat other ocular conditions. When a specific wavelength of EMR is shined on a small area of the implant for a short period of time the impermeable outer core is broken and the entrapped drug, including a dye for visual confirmation, is released [133]. This implantable light-activated system provides a platform for controlled drug delivery of many types of drugs in the treatment of posterior segment diseases, although its non-biodegradable nature may lead to implant accumulation over time. The ODTx system is still in pre-clinical trials and the feasibility for its use in treating posterior eye segment conditions has not been fully evaluated. In addition, due to the non-biodegradable nature of the implant, it is also important to investigate its fate as the accumulation of non-biodegradable implants in the vitreous humour may lead to long-term complications.

### 1.5.2 Micro electro mechanical system (MEMS)-based implants

MEMS actuate to perform their function, with actuation achieved by various stimuli including temperature [134], electrical stimulus [135], magnetic field [63] or osmotic pressure [136]. They consist of one or more drug reservoirs, containing either a single drug or multiple drugs in different reservoirs and actuators, which are responsible for pushing the drug out of the reservoir by mechanical means in response to the stimulus. A cannula is sometimes used to deliver the drug, with or without a check valve, to the target site [137]. Such implants have been explored for the purpose of drug delivery to the posterior segment of the eye with manual mechanical, electrolysis-based mechanical and magnetic actuation.

A MEMS-based implant for ophthalmic drug delivery was investigated by Lo et al. [138], where stimuli-responsive phenylephrine delivery was observed in-vitro, ex-vivo and in-vivo (in male Dutch-Belted pigmented rabbits). The device was fabricated by moulding PDMS to form a refillable drug reservoir and featured manual mechanical actuation to force the drug out of the reservoir through a cannula. A check valve was placed into the cannula to ensure one-way flow of the drug, only allowing the drug to pass when the pressure was high enough to open the valve. The reservoir was refilled using a 30-gauge needle by simply puncturing the PDMS layer, with leakage observed after 12 injections. In-vivo experiments revealed that drug delivered through the device using manual mechanical actuation showed better clinical outcomes compared to conventional intravitreal injection, indicating that the device was able to deliver the drug effectively. However, manual actuation did not provide precise control over the amount of drug released. Later, this system evolved to using a mini drug pump featuring hydrolysis-based actuation, where the conversion of water into gas results in a volume expansion that increases the pressure on the drug reservoir, thus pushing more drug out through the cannula and into the target area (Figure 1.5) [21, 139]. This implant provided precise control over the
amount of drug released in response to the stimulus, exhibiting more accurate drug delivery. Low power requirements (µA-mA) enabled the activation of electrolysis through wireless means, making this implant clinically practical.

Figure 1.5: a) MEMS-based mini drug pump concept, b) potential location in the eye and c) volume of drug pumped in the presence and absence of an electrical current, adapted with permission from [21, 139, 140].

However, there were still many unanswered questions related to the safe and reliable long-term use of this technology. Back then, this technology featured a very small reservoir that only lasted four to six weeks; therefore, another procedure was frequently required to refill the reservoir. Moreover, the electrolysis area is separate from the drug reservoir; however, both are in a very confined space. Thus, any attempt to refill in which the needle punctures the electrolysis compartment may damage the integrity of the system. Finally, this system only had a lifetime of nine months, after which it was prone to leakage and needed surgical replacement. However, the MEMS-based mini drug pump has since evolved into the MicroPump™ System (Replenish) for clinical use in humans and is currently under safety testing for the treatment of various ocular conditions with a lifetime of up to five years [97, 141]. In addition to the original features it now contains a fluidic flow sensor, a bi-directional telemetry system for wireless programming and a microcontroller allowing the pre-programmed administration of nanolitre-sized doses. It can be refilled using a proprietary 31-gauge needle kit, and it is envisaged that this technology could serve as a closed-loop system in the future, where an increase in intraocular pressure could initiate drug release to treat glaucoma [20, 97].

Another MEMS-bases ocular implant is the implantable device investigated by Pirmoradi et al. [63] exhibiting magnetic stimuli-responsive drug release to the posterior segment of the eye. This device contains docetaxel intended for the treatment of PVR. It is based on polydimethylsiloxane (PDMS) with one side of the device having a magnetic field-responsive membrane incorporating iron oxide nanoparticles in the PDMS matrix. An aperture of approximately 100×100 µm² was introduced in this membrane by laser light to facilitate drug release (Figure 1.6). A 64-fold increase in drug release was observed in the presence of a magnetic field (213 mT magnetic field strength) compared to spontaneous release through the aperture. This device provided precise control over drug release, dependent upon the strength and duration of the magnetic field applied, and is a promising example of stimuli-responsive implantable DDS where release is controlled through application of a small magnetic
field. However, the long-term use of such an implantable system is questionable and this will be further discussed in the next section.

Figure 1.6: a) Magnetically responsive MEMS-based implant and b) docetaxel release in the presence and absence of a magnetic stimulus, adapted with permission from [63].

1.5.3 Magnetically modulated implantable systems

Magnetically modulated polymeric implants for drug release have been reported over the last four decades [142]. These systems can be divided into two types: matrix- and reservoir-based systems. In matrix systems, tiny magnetic beads are incorporated into the polymer-drug matrix to modulate the drug release with the application of an external magnetic field. These magnets oscillate back and forth when an external magnetic field is applied, interacting with the polymer to release more drug out of the polymer pores [143]. It was also suggested that when an external magnetic field is applied, these small magnetic beads break the polymer chains allowing the solvent to penetrate, dissolving some portion of the embedded drug and swelling the polymer, which in turn increases the overall drug release [144].

Drug release from such polymeric systems depends on the mechanical strength of the polymer, the strength of the external magnetic field, the strength of the implanted magnets and the orientation of the implanted magnets against the external magnetic field [143, 145].

Matrix systems have been explored for magnetically responsive drug release composed of one or more polymers with drug and tiny magnets incorporated into the matrix system. EVA is the most widely studied polymer for this purpose as it is biocompatible and offers easy fabrication to the desired shape, while incorporated magnetic beads have the ability to interact with the polymer to enhance drug release upon the application of a suitable stimulus. The vinyl acetate portion of the polymer affects the magnetically responsive drug release from this matrix system. A higher vinyl acetate portion leads to a weaker mechanical strength of the polymer structure (as measured by Young’s modulus), which leads to higher magnetically responsive drug release [142]. A matrix system containing insulin has been studied for its possibility to release insulin on demand in response to an external magnetic field in the case of high blood glucose levels. Kost et al. [146] fabricated such a system by incorporating insulin in the EVA polymer matrix (40% w/w) with a magnet embedded between two layers of the polymer. It was
observed that the presence of a magnetic field triggered insulin release, resulting in a decrease in blood glucose levels of up to 50% in a rat model when compared to those implants where no magnetic field was applied. To date, no magnetically responsive matrix system has been explored in the area of ophthalmic drug delivery; however, the low invasiveness of magnetic stimulation makes it an area of future interest.

Magnetically responsive reservoir systems contain one or more magnetically responsive components which control drug release. One such implantable device for the purpose of drug delivery to the posterior segment of the eye has been investigated, containing docetaxel for the treatment of PVR, with the fabrication discussed above [63]. The long-term performance of implants responding to an external magnetic stimulus has not yet been established. Previous research has shown that repeated application of external magnetic stimulus may result in variations in drug release with a decrease after a few high-frequency pulses, possibly due to the rapid depletion of the magnetic response of the small magnets against these high frequencies due to a loss of polarity [124]. This renders the long-term use of these implants questionable, especially in terms of achieving reproducible drug-release profiles. Moreover, magnetic field stimuli are used in a number of medical procedures such as computerised tomography (CT) and magnetic resonance imaging (MRI) which pose problems in patients with a magnetically responsive drug delivery device. Therefore, additional research is required to determine the suitability of these systems for the purpose of long-term stimuli-responsive drug delivery to the posterior segment of the eye.

1.6 Conducting polymers

CP are organic materials that possess both polymer- and metal-like properties [25]. Since their first description in 1977, this interesting group of polymers has been explored for various biomedical purposes, including electrically tuned drug release [147-150]. Polypyrrole (PPy), poly(3,4-ethylenedioxythiophene) (PEDOT) and N-methylpyrrole are the most widely studied and utilised CP for drug delivery. They are prepared by either chemical or electrochemical oxidation of monomer units, using chemical oxidants or an oxidising current.

1.6.1 Conducting polymers in drug delivery

CP offer exciting opportunities for use in stimuli-responsive implantable DDS to treat posterior eye conditions. These polymers are biocompatible, non-toxic and offer fine control over drug release through electrochemical stimulation [26]. This stimulation typically alters the redox state of the polymer, which is accompanied by changes in polymer charge, volume, molecular permeability and hydrophobicity, all of which can be exploited to alter drug release. CP are typically non-biodegradable; however, they can be modified into biodegradable forms [151], although there may be a trade-off in conductivity, release rate, actuation and other properties.
CP have been used for over two decades to fabricate DDS in the form of reservoirs [152], microchips [153] and actuating devices [154]. Drugs can be doped directly into the CP [155]; however, to increase the drug carrying capacity, reservoir systems have also been utilised [152, 156]. These systems are prepared by using hard or soft templates with the conducting polymer grown on top [152, 157]. The template may hereby act as a drug reservoir itself or may be sacrificial and when removed may leave behind porous structures suitable for drug loading (Figure 1.7). Through the incorporation of drug into a CP reservoir, loading can be increased compared to simple drug-doped CP films. Sharma et al. [152] fabricated porous inverse opal PPy structures using poly methylmethacrylate (PMMA) colloids as a sacrificial template, almost doubling the drug loading compared to non-template PPy films. These systems could therefore be explored for tuneable drug delivery to the target site, with passive release via diffusion occurring in the absence of electrical stimulation and increased doses achievable upon application of an electrical stimulus (Figure 1.7).

Figure 1.7: a) Process of template-based porous polymer structure formation and drug loading, b) SEM images of template, inverse opals and inverse opals loaded with drug, c) drug release in the presence and absence of an electrical stimulus, adapted with permission from [152].

Microchips have also been prepared by electrochemical deposition of drug-doped CP films onto metal microelectrodes that were fabricated on a silicon substrate. Ge et al. [153] developed microchips using gold to make microelectrode arrays with the drug released upon electrical stimulation. Spontaneous release was minimised by electro-polymerisation of a second layer of CP, enabling precise amounts of drug to be released upon application of a defined electrical stimulus. These microchips offered stimuli-responsive drug release without passive diffusion and could, therefore, be used in conditions where spontaneous drug release is not required.
Actuating devices such as peristaltic or micro-pumps, electro-conductive hydrogels and microneedle-based nano-actuators (MNBNA) have been fabricated utilising the volume changes observed in CP upon redox switching [154]. Peristaltic or micro-pumps have been prepared using a CP component as the actuator, with a drug reservoir and an electrode array to control actuation. Electrical stimulation caused volume changes in the CP, leading to expulsion of drug from the reservoir, giving precise control over the amount of drug being released. PPy, PPy-derivatives and polyaniline are suitable for such systems as they require only a small potential for stimulation similar to devices already in clinical use, including pacemakers or cochlear implants [158-160]. The peristaltic or micro-pump technology could also be used for the preparation of ocular implants placed into the sub-conjunctival space, releasing precise amounts of drug upon electrical stimulation via a trans-scleral cannula directly into the vitreous. A combination of CP and hydrogels is also very interesting, as CP possess electrochemical properties and hydrogels offer swelling/deswelling properties, providing a unique combination of increased drug loading and desirable release characteristics. Tsai et al. [161] developed such a DDS incorporating indomethacin as a model drug, prepared by co-blending the CP polyaniline with PVA and cross-linking it with diethyl acetamidomalonate. The drug entrapment efficiency was 65—70% and there was an increase in the amounts of drug released with the application of electrical potential (+1.5 V), which suggested the swelling of the hydrogel, as the CP actuated in response to the electrical stimulus. Such behaviour would be suitable for injectable stimuli-responsive devices based on biodegradable CP. An MNBNA-based device was developed as a DDS for stimulated transdermal drug delivery that could also be tailored towards a sub-conjunctival implant. It consisted of electro-deposited PPy on a gold-sputtered polycarbonate (PC) membrane, with PPy acting as an electrochemical actuator opening and closing membrane pores for stimuli-responsive drug release (Figure 1.8). Application of a negative potential resulted in the closure of the membrane pores, while drug release was observed with a positive potential when the pores were opened [154].

![Figure 1.8: a) Schematic representation of the microneedle-based nano-actuator (MNBNA) system. The main components are i) hollow microneedle array, ii) gold-sputtered polycarbonate membrane electrodeposited with dodecylbenzenesulfonate-doped polypyrrole (PC/Au/PPy/DBS) and iii) polydimethylsiloxane (PDMS) reservoir, b) Schematic illustration of the assembled dual-channel drug delivery system outlining the reservoirs for iv) drug 1 and v) drug 2, c) Schematic of the main components of a single microneedle during drug delivery: i) reservoir, ii) lumen, iii) hollow microneedle, iv) Au/PPy/DBS nanoporous membrane, v) PC membrane and vi) drug, adapted with permission from [154].]
CP-based implants delivering drugs to the posterior segment of the eye in response to electrical stimulation have not been reported in the literature to date. However, the fabrication of such a system, in the form of a non-biodegradable refillable implant that could be used for prolonged drug delivery (tunable by the application of a small electrical stimulus) would provide better control over drug release. This could be achieved through wireless means, using a technology similar to an implant currently in phase one clinical trials for human parathyroid hormone fragment delivery in the treatment of anabolic osteoporosis [162]. This technology is based on electrical energy transfer across the skin by induction, where a change in the magnetic field of an external coil (primary coil) induces current in the internal coil (secondary coil) [163]. Among the CP suitable for drug delivery, PEDOT is a strong candidate due to its stable, reproducible electroactive nature and good biocompatibility and is to be further explored for the fabrication of such DDS system which could be further developed into an implant to deliver drug over longer periods of time.

1.6.2 PEDOT

PEDOT is composed of 3,4-ethylenedioxythiophene (EDOT) monomers units and is a conjugated polymer that carries a positive charge on almost every three monomer units, similar to other CP (Figure 1.9). The extended ring containing two oxygen atoms gives PEDOT its superior protection against over-oxidation by donating electrons to further stabilise the doped bipolaronic state. In addition, this extended ring also contributes towards the easy processibility of EDOT for polymerisation while retaining high conductivity [164]. Due to its stable nature, PEDOT is considered a promising candidate for use in devices for the treatment of chronic conditions [165]. PEDOT has shown good biocompatibility and has thus been widely studied for various biomedical applications including drug delivery [166].

![Figure 1.9: Chemical structure of PEDOT.](image)
1.6.3 Properties of PEDOT

1.6.3.1 Ion exchange

In the oxidised form, PEDOT exhibits one net positive charge over approximately every three monomer units that is balanced by a negatively charged molecule referred to as counter-ion or dopant (Figure 1.10) [25]. This negatively charged dopant molecule can be expelled out of the polymer structure when the polymer is reduced due to the overall negative charge and attracted back into the polymer when it is oxidised due to the positive charge of the polymer backbone (Figure 1.10). Some dopant molecules may move freely between the polymer structure, and the solution (mobile counter-ions), whereas other dopant molecules once incorporated into the polymer during the polymerisation do not move out of the polymer, even when the CP is reduced (immobile counter-ions) [167]. The movement of the counter-ions (primary dopant) incorporated into the CP structure during polymerisation and the anions (secondary dopant) present in the electrolyte solution depends on the molecular weight and size of the counter-ion as well as the anion [168]. The exchange of the dopant (counter-ion) with another anion present in the solution can happen in the presence and in the absence of an electrical stimulus as demonstrated by Dobbelin et al. [169]. Ion exchange has also been explored for using PEDOT as an ion sensor, with potential applications at the interface of ionic and electric signals (as in bioelectrodes) [166, 170]. Ion exchange also offers the potential to explore PEDOT for use as an ion-exchange resin for drug delivery. Ion exchange resins have been employed for the delivery of drugs that are ionic and usually have shorter half-lives [171]. Negatively charged drugs can thus be loaded via ion exchange, where the application of a suitable electrical stimulus would release the loaded drug.

![Figure 1.10: Mechanism of ion-exchange from PEDOT, the dopant is expelled out of the polymer during reduction and attracted back into the polymer during oxidation due to electrostatic interactions.](image)

1.6.3.2 Actuation

The phenomenon of actuation due to reduction and oxidation has been extensively explored in CP including PEDOT. It has been described as the function of ions either leaving or entering into the polymer structure that leads to volume changes [172]. For relatively mobile dopants, when the dopant molecules leave the polymer during reduction it causes the polymer structure to shrink. Continued
reduction of the CP leads to swelling, as the cations present in the solution are attracted to the CP structure to balance the charge. On the other hand, when CP oxidise these cations are expelled out, leading to a decrease in volume before the oxidation reaches a level where counter-ions are attracted back into the polymer structure, leading to an increase in the overall volume [173, 174]. The mobility of the counter-ions incorporated in the CP structure as well as the mobility of the cations present in the electrolyte solution hereby have a significant effect on the actuation. Counter-ions that are more mobile within the polymer and the electrolyte solution tend to produce less reproducible actuation, as they may be replaced by other anions in the electrolyte solution. On the other hand, large immobile counter-ions incorporated into the CP structure are not expelled during reduction; therefore, the volume change depends solely on the movement of cations present in the solution. Similarly, larger cations present in the solution are difficult to incorporate into the CP structure during reduction. Hence they do not result in reproducible actuation, whereas small cations in the solution provide better reproducibility [174, 175]. Overall, the exchange of anions and cations between the polymer and the surrounding solution is a competitive process, which in addition to mobility is also influenced by the concentrations of these anions and cations. PEDOT-based drug delivery systems have previously been explored where an applied stimulus produces mechanical force through actuation, thus pushing the drug out to achieve stimuli-responsive release [176].

1.6.3.3 Conductivity and stability
PEDOT is a conductive polymer due to the availability of uninterrupted π electrons in the backbone of this conjugated polymer. It is often used in combination with a dopant called polystyrene sulfonate (PSS); however, this combination decreases the conductivity of PEDOT when compared to PEDOT produced by other dopants [177]. It has been learned that different solvents may also affect the conductivity of PEDOT and the introduction of a secondary dopant can increase the conductivity and stability of this polymer [178, 179]. PEDOT polymerised with electrochemical polymerisation (ECP) has been reported to have conductivities of up to 10 S/cm at room temperature while PEDOT polymerised with vapour-phase polymerisation (VPP) has shown conductivities above 10^3 S/cm under the same conditions [180, 181]. This could be attributed to the highly ordered structure of PEDOT polymer produced via VPP when compared to ECP [180]. PEDOT is considered more stable and robust when compared to other CP as demonstrated by Che et al. [182], who observed a minimal charge capacitance loss within the first 12 days of soaking PEDOT in glutathione solution to analyse the effect of biological reducing agents on long-term reproducible in-vivo electro-activity. In addition, PEDOT has also demonstrated superior long-term electrochemical reproducibility when compared via CV. Venkatraman et al. [183] evaluated PEDOT-coated electrodes for long-term in-vitro and in-vivo stimulation. They found that PEDOT-coated electrodes offered less impedance and higher response against the stimulus when compared to bare Platinum-Iridium electrodes. PEDOT-coated electrodes also exhibited fewer variations in the CV, impedance and charge storage capacity before and after the in-vitro and in-vivo analysis in comparison to the bare Platinum-Iridium electrodes.
1.6.3.4 Biocompatibility

CP have been shown to be biocompatible as they lack any immunogenic reaction and cytotoxicity, both pre-requisites for biomaterials [184]. Due to the superior electroactive properties and stability of PEDOT, there is great interest in using this polymer for various long-term biomedical applications, including drug delivery. Therefore, the biocompatibility of PEDOT fabricated through various polymerisation techniques has already been established in the literature [185-187]. Furthermore, the effect of PEDOT doped with biological dopants such as heparin or nerve growth factor has been investigated, revealing that PEDOT retains good electro-activity and reproducible electrochemical properties without any toxic effects, thus making it an ideal electro-active polymer for biomedical use [188-190].

1.6.4 PEDOT in drug delivery

The potential of PEDOT to be used in DDS has been investigated for over a decade [191]. Previous studies have looked at PEDOT in conjunction with other drug delivery materials to develop electrically responsive DDS in order to achieve higher levels of drug loading. One of the early reports to successfully achieve long-term electrically responsive drug delivery from PEDOT was published by Abidian et al. [176], who were able to demonstrate an increase in the amount of drug released from PEDOT-coated PLGA nanotubes in response to an electrical stimulus for up to two months. Dex-loaded PLGA nanotubes were coated with PEDOT and drug release was observed in the absence and presence of an electrical stimulus (Figure 1.11). Minimal passive drug release was observed in the absence of the electrical stimulus, while a surge in drug release was observed when an electrical stimulus was applied. When compared to PLGA-only nanotubes loaded with dex, the rate of drug release was more controlled and was tunable by the applied stimulus. The main mechanism involved in the release of dex in response to the electrical stimulus was hypothesised to be the actuation of PEDOT during the redox reaction; this would push the drug out of the PLGA nanotubes [176]. However, application of redox stimulus has also been associated with the formation of cracks in PEDOT coatings; therefore, these cracks could also contribute to release of more drug into the release medium [192].
Doping is another approach that has been explored to load anionic drugs into CP [193]. As described previously in Section 1.3, dexP is a potent corticosteroid that requires comparatively small amounts of drug to exert its action. It has been used several times as a dopant in CP systems. Xiao et al. [194] reported on carbon nanotubes immobilised on gold electrodes with PEDOT polymerised on the surface using dexP as a dopant via ECP. Carbon nanotubes were used as a template to enhance the surface morphology of PEDOT in order to achieve a higher surface area and thus lower resistance. DexP was released by cyclic voltammetry (CV) in phosphate buffered saline (PBS) solution. When the polymer was reduced, the dopant was expelled due to the overall negative charge, and drug release continued until equilibrium was reached between loaded drug and the anionic component in the release medium (phosphate). The release rate of drug loaded as a dopant into the polymer backbone was also investigated in this study, indicating that there is an initial burst release, and the release rate later decreases as more drug has moved out into the release medium. Drug loading and release via this method rely solely on the electrostatic interaction between the anionic drug and the polymer and may offer better control over spontaneous release as the drug is strongly bound to the polymer backbone.

Manipulation of the surface morphology to increase drug loading and to enhance electrochemical properties of CP has also been investigated. High surface area structures offer increased exchange of anions between the CP structure and the surrounding solution [195]. This phenomenon has been
Introduction

applied to fabricate CP structures with higher sensitivity, which can be utilised to achieve higher drug loading via ion-exchange in the case of anionic drugs. Highly porous CP structures have also been used as drug reservoirs, with drug being released in a stimuli-responsive fashion after sealing of the pores [152]. However, these strategies have not yet been explored to fabricate porous PEDOT structures for ion-exchange in the case of an anionic drug or for the purpose of physical entrapment within the pores; therefore, these strategies could be trialled for PEDOT. Furthermore, CP membranes also offer a high surface area, and these membranes have previously been explored as a switchable device to control drug delivery in response to an electrical stimulus [196]. The ionic permeation across a CP membrane shifts when the redox state is changed, making it possible to control the permeation in response to an external stimulus [197]. Previous studies have revealed that the diffusion rate across a PEDOT membrane may vary up to four folds depending upon the redox state of the polymer [198]. To achieve stimuli-specific release from such PEDOT membranes, they need to be developed in a way that achieves a notable increase in drug release upon the application of a suitable electrical stimulus.

1.7 Aim and hypothesis of this PhD thesis

Unmet drug delivery needs for the treatment of chronic back-of-the-eye conditions have partially been addressed with the development of sustained drug release ocular implants. However, such ocular implants that are either already available on the market, or are currently in the development phase, release drug at a pre-determined rate. Furthermore, the amount of drug required for the effective treatment of the disease or the severity of the adverse effects may differ from patient to patient and over time. Therefore, individualisation of the dose may be required for each patient depending on the disease progression and the adverse effects. Stimuli-responsive implants can provide this dose individualisation to achieve specific therapeutic outcomes among individual patients.

PEDOT is a stable and biocompatible CP, which has been explored for use in drug delivery devices to achieve stimuli-responsive drug delivery. Dex is a potent corticosteroid that is widely used either alone or in combination with other anti-inflammatory drugs for the treatment of various back-of-the-eye conditions. Ozurdex, currently the only available dex-containing implant on the market, is biodegradable, meaning that drug release from this implant may vary over time depending on the rate of degradation. Moreover, it has only shown efficacy for up to six months, thus developing an implant that can hold larger amounts of drug and provide better control over release would be of advantage. In addition, efficacy and implant lifetime could also be enhanced by loading more than one drug into the implant. Thus, a CP-based implant loaded with more than one drug would be an ideal candidate for on-demand ocular drug delivery to treat chronic posterior eye conditions.

The overall aim of this PhD is to fabricate a PEDOT-based stimuli-responsive DDS which could be further developed as an implant to provide on-demand drug delivery to the back-of-the-eye. DexP will be explored as a hydrophilic drug that can be loaded into the polymer structure as a dopant during polymerisation or via ion-exchange after polymerisation. On the other hand, dex will be investigated as a hydrophobic drug that can be physically entrapped into the porous polymer voids. The release of these drugs in response to a suitable electrical stimulus will then be explored, where dexP is released
mainly as a result of ion-exchange and dex due to actuation as well as permeation through the PEDOT structure. In order to determine the permeability of the loaded drugs through the PEDOT sealing layers, PEDOT-coated cellulose membranes will also be fabricated and characterised.

The overall hypothesis being tested in this project is that a PEDOT based ocular implant could enable stimuli-responsive release of dex and/or dexP into the eye, ideally for up to six months. It is suggested that template-based highly porous PEDOT structure would allow a higher surface area for drug loading [152], where dexP (anionic drug) could be loaded as a dopant onto the positively charged polymer backbone and dex (neutral drug) could be physically entrapped into the created pores. In theory, sealing of the drug loaded porous PEDOT structure would restrict unwanted drug leakage. It is assumed that drug release from the implant would be higher in the presence of a stimulus due to oxidation of the polymer and pore wall swelling/polymer actuation. To avoid any tissue interaction, the final structure would then be encased into a biocompatible implant casing which would support the functioning of the implant assembly and avoid any influence over the proposed stimuli-responsive release. On the basis of overall aim and hypothesis, specific objectives of this PhD project covered in the upcoming chapters are as follows:

- **Chapter 2:** To develop a stability indicating HPLC method for the simultaneous detection and quantification of dex and dexP from *in-vitro* medium and vitreous humour.

- **Chapter 3:** To fabricate and characterise PEDOT structures, including PEDOT fabrication via different polymerisation techniques, template-based porous PEDOT fabrication and the fabrication of PEDOT-coated cellulose membranes.

- **Chapter 4:** To load dexP via ion-exchange and observe *in-vitro* release from porous and non-porous PEDOT structures. To load dexP via physical entrapment and observe *in-vitro* release from the pores of porous PEDOT structures. Furthermore, to analyse PEDOT-coated cellulose membranes for their permeability.

- **Chapter 5:** To design, fabricate and characterise a prototype implant for *in-vitro* drug release.

- **Chapter 6:** To provide overall summary of this thesis, future directions and a final thesis conclusion.
CHAPTER 2: ANALYTICAL METHOD DEVELOPMENT

2.1 Introduction

Dexamethasone (dex) and its derivative dexamethasone sodium phosphate (dexP) are used for the treatment of chronic back-of-the-eye conditions, as discussed in Section 1.3. For the purpose of developing an ocular implant, combining a hydrophobic drug (dex) and a hydrophilic drug (dexP) may provide a more versatile and flexible release profile and thus superior therapeutic outcomes. For example, in the case of biodegradable implants containing only a hydrophobic drug, sufficient drug amounts may not be released until the implant starts degrading, at which point the amount being released may exceed the required therapeutic concentration, leading to excessive drug dumping and potential adverse effects [51]. Therefore, adding a hydrophilic drug may improve the release profile overall by providing an initial drug burst to rapidly achieve effective therapeutic concentrations, while in the later stages the hydrophobic drug would provide a sustained prolonged effect by dissolving slowly in the body fluids [199, 200].

In order to effectively treat chronic back-of-the-eye conditions using sustained release implants, the ideal duration of action should be from six months at least up to several years to offer a superior alternative to intravitreal injections [87]. However, in the case of such implants and other long-term drug delivery devices, the loaded drug may chemically degrade—resulting in lower efficacy and concerns over safety during the course of therapy. Analytical methods used to quantify drug concentrations after prolonged periods of release should therefore be able to separate and quantify the intact drug from any degradation products, as well as from excipients and any other interferences such as tissue components. Therefore, to quantify the amount of drug being released over prolonged periods of time, a stability indicating high-performance liquid chromatography (HPLC) method is required [201]. Regulatory guidelines suggest exposing the drug to thermal hydrolysis, acidic hydrolysis, basic hydrolysis and oxidative hydrolysis in order to produce the possible degradation products and develop a stability indicating HPLC method [202].

Dex and dexP have previously been separated and quantified using HPLC in various ophthalmic formulations [203]. However, separate HPLC methods have previously been developed for the purpose of extraction and quantification from the vitreous humour [204]. A single combined stability indicating HPLC method to simultaneously extract, quantify and separate both of these drugs from each other, as well as the degraded products and other components, has not previously been reported. Therefore, this part of the study investigated a stability indicating HPLC method for the simultaneous detection and quantification of dex and dexP from synthetic medium and vitreous media. The method was adopted from a previous study [203]; however, an increase in peak resolution was desired to obtain a
Analytical method development

stability indicating HPLC method which would allow simultaneous quantification of small drug amounts in biological fluids.

2.2 Aim and objectives

The overall aim of the work in this chapter was to optimise various parameters to develop a stability indicating HPLC method for separation and simultaneous quantification of dex and dexP from in-vitro release medium and bovine vitreous humour. Specific objectives were:

- To separate dex and dexP (with high peak resolution) by optimising chromatographic conditions, followed by method validation to obtain acceptable linearity, precision and accuracy.
- To separate EDOT from dex and dexP in order to avoid any interference with the drug release samples in case of unreacted EDOT monomers present in the polymer.
- To separate degradation products following forced degradation of dex and dexP.
- To recover and simultaneously quantify dex and dexP from the vitreous humour.

2.3 Materials

DexP was purchased from Jai Radhe Sales (India). Dex was purchased from CFM Oskar Troitzsch (Germany). Methanol was purchased from Scharlau Chemicals (Spain). All other chemicals were obtained from Sigma-Aldrich (Australia) and used without any further purification. Analysis was done using an Agilent (USA) 1200 Series HPLC equipped with a quaternary pump, an autosampler and a DAD detector. HPLC data was collected and analysed by ChemStation® Software, Agilent Inc. (USA).

2.4 Methods

2.4.1 Mobile phase

The initial mobile phase composition was obtained from a previous study that simultaneously separated and quantified dex, dexP and chloramphenicol from combination ophthalmic products, comprising 44.4% buffer (48 mmol sodium dihydrogen phosphate, pH: 5.4), 29.8% acetonitrile and 25.7% methanol [203]. However, using this composition for analysing dex and dexP samples resulted in poor peak resolution (R= 0.34). Various buffer strengths were therefore trialled, with and without adjusting the pH, to separate dex (pKa=12.14) and dexP (pKa=6.8). The composition was optimised to
50% buffer (24 mmol sodium dihydrogen phosphate, pH: 4.9 not adjusted), 27.3% acetonitrile and 22.7% methanol.

2.4.2 Standard solution

A solvent mixture of water:acetonitrile:methanol (50:27.3:22.7) was prepared to solubilise both drugs. Dex and dexP (100 mg of each) were separately dissolved in a 100 mL of this mixture to obtain a concentration of 1 mg/mL (standard solution). The same solvent mixture was then used to further dilute the standard solution to obtain working concentrations in the range of 0.5 µg/mL to 100 µg/mL. Lower limit of quantification (LLOQ) and limit of detection (LOD) were determined by further diluting the working concentrations in the range of 50 ng/mL to 500 ng/mL. LOD was determined by the drug concentration where the signal to noise ratio was greater than 3, whereas LOQ represented the concentration where the signal to noise ratio was greater than 10.

2.4.3 Chromatographic conditions

A Kinetix series C18 column was used (dimensions: 250 x 4 mm) packed with silica (average particle size 5 Å) (Phenomenix, USA). According to a previous study an injection volume of 10 µL provided a good peak area and resolution [203]. Similarly, in the previously reported method the flow rate was 0.5 mL/min, which resulted in dexP eluting around 6 min and dex at around 11 min. Therefore, in order to decrease these retention times, the flow rate was slightly increased from 0.5 mL/min to 0.6 mL/min. However, this resulted in dexP eluting at around 3 min and dex at around 7 min, with the solvent front eluting at around 2.5 min. To move dexP slightly away from the solvent front, the column temperature, previously reported to be 40°C, was reduced to 30°C. The wavelength for the detection was 254 nm, which was chosen as advised in the United States Pharmacopeia 2002 (USP 25) for both drugs [205]. Interestingly, the maximum absorption ($\lambda_{\text{max}}$) for dex and dexP is 240 nm; therefore, this wavelength was also trialled. However, superior peak purity was obtained at 254 nm, which could be due to less interference at higher wavelength when compared to 240 nm.

2.4.4 Separation of EDOT

One recent report investigated various analytical methods for quantifying the amount of dexP released from PEDOT [206]. It showed that non-polymerised EDOT monomer entrapped within the polymer during ECP can also be released with the dopant when a stimulus is applied and has the same wavelength of absorption under ultra-violet (UV) radiations. This may interfere with and mislead quantification of the amount of drug released. Therefore, EDOT (10 µg/mL) was injected alone, as well as with dex (10 µg/mL) and dexP (10 µg/mL), to observe any interference of the EDOT peak with the dex and dexP peaks.
2.4.5 Linearity
Triplicate dilutions were performed to get standard working solutions in the range of 0.5-100 µg/mL for dex and dexP. A calibration curve was plotted (using average values ± SD) with the concentration on the x-axis and the absorbance of the peak on the y-axis.

2.4.6 Precision and accuracy
Dex and dexP intra-day precision was determined by injecting triplicates of five different concentrations (5, 10, 25, 50 and 100 µg/mL) during a day and then comparing %RSD. Similarly, inter-day precision was also measured by injecting triplicates of five different concentrations over three consecutive days and comparing the %RSD. Accuracy was determined by spiking known concentrations of both drugs, prepared by diluting the stock solution with the release medium, and comparing them with the results obtained.

2.4.7 Forced degradation
Forced degradation was performed by separately exposing dex and dexP solutions to hydrolytic, oxidative and temperature stressors. These conditions were selected to replicate the same stressors these drugs could be exposed to during implant development as well as during in-vivo studies. Triplicates for each drug solution for testing under each stress condition were prepared by taking 200 µL of stock solution (100 µg/mL) and adding 200 µL of acidic, basic or oxidative stock solution. MilliQ water was added to make up the volume to 2 mL with a final concentration of 10 µg/mL. For acidic hydrolysis, a standard working solution of HCl (1 N) was added to the dex and dexP samples to obtain a concentration of 0.1 N HCl. Similarly, a standard working solution of NaOH (1 N) was added to the dex and dexP samples to obtain a concentration of 0.1 N NaOH for basic hydrolysis. For oxidative hydrolysis, a standard working solution of H₂O₂ (3% w/v) was added to the dex and dexP samples to obtain a concentration of 0.3% H₂O₂. Two sets of triplicate samples were prepared for each condition with one set kept at room temperature while the other set was kept at 60°C to accelerate the degradation process. In order to observe the effect of elevated temperature on hydrolysis, dex and dexP samples were prepared from the standard working solutions by dilution with the mobile phase to obtain a concentration of 10 µg/mL and were kept at 60°C to observe any drug degradation.

Samples were collected at 1, 2, 3, 4, 24, 72 and 120 h, centrifuged and the supernatant analysed (10 µL injection volume). Blanks for HCl, NaOH, H₂O₂ and the diluent (MilliQ water) were also injected to identify any interference with the dex or dexP chromatogram. Peak purity was measured by the ChemStation® Software to account for any interference of the degradation product peaks with the pure drug peaks.
2.4.8 Recovery and quantification from vitreous humour

Stock solutions of 500 µg/mL of both drugs were diluted in triplicate with bovine vitreous humour to obtain concentrations of 50, 25, 12.5, 6.25 and 3.125 µg/mL. A volume of 200 µL from each of the triplicate samples was further diluted with 500 µL of acetonitrile (to precipitate vitreous proteins) and 300 µL of MilliQ water, centrifuged at 13,400 rpm for 10 min at 20°C and the supernatant was analysed (injection volume 10 µL). Standard working solutions were prepared for the exact same concentrations by diluting the stock solutions (500 µg/mL) in triplicate with MilliQ water; further dilution for these samples was done by adding in the exact same ratio of acetonitrile and MilliQ water.

2.5 Results and discussion

The optimised composition of the mobile phase resulted in sharp symmetrical peak shapes for dexP (eluted at 4.8 min) and dex (eluted at 8.7 min). The resulting peak resolution increased from 12.5 (as reported in the previous study [203]) to 26.4. This resulted in both drug peaks remaining pure when injected together, which revealed a good separation and absence of any interference between these drugs (Figure 2.1). Peak purity was determined via ChemStation® Software by comparing 5 UV spectra across the dex and dexP peaks. Two spectra were taken before the apex of the peak, one spectrum at the apex and two spectra after the peak apex; if all spectra matched the dex or dexP spectrum, as determined by the ChemStation® Software, this would indicate that the peak was pure [207]. The UV scans have been inserted in all of the figures displaying peak purity. There was a noticeable difference in the retention time between dex and dexP peaks, which provided with a good peak resolution. When compared to the peak resolution obtained by replicating the previously reported method, there was a ten-fold increase in peak resolution. Therefore, further method validation was carried out. The LLOQ was determined to be 250 ng/mL for both drugs and LOD was determined to be 75 ng/mL.
Figure 2.1: Chromatograms of a) dexP injected alone, b) dex injected alone and c) both dex and dexP injected together. Dex and dexP peaks were pure, as displayed by the UV scans inserts.

2.5.1 Separation of EDOT from dex and dexP

PEDOT is a polymer that consists of EDOT monomers and may contain unreacted EDOT monomers. These unreacted EDOT monomers could be released upon the application of an electrical stimulus, as observed by Boehler et al. [206]. Therefore, the HPLC method should be able to separate the EDOT peak from the dex and dexP peaks, in order to avoid any interference. To assess the HPLC method for such a purpose, EDOT spiked samples were analysed; however, they did not show any interference between the dex and dexP peaks and the EDOT peak (eluting after 7.3 min), as displayed in Figure 2.2. This indicated that the method developed here was capable of separating the EDOT monomer from the released drugs to avoid any error in the quantification of drug concentrations from the release samples. Dex and dexP peaks were pure when injected with EDOT (Figure 2.2). EDOT peak was also pure when injected alone or with these drugs.
Analytical method development

2.5.2 Linearity

Linearity is the measure of response over various concentrations against the area recorded, with the variation in concentration expected to produce a proportional increase or decrease in the area. Linearity is usually measured by injecting different concentrations of the active, plotting the area against each concentration injected and then measuring the goodness-of-fit ($R^2$). Triplicates of seven different concentrations (range: 0.5-100 µg/mL) of dex and dexP were injected, and the goodness-of-fit was calculated to be 0.9998 for dexP and 0.9999 for dex (Figure 2.3).
Figure 2.3: The linearity of dex and dexP (values represent the average ± SD, n=3), an acceptable goodness-of-fit was calculated for both drugs.

### 2.5.3 Precision and accuracy

Precision is a measure of the repeatability of an analytical method. It is measured by repeatedly injecting a range of concentrations over a short period of time (usually within a day) and calculating the %RSD (relative standard deviation) in the peak area which is called intra-day precision. Inter-day precision is measured in the same way, except the analysis is done over a number of days and the %RSD values are compared. Table 2.1 and Table 2.2 display that the %RSD for all intra- and inter-day precision was well within the acceptable limit (2%) for both dex and dexP.

![Graph showing linearity of dex and dexP](image)

The accuracy of the analytical method can be defined as the closeness of the test results to the true values. It is determined by spiking the samples with known concentrations of active and analysing it. The results obtained here revealed that the accuracy of this method was within the acceptable limit of 100 ± 5%, as displayed in Table 2.1 and Table 2.2 for dex and dexP respectively.
Table 2.1: Precision and accuracy calculated for dex (n=3).

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intra-day</th>
<th></th>
<th></th>
<th>Inter-day</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average accuracy (%)</td>
<td>Precision (%RSD)</td>
<td>Average accuracy (%)</td>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>95.4</td>
<td>0.2</td>
<td>95.7</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>95.9</td>
<td>0.1</td>
<td>96.5</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>97.2</td>
<td>0.1</td>
<td>96.6</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>101.6</td>
<td>0.4</td>
<td>100.8</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>98.3</td>
<td>0.2</td>
<td>96.9</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2: Precision and accuracy calculated for dexP (n=3).

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intra-day</th>
<th></th>
<th></th>
<th>Inter-day</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average accuracy (%)</td>
<td>Precision (%RSD)</td>
<td>Average accuracy (%)</td>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>96.4</td>
<td>0.6</td>
<td>95.7</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>96.5</td>
<td>0.8</td>
<td>96</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>99.6</td>
<td>0.1</td>
<td>99.8</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>102.5</td>
<td>0.2</td>
<td>102.3</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>97.4</td>
<td>0.1</td>
<td>97.8</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.5.4 Forced degradation

Both dex and dexP were exposed to acidic, basic and oxidative stress at room temperature as well as an elevated temperature. Results revealed that there was no interference from the degraded products with the dex and dexP peaks (Figure 2.4, Figure 2.6, Figure 2.5 and Figure 2.7). Table 2.3 lists the degree of degradation under various stress conditions for both dex and dexP; it is obvious from the data that dex is much more stable under hydrolysis at high temperature than dexP. However; dex is more likely to be degraded via basic hydrolysis, where ~93% degradation was observed after 1 h in 0.1 N NaOH solution at 60°C. On the other hand, dexP was found to be more stable under acidic
hydrolysis, even at elevated temperature (60°C); nevertheless, it was more likely to be degraded via oxidative hydrolysis when compared to dex.

Table 2.3: Forced degradation of dex and dexP under various stress conditions (values represent the average ± SD, n=3).

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Time (h)</th>
<th>Dex (% remaining)</th>
<th>Time (h)</th>
<th>DexP (% remaining)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (60°C)</td>
<td>120</td>
<td>95.9 ± 0.2</td>
<td>120</td>
<td>43.5 ± 0.4</td>
</tr>
<tr>
<td>0.1 N NaOH (25°C)</td>
<td>1</td>
<td>22.2 ± 2.5</td>
<td>120</td>
<td>57.6 ± 0.3</td>
</tr>
<tr>
<td>0.1 N NaOH (60°C)</td>
<td>1</td>
<td>7.3 ± 1.9</td>
<td>72</td>
<td>27.8 ± 0.5</td>
</tr>
<tr>
<td>0.1 N HCl (25°C)</td>
<td>120</td>
<td>97.8 ± 0.2</td>
<td>120</td>
<td>95.0 ± 0.6</td>
</tr>
<tr>
<td>0.1 N HCl (60°C)</td>
<td>120</td>
<td>66.7 ± 1.1</td>
<td>120</td>
<td>78.0 ± 2.2</td>
</tr>
<tr>
<td>0.3% H₂O₂ (25°C)</td>
<td>120</td>
<td>93.9 ± 0.8</td>
<td>120</td>
<td>90.5 ± 1.2</td>
</tr>
<tr>
<td>0.3% H₂O₂ (60°C)</td>
<td>120</td>
<td>96.8 ± 0.5</td>
<td>120</td>
<td>75.0 ± 2.3</td>
</tr>
</tbody>
</table>

2.5.4.1 Temperature stress

Exposure to elevated temperatures increases the rate of reaction for chemical substances; thus, elevated temperature degradation is used to predict the shelf-life of drugs under various storage conditions [208]. Exposure to a high temperature resulted in only a slight degradation of hydrophobic dex, whereas higher degradation was observed for hydrophilic dexP. When kept at 60°C, there was almost 96% dex remaining; however, only 43.5% of dexP was detected under the same conditions after 120 h. This suggests that dex is more resistant to degradation by hydrolysis than dexP, with the same observation reported by previous studies [209, 210]. No significant degradation product peaks were observed for dexP, which suggests that the dexP degraded products didn’t absorb at the selected wavelength here (λ= 254 nm). On the other hand, a degradation product peak was detected at 3.4 min for dex (Figure 2.4). Dex has been reported to degrade into dex-21-oic-acid under heat, with this degradation product eluting at almost half the retention time of dex; thus being responsible for the peak at 3.5 min in Figure 2.4a [209].
Figure 2.4: Chromatograms of temperature-stressed samples a) Dex and b) dexP and c) the blank MilliQ water (diluent), kept at 60°C. Dex and dexP peaks were pure, as displayed by the UV scans inserts.

2.5.4.2 Basic stress

Basic stress to dex and dexP showed that these drugs are rather unstable in alkaline conditions. Both drugs showed high susceptibility to degradation in 0.1 N NaOH at room temperature, which escalated with an increase in temperature. Dex degraded almost fully within 1 h at 60°C in 0.1 N NaOH solution (only 7.3% remaining), whereas there was still about 70% dexP detected after 120 h under the same conditions. At room temperature, the rate of degradation was relatively slow but still only 22% of dex was detected after 1 h compared to 57% of dexP detected after 120 h. Degradation products were detected at 4.6 and 4.9 min for dex and at 4.1 min for dexP (Figure 2.5). Dex has been reported to degrade into dex-enol-aldehyde under alkaline conditions, which has two isomeric forms [211]. Both isomers elute close to each other and may correspond to the peaks at 4.6 and 4.9 min. DexP has been reported to degrade into 6-β-hydroxydexamethasone phosphate by the introduction of the OH group at position 6, eluting at 4.1 min [210]. In general, the phosphate group in dexP is responsible for protecting dexP from degrading into dex-enol-aldehyde, whereas dex is more susceptible to alkaline degradation.
2.5.4.3 Acidic stress

Acidic stress to dex and dexP revealed that both drugs are quite stable under acidic conditions at room temperature; however, degradation was observed at an elevated temperature (60°C). At room temperature 98% of dex and 95% of dexP was detected after 120 h, proving that these drugs are resistant to acidic degradation. However, at the elevated temperature, only 66.7% of dex and 78% of dexP were detected after 120 h. For dex, degradation products were detected at 10.5 min, while no significant degradation product peaks were observed for dexP (Figure 2.6). A comparison with the chromatogram of blank 0.1 N HCl showed that an HCl peak eluting at 4.8 min; however, no interference was detected with the peak purity of dexP, possibly due to the lower intensity of this peak. Dex has been reported to lose the OH group at position 17 with the resulting degradation product eluting at 1.2 times the retention time of dex, which may correspond to the degradation product eluting at 10.5 min here [212].
Figure 2.6: Chromatograms of acid-stressed samples a) Dex and b) dexP and c) blank 0.1 N HCl, kept at 60°C. Dex and dexP peaks were pure, as displayed by the UV scans inserts.

2.5.4.4 Oxidative stress

Oxidative stress studies concluded that dex and dexP both are quite stable under oxidative degradation, with 94% of dex and 91% of dexP remaining after 120 h at room temperature. The increase in temperature did not seem to affect the oxidative degradation of dex with 97% still detected after 120 h at 60°C in 0.3% H₂O₂ solution. However, only 75% of dexP was still detected after 120 h of oxidative stress under elevated temperature (60°C). This suggests that dexP is relatively unstable under oxidative stress when compared to dex. No degradation product peaks were observed for dex suggesting that the degraded products didn’t absorb at the selected wavelength here (λ= 254 nm). However, an additional peak was observed at 8.7 min for dexP, which indicated that dexP was degrading into dex as a result of oxidative hydrolysis (Figure 2.7). This confirmed that dexP is more susceptible to oxidative stress than dex.
2.5.4.5 Rate of degradation

The drug degradation first-order rate constants, $-k_{obs}$, were calculated for dex and dexP under each stress condition at room temperature and at an elevated temperature, from the slope of the semi-logarithmic graph of the fraction of drug remaining versus time, as described by the following equation [213]:

$$\ln \left( \frac{c_t}{c_0} \right) = -k_{obs} t$$

Where $c_t$ is the concentration of the drug (dex or dexP) remaining at time $t$, and $c_0$ is the initial concentration of the drug (dex or dexP). The degradation rate constant provides information about the stability of these drugs (dex and dexP) as well as the shelf-life under each condition. Figure 2.8 and Figure 2.9 show the degradation profiles of dex and dexP respectively. Table 2.4 lists the degradation rate constants ($k_{obs}$) calculated for the degradation of dex and dexP, as well as the goodness-of-fit for the first-order degradation reactions. However, less than five data points were available for the basic degradation of dex kept at 25°C and 60°C (Figure 2.8); therefore, degradation rate constants were not calculated for these profiles.
Figure 2.8: First-order plots illustrating the degradation of dex under different stress conditions (values represent the average ± SD, n=3).

Figure 2.9: First-order plots illustrating the degradation of dexP under different stress conditions (values represent the average ± SD, n=3).
Table 2.4: First-order degradation rate constants ($k_{obs}$) and goodness-of-fit ($R^2$) calculated for the degradation of dex and dexP under various stress conditions (values represent the average ± SD, n=3).

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Dex</th>
<th>R²</th>
<th>DexP</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (60°C)</td>
<td>1.04 ± 0.05 x 10⁻⁵</td>
<td>0.778</td>
<td>2.92 ± 0.50 x 10⁻⁴</td>
<td>0.997</td>
</tr>
<tr>
<td>0.1 N NaOH (25°C)*</td>
<td>-</td>
<td>-</td>
<td>1.90 ± 0.38 x 10⁻⁴</td>
<td>0.991</td>
</tr>
<tr>
<td>0.1 N NaOH (60°C)*</td>
<td>-</td>
<td>-</td>
<td>1.57 ± 0.13 x 10⁻³</td>
<td>0.867</td>
</tr>
<tr>
<td>0.1 N HCl (25°C)</td>
<td>3.07 ± 0.60 x 10⁻⁶</td>
<td>0.489</td>
<td>1.96 ± 0.39 x 10⁻⁵</td>
<td>0.806</td>
</tr>
<tr>
<td>0.1 N HCl (60°C)</td>
<td>1.40 ± 0.10 x 10⁻⁴</td>
<td>0.997</td>
<td>8.67 ± 0.17 x 10⁻⁵</td>
<td>0.971</td>
</tr>
<tr>
<td>0.3% H₂O₂ (25°C)</td>
<td>1.80 ± 0.60 x 10⁻⁵</td>
<td>0.925</td>
<td>3.02 ± 0.60 x 10⁻⁵</td>
<td>0.876</td>
</tr>
<tr>
<td>0.3% H₂O₂ (60°C)</td>
<td>9.45 ± 0.28 x 10⁻⁶</td>
<td>0.891</td>
<td>9.34 ± 0.45 x 10⁻⁵</td>
<td>0.988</td>
</tr>
</tbody>
</table>

*Less than five data points were available for basic degradation of dex kept at 25 and 60 °C; therefore, degradation rate constants were not calculated for these profiles.

The $R^2$ value shows that the rate of degradation for dex under acidic hydrolysis at 60°C is pseudo-first order ($R^2$ value above 0.95), whereas, it did not follow first-order kinetics under acidic hydrolysis at 25°C. On the other hand, the $R^2$ values for dexP show that the rate of degradation followed first-order kinetics under all conditions; however, a pseudo-first order rate of degradation was observed under acidic hydrolysis (at 25°C), basic hydrolysis (at 60°C), oxidative hydrolysis (at 60°C), as well as for hydrolysis at an elevated temperature (60°C).

2.5.5 Recovery and quantification from vitreous humour

The developed HPLC method was able to separate and quantify both dex and dexP without any interference from the vitreous humour components, although a peak from blank vitreous humour components was observed at 3.3 min (Figure 2.10).
Figure 2.10: Chromatograms of a) blank vitreous humour showing a peak at 3.3 min, b) dex and dexP recovered from the vitreous humour. Dex and dexP peaks were pure, as displayed by the UV scans inserts.

Dex and dexP were extracted and quantified simultaneously from bovine vitreous humour. The area of peak was plotted against the injected concentrations to measure the goodness-of-fit ($R^2$). Triplicates of five different concentrations ranging from 3.125 µg/mL to 50 µg/mL were injected and the goodness-of-fit was found to be 0.9997 for dex and 0.9994 for dexP (Figure 2.11).

Figure 2.11: Linearity of dex and dexP in vitreous humour (values represent the average ± SD, n=3), an acceptable goodness-of-fit was calculated for both drugs.
Table 2.5 describes the accuracy and precision of the developed HPLC method for the extraction of three different concentrations of dex and dexP from the bovine vitreous humour. As per the FDA guidelines with regard to the bioanalytical HPLC method development and validation, a variation of up to 15% is acceptable for accuracy and precision [214]. The values of accuracy and precision displayed in Table 2.5 were within the accepted range.

Table 2.5: Accuracy and precision of dex and dexP extracted from the bovine vitreous humour (Intra-day only; n=3).

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Dex</th>
<th></th>
<th>DexP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Precision (% RSD)</td>
<td>Average</td>
<td>Precision (% RSD)</td>
</tr>
<tr>
<td></td>
<td>accuracy (%)</td>
<td></td>
<td>accuracy (%)</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>112.5</td>
<td>0.65</td>
<td>85.7</td>
<td>2.9</td>
</tr>
<tr>
<td>25</td>
<td>110.4</td>
<td>0.87</td>
<td>88</td>
<td>1.9</td>
</tr>
<tr>
<td>50</td>
<td>106</td>
<td>0.37</td>
<td>92</td>
<td>0.3</td>
</tr>
</tbody>
</table>

2.6 Conclusion

In conclusion, a stability indicating HPLC method was developed that was capable of separating and simultaneously quantifying dex and dexP from the in-vitro release medium and the vitreous humour, with an acceptable goodness-of-fit for both drugs. A separate EDOT monomer peak was observed with no interference with the dex and dexP peaks, thus eliminating any error in the quantification of these drugs in the release medium. The accuracy of this method was within 100% ± 5% and the precision (expressed as %RSD) was below 2%. Forced degradation of dex and dexP revealed that both drugs are stable under oxidative hydrolysis; however, these drugs degraded quickly via basic hydrolysis. Forced degradation also provided degradation kinetics for both drugs, where a first-order rate of degradation was observed under most of the stress conditions. Recovery from the vitreous humour showed that the extraction and simultaneous quantification of dex and dexP within an acceptable variation of ±15% for accuracy and precision and without compromising the peak purity for both drugs was possible by this method. Therefore, this method was deemed suitable for the quantification of dex and dexP from release samples, from the in-vitro release medium, and from bovine vitreous humour.
CHAPTER 3:  FABRICATION AND CHARACTERISATION OF PEDOT STRUCTURES

3.1 Introduction

3.1.1 Fabrication of PEDOT

PEDOT is fabricated by the polymerisation of EDOT monomers (Figure 3.1), achieved through either chemical or electrochemical oxidation these monomers [164]. There are three polymerisation techniques typically employed for the fabrication of PEDOT and other CP. Electrochemical polymerisation (ECP) uses an electrical current as the oxidant for the polymerisation process. On the other hand, chemical polymerisation (CHP) and vapour-phase polymerisation (VPP) utilise a chemical oxidant for the polymerisation process [164]. In VPP, the monomer is converted into the vapour phase either by application of a vacuum, heat, or both, and these monomer vapours are then polymerised into PEDOT once in contact with the oxidant, resulting in solid polymer formation [215]. CHP is a simple technique for the polymerisation of PEDOT where the oxidant solution comes directly in contact with the monomer units to oxidise them and produce the polymer [216]. To fabricate PEDOT for the purpose of biomedical applications, ECP is the most explored polymerisation technique to date [26, 217].

Fabrication of PEDOT via ECP is usually achieved in two different ways, either by application of a potentiostatic or galvanostatic continuous positive potential to oxidise the monomer or via cyclic voltammetry (CV), where a potential is cycled between positive and negative values and the monomer oxidises during the positive sweep [217, 218]. An electrically conductive surface is required to produce PEDOT via this technique, restricting it to conductive substrates only. ECP happens within an electrolyte solution; therefore, the negatively charged molecule (i.e. anionic drug molecule) in the solution is usually incorporated into the polymer as a dopant [206]. Moreover, various dopants and solvents have been reported for the ECP PEDOT for the purpose of drug delivery and other biomedical applications (Table 3.1). However, the polymerisation process in ECP happens in a solution, which can be a disadvantage for the purpose of physically entrapping a drug within the polymer structure as the drug may wash away [219]. PEDOT is insoluble in organic and aqueous solvents, but it is often combined with polystyrene sulfonate (PSS) to improve its solubility [164]. For the purpose of biomedical applications and drug delivery, previous studies have only reported ECP of PEDOT being carried out with small surface area electrodes or using a PEDOT/PSS combination [176]. ECP is a
Fabrication and characterisation of PEDOT structures

good technique for controlling the amount of polymer being produced, as the amount of charge passed during polymerisation is directly proportional to the amount of polymer formed [220]. The possibility of fabricating templated-based porous PEDOT using dexP as a dopant will be explored in this chapter with the possibility of achieving higher drug loading than for non-porous structures.

VPP occurs within either a vacuum chamber or a container where pressure, heat and various other parameters such as the humidity are well-controlled. It is a dry process compared to ECP although an oxidant solution is needed for the polymerisation process to occur [221]. Fe$^{3+}$ is the most widely used chemical oxidant for VPP and CHP, usually in the form of iron chloride or iron tosylate. The negatively charged counter-ion (chloride or tosylate) is incorporated into the CP structure as a dopant during polymerisation [215]. The polymer only forms at locations where oxidant solution is available; therefore VPP is a good technique for controlling the shape of PEDOT and can be performed on any surface irrelevant of the conductive nature of that surface [219, 222]. This polymerisation technique has also been explored to print PEDOT, where a desired oxidant pattern is first produced over which PEDOT polymerises [223]. Manipulation of the area has been previously attempted with VPP to achieve a higher surface area; however, there are no previous reports on the fabrication of template-based porous PEDOT via VPP [224-226]. Therefore, the fabrication of template-based porous PEDOT via VPP could be attempted here via selecting a suitable template as well as a suitable process for this purpose.

Figure 3.1: Polymerisation of EDOT to PEDOT; the polymer is formed in the oxidised state and requires negatively charged counter-ions (dopant, indicated by A$^-$) to stabilise the charge.
Fabrication and characterisation of PEDOT structures

Table 3.1: Electrochemical polymerisation of EDOT for drug delivery and biomedical applications.

<table>
<thead>
<tr>
<th>C_{EDOT}</th>
<th>C_{Dopant}</th>
<th>Solvent</th>
<th>Synthesis Conditions</th>
<th>Electrode System</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 M</td>
<td>0.01 M DexP</td>
<td>Aqueous</td>
<td>80 µA/cm² for 20 min</td>
<td>Three WE: Pt</td>
<td>[206]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>(100 mC/cm²)</td>
<td>(Only WE given)</td>
<td></td>
</tr>
<tr>
<td>0.01 M</td>
<td>0.01 M PSS</td>
<td>Aqueous</td>
<td>120 µA/cm² for 20 min</td>
<td>Two WE: Au-Pd</td>
<td>[227]</td>
</tr>
<tr>
<td></td>
<td>1 M PBS</td>
<td>PBS</td>
<td></td>
<td>CE: Pt</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Three WE: silicon probe</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(over Au)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CE: Pt</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RE: Saturated calomel electrode</td>
<td></td>
</tr>
<tr>
<td>0.01 M</td>
<td>0.01 M LiClO₄</td>
<td>Aqueous</td>
<td>160 µA/cm²</td>
<td>Three WE: SnO₂/F</td>
<td>[228]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+0.8 V for 5 min</td>
<td>CE: Pt</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(25 mC/cm²)</td>
<td>RE: Ag/AgCl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-ethyl-3-methylimidazolium bis(perfluoroethylsulfonyl) imide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 M</td>
<td></td>
<td>Aqueous</td>
<td></td>
<td>Three WE: SnO₂/F</td>
<td>[228]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CE: Pt</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RE: Ag/AgCl</td>
<td></td>
</tr>
<tr>
<td>0.06 M</td>
<td>0.1 M KCl</td>
<td>Aqueous</td>
<td>200 µA/cm²</td>
<td>Three WE: Pt</td>
<td>[230]</td>
</tr>
<tr>
<td></td>
<td>0.1 M NaPSS</td>
<td></td>
<td>(0.72 C/cm²)</td>
<td>CE: Pt</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RE: Ag/AgCl</td>
<td></td>
</tr>
<tr>
<td>0.1% w/v</td>
<td>0.2% w/v PSS</td>
<td>Aqueous</td>
<td>5 µA/cm² (0.93 mC)</td>
<td>Two WE: Cochlear implant (Pt wire with Teflon coating)</td>
<td>[231]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CE: Pt wire</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PBS: Phosphate buffer saline, LiClO₄: Lithium per chlorate, NaPSS: Sodium polystyrene sulfonate, WE: Working electrode, CE: Counter electrode, RE: Reference electrode

3.1.2 Characterisation of PEDOT

3.1.2.1 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) is a technique where the sample is exposed to a monoenergetic beam of x-rays in order to displace inner-valence electrons. The kinetic energy of these electrons varies depending on the nature of the substance and the surroundings of the atom. These electrons are recorded by the detector and data is plotted as binding energy versus energy counts.
This plot can be further analysed to determine the chemical composition of the exposed sample [232]. XPS is a semi-qualitative technique that can provide information about the surface chemical composition as well as the nature of interactions among the atoms in the surface of the sample [232, 233]. XPS has been used to analyse the chemical composition of PEDOT and other CP, in order to track any changes in the chemical composition under various physical and redox conditions [234]. Khan et al. [234], reported the treatment of VPP PEDOT doped with tosylate using different pH solutions, where they observed a shift in the tosylate peak after treatment with acidic or basic solution that was due to the exchange of the tosylate with either Cl\textsuperscript{−} or OH\textsuperscript{−} respectively. Therefore, XPS can also be utilised to estimate the level of doping and to observe any changes when PEDOT is doped with tosylate (see Section 4.5.3.2 where such analysis has been performed to identify the exchange of tosylate with dexP).

### 3.1.2.2 Atomic force microscopy

Atomic force microscopy (AFM) is a technique where a sample is imaged with the help of a tiny probe, either via contact, non-contact or tapping mode [235, 236]. In contact mode the tiny probe is continuously in touch with the surface, whereas in the tapping mode the tiny probe regularly touches the surface for short periods of time. On the other hand, the non-contact mode images the sample surface utilising the inter-atomic forces between the tiny probe and the sample surface. The position of the probe is recorded by the angle of deflection of a laser light recorded by a detector, and this data is plotted to image the surface of the sample. Although the lateral resolution of AFM is low (~30 nm) when compared to SEM, the vertical resolution is higher (~0.1 nm) [237]. For PEDOT and other CP, AFM has been extensively used to study the surface morphology under normal conditions, as well as to observe any changes during various redox states [238]. AFM can also be used to characterise the mechanical properties of PEDOT and to evaluate the growth of PEDOT over different templates [239].

### 3.1.2.3 Scanning electron microscopy

Scanning electron microscopy (SEM) is a technique used to image the surface of samples at high resolution, generally ~1.5 nm [240]. To image a sample a beam of electrons is fired at the sample, which excites the atoms at the surface of the sample. While some electrons are reflected, the excited atoms in the sample emit electrons when they go back to the normal state. These reflected and emitted electrons are recorded by detectors, and the recorded data is analysed to plot an image of the surface of the sample [240, 241]. SEM has been used extensively to characterise the surface morphology of PEDOT, especially to observe any variation in the polymerisation parameters [242]. In the case of template-based porous structures, this technique has been employed for extensive morphological characterisation [226].
3.1.2.4 Cyclic Voltammetry

Cyclic voltammetry (CV) is a technique where a material is cycled between a positive (oxidation) and a negative (reduction) potential and the resulting current passed through is recorded. When this current is plotted against the applied voltage, the resulting graph is called a cyclic voltammogram [243]. Various electrically conductive substrates have a characteristic cyclic voltammogram often used to characterise them electrochemically by identifying oxidation and reduction events, which usually indicate the doping stages for a particular compound [244]. A reproducible CV indicates a reversible redox process for a substrate. It can be used to evaluate the stability of a reversible redox process over a number of CV cycles [245]. CV is also employed to determine the electrochemically active surface of a substrate, where a higher area under the curve corresponds to a higher electrochemically active surface area and vice versa [246]. PEDOT cyclic voltammograms usually show multiple oxidation and reduction peaks, which can be explained by the different doping states of this polymer [244].

3.1.2.5 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is a technique used to measure the dielectric properties of a material as a function of frequency [247]. Impedance is defined as the resistance offered to an alternating current (AC), which depends upon the resistance as well as the capacitance and inductance arising from AC [247]. Depending upon the frequency of AC, impedance changes have been used to analyse the energy storage and dissipation properties of a sample over a range of frequencies. An impedance spectrogram is a graph of frequency versus impedance, which may differ if the composition, surface morphology and area of the sample is changed [248]. Therefore, impedance has been employed to detect the presence of impurities such as corrosion in metals and for label-free detection of bacteria in biosensors [249-251]. When using a CP e.g. PEDOT in implantable devices impedance can be used to characterise the electrochemical properties in order to obtain the power requirements for such devices [252].

3.2 Aim and objectives

The aim of the work presented in this chapter was to fabricate and characterise non-porous (plain) PEDOT, template-based porous PEDOT and PEDOT-coated cellulose membranes. Specific objectives were:

- To explore ECP and VPP for the fabrication of non-porous (plain) and template-based porous PEDOT.

- To characterise the surface morphology, electrochemical behaviour and biocompatibility of fabricated non-porous (plain) and template-based porous PEDOT.
• To fabricate PEDOT-coated cellulose membranes.

• To characterise the surface morphology, *in-situ* electrochemical behaviour and biocompatibility of fabricated PEDOT-coated cellulose membranes.

### 3.3 Materials

Water was obtained from a MilliQ ultra-pure water-producing system by reverse osmosis (Millipore, USA). EDOT monomer, lithium perchlorate, N,N-dimethylformamide (DMF), phosphate buffer saline (PBS), PEG-PPG-PEG (mw: 5800 Da) and dialysis cellulose tubing (MECO: 14000 Da) were purchased from Sigma-Aldrich (USA). Dexamethasone phosphate (dexP) was purchased from Jai Radhe Sales (India). Methanol was obtained from Scharlau Chemicals (Spain) and acetonitrile was purchased from Merck Inc. (USA). Iron tosylate (CB-40 V2) (40% w/v in butanol) was purchased from Heraeus Chemicals (Germany). Indium tin-oxide (ITO)-coated glass slides were purchased from Delta Technologies (USA). Stainless-steel mesh was purchased from Ted Pella Inc. (USA). Kapton tape was purchased from Shercon Inc. (USA). Borosilicate glass slides were purchased from Paul Marienfeld GmbH & Co. KG (Germany). Human ARPE-19 cells 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 Thermo Fisher Scientific (USA). All chemicals were reagent grade and were used as received without any further purification.

### 3.4 Methods

#### 3.4.1 Electrochemical polymerisation of PEDOT

ECP of EDOT was carried out using a three-electrode, one-compartment setup, where either an ITO-coated glass slide or a stainless-steel sheet was used as the working electrode. Stainless-steel mesh was utilised as the counter electrode and Ag/AgCl was used as the reference electrode for all ECP experiments. ECP of PEDOT was conducted using an eDAQ Pty Ltd. model EA161 potentiostat and E-corder 410 with Chart, E-Chem and Scope software (Australia).

MilliQ water, acetonitrile and a mixture of methanol/water (50/50 v/v, pH: 7.0 adjusted with 1 N NaOH) (50/50) were explored as solvents to carry out the polymerisation. The EDOT concentration in MilliQ water was 0.01 M, while it was 0.1 M in acetonitrile and methanol/water due to having higher solubility in organic solvents. LiClO₄ (0.1 M) was explored as a dopant in MilliQ water as well as in acetonitrile. DexP (0.01 M) was also explored as a dopant in MilliQ water and methanol/water for incorporation into the PEDOT structure during polymerisation.

Galvanostatic electrochemical polymerisation was only explored using LiClO₄ as a dopant, where a constant current of 80 µA/cm² was initially used to carry out the polymerisation of PEDOT as adopted from a previous report [206]. The voltage that emerged from this applied current was 0.9 V (Figure
3.2a); however, due to the large surface area of the working electrode for polymerisation, it took 3 h to form thin PEDOT films. Therefore, the current density was increased to 200 µA/cm² (adopted from another previous report [230]). A voltage of 1.4 V was observed after the application of this current, resulting in the formation of nice PEDOT polymer films in 1 h using an ITO-coated glass slide or a stainless-steel sheet (Figure 3.2b). For the ITO-coated glass slide, the exposed area for polymerisation was kept to 2 cm² whereas for the stainless-steel sheet (due to the ease to cut into the desired shape) it was 1 cm². Kapton tape was used to cover the remaining area and the back side of the stainless-steel where polymerisation was not desired.

![Voltage plots](image)

Figure 3.2: Voltage emerged after the application of a) 80 µA/cm² (without any template), b) 200 µA/cm² (without any template) and c) 200 µA/cm² (with PS template deposited).
3.4.2 Vapour-phase polymerisation of PEDOT

VPP was carried out under vacuum at 40°C using a vacuum oven (Jiotech Inc., USA). An oxidant solution composed of PEG-PPG-PEG (23% w/w), iron tosylate (30.7% w/w) and ethanol (46.3% w/w) was used. This composition was adopted from our collaborators at the University of South Australia who have pioneered the VPP methodology to fabricate PEDOT layers that can be peeled off the substrate and stand free in a liquid medium [253]. The oxidant solution was spin-coated onto borosilicate glass slides at 1500 rpm for 20 s using a spin coater (Laurel tech, USA). Glass slides were then kept at 70°C for 30 s and then at 40°C until being placed into the vacuum oven for polymerisation. The vacuum oven was pumped down to 40 mbar, operating at 40°C during the course of polymerisation. The polymerisation time was optimised to 3 h for all VPP PEDOT films except during the initial attempts and when the growth of PEDOT was investigated over a PS template, which was carried out at the University of South Australia. After polymerisation, the glass slides were removed immediately, washed with methanol and air-dried.

3.4.2.1 Fabrication of porous PEDOT

As previously reported, a sacrificial template-based approach was used to fabricate porous PEDOT [254]. Polymethylmethacrylate (PMMA) beads (diameter: 350 nm) were initially used as a sacrificial template. Template beads were deposited on a substrate (either an ITO-coated glass slide or a stainless-steel sheet) using a slow vertical deposition method, where a PMMA beads suspension was poured into a clean glass beaker. Clean glass slides were vertically immersed into the glass beaker, and a peristaltic pump was employed to slowly remove the PMMA suspension, resulting in a thin uniform deposition of PMMA beads on the glass slides [255]. This deposition was heat-cured at 120°C for 10 min. Initially, ECP was attempted to form PEDOT on top of the deposited template in an aqueous solution of EDOT (0.01 M) and LiClO₄ (0.1 M). However, this attempt failed as there was no polymer formed on top of the deposited template, which could be attributed to the lower concentrations of EDOT due to its low solubility in aqueous media. To overcome this problem a solvent of methanol/water was used to obtain higher concentrations of dissolved EDOT to facilitate the polymerisation process; however, the attempt to fabricate porous PEDOT via ECP failed at the constant current density of 200 μA/cm² and resulted in hydrolysis of the electrolyte solution. This could be due to the PS template deposited over the conductive substrate increasing resistance, and thus a higher voltage is required to achieve the required current density (Figure 3.2c).

Polystyrene (PS) beads (diameter ~350 nm) were subsequently selected as a sacrificial template as they were resistant to the solvents used during polymerisation. They were deposited by slow vertical deposition on a stainless-steel sheet and an ITO-coated glass slide for ECP. The deposition was heat-cured at 90°C for 1 h. Methanol/water was used as a solvent to obtain higher concentrations of dissolved EDOT to facilitate the polymerisation process; however, the attempt to fabricate porous PEDOT via ECP failed at the constant current density of 200 μA/cm² and resulted in hydrolysis of the electrolyte solution. This could be due to the PS template deposited over the conductive substrate increasing resistance, and thus a higher voltage is required to achieve the required current density (Figure 3.2c).
Therefore, VPP was trialled for the fabrication of porous PEDOT. The oxidant solution was spin-coated onto the heat-cured template deposited on the borosilicate glass slides. After polymerisation, the polymer film was peeled off in methanol using tweezers and turned upside down. This film was placed on another glass substrate, dried and immersed in DMF overnight to dissolve the PS template. Figure 3.3 shows the process of fabricating porous PEDOT by VPP. Initial attempts to fabricate porous VPP PEDOT were carried out under slightly different polymerisation conditions, which resulted in a higher rate of polymerisation, to help investigate the growth of PEDOT over the template. The vacuum oven was pumped down to 45 mbar while operating at 35°C; however, the monomer was kept at 45°C. The polymerisation time was 1 h for the initial attempts; however, to investigate the growth of PEDOT over the PS template over time, polymerisation times of 12.5, 25 and 50 min were chosen.

![Fabrication process](image)

Figure 3.3: (1) The PS template was deposited over the substrate, (2) the template was spin-coated with oxidant solution, (3) polymerisation progressed through the deposited beads, (4) the template polymerised for 3 h and (5) the polymerised film was peeled off, turned upside down and placed on another substrate to dissolve the template in DMF overnight to obtain the inverse opal PEDOT structure.

### 3.4.2.2 Fabrication of PEDOT-coated cellulose membranes

Dialysis cellulose tubing was cut into pieces with an area of 12 cm² (4 cm x 3 cm), pieces were immersed in PBS overnight, and the two layers were separated. Each single layer was dip-coated with oxidant solution on both sides, kept at 70°C for 3 min and then placed into the vacuum oven pumped down to 40 mbar and operating at 40°C. VPP was carried out for 3 h for each sample to deposit a
PEDOT layer over both sides of the cellulose membrane. This procedure was later optimised and performed three times to deposit three PEDOT layers on both sides of the cellulose membrane. PEDOT-coated cellulose membranes were then soaked in methanol to wash away any excess oxidant solution, and were later removed from the methanol and air-dried.

3.4.3 Characterisation

Due to the failure of fabricating porous PEDOT via ECP, which was highly desired to entrap drug physically into the pores, this technique was not explored any further with the exception of semi-qualitative XPS analysis to compare the quality of non-porous VPP PEDOT to that of ECP PEDOT. However, porous VPP PEDOT was not characterised by XPS due to the limited penetration of this technique. Therefore, non-porous and porous VPP PEDOT structures were further characterised and compared via AFM, SEM, CV, EIS and biocompatibility studies. PEDOT-coated cellulose membranes were also characterised by SEM, in-situ CV and biocompatibility studies.

3.4.3.1 X-ray photoelectron spectroscopy

Elemental analysis of the PEDOT films was performed using an X-ray photoelectron spectroscope (XPS; SPECS, SAGE, Phoibos 150-HAS, Germany) fitted with a non-monochromated Al anode, a power of 200 W and with a base pressure of $2 \times 10^{-8}$ mbar. Samples were mounted on the stage, and the polymer film was earthed to minimise charging effects. The take-off angle was 90° with an incident beam angle of 45°. The sampling depth was approximately 10 nm. Curve fitting was performed with CasaXPS (Neil Fairley, UK) using a linear background. Spectra were charge corrected relative to the aliphatic carbon peak at 285 eV. XPS analysis was performed for freshly prepared non-porous VPP PEDOT as well as for ECP PEDOT films polymerised with dexP as the dopant. However, porous VPP PEDOT films were not characterised with XPS due to the limited sample depth of this technique.

3.4.3.2 Atomic force microscopy

The morphology of PEDOT surfaces in air was characterised using AFM in tapping mode. A MultiMode® 8 instrument with a Nanoscope V controller (Bruker, USA) was used to perform imaging in ScanAsyst modes in an air environment. The AFM base was placed on an active antivibration table (Vision IsoStation, Newport), and equipped with a vertical engagement scanner “J” (maximum scan range 125 µm in the X and Y directions, and nominal 5 µm in the Z direction). For ScanAsyst in air silicon nitride triangular probes of a resonance frequency between 70 and 95 kHz, a spring constant between 0.4 and 0.8 N/m, and a silicon nitride tip with the nominal radius of 2 nm (ScanAsyst AIR, Bruker) were used. All AFM experiments were conducted inside a clean room (Class 1000) at a temperature of 22 ± 1°C and a relative humidity of 39 ± 2%. The images were processed using WSxM 5.0 Develop 6.5 [257]. To investigate the growth of PEDOT over the template, samples were polymerised for different time periods and immediately removed, turned upside down to dissolve the
Fabrication and characterisation of PEDOT structures

3.4.3.3 Scanning electron microscopy
The surface morphology of the PEDOT films was further investigated using a Philips XL30S field emission gun scanning electron microscope (FEGSEM). Samples were mounted on aluminium studs using adhesive graphite tape and lightly sputter-coated with platinum (Polaron SC 7640 sputter coater). Cross-sectional images were recorded after the films were freeze-fractured under liquid nitrogen. For morphology evaluation of PEDOT-coated cellulose membrane samples, a ‘bench-top’ SEM model: TM3030Plus (Hitachi corp., Japan) with an electron gun was used. These samples were mounted onto aluminium studs using adhesive graphite tape and left uncoated.

3.4.3.4 Cyclic voltammetry
A three-electrode, one-compartment electrochemical setup was used to measure the redox behaviour of porous and non-porous VPP PEDOT films via cyclic voltammetry (CV). Electrical contact with the films was made using copper tape, allowing them to be cycled between −0.9 and +1.4 V against Ag/AgCl at a rate of 100 mV s⁻¹ in 0.1 M LiClO₄ aqueous solution using stainless-steel mesh as a counter electrode. Measurements were carried out using an eDAQ Pty Ltd. Model EA161 potentiostat and E-corder 410 with Chart, E-Chem and Scope software (Australia). Non-porous and porous PEDOT films were compared via CV, and the dimensions of PEDOT films (as working electrodes) were kept constant for this comparison. For PEDOT-coated cellulose membranes, CV was performed in Franz-cells, with PEDOT-coated cellulose membranes separating the two compartments used as working electrode, stainless-steel mesh as a counter electrode inserted from the sampling port in the receiver compartment and Ag/AgCl as reference electrode placed in the donor compartment. PBS was used as the electrolyte solution for the CV of PEDOT-coated cellulose membranes. In order to calculate the active electrochemical surface area of porous and non-porous VPP PEDOT films, CV was performed in 5 mM potassium ferrocyanide solution at different scan rates (100, 200 and 400 mV/s).

3.4.3.5 Electrochemical impedance spectroscopy
EIS was performed to analyse the behaviour of VPP PEDOT films against changing voltages (i.e. pulse stimulus) and to compare porous and non-porous PEDOT films. These films were characterised by electrochemical impedance in a one-compartment, three-electrode setup where PEDOT films were used as the working electrodes (geometrical area exposed to the solution: 3.75 cm²). Stainless-steel mesh was used as a counter electrode and Ag/AgCl was used as a reference electrode. EIS was carried out in a 5 mM potassium ferrocyanide electrolyte solution. EIS was observed over the range of 1 to 5 KHz. Data was analysed by EC-Lab® software (Bio-Logic, USA).
3.4.3.6 Cytotoxicity studies

PBS extracts from PEDOT structures were used to evaluate any potential toxicity on a retinal pigment epithelium cell line (ARPE-19). PBS extracts were obtained from porous VPP PEDOT films, non-porous VPP PEDOT films and PEDOT-coated cellulose membranes by exposing 3.75 cm² area (for each sample) to 10 mL of PBS. In order to eliminate any chances of interference with the cytotoxicity assay by PBS, blank PBS was also analysed. Positive control cells were treated with 1% Triton X-100 in DMEM/F-12, GlutaMAX to kill the cells (0% cell viability) while negative control cells were treated with DMEM/F-12, GlutaMAX only (100% cell viability). ARPE-19 cells were seeded into 96-well plates in DMEM/F-12, GlutaMAX at a concentration of 1 × 10⁴ cells/well and allowed to adhere for 24 h at 37°C in a 5% CO₂ humidified atmosphere. The cell culture medium was then replaced with PBS extracts from each sample in DMEM/F-12, GlutaMAX (1:1). Plates were incubated at 37°C and 5% CO₂ for 1 h. After the incubation period, the solution in each well was removed and replaced with MTT/PBS solution (0.5 mg/mL) and incubated for 4 h at 37°C and 5% CO₂ [258]. The MTT/PBS solution was then removed and replaced with HCl-isopropanol solution (0.04 M) to dissolve the formed formazan. The intensity of the purple colour was quantified by measuring the absorbance at 570 nm with correction of any interference at 650 nm (BioTek Synergy HT), and the percentage cell viability was calculated based on the negative control. Each group was tested in triplicate.

3.5 Results and discussion

3.5.1 Electrochemically polymerised PEDOT

ECP of PEDOT using a large surface area electrode, compared to most of the previously reported studies using a small surface area electrode, presented many problems during the fabrication process. ECP on ITO-coated glass slides was comparatively straightforward with 0.01 M EDOT and 0.1 M LiClO₄ in water. PEDOT films formed nicely on ITO-coated glass slides (Figure 3.4a). However, using stainless-steel sheet as a substrate resulted in polymerisation happening only at certain focal points on the exposed working electrode surface with 0.01 M EDOT and 0.1 M LiClO₄ in water (Figure 3.4b). In order to achieve polymerisation on the stainless-steel sheet, methanol was subsequently used as a co-solvent to achieve a 0.1 M EDOT concentration, which resulted in higher PEDOT fabrication (Figure 3.4c). Our lab has previously achieved dexP incorporation as a dopant during the polymerisation process by manipulating the pH of the solution while using pyrrole [254]. The same manipulation technique was trialled for the polymerisation of EDOT with dexP as a dopant. In order to dissolve dexP and EDOT, a mixture of water/methanol was used as described under methods. However, the polymerisation process was only successful under neutral pH as any changes to acidic (pH: 3.0) or basic (pH: 11) resulted in no polymerisation at all. Adjustment of pH for the polymerisation of PEDOT has not been reported previously, as given in Table 3.1. Therefore, it could be assumed that electrochemical polymerisation of PEDOT with dexP as a dopant happens only at neutral pH.
Fabrication and characterisation of PEDOT structures

In order to fabricate porous PEDOT with ECP, polymerisation was attempted on ITO-coated glass slides with deposited PS spheres in 0.1 M EDOT and 0.1 M LiClO₄ in water/methanol. However, this polymerisation could not be achieved due to the higher voltage resulting in hydrolysis of the electrolyte solution during the polymerisation process. This could be attributed to the surface area of the working electrode being covered with a non-conducting substance (PS spheres); ultimately increasing the voltage required to deliver the amount of current needed for EDOT polymerisation (Figure 3.2c). Due to the difficulty in the polymerisation process and the inability to manipulate the surface morphology of PEDOT, ECP was not investigated any further.

3.5.2 Vapour-phase polymerised non-porous PEDOT

As ECP was unsuccessful in fabricating porous PEDOT structures, VPP PEDOT was chosen as the main fabrication method. This technique was adopted from our collaborators who previously reported superior mechanical strength of PEDOT layers produced via this technique [253]. First, the successful fabrication of non-porous (plain) VPP PEDOT was achieved over a glass substrate where colour and surface features appeared similar under an optical microscope to what previously been reported for the non-porous VPP PEDOT (Figure 3.5) [259, 260]. The dark lines appearing on the surface have been attributed to the liquid-like behaviour of the oxidant solution induced by the tri-block co-polymer (PEG-PPG-PEG) used. However, these films were mechanically strong enough to be peeled off as a sheet and could be placed intact onto another substrate.
3.5.2.1 XPS elemental analysis to compare with ECP PEDOT

XPS is a good technique for analysing the surface elemental composition of a material. In the case of PEDOT, XPS was performed to compare the quality of non-porous VPP PEDOT with that of ECP PEDOT. Surface elemental analysis was carried out via XPS, where freshly prepared non-porous VPP PEDOT was doped with tosylate and ECP PEDOT doped with dexP (Figure 3.6). Table 3.2 provides information on the elemental composition of VPP PEDOT where the ratio of C:O:S was 5.7:1.5:1. The chemical composition of the EDOT monomer is \([C_6O_2S]\); however, tosylate \([C_6H_4SO_2]\) (loaded as a dopant during VPP) also contains sulphur, which could contribute to the slightly higher ratio of sulphur for this sample compared to the expected 1 S to 6 C. On the other hand, for the freshly prepared ECP PEDOT containing dexP, the atomic weight percentage of sulphur was much lower compared to freshly prepared VPP PEDOT (doped with tosylate). This could be attributed to the use of dexP as a dopant for the ECP process. The elemental ratio of C:O:S was 14.7:4.4:1 (Table 3.2), which correlated well with the ratio of carbon to oxygen in PEDOT. In addition, the ratio of F to P was 1.2:1, which correlated well with the elemental ratio of these elements in dexP. Moreover, this comparison confirmed that the quality of PEDOT produced via VPP was comparable in elemental composition to that of ECP PEDOT.
Figure 3.6: XPS surface elemental analysis of a) non-porous VPP PEDOT doped with tosylate and b) ECP PEDOT doped with dexP.

Table 3.2: Elemental composition of VPP PEDOT doped with tosylate and ECP PEDOT doped with dexP.

<table>
<thead>
<tr>
<th>ELEMENT (DOPED WITH TOSYLATE)</th>
<th>AT %</th>
<th>ELEMENT (DOPED WITH DEXP)</th>
<th>AT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O 1s</td>
<td>18.05</td>
<td>O 1s</td>
<td>21.2</td>
</tr>
<tr>
<td>C 1s</td>
<td>69.83</td>
<td>C 1s</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 1s</td>
<td>2.8</td>
</tr>
<tr>
<td>S 2p</td>
<td>12.12</td>
<td>S 2p</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P 2p</td>
<td>2.2</td>
</tr>
</tbody>
</table>
3.5.3 Vapour-phase polymerised porous PEDOT

Template-based porous PEDOT structures have not previously been fabricated via VPP; however, there is one report where polypyrrole was polymerised via VPP with the porosity of the resulting films manipulated by a variation in oxidant solution concentrations [224]. Another previous report described the fabrication of a single-layer, template-based porous structure via chemical vapour deposition (CVD) [226]. CVD differs from VPP in that the oxidant is sublimed from solid to the gas state, compared to VPP where the oxidant is dissolved in a solution. In addition, both the monomer (EDOT) and the oxidant react in the vapour phase to form solid PEDOT whereas in VPP only the monomer (EDOT) is in the vapour phase. To our knowledge, this was the first successful attempt to fabricate porous PEDOT via VPP as (displayed in the next sections), with this only being possible by peeling-off the PEDOT polymerised over the PS template, turning it upside down, and then etching away the template to obtain the porous PEDOT.

3.5.4 Characterisation of non-porous and porous VPP PEDOT

3.5.4.1 AFM characterisation

The PS beads used as a template for the fabrication of porous PEDOT had an average diameter of ~350 nm, thus any porous structure formed from this template was only visible with high-resolution techniques such as AFM. The porous PEDOT films fabricated via VPP were first characterised by this technique. After the initial attempts to polymerise for 1 h, the top-side of these films looked quite similar to non-porous PEDOT as reported previously (Figure 3.7) [261]. On the other hand, the bottom side showed regular pores, suggesting ‘honeycomb’ morphology. However, previous reports have suggested that the formed PEDOT sinks into the oxidant solution [262]. Furthermore, the use of template here allowed visualisation of the sinking of PEDOT, which could be observed via AFM. Therefore in order to optimise the fabrication of porous VPP PEDOT structures, the growth of PEDOT over the PS template was further analysed by terminating the polymerisation process at different time intervals and immediately observing the bottom side of these PEDOT films via AFM.
Fabrication and characterisation of PEDOT structures

In order to observe the growth of PEDOT over the PS template with a change in polymerisation time, three timeframes were chosen (12.5, 25 and 50 min). Immediately after polymerisation the films were made free-standing and were turned upside down to observe the morphology. The alterations in the morphology can be observed in the AFM images in Figure 3.8. It can be clearly seen that the honeycomb structure becomes more prominent with increasing polymerisation time, as only minimal imprinting occurred in the 12.5-min sample (Figure 3.8a-i) compared to an obvious imprint in the 50-min sample (Figure 3.8a-iii). It is assumed that the polymer growth occurs above the PS spheres (suspended on top of the oxidant solution), but whether the polymer film ‘sinks’ into the oxidant solution or whether the thickness with growth is responsible for the imprinting is still unclear. To better understand the process, a PEDOT film was synthesised for 12.5 min and left to stand for 1 h after polymerisation before washing and delamination. This experiment was expected to allow ample time for the PEDOT film to ‘sink’ if this was the process in which the imprinting was occurring.

The hypothesis of the CP ‘sinking’ into the oxidant solution was confirmed by the increased imprinting present in Figure 3.8c when compared to Figure 3.8a-i. The polymer ‘sinking’ mechanism is supported by previous investigations of the VPP mechanism which is proven to be a ‘bottom-up’ process where the oxidant diffuses up through the initially formed CP film [262]. Therefore, it makes sense that if the oxidant diffuses up through the CP film; the CP is essentially ‘sinking’ into the oxidant solution. This is a reasonable result as the film has ‘sunk’ onto a rough surface, and, as a consequence produced a porous PEDOT surface. This is the first successful report of fabricating template-based porous PEDOT structures and to visualise the mechanism by which PEDOT film sinks into the oxidant solution over time.

Figure 3.7: AFM image showing a) the top-side and b) the bottom-side of VPP PEDOT after peeling off, turning the film upside down, and etching away the PS template with pores being visible.
Figure 3.8: a) AFM images of the bottom side of VPP PEDOT films fabricated on a PS template-deposited glass slides showing a progression of imprints from the PS beads over the polymerisation times of i) 12.5 min, ii) 25 min and iii) 50 min. b) Cross-sections of the AFM images showing the depth of the honeycomb structure within the PEDOT film, c) the bottom of a VPP PEDOT film fabricated over PS beads with a polymerisation time of 12.5 min with a 1 h wait period between the termination of the polymerisation and turning upside down and removing the template and d) Cross-sections of the corresponding AFM images showing the PS sphere imprints on the PEDOT film.

After the optimisation of the VPP process, in which a balance was achieved between the rate of polymerisation happening at the interface of the oxidant solution and the monomer vapours and the rate at which the formed PEDOT film sinks into the PS template, the surface morphology was further characterised by AFM by moving the tiny probe in three different directions. Non-porous VPP PEDOT film morphology was the same as previously reported for VPP PEDOT (Figure 3.9a) [261]. It can also
be seen that non-porous VPP PEDOT featured irregular cross-sections, which suggested a rough surface morphology. On the other hand, AFM cross-sections for porous VPP PEDOT revealed a highly regular pattern of pores in all directions, which suggested a regular porous honeycomb structure (Figure 3.9b). Furthermore, these AFM cross-sections also revealed that the average diameter of these pores was approximately 330 nm, which correlated well with the diameter of the PS beads (~350 nm). This may be advantageous for applications requiring increased surface area electrodes, as the bottom side of VPP PEDOT fabricated on PS spheres exhibited increased surface area (revealed by AFM cross-sections) compared to PEDOT created without the PS template.

Figure 3.9: AFM images of a) non-porous (plain) VPP PEDOT, b) cross-sections revealing an irregular surface, c) porous VPP PEDOT and d) cross-sections revealing a highly regular porous structure.
3.5.4.2 SEM characterisation

In order to further analyse and understand the morphology of VPP PEDOT, SEM was employed to image the top-side and a cross-section of non-porous VPP PEDOT, PS template, VPP PEDOT with the template (only the cross-section to better visualise the penetration of PEDOT into the PS template). SEM was further used to study the cross-section and the bottom-side of non-porous VPP PEDOT after etching away the PS template. Figure 3.10a shows the top-side of non-porous VPP PEDOT with no special features at the surface and appearing similar to what has previously been reported [221, 263]. Figure 3.10b shows a cross-section of the non-porous VPP PEDOT, which suggested the total thickness of these films to be \(\sim 2\ \mu m\). There were no pores observed for non-porous VPP PEDOT as revealed here by the SEM.

![SEM images of non-porous VPP PEDOT](image)

Figure 3.10: SEM images of non-porous VPP PEDOT. a) The top-side showed a regular VPP PEDOT structure and b) the cross-section revealed the total thickness of non-porous VPP PEDOT film to be \(\sim 2\ \mu m\).

PS beads were also imaged using SEM, where the top-side of deposited beads on the glass substrate (Figure 3.11a) revealed a highly regular pattern for the deposited beads. Furthermore, the cross-section image confirmed that multi-layer deposition was achieved with the slow vertical deposition method (Figure 3.11b). Accordingly, after VPP polymerisation of PEDOT, the cross-section SEM image confirmed the formation of PEDOT all around the PS template, as displayed in Figure 3.11c. Furthermore, this SEM image also revealed that the top-side had a slightly thicker layer of PEDOT on top of the PS template deposition; however, this cross-section image also revealed that PEDOT was able to penetrate all the way into the PS template. This explained why the PS sphere template remained attached to the PEDOT film while peeling it off the glass substrate as PEDOT penetrated well into the PS template. This penetration of PEDOT into the PS template should result in a highly porous, multi-layered structure if the template is successfully etched away. As expected, SEM images confirmed the formation of a highly regular porous PEDOT structure fabricated via VPP (Figure 3.11d). Furthermore, when compared to the deposited PS template (Figure 3.11a), porous PEDOT appears to be the reverse-opal of the PS template (Figure 3.11d). In addition to the formation of a highly regular structure, the cross-section SEM image confirmed that the PEDOT layer penetrated multiple layers of the deposited PS template, as evident in Figure 3.11e after the removal of PS template.
Fabrication and characterisation of PEDOT structures

Figure 3.11: SEM image of a) the deposited PS template top-side showing highly regular pattern, b) the deposited PS template cross-sections showing multi-layer deposition, c) cross-section image of VPP PEDOT with PS template where the PS beads are all covered with PEDOT, d) porous VPP PEDOT where the flat section (bottom-side of PEDOT film) shows the formed inverse opals and e) porous VPP PEDOT cross-section confirming the multi-layered porous structure.
3.5.4.3 Electrochemical characterisation

Cyclic voltammograms for non-porous and porous VPP PEDOT films are shown in Figure 3.12. The currents observed during potential cycling gradually decreased over the first 40 cycles and then stabilised out to 100 cycles. The 20th cycle in both, Figure 3.12 a and b, displayed two oxidation peaks at 0.15 V and 0.9 V as well as two reduction peaks at 0 V and -0.6 V. The presence of two oxidation and two reduction peaks for PEDOT is due to the different oxidation states of PEDOT, which may lead to different doping levels ultimately responsible for these redox peaks [244, 264]. However, more than two oxidation peaks have also been reported for PEDOT depending on the presence of a certain anion in the solution [265]. After the 40th cycle an additional oxidation peak can be observed at 1.1 V, which could have been overlapped by the other oxidation peaks during the early cycles. Furthermore, LiClO4 has previously been reported to produce additional oxidation/reduction peaks due to doping/de-doping of ClO4 [266]. Therefore, the additional peak at 1.1 V also could be attributed to the doping/dedoping of LiClO4. However, the absence of an additional reduction peak would suggest that doping of LiClO4 is easier than to its de-doping.

Both films (porous and non-porous VPP PEDOT) have exhibited good reproducible redox behaviour over several cycles indicating their redox stability. Comparing the cyclic voltammograms of porous and non-porous films, it is obvious that the currents evolved were higher for porous PEDOT than for the non-porous film. This is a clear indication of the higher surface area of these porous PEDOT structures, which can withdraw much higher currents under the same conditions. A higher surface area facilitates the interchange of electro-active species between the solution and the polymer film, which may ultimately yield higher current passing through the polymer film [267].
In order to calculate the surface area of porous and non-porous VPP PEDOT films as working electrode, CV was performed in 5 mM potassium ferrocyanide. The area of the electrode can be calculated by the Cottrell equation [268]:

\[
i = nFAD^{1/2}c_0\pi^{-1/2}t^{-1/2}
\]

Where \(i\) is the current in ampere (A), \(F\) is the Faraday constant (96,485 C/mol), \(A\) is the electrode surface area in cm\(^2\), \(D\) is the diffusion coefficient in cm\(^2\)/s for potassium ferrocyanide 6.2 \(\times\) 10\(^{-6}\) cm\(^2\)/s, \(t\) is time in s, \(n\) is the number of electrons required to reduce/oxidise one molecule of analyte and \(c_0\) is the initial analyte concentration in mol/cm\(^3\). The equation can be simplified as,

\[
i = kt^{-1/2}
\]

Furthermore, the (scan rate\(^{1/2}\)) is usually used in place of \(t^{-1/2}\). Thus, \(k\) is equivalent to:

\[
k = nFAD^{1/2}c_0\pi^{-1/2}
\]

\(k\) can be calculated for porous and non-porous VPP PEDOT by measuring the oxidation currents as a function of the scan rate. CV was performed at different scan rates and graphs were plotted for the current as a function of the square root of the potential scan rate (Figure 3.13). The value of \(k\) was then calculated from the slope and the electrochemically active surface area was calculated for porous and non-porous VPP PEDOT films:
Fabrication and characterisation of PEDOT structures

\[
A_{\text{porous}} = 12.71 \text{ cm}^2 \\
A_{\text{non-porous}} = 4.43 \text{ cm}^2
\]

When compared to the geographical area of the films exposed to the electrolyte solution (3.75 cm²), the roughness factor was calculated to be 3.75 for porous films and 1.18 for non-porous films. These results show that the active electrochemical surface area is ~3.4 times higher for porous films and ~1.2 times higher for non-porous films when compared to the geographical area. Area calculations confirmed the anticipated difference in the active electrochemical surface area of non-porous and porous VPP PEDOT, as observed by the AFM and SEM. It revealed that porous VPP PEDOT has a higher surface area leading to more electrolyte solution-polymer interface when compared to non-porous VPP PEDOT. The slight increase in the active surface area of non-porous VPP PEDOT from the geographical area exposed to the electrolyte solution could be attributed to the surface roughness that can be observed in AFM cross-sections (Figure 3.9b).

![Graph showing current plotted as a function of the square root of the scan rate for porous and non-porous VPP PEDOT films.](image)

**Figure 3.13:** Current plotted as a function of the square root of the scan rate for porous and non-porous VPP PEDOT films.

EIS was performed to further electrochemically characterise porous and non-porous VPP PEDOT and revealed that porous structures offer less impedance than non-porous structures (Figure 3.14). With regards to the final application of these structures into an implant that will be powered by transcutaneous wireless electricity, 100 KHz seems to be the optimal frequency at which transcutaneous wireless electricity operates [269]. Therefore, it is important to compare the impedance offered by these structures at 100 KHz as it may ultimately determine the powering requirements for
the final implant. Lower impedance means that less power would be required to stimulate the PEDOT structures which would be better for use in the final implant. Figure 3.14 shows that the impedance offered by porous VPP PEDOT is 80 Ohm compared to 105 Ohm offered by non-porous VPP PEDOT. These values reveal a difference in the active surface area of approximately ~25%. This difference indicates that there is a variation in the active surface area of porous and non-porous structures, which could be correlated to the electrochemically active surface area difference calculated by the CV. It also shows that porous VPP PEDOT would be more suitable for use in the implant due to the lower impedance offered to transcutaneous wireless electricity; therefore, less power is required for stimulation (to release drug) which would in turn result in a reduced frequency to recharge the battery circuitry.

![Electrochemical impedance spectrograms for porous and non-porous PEDOT structures.](image)

**Figure 3.14:** Electrochemical impedance spectrograms for porous and non-porous PEDOT structures.

### 3.5.4.4 Cytotoxicity studies

MTT assay was used to measure the cellular metabolic activity that indicates how cells behave in the presence of certain toxic substances. If the metabolism is affected by a substance, that substance is deemed toxic and *vice versa*. MTT assay has previously been employed to assess the cytotoxicity of PEDOT either via direct contact between the cells and PEDOT or by using extracts from PEDOT structures [270]. PBS extracts were employed here to evaluate the cell viability of PEDOT structures, as in the final implant these PEDOT structures will not be in direct contact with the surrounding cells. Instead, PEDOT structures will be enclosed in a biocompatible non-biodegradable polymer casing. However, the MTT assay was performed with the PBS extracts obtained from porous and non-porous VPP PEDOT structures in order to assess the leaching of any toxic/non-drug substances from these PEDOT structures. PBS extracts were obtained either after the application of an electrical stimulus (50
CV cycles, -0.9 V to +1.4 V at 100 mV/s) or simply by immersing them into the PBS overnight. MTT results revealed that the PBS control resulted in almost the same cell viability as the negative control (DMEM/F-12, GlutaMAX only). On the other hand, PBS extracts obtained from the porous and non-porous VPP PEDOT films in the absence or presence of an electrical stimulus showed slightly reduced cell viability, though still over 80% (Figure 3.15). Interestingly, there was no difference in the cell viability of porous VPP PEDOT PBS extracts in the absence or presence of an electrical stimulus. On the other hand, a noticeable decrease in cell viability was observed for stimulated non-porous VPP PEDOT PBS extracts when compared to no stimulation. This could be attributed to any entrapped unwashed oxidant solution components still present within these structures, which would be better washed away for porous VPP PEDOT structures due to their higher surface area. Furthermore, a slight decrease in the cell viability has previously been reported for VPP PEDOT; however, it was reported to be insignificant [271]. Therefore, significance was determined here through statistical analysis by performing two-tailed unpaired Student's t-tests to compare porous and non-porous VPP PEDOT films (control and stimulated) to the PBS control. However, there was no statistically significant difference found (p-value >0.05). Therefore, it was concluded that these structures did not leach any cytotoxic substances into the PBS in the absence or presence of any stimulus and are suitable for use in the eye for the final implant application. However, thorough washing of these structures would be performed to wash away any remaining oxidant solution components entrapped within these structures, especially in the case of non-porous VPP PEDOT structures.

![Figure 3.15: Cell viability of PBS extracts where PS: porous stimulated, NPS: non-porous stimulated, PC: porous control, NPC: non-porous control, NEG.: negative control, POS.: positive control and PBS: PBS control (values represent average + SD, n=3).](image-url)
3.5.5 PEDOT membranes

PEDOT was successfully coated over cellulose membranes with initially only one layer that was later increased to three layers. Figure 3.16 shows the formed PEDOT-coated cellulose membranes, which appeared darker with three layers compared to a single layer of PEDOT. These membranes were later cut to fit perfectly between the donor and the receiver compartment in the Franz-cell; an extension was left to make the connection with the electrode clip for in-situ stimulation (Figure 3.16d). These membranes were further characterised by CV, SEM, XPS and cytotoxicity studies.

![Figure 3.16: a) single layer of PEDOT on cellulose compared with b) three layers of PEDOT on cellulose, which appear slightly darker, c) the donor compartment of the Franz-cell (average diameter: 2.7 cm) and d) PEDOT-coated cellulose membranes cut accordingly to fit in the Franz-cell (this picture was taken after membrane removed from the Franz-cell).](image)

CV of these PEDOT-coated cellulose membranes was performed within the Franz-cell setup in order to replicate the environment of a release study (Figure 3.17); however, there was some leakage with the use of the glass donor compartment. Therefore, a plastic donor compartment was used that had slightly elongated edges and was able to stop the leakage while in-situ application of stimulus was still achievable (Figure 3.16c).

![Figure 3.17: a) PEDOT-coated cellulose membrane mounted on to the Franz-cell connected to the working electrode with copper tape and b) the reference electrode dipped in the liquid in the donor compartment.](image)
These membranes were subjected to 100 CV cycles, where a decrease in current flow was observed from cycle 1 to 25 before stabilising between cycles 25 and 100 (Figure 3.18). The electrical activity remained stable during the CV, which indicates the durable electrochemical nature of these PEDOT-coated cellulose membranes. High stability under electrical stress is advantageous as it would allow for PEDOT-based implants to operate for a longer period of time before requiring replacement. Two oxidation peaks were observed at -0.2 V and 0.6 V and two reduction peaks at 1 V and 0.4 V during the first cycle. However, as the flow of current decreased up to cycle 25, there was only one broad oxidation peak at 0 V and one broad reduction peak at 0.2 V. This shift in peak position could be attributed to the de-doping of tosylate and doping of the anions present in the PBS solution [266]. When compared to the porous and non-porous VPP PEDOT, there was a shift in the position of the oxidation and reduction peaks with the PEDOT-coated cellulose membrane, which could be influenced by the different electrolyte solution (PBS) used for CV here [272].

In addition, the amount of current passing through these membranes during CV was higher than for porous and non-porous VPP PEDOT structures, which could also be attributed to the different electrolyte solution (PBS) used here as well as the difference in surface area exposed to the electrolyte solution. PBS contains \( \text{PO}_4^{3-} \) and \( \text{Cl}^- \) anions, which are smaller in size compared to \( \text{ClO}_4^- \); therefore, they can move in and out of the polymer structure more easily [266]. Indeed, the shift of the peaks in

![Figure 3.18: Cyclic voltammogram of PEDOT-coated cellulose membrane mounted on a Franz-cell; arrows indicate the direction of current flow (geographical area exposed to the electrolyte solution: 1.77 cm²).](image)
Figure 3.18 also indicates that doping/de-doping is happening at an earlier stage when compared to Figure 3.12. Furthermore, the additional oxidation peak in Figure 3.12 at 1.1 V was not observed with PEDOT-coated cellulose membranes. This peak was observed with porous and non-porous VPP PEDOT CV performed in LiClO₄ solution (Section 3.5.4.3); therefore, it can be assumed that the additional peak was influenced by the doping/de-doping of LiClO₄. It also suggests that LiClO₄ could be doped at a higher doping level (at 1.1 V) for PEDOT [273].

SEM was employed to study the surface morphology of PEDOT-coated cellulose membranes before and after the application of in-situ CV stimulation. Before the application of the CV stimulus, the membrane surface morphology appeared to be similar to that of non-porous VPP PEDOT, which could be due to the method of fabrication (VPP). However, at lower magnification, PEDOT-coated cellulose membranes featured an inhomogeneous and highly rough surface, which could be attributed to the dip-coating method employed to coat these membranes with oxidant solution (Figure 3.19). Previous PEDOT fabrication studies have revealed that dip-coating leads to rough and inhomogeneous surfaces when compared to spin-coating [274]. In addition, previous reports have also shown that PEDOT fabricated via other polymerisation techniques (i.e. ECP) using other membrane substrates featured rough surface morphology [275]. Therefore, the surface properties of PEDOT-coated cellulose membranes could also be influenced by the substrate (cellulose) when compared to non-porous VPP PEDOT, where borosilicate glass slides were used as a substrate.

Figure 3.19: SEM image of the PEDOT-coated cellulose membrane obtained using bench-top SEM that reveals inhomogeneous and highly rough surface morphology.
SEM was further employed to look at the surface morphology of the PEDOT-coated cellulose membranes after the in-situ application of CV stimulus. Figure 3.20a shows a SEM image of a PEDOT-coated cellulose membrane following CV that revealed cracks along the surface. In response to the changes in redox state, actuation of the polymer could cause these cracks as the polymer expands and contracts. PEDOT actuation has previously been shown to cause cracks over a number of substrates [276-278]. Figure 3.20b shows the SEM image obtained from the bench-top SEM, where another PEDOT layer is visible beneath a large crack. Therefore, it could be assumed that the upper layer of PEDOT exhibited more actuation compared to the layers beneath and thus cracks were formed on the surface of PEDOT-coated cellulose membranes. It could also be attributed to the difference in tensile strength between the PEDOT layer and the cellulose membrane. Formation of these cracks presents a challenge for the use of PEDOT as a sealing layer structure in the final implant, as the crack formation would remove any control on the release rate of the drug. Furthermore, previous studies have suggested strategies to eliminate these cracks by the use of a stretchable substrate (to match the tensile strength of the PEDOT layer and the substrate), where actuation of the PEDOT layer would cause the substrate to expand and shrink [192]. However, due to the use of the Franz-cell, these membranes were tightly sandwiched between the donor and the receiver compartment; therefore, such strategies could not be applied here for in-situ electrochemical characterisation.

![Figure 3.20: SEM images showing the formation of cracks in PEDOT-coated cellulose membranes after the application of an in-situ CV stimulus: a) small cracks and b) one large crack.](image)

The cell viability of the PBS extracts obtained from the PEDOT-coated cellulose membranes was assessed the same way as described for porous and non-porous VPP PEDOT structures (Section 3.5.4.4). Non-stimulated PEDOT-coated cellulose membrane PBS extracts resulted in 87% cell viability compared to 73% cell viability from stimulated PBS extracts (Figure 3.21). This could be attributed to any unwashed oxidant solution components still present within the PEDOT layer, which could leach out upon the application of the stimulus. In order to determine if there was a significant difference in cell viability, statistical analysis was performed using a two-tailed unpaired Student's t-test to compare the PEDOT-coated cellulose membrane (stimulated and control) to the PBS control; however, no
A statistically significant difference was found ($p$-value >0.05). Therefore, it can be concluded that PEDOT-coated cellulose membranes didn’t leach any cytotoxic substances in the absence or presence of electrical stimulus. The cell viability here is acceptable for use in the eye for the final implant.

Figure 3.21: Cell viability of PBS extracts where MS: membrane stimulated, MC: membrane control, NEG.: negative control, POS.: positive control and PBS: PBS control (values represent average ± SD, $n=3$).

### 3.6 Conclusion

ECP and VPP were both explored for the fabrication of the desired PEDOT structures. ECP presented many challenges for the fabrication of large surface area PEDOT electrodes and for the manipulation of the surface morphology of PEDOT. Therefore, VPP was chosen as the preferred technique due to the ease in polymerisation over any surface and successful fabrication of porous PEDOT structures. To our knowledge, this is the first successful fabrication of template-based porous PEDOT structures via VPP. The chemical composition of non-porous VPP PEDOT was analysed via XPS, which revealed no major differences in the quality and elemental composition of non-porous VPP PEDOT compared to the ECP PEDOT. The surface morphology of VPP PEDOT was characterised by AFM and SEM, which revealed the honeycomb porous nature of porous VPP PEDOT structures. CV and EIS were performed for electrochemical characterisation and confirmed a higher electrochemically active surface area for porous VPP PEDOT. The biocompatibility was determined through an MTT assay, which revealed that porous and non-porous VPP PEDOT film extracts exhibited acceptable biocompatibility in the absence and presence of an electrical stimulus. PEDOT-coated cellulose membranes were successfully fabricated by coating PEDOT over cellulose membranes via VPP. These PEDOT-coated cellulose
membranes exhibited a rough surface morphology as indicated by SEM analysis. A reproducible electrochemical behaviour was observed via CV by *in-situ* stimulation within the Franz-cell; however, cracks were observed on the surface of PEDOT-coated cellulose membranes after the application of a stimulus, which could affect their use as sealing layers to control drug release. The biocompatibility of PEDOT-coated cellulose membranes was also determined via an MTT assay with no significant difference observed when compared to the control PBS. Further analysis of these structures with respect to their use for stimuli-responsive drug delivery is presented in Chapter 4.
CHAPTER 4: DRUG LOADING AND RELEASE FROM PEDOT STRUCTURES

4.1 Introduction

There are a number of ways to load and release drug molecules from PEDOT structures, which have been discussed in Chapter 1. Parameters for drug loading and release may vary depending upon the PEDOT polymer morphology, the polymerisation technique and the nature of drug being loaded or released. VPP is a technique that has been used to fabricate PEDOT structures during this PhD due to its ease to fabricate porous PEDOT structures [215, 223]. However, loading an anionic drug as a dopant into these PEDOT structures during polymerisation is usually not an option with VPP as the negatively charged molecule within the oxidant solution (usually chloride or tosylate) is attracted as a dopant into the polymer structure [253]. To overcome this disadvantage, ion-exchange can be performed to exchange a previously loaded dopant molecule with an anionic drug. Previous ion-exchange studies have looked into the exchange of ions in the absence of any stimulus (passive ion-exchange) as well as when a stimulus was applied (active ion-exchange) [230]. For PEDOT and other conducting polymers, an anionic drug can be loaded and released via ion-exchange; therefore, it offers the potential to explore fabricated PEDOT structures as stimuli-responsive ion-exchange resins. Furthermore, it also offers an interesting opportunity to analyse the effect of surface area for ion-exchange by comparing drug loading and release from the non-porous and porous VPP PEDOT structures.

Physical entrapment of drug into the polymer structure has previously been extensively studied [152]. However, it has previously involved combining PEDOT with another polymer used extensively for drug delivery applications. Porous VPP PEDOT structures fabricated during this PhD offer high surface area and provide physical space to entrap additional drug. Following the physical entrapment of drug, VPP could then be further employed to seal these porous structures to avoid leakage. It is anticipated that VPP will allow more drug to stay entrapped during the sealing process due to working in the vapour phase (dry environment) rather than an aqueous environment (as seen with ECP) where the drug could easily leach out [152]. In this way, neutral drug molecules could be loaded and sealed into the pores without the need of ion-exchange. Overall, it is important to consider that the permeability of such PEDOT sealing layers still plays an important role during the release studies.
4.2 Aim and objectives

The aim of the work presented in this chapter was to investigate drug loading and release for the fabricated VPP PEDOT structures (non-porous and porous). Specific objectives were:

- To investigate ion-exchange for drug loading and release from the non-porous and porous VPP PEDOT structures employing dexP as the anionic dopant.
- To explore physical entrapment as a way to load drugs into the voids of porous VPP PEDOT structures using dex as a neutrally charged drug.
- To seal the physically entrapped drug-loaded structures with PEDOT via VPP and evaluate the permeability of the sealing PEDOT layer.

4.3 Materials

Materials used for the fabrication of PEDOT have been described in Chapter 3. DexP was purchased from Jai Radhe Sales (India) and Dex was purchased from CFM Oskar Tropitzsch (Marktredwitz, Germany).

4.4 Methods

4.4.1 Drug selection

The use of dex and dexP to treat chronic back of the eye conditions is well established, with one dex implant (Ozurdex®) currently available on the market for the long-term treatment of DR, as discussed in Section 1.2.2. At a physiological pH, dex (pKa 12.14) is uncharged while dexP (pKa 6.8) is anionic. DexP can be loaded into the CP structure as a dopant during polymerisation (in the case of ECP) or via ion-exchange after polymerisation (in the case of VPP). On the other hand, dex can be physically entrapped into the polymer pores. Previous research with CP systems has shown that porous structures are able to load more drug than non-porous ones [152]. Both of the selected drugs offer versatility in terms of drug loading and release as well as different solubility in the release medium. Moreover, these drugs have distinct pharmacokinetic profiles allowing release profiles to be tailored.

4.4.2 Drug solubility in release media

The solubility of dex and dexP was determined in the respective release media in order to maintain sink conditions during the drug release studies. Sink conditions must be maintained in order to have ample release medium available to dissolve the released drug. Under sink conditions the concentration
Drug loading and release from PEDOT structures

of drug must remain at less than one-third of the saturated concentration [279, 280]. For dexP alone, 0.01 M PBS (pH 7.4) was used as a release medium and drug solubility was determined by adding excess amounts of dexP and was further treated as outlined below:

- Vortexed (IKA Inc., Germany) for 15 min.
- Sonicated (Elma Schmidbauer GmbH, Germany) for 30 min.
- Placed in a shaking water bath (Thermo Fisher Scientific, USA) operating at 100 rpm at 37°C for 24 h.
- Centrifuged (Eppendorf Inc., Germany) at 10,000 rpm at 20°C for 10 min.
- Supernatant collected and diluted based on the standard curve from the expected solubility.

For dex, the release medium consisted of 0.01 M PBS (pH 7.4) and methanol (50:50, v/v). Dex solubility was determined by adding excess amounts of dex to the release medium and treating it exactly the same way as described above for dexP.

4.4.3 Evaluation of drug stability in release media

Determining the stability in the release medium is an important step in ensuring that drug is not degrading during the course of the release studies. The stability of dexP in PBS was determined by dissolving 50 µg/mL and storing at 4, 25 and 37°C. Samples were taken at 1, 2, 6, 24, 48, 72, 120 and 168 h and analysed via HPLC to observe any degradation of dexP. Triplicates were analysed for each timepoint and each condition.

The stability of dex was determined in the same fashion; however, a mixture of PBS and methanol (50:50, v/v) was used in the place of PBS.

4.4.4 Drug loading and release: Ion-exchange of dexP

All drug loading and release experiments were performed in a one-compartment, three-electrode setup, where stainless-steel mesh was used as the counter electrode and Ag/AgCl as the reference electrode. PEDOT films were connected to the working electrode via a copper tape (Figure 4.1).
4.4.4.1 Drug loading

VPP was performed as described in Section 3.4.2. Non-porous and porous VPP PEDOT films were cycled in a 0.1 M dexP aqueous solution between -0.9 V to +1.4 V to perform active ion-exchange (also referred to as active drug loading in this thesis) at a scan rate of 100 mV/s. Extreme care was exercised to ensure that a precisely 3.75 cm² area (dimensions: 2.5 cm x 1.5 cm) of film was exposed to the 0.1 M dexP aqueous solution for the drug loading of all porous and non-porous structures via ion-exchange (Figure 4.1). CV is a good measure of ion-exchange; therefore, it was selected and used for active drug loading. The CV parameters for active drug loading were selected to maximise the exchange of tosylate with dexP [266]. As discussed in Section 3.5.4.3, three oxidation peaks were observed with VPP PEDOT; therefore, +1.4 V was selected to cover the oxidation event occurring at +1.1 V and to attract maximum amounts of dexP during oxidation. On the other hand, -0.9 V was selected to cover the reduction event occurring at -0.6 V and to expel the maximum amount of tosylate into the solution. Similar parameters have previously been used to release drug from PEDOT-based systems; therefore, those parameters were modified accordingly for the purpose of drug loading via ion-exchange onto VPP PEDOT films [281]. To exchange tosylate with dexP, 50 CV cycles were performed. At the end of 50 cycles, the samples were held at +1.4 V for 2 min to retain as much of the loaded dexP in the polymer structure as possible, with this drug loading process taking 45 min in its entirety. In addition to the active drug loading (CV-facilitated active ion-exchange), passive drug loading (spontaneous ion-exchange) was also performed where VPP PEDOT films were immersed in 0.1 M dexP aqueous solution for 45 min to provide the same contact time with the drug solution; however, no stimulus was applied in this case. Following removal from the drug solution, all samples were carefully washed with MilliQ water and air-dried. This wash process was repeated three times to ensure that any loosely bound drug was removed from these films.

PEDOT is a non-biodegradable CP that does not dissolve in any solvent; therefore, there is no direct way to calculate drug loading which is usually done by dissolving the polymer for other dosage forms. Another way to measure drug loading is by calculating the difference in the drug concentration in
solution before and after loading; however, the concentration of drug was very high in the solution (0.1 M dexP) compared to the amount loaded onto the PEDOT structures; therefore, no significant difference was observed. The only way to estimate loading was to release all loaded drug into the release medium and measure the amount of drug released.

4.4.4.2 XPS analysis
Survey and sulphur peak analysis were performed using XPS (for methodology, please see Section 3.4.3.1) to observe the extent of spontaneous and facilitated exchange of tosylate with dexP. Due to the limited penetration of XPS (only 10 nm), this analysis was only performed for non-porous VPP PEDOT. Sulphur peak curve fitting was performed using Magicplot Pro (Magicplot systems, Russia).

4.4.4.3 Drug release
Drug release from active and passive dexP-loaded PEDOT samples was evaluated in the absence and presence of a stimulus, which will be referred to as non-stimulated and stimulated release, respectively. For the purpose of stimulated drug release, parameters used are listed in Table 4.1. Initially, a stimulus of 50 CV cycles was applied between +0.9 V to -0.6 V at a rate of 100 mV/s, this being adopted from a previous study [206]. Drug release from active dexP-loaded films was carried out via a CV being evaluated as a release stimulus (~25 min for each sample) applied using an eDAQ Pty Ltd. model EA161 potentiostat and E-corder 410 with E-Chem software (Australia). However, one CV stimulus of 50 cycles was not able to release all of the drug, as more drug release was observed after the application of another 50 cycles of CV stimulus.

Pulse voltammetry (referred to as ‘pulse’ from here on) was selected and used instead of CV from there on, due to its suitability for the final implant application as it offers easy integration with the wireless electricity technology [269, 282]. Furthermore, the effect of various redox states was also analysed in order to optimise the release stimulus. To observe the effect of different redox states of the polymer on drug release, 50-mL falcon tubes were used as the vessels. These were kept in a ThermoMixer® (Eppendorf Inc., Germany) operating at 37°C and 300 rpm (Figure 4.2). The release medium was 20 mL PBS and the sample volume for each timepoint was 200 µL, which was replaced with fresh PBS. Samples were collected at time zero and then every 5 min, up to 30 min. Active dexP-loaded VPP PEDOT films (porous and non-porous) were kept under these conditions:

- Constantly oxidised at +1 V for 30 min, or
- Constantly reduced at -1 V for 30 min, or
- A continuous pulse of ±1 V applied for 30 min at 0.5 Hz.
Statistical analysis was performed using a two-tailed unpaired Student’s t-test where the significance in the amount of drug release was determined upon the application of an oxidation, reduction or pulse stimulus for both porous and non-porous VPP PEDOT films. A comparison between porous and non-porous films was also performed. Finally, a student’s t-test (two tails, unpaired) was also performed to compare the total dexP loading in Section 4.5.3.5 and to compare the amount of drug release as a function of the stimulus applied in Section 4.5.3.6. Significance was only established where the p-value was less than 0.05.

Figure 4.2: Setup showing the in-situ stimulus applied to observe drug release of different redox states in a ThermoMixer operating at 37°C and 300 rpm.

For all the subsequent release experiments, a stimulus of ±1 V was applied as a continuous pulse at a frequency of 0.5 Hz for either 2 or 17 min. These two timepoints were selected to cycle the drug-loaded VPP PEDOT films between the positive and the negative potentials (+1 V and -1 V) either ~50 times or ~500 times respectively. The 2-min release stimulus was used for the multi-day drug release experiments whereas the 17-min release stimulus was used to determine the drug loading, as the in-situ stimulation indicated that this should be able to release all of the loaded drug.
In order to observe the release of dexP from drug loaded (active or passive) VPP PEDOT samples, 50-mL falcon tubes were used as vessels containing 20 mL PBS as release medium and kept in a shaking water bath operating at 37°C and 100 rpm. For the initial drug release experiments via CV, release samples were collected at 24, 25, 48, 49 and 72 h (replaced with fresh PBS) with the CV stimulus applied at 24 and 48 h. However, some drug release was observed before the application of the stimulus. Therefore, additional timepoints were added before and after the application of the stimulus to obtain a better understanding of the release trend. For the 17-min release stimulus, samples were taken at 1, 2, 6, 23, 24, 26, 27, 47 and 48 h with the stimulus applied at 24 h for stimulated samples. For the multi-day release, release samples were collected at 1, 2, 6, 23, 24, 26, 27, 47, 48, 50, 51, 71, 72, 74, 75 and 96 h with a release stimulus of 2 min applied at 24, 48 and 72 h for stimulated samples.

A 200 µL sample volume was collected for all drug release experiments and replaced by fresh PBS at each timepoint. All release media samples were collected and centrifuged at 11,400 rpm at 20°C for 10 min before analysing the supernatant using the validated HPLC method described in Chapter 2.

### 4.4.5 Physical entrapment of dex and dexP

Dex was loaded into the pores by direct application of the drug solution (12.5 mg/mL in a methanol-PBS mixture, 50:50 v/v). A volume of 215 µL was applied onto each porous VPP PEDOT film and air-dried overnight (total drug loaded: 2.68 mg). To seal these structures, the oxidant solution (composition of this solution as described in Section 3.4.2) was spin-coated on top and then polymerised for 3 h (Figure 4.3). To optimise the sealing of dex in the pores, this process was repeated 3 or 5 times in order to form either 3 or 5 sealing layers on top of the dex-loaded VPP PEDOT porous films. These sealed films were then washed with methanol, and drug release studies were performed in 50-mL falcon tube vessels placed in a shaking water bath operating at 100 rpm and 37°C. Each tube
Drug loading and release from PEDOT structures contained 20 mL of PBS/methanol (50:50, v/v) as release medium. Samples were taken at 1, 2, 6, 23, 24, 26, 27, 47 and 48 h (sample volume: 200 µL, replaced with fresh methanol/PBS) while a stimulus of ±1 V at 0.5 Hz was applied as a continuous pulse for 17 min at 24 h for stimulated samples.

Figure 4.3: Schematic diagram of loading drug into the pores and sealing of the VPP PEDOT porous structures.

In order to also observe the behaviour of a hydrophilic drug loaded into the pores, dexP solution (40 mg/mL in PBS) was applied directly onto the porous VPP PEDOT films. A volume of 215 µL was applied and dried overnight (total drug loaded: 8.6 mg). To seal these dexP-loaded films, the same procedure was performed as described earlier for dex to obtain five sealing layers (Figure 4.3). The sealed films were washed with MilliQ, and drug release was carried out in exactly the same way as for dex, except that PBS was used as the release medium for dexP instead of PBS/methanol (50:50, v/v). As all of the loaded drug was assumed to be incorporated into the pores during physical entrapment, drug release was expressed as a cumulative % over time rather than an absolute value as for other parts of the study where the amount of drug loading was unknown.

4.4.6 PEDOT membranes as drug release rate controllers

To further evaluate the permeability of PEDOT layers, for example as sealing layers on top of the porous film, PEDOT-coated cellulose membranes were prepared (Section 3.4.2.2) and analysed. The
membranes were evaluated in Franz-cells to separate the donor and receiver compartments (Figure 4.4). DexP solution in PBS (40 mg/mL) was used in all experiments in the donor compartment (volume: 3.5 mL). Freshly prepared PBS was used as the release medium in the receiver compartment (volume: 11.8 mL). As membranes were prepared by polymerisation of PEDOT on top of cellulose membranes, a comparison was carried out between simple and PEDOT-coated cellulose membranes.

![Figure 4.4: Franz-cell setup with a PEDOT-coated cellulose membrane separating the donor and receiver compartments to study the permeation of drug through the membrane.](image)

In order to evaluate the effect of different redox states on drug permeation across PEDOT-coated cellulose membranes, these membranes were used ‘as prepared’ or either oxidised (kept at +1.4 V for 2 min) or reduced (kept at -0.9 V for 2 min). Samples (volume: 200 µL, replaced with fresh PBS) were collected at 1, 2, 3, 4, 5, 6 and 24 h. Further analysis of the surface morphology of ‘as prepared’, oxidised and reduced PEDOT-coated cellulose membranes was performed on a bench-top SEM as per the methodology described in Section 3.4.3.3. As the total amount of drug loaded into the donor compartment was known, the amount of drug permeated was expressed as a %age of the total drug loaded.
4.5 Results and discussion

4.5.1 Drug solubility in release media
The saturated solubility of dexP in PBS was calculated to be $40 \pm 0.5$ mg/mL, which is far in excess of the amount of drug released, thus allowing us to conclude that sink conditions were maintained during the course of the release studies. The previously reported maximum saturated solubility of dexP in water is approximately 50 mg/mL [74, 283]; however, re-crystallisation of dexP was observed while attempting to make the same concentrated solution of dexP in PBS. This could be due to the presence of phosphate ions in the PBS solution, which could compete with phosphate ions present in dexP.

The saturated solubility of dex in PBS/methanol (50:50, v/v) was calculated to be $12.5 \pm 0.3$ mg/mL. The saturated solubility of dex in pure methanol is reported to be 25 mg/mL in the United States Pharmacopeia (USP); therefore, the solubility calculated here in 50% methanol aligns well with this value [205]. The solubility determined here is far in excess of the drug released, allowing us to conclude that sink conditions were maintained during the course of the release studies.

4.5.2 Drug stability in release media
DexP dissolved in PBS and stored at 4, 25 or 37°C was stable up to 168 h (1 week). The amount of dexP remained at $100 \pm 2\%$ for all timepoints. Therefore, PBS was deemed as a suitable release medium for dexP release studies.

Dex dissolved in PBS/methanol (50:50, v/v) and stored at 4, 25 or 37°C was also stable at timepoints of up to 168 h (1 week). The amount of dex remained at $100 \pm 2\%$ for all timepoints. Therefore, PBS/methanol (50:50, v/v) mixture was deemed to be a suitable release medium for dex release studies.

4.5.3 Drug loading and release: Ion-exchange of dexP

4.5.3.1 DexP loading via CV
Ion-exchange has been deemed to be the only way to load dexP as a dopant onto the VPP PEDOT films to substitute tosylate, which is incorporated as a dopant during polymerisation process. This ion-exchange for drug loading was investigated in the presence of a stimulus (active drug loading) as well as in the absence of any stimulus (passive drug loading). Initially, active drug loading was performed to load dexP onto the VPP PEDOT films. CV of porous and non-porous VPP PEDOT has been discussed in Section 3.5.4.3, where significant changes in the CV cycle were observed for up to 40 cycles, after which it stabilised. CV is also a good measure of the extent of ion-exchange; i.e. it stabilises as the exchange of counter-ions in the electrolyte solution reaches an equilibrium with the dopant ions present.
Drug loading and release from PEDOT structures in the polymer structure [230, 284, 285]. Therefore, CV of PEDOT was performed in a 0.1 M dexP aqueous solution for porous and non-porous VPP PEDOT in order to obtain the optimum number of cycles required to exchange tosylate with dexP (Figure 4.5a and b). These cyclic voltammmograms showed a similar trend where they stabilised after 30-40 cycles; thus 50 cycles of CV were deemed sufficient to allow the maximum exchange of tosylate with dexP in the presence of a stimulus (active drug loading).

Interestingly, the cyclic voltammograms of porous and non-porous PEDOT in a 0.1 M dexP aqueous solution (pH 7.3) also revealed that these structures drew higher currents when compared to the CV of the same structures in 0.1 M LiClO₄ solution (pH 7.5) (Figure 3.12). This could be attributed to the slight difference in the pH of both solutions; however, this difference is quite small compared to the difference in the amounts of current being drawn [286]. Therefore, it is more likely attributed to the counter-ions present in the solution. Previous reports have suggested that large counter-ions tend to draw more current than smaller counter-ions, explaining the larger currents drawn by porous and non-porous VPP PEDOT structures in dexP solution compared to the LiClO₄ solution [266]. Furthermore, there was no additional oxidation peak observed at +1.1 V here when compared to the CV of these structures in LiClO₄ solution, aligning well with the CV findings of PEDOT-coated cellulose membranes (as discussed in Section 3.5.4.3). Therefore, it could be concluded that the additional peak at 1.1 V was due to the doping of LiClO₄, which was not observed with other counter-ions. Moreover, the difference in the active electrochemical surface area was calculated to be approximately three times higher in porous compared to non-porous VPP PEDOT films (Section 3.5.4.3). A similar trend was observed here (Figure 4.5) when comparing the area of cyclic voltammograms for the porous and non-porous VPP PEDOT films in a 0.1 M dexP aqueous solution.
4.5.3.2 XPS surface analysis to confirm dexP loading

XPS analysis was carried out to observe the exchange of ions and the surface drug loading of dexP to later compare with the drug release data produced using HPLC quantification. As XPS is a surface technique, any variation in the surface area may affect the results; therefore, XPS analysis was only carried out for non-porous VPP PEDOT films due to the limited penetration (~10 nm) of this technique. Figure 4.6a displays the XPS spectrum of freshly prepared non-porous VPP PEDOT, where the ratio C:O:S was 6.8:2.3:1, respectively. This ratio was well reflective of PEDOT, as reported previously [287]. In comparison, the XPS spectrum of passive dexP-loaded non-porous VPP PEDOT (Figure 4.6b) exhibited a decrease in the percentage of sulphur with the appearance of fluorine and phosphorous peaks (Table 4.2). The decrease in the percentage of sulphur was further noticed when compared with the XPS spectrum of non-porous VPP PEDOT where active dexP loading was performed (Figure 4.6c). This was a clear indication that active drug loading was able to load more dexP onto the VPP PEDOT films as determined by the XPS elemental analysis (Table 4.2).
Drug loading and release from PEDOT structures

Figure 4.6: XPS spectrogram of a) freshly prepared non-porous VPP PEDOT, b) non-porous VPP PEDOT immersed in 0.1 M dexP aqueous solution for 45 min (passive drug loading) and c) non-porous VPP PEDOT after 50 CV cycles in 0.1 M dexP aqueous solution (active drug loading).
Table 4.2: Surface elemental analysis for blank (not loaded), passive and active dexP-loaded non-porous VPP PEDOT films.

<table>
<thead>
<tr>
<th>Element</th>
<th>At %</th>
<th>Element</th>
<th>At %</th>
<th>Element</th>
<th>At %</th>
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<tbody>
<tr>
<td>O 1s</td>
<td>22.81</td>
<td>O 1s</td>
<td>21.21</td>
<td>O 1s</td>
<td>21.92</td>
</tr>
<tr>
<td>C 1s</td>
<td>67.38</td>
<td>C 1s</td>
<td>70.89</td>
<td>C 1s</td>
<td>72.49</td>
</tr>
<tr>
<td>F 1s</td>
<td>-</td>
<td>F 1s</td>
<td>0.90</td>
<td>F 1s</td>
<td>1.36</td>
</tr>
<tr>
<td>S 2p</td>
<td>9.81</td>
<td>S 2p</td>
<td>5.89</td>
<td>S 2p</td>
<td>2.38</td>
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<tr>
<td>P 2p</td>
<td>-</td>
<td>P 2p</td>
<td>1.11</td>
<td>P 2p</td>
<td>1.85</td>
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XPS is a unique technique as it offers the advantage of identifying and quantifying elements in different energy states. Sulphur is present as a sulfonate in tosylate (SO$_3$^-) while in the thiophene ring of EDOT/PEDOT (Figure 4.7) [288]. Therefore, detailed sulphur peak analysis was performed in order to observe changes in the sulphur peak after passive and active dexP loading and compare it to the freshly prepared blank non-porous VPP PEDOT film.

Figure 4.7: Chemical structures of a) tosylate (sulphur present as sulfonate) and b) EDOT monomer (sulphur present in the thiophene ring as sulphide).

Figure 4.8a shows the sulphur peak analysis for freshly prepared non-porous VPP PEDOT. It is clear that the sulphur-related peaks arising from PEDOT are dominant; however, the sulfonate peak from tosylate appeared clearly distinct from the PEDOT peaks. A clear shift in sulphur peaks was observed.
Drug loading and release from PEDOT structures after the active and passive dexP loading due to the replacement of tosylate with dexP (displayed Figure 4.8b and Figure 4.8c).

Figure 4.8: XPS spectrogram peak fitting and quantification for sulphur peaks of a) the freshly prepared, b) passive dexP-loaded and c) active dexP-loaded non-porous VPP PEDOT.
As anticipated, after passive dexP loading via spontaneous ion-exchange of tosylate with dexP was carried out by keeping the non-porous VPP PEDOT film in 0.1 M dexP aqueous solution for 45 min, there was only 34.5% sulphur left in sulfonate form (Figure 4.8b). This shift in the peak area suggested a reduction in tosylate doping of PEDOT in comparison to the freshly prepared PEDOT. However, the overall comparison revealed that there was an increase in doped PEDOT with the disappearance of the neutral PEDOT peak at 159 eV. The higher level of doping could be attributed to the excessive availability of dexP anions in the solution. This correlated well with the surface elemental analysis of potentiometric dexP-loaded non-porous VPP PEDOT, where fluorine (F) and phosphorus (P) peaks appeared (due to dexP doping) and a decrease in the percentage of sulphur (S) was seen.

The active dexP-loaded non-porous VPP PEDOT displayed an even lower proportion of sulfonate (26.8%) when compared to the passive dexP-loaded non-porous VPP PEDOT (Figure 4.8c). Active dexP loading would involve the active exchange of tosylate with dexP on the surface as well as in the bulk of the polymer film. Moreover, the additional sulphur peak at 168 eV could be assigned to the free tosylate [288]. Therefore, it could be concluded that the application of a suitable stimulus exchanged tosylate at the surface as well as in the bulk of the polymer, where tosylate was present in the free-state as sulphonic acid. This clear shift in sulphur peaks indicated that the exchange of tosylate with dexP was carried out successfully and was higher in ‘active’ versus ‘passive’ dexP loading.

4.5.3.3 DexP release via a CV stimulus

Initial experiments used CV as a stimulus to release dexP from drug-loaded porous VPP PEDOT films. Stimulation was achieved for the stimulated samples by the application of 50 CV cycles (equivalent to the drug-loading stimulus) in PBS with the aim of releasing all loaded drug; however, another burst of drug release was observed at the application of a second 50 CV stimulus, implying that there was still drug remaining after the application of the first 50 CV cycles. The inability of a single 50 CV cycles stimulus to release all the loaded drug (keeping in mind that 50 CV cycles were used for drug loading) could be attributed to the competitiveness of the ion-exchange mechanism here, which could differ for different counter-ions present in the solution as well as for different dopant molecules loaded onto the polymer. As discussed in Section 1.6.3.1, small dopant molecules are easily expelled out of the polymer during ion-exchange, whereas large dopant molecules are relatively difficult to expel [168]. Therefore, these findings suggested that during drug loading tosylate was easily expelled from the polymer structure. However, due to the high concentration of dexP in the drug loading solution (0.1 M dexP aqueous solution), dexP was readily available to be loaded onto the polymer structure. On the other hand, the release of dexP could be different due to the large size of this dopant molecule making it harder to be expelled out of the polymer, as well as the nature and concentration of the counter-ions present in PBS. Figure 4.9 shows another burst in dexP release upon the application of a second 50 CV cycles stimulus. However, the total amount of drug released against each 50 CV cycle stimulus decreased upon the application of a second 50 CV cycles.
Figure 4.9: Drug release from the active dexP-loaded porous VPP PEDOT films. A burst of drug release was observed upon the application of a stimulus represented by lightning bolts (50 CV cycles from -0.6 V to 0.9 V at 100 mV/s) at 24 and 48 h. Release from non-stimulated films is included for reference (values represent average ± SD, n=2).

As described previously, the CV is a good measure of the extent of ion-exchange. The CV of porous VPP PEDOT during release revealed that these films remained relatively electrochemically stable over the period of the study from 10 up to 50 cycles (Figure 4.10). In addition, there was only one broad oxidation peak and one broad reduction peak; however, no additional peaks were observed during the course of drug release via CV. This could be due to the parameters for CV (-0.6 V to 0.9 V) where PEDOT showed only one oxidation and one reduction event. The relatively stable CV during drug release confirmed that dexP was only expelled out of the polymer during the initial CV cycles, after a while, the counter-ions present in PBS were loaded and released upon oxidation and reduction of the polymer, respectively. This could be the reason why less amount of drug was released by the application of a second 50 cycles CV stimulus when compared to the first 50 CV cycles stimulus.
4.5.3.4 Effect of redox state on dexP release

In order to optimise the release stimulus, the effect of different redox (oxidation and reduction) states on the drug release from active dexP-loaded porous and non-porous PEDOT films was observed and compared with the drug release in response to a continuous pulse stimulus. Figure 4.11a shows the dexP release from the oxidised samples. There was no further drug release observed when an oxidation potential of +1 V was applied continuously for 30 min. This showed that the application of an oxidation potential can hold the dopant (dexP) inside the polymer structure, minimising the release of dexP from both porous and non-porous VPP PEDOT structures. Similar trends have previously been reported where the application of an oxidation potential was able to hold the release of the loaded dopant molecule from the polymer structure, with a burst in drug release observed when a reduction potential was applied [148, 206].

Figure 4.11b shows the dexP release from the reduced VPP PEDOT films. As expected, the application of a -1 V reduction stimulus pushed the drug out of both porous and non-porous VPP PEDOT films. Interestingly, the surge in drug release was noticeable only within 5 min after the application of the reduction stimulus in porous VPP PEDOT films, after which there was no further increase in the amount of drug released. On the other hand, for non-porous VPP PEDOT films, a gradual increase was observed in the amount of drug released for up to 15 min after the application of
Drug loading and release from PEDOT structures

reduction stimulus. The rapid release from porous VPP PEDOT films could be attributed to the higher surface area where released drug rapidly diffused out of the polymer into the release medium. Overall, more dexP was released from the porous VPP PEDOT films when compared to non-porous VPP PEDOT films. Again, this could be attributed to the higher surface area resulting in a higher interface between the release medium and the porous VPP PEDOT film surface, thus more drug being readily pushed out from the polymer surface into the release medium. Comparatively, as a smaller surface area is exposed to the release medium for non-porous VPP PEDOT films, thus drug expelled of the polymer surface may also be less when compared to porous VPP PEDOT films.

Figure 4.11: Drug release from active dexP-loaded porous and non-porous VPP PEDOT films, an application of a) oxidation stimulus (+1 V) for 30 min holding the dexP release in comparison to when no stimulus was applied, b) a reduction stimulus (-1 V) able to release the drug faster in comparison to when no stimulus is applied and c) a continuous pulse stimulus (±1 V at 0.5 Hz) for 30 min released higher amounts of dexP when compared to reduction stimulus (values represent average ± SD, n=3).
Figure 4.11c shows the release of drug upon the application of a pulse stimulus (±1 V at 0.5 Hz), where most of the loaded drug was released within 15 min from non-porous VPP PEDOT films while a comparatively faster release rate was observed from porous VPP PEDOT films, releasing most of the loaded drug within 10 min. However, it took almost 15 min for the drug to reach peak levels for both porous and non-porous VPP PEDOT films, which could be due to the time taken for the drug to diffuse out of the polymer and into the release medium. The quick movement of dexP out of the porous VPP PEDOT suggests that a higher surface area may produce a quicker release response upon application of the stimulus due to the higher interface between the medium and the polymer, especially when dexP molecules diffuse out of the polymer bulk and into the medium. A similar trend was observed from Figure 4.11b, where upon the application of the reduction stimulus, porous films exhibited a much higher burst within 5 min releasing more dexP out of the polymer compared to non-porous films.

A comparison of the amount of drug released in response to each stimulus i.e. oxidation, reduction or pulse for up to 15 min after the application of the stimulus is shown in Figure 4.12. This duration was selected because there was no further dexP released after 15 min from any in-situ stimulated samples (Figure 4.11). Two-tailed unpaired Student’s t-tests were performed in order to determine the significant difference in the amounts of drug released upon the application of reduction, oxidation or pulse stimulus and to compare porous and non-porous films. There was no significant difference between porous and non-porous films; they both behaved similarly against each stimulus. However, significant (p-value <0.05) differences were observed between oxidised, reduced and pulse-stimulated samples (Figure 4.12).
Drug loading and release from PEDOT structures

Figure 4.12: Comparison of the amount of drug released for up to 15 min after the application of each stimulus. Capital letters identify differences between porous samples and lower-case letters identify differences between non-porous samples (p-values included for relevant comparisons of significance).

4.5.3.5 DexP release via a pulse stimulus

The only available way to determine DexP loading (achieved via ion-exchange) from these VPP PEDOT structures was by releasing all loaded drug. Therefore, in order to obtain the amount of drug loaded onto the VPP PEDOT films, a ±1 V continuous pulse stimulus was chosen and applied at a frequency of 0.5 Hz for 17 min, based on the fact that application of a continuous pulse provided the maximum drug release in Section 4.5.3.4. Furthermore, this stimulus would cycle the DexP-loaded samples in between the positive and the negative potential 500 times, which was 10 times more cycling between the positive and the negative potential when compared to the active DexP loading stimulus. This should have ensured that all of the loaded DexP would be exchanged with the ions available in the release medium; thus drug loading could be determined via releasing all of it.

As observed with XPS (Section 4.5.3.2), DexP loading was confirmed for the passive DexP loaded non-porous VPP PEDOT samples. Therefore, a 17-min pulse stimulus was applied to quantify DexP-loaded onto these structures. Figure 4.13a shows that 20 ± 10 µg of DexP was released upon the application of this stimulus. This confirmed that successful passive DexP loading was achieved for the non-porous VPP PEDOT films. In order to determine the effect of surface area on passive DexP loading, the selected stimulus here was then applied to passive DexP-loaded porous VPP PEDOT films. Figure 4.13b shows that 50 ± 34 µg of DexP was released from these samples after the application of the stimulus. This difference in the amounts of DexP loading could be attributed to the difference in the
surface area, where a greater surface area was exposed for the passive exchange of tosylate with dexP with the porous VPP PEDOT films when compared to non-porous VPP PEDOT films, even though the geographical area (3.75 cm², dimensions: 2.5 x 1.5 cm) remained equivalent. Previous reports of ion-exchange resins have shown that an increase in the surface area leads to higher ion-exchange in porous structures [289], which explains the higher passive drug loading in the porous VPP PEDOT films. This also aligns well with the CV findings where the active electrochemical surface area calculated for porous VPP PEDOT films was almost three times more when compared to non-porous VPP PEDOT films (Section 3.5.4.3). However, the high standard deviation for the passive dexP loaded porous VPP meant that statistically there was no significant difference ($p$-value = 0.12) in drug loading when compared to passive drug loaded non-porous PEDOT.

The amount of dexP loaded with passive loading was expected to be less compared to the active loading, which was indicated by the XPS analysis (Figure 4.8). Therefore, the selected stimulus was applied to determine the exact drug loading for active dexP-loaded non-porous VPP PEDOT films. Figure 4.13c shows the amount of drug loading via active means to be $140 \pm 20 \, \mu$g. When compared to the passive dexP-loading for non-porous VPP PEDOT films (Figure 4.13a), there was a significant (seven-fold) increase in the amounts of drug loading via active means ($p$-value < 0.0001). This aligns well with the previous reports where application of a redox stimulus could enhance the active exchange of ions between the polymer and the counter-ion solution, even at lower concentrations of counter-ions, when compared to the passive ion-exchange [230]. However, here the concentration of counter-ions was kept constant (0.1 dexP aqueous solution) for both passive and active dexP loading, which indicated that the increase in the amounts of drug loaded via active dexP loading could only be attributed to the active loading stimulus (50 CV cycles from -0.9V to +1.4 V at 100 mV/s).
Drug loading and release from PEDOT structures

In order to compare active dexP loading between the porous and non-porous VPP PEDOT films, the selected stimulus was also applied to the active dexP-loaded porous VPP PEDOT films. Figure 4.13d shows the amount of drug released from the active dexP-loaded porous VPP PEDOT films over 48 h. The amount of drug loading was calculated to be 140 ± 17 µg; this was comparable to the amount of the drug loading achieved for non-porous VPP PEDOT (Figure 4.13c) with no significant difference (p-value= 0.84). These results showed that active drug loading was not dependent on the surface area; however, it could be influenced by the total amount of polymer formed, as there should be one dexP molecule for every three monomer units. Therefore, it could be assumed that same quantity of polymer was formed for the porous and non-porous PEDOT films after 3 h of polymerisation (as detailed in Section 3.4.2); thus, 50 CV cycles should load the same amount of drug irrespective of the surface area given that a saturated level of drug loading is achieved via facilitated drug loading. However, a difference in passive drug loading observed between non-porous and porous VPP PEDOT films indicated that passive ion-exchange could be affected by the surface area.

The total amount of dexP released was ~140 µg for both non-porous and porous films after the application of a 17-min pulse stimulus (Figure 4.13c and Figure 4.13d). In comparison, a total of 118 ±
9 µg of dexP was released upon the application of a continuous in-situ pulse stimulus for porous films and 109 ± 11 µg for non-porous films (Figure 4.11). This could be attributed to the amount of time given for the released drug to diffuse out of the polymer and into the release medium. It is apparent from the previous comparison of porous and non-porous films that porous films release drug much faster into the release medium when compared to non-porous films (Figure 4.11). However, when a release stimulus is applied, dexP molecules move from the bulk of the polymer film to the surface due to the electrostatic expulsion from the polymer backbone. Therefore, additional time may be required for the all the expelled drug molecules from the polymer to diffuse into the release medium. This additional time was given for drug release samples in Figure 4.13c and Figure 4.13d; however, that was not the case for the continuous application of in-situ pulse stimulus (Section 4.5.3.4).

4.5.3.6 DexP release via multiple pulse stimuli

In order to observe multi-day release from the actively loaded dexP films, a stimulus of ±1 V was applied for 2 min as a continuous pulse at 0.5 Hz. Figure 4.14 shows the drug release from the porous and non-porous active dexP-loaded films over a period of four days. Interestingly, the amount of drug released by porous and non-porous films was comparable at the end with both releasing almost 40 µg. However, the pattern of drug release was different. Drug released from the porous VPP PEDOT films seemed to diffuse out of the polymer before the next sampling time (within 2 h) when compared to non-porous VPP PEDOT films, where it took longer for the drug molecules to diffuse into the release medium (3 h). This aligned well with the previous findings here, where dexP molecules were expelled out during the application of a stimulus as a higher surface area provided more exposure to the release medium, thus dexP diffused out faster.

A decrease in the amount of drug released against the application of each stimulus was previously observed with the CV, with ~50 µg released against the first 50 CV cycles stimulus and ~20 µg of dexP released against the application of a second 50 CV cycle stimulus (Figure 4.9). A similar trend was observed here with the application of a pulse stimulus for 2 min, with a total of 40 µg of drug released upon the application of the stimulus at three different timepoints. However, a decrease in the amount of drug being released against each stimulus was observed, with the exception of the 72-h timepoint for non-porous VPP PEDOT films. Such release trends have previously been reported, where a gradual loss in the amount of dexP release was observed when dexP was incorporated into the PEDOT structure as a dopant and multiple release stimuli were applied to release the dopant drug [206]. This could be due to the exchange of dexP with the counter-ions present in the release medium (PBS) during the initial stimulus application, where subsequent stimulus applications would release these PBS counter-ions as well as dexP, thus favouring this gradual loss in dexP release. Moreover, an extended application of the release stimulus would only be able to release the loaded drug, as determined by the application of 17-min pulse stimulus in Section 4.5.3.5. However, the amount of drug released as a function of the stimulus applied at 24, 48 and 72 h was not significantly different for porous VPP PEDOT with p-values of 0.05, 0.70 and 0.93 respectively as well as for non-porous VPP PEDOT with p-values of 0.07, 0.69 and 0.21, respectively compared to unstimulated films. This shows that a 2-min pulse stimulus may be insufficient to release significant amounts of drug.
Drug loading and release from PEDOT structures

Figure 4.14: Multi-day drug release from active dexP-loaded a) non-porous and b) porous VPP PEDOT films. Release from non-stimulated samples is also included for comparison. A stimulus of ±1 V was applied at 24, 48 and 72 h for stimulated samples, as a continuous pulse at 50 Hz for 2 min represented by lightning bolts (values represent average + SD, n=3).

4.5.3.7 Mechanism of drug release

The results obtained here led to the hypothesis about the proposed mechanism of dexP release (loaded as a dopant molecule). DexP release is dependent upon the stimulus applied, the duration for which the stimulus is applied and the surface morphology of the polymer. Figure 4.15 shows the release of dopant molecules upon the application of different stimuli. An oxidation stimulus holds the dopant molecules strongly at the surface as well as within the bulk of the polymer due to electrostatic attraction. Therefore, no dopant molecules are released happens upon the application of an oxidation stimulus (Figure 4.11a and Figure 4.15a). On the other hand, a reduction stimulus expels the dopant molecules present at the surface of the polymer into the release medium due to the electrostatic repulsion; however, the dopant molecules bound to the bulk of the polymer seems to remain within the polymer structure (Figure 4.15b). This was observed by the surge in dexP release upon the application of the reduction stimulus, resulting in a rapid release of dexP within 5 min from porous VPP PEDOT films. However, comparably slow release rate and lower amounts of dexP were released out of the non-porous VPP PEDO; both of these could be attributed to the smaller surface area of non-porous VPP PEDOT films ultimately leading to a smaller polymer-release medium interface (Figure 4.11b). Furthermore, the application of a pulse stimulus cycles the polymer between a negative and a positive potential; therefore, dopant molecules are released from the surface as well as bulk of the polymer (Figure 4.15c). However, it may take longer for the non-porous VPP PEDOT films to release all of the dopant molecules into the release medium due to the smaller polymer-release medium interface. On the other hand, a greater polymer-release medium interface would facilitate the fast release of dopant molecules from the porous VPP PEDOT films into the release medium, as observed in Figure 4.11c.
The effect of the duration for which a stimulus is applied to release dexP has been discussed in Sections 4.5.3.3 to 4.5.3.6. Noticeable drug leakage was also observed for dexP loaded as a dopant as seen in Figure 4.9, Figure 4.11, Figure 4.13 and Figure 4.14. This dexP leakage could be attributed to the tendency of the CP to equilibrate with the surrounding medium, i.e. in this case the synthetic release medium [290]. Therefore, some dexP anions are passively exchanged with the phosphate anions present in the release medium. Such leakage may be unavoidable, even \textit{in-vivo}; however, the application of a constant small oxidation potential may be able to avoid this passive exchange as shown in Figure 4.11a and ultimately reduce the leakage of dexP from these PEDOT structures. Figure 4.14 also shows that fine control over the amount of dexP release against the application of each stimulus was not achieved however, such control would be required for the long-term clinical application of these structures in the final implant.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{Figure4_15.png}
\caption{Mechanism of release for dopant (dexP) molecules (represented here by circled dots) from the dexP-loaded VPP PEDOT films: a) no dexP release observed for the oxidation stimulus, b) dexP released from the surface only, for the reduction stimulus and c) dexP released from the surface and bulk of the polymer for the pulse stimulus.}
\end{figure}
4.5.4 Physical entrapment of dex and dexP

DexP loading and release attempted via ion-exchange showed promising results for stimuli-responsive release from the fabricated polymer structures. However, the amount of drug loaded was limited to the degree of doping in the polymer. Therefore, the ability to increase the amount drug loaded by physically entrapping another drug into the pores was explored; dex was physically entrapped into the pores by applying dex solution (12.5 mg/mL) onto the pores. The amount of dex loaded was 2.68 mg by applying 215 µL of this solution onto the porous VPP PEDOT films (geographical area: 3.75 cm²). These dex-loaded samples were then sealed by polymerising PEDOT on top of the porous PEDOT films via VPP. During drug release, it was observed that only 60% of the loaded dex was released from these films, all within the first hour. Figure 4.16 shows that no further release was observed following the 1 h mark, even after the application of a stimulus at 24 h. This prompted the investigation to observe drug loss during spin-coating for sealing.

![Cumulative release vs. time](image)

Figure 4.16: Dex base release from the sealed porous VPP PEDOT films. At 24 h a continuous pulse of ±1 V was applied at a rate of 0.5 Hz for 17 min represented by a lightning bolt; however, no increase in drug release was observed (values represent average + SD, n=3).

To estimate the potential loss of drug during the sealing procedure via spin-coating, dex-loaded porous VPP PEDOT structures were spin-coated with oxidant solution (composition described in Section 3.4.2), dried and then immersed in the release medium to measure the remaining drug. In order to restrict the leakage of loaded dex out of the pores, the number of sealing layers was later increased to five. Table 4.3 shows that ~40% of the drug was lost during spin-coating and the remaining ~60% was released within the first hour—even with five sealing layers.
Table 4.3: Physically entrapped drug lost during the spin-coating process and leaked during the first hour in the release medium (values represent average ± SD, n=3).

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<th>Dex</th>
<th>DexP</th>
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<td>% lost during spin-coating</td>
<td>39.8 ± 1.6</td>
<td>32.6 ± 1.7</td>
</tr>
<tr>
<td>% released within the first hour</td>
<td>60.6 ± 8.3</td>
<td>67.3 ± 1.7</td>
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These results indicated that a high proportion of the dex loaded into the pores was lost during the spin-coating process. In addition, the highly permeable nature of PEDOT sealing layers resulted in all the drug being released within the first hour. Due to the solubility of dex in organic solvents, this drug may have dissolved in the organic solvent of oxidant solution and was then lost during the spin-coating process. Therefore, dexP was loaded into the pores to observe the behaviour of a hydrophilic drug in the pores. Table 4.3 also shows that almost ~33% of the dexP loaded into the pores was lost during the spin-coating process and ~67% was released during the first hour from the five-layer sealed structure. When compared to dex, these numbers were not significantly different, which suggested that the loss of drug loaded into the pores during spin-coating was not related to the chemical nature of the drug and could be attributed to the physical displacement with the oxidant solution during spin-coating. The high permeability of these drugs through up to five sealing layers suggested that VPP PEDOT may not be able to hold the drug in its pores. Therefore, the permeability of PEDOT as a layer was further investigated using PEDOT-coated cellulose membranes.

### 4.5.5 Drug permeation through PEDOT-coated cellulose membranes

Cellulose membranes are commonly used in conjunction with Franz-cells to evaluate the permeability of a drug from the donor to the receiver compartment. Coating PEDOT onto the cellulose membrane offers a good way to evaluate the permeability of drugs across this polymer in comparison to a plain cellulose membrane. Drug release through PEDOT-coated cellulose membranes was also carried out under different redox states in order to evaluate the effect of reduction/oxidation on the permeability of drugs through PEDOT. Figure 4.17 shows the permeation of dexP across PEDOT-coated cellulose membranes in different redox states compared to the plain cellulose membranes. It was observed that all drug permeated through plain cellulose within 2 h. On the other hand, approximately 30% of drug permeated through the PEDOT-coated cellulose membranes. There was no significant difference between the freshly prepared, reduced or oxidised PEDOT-coated cellulose membranes. However,
Drug loading and release from PEDOT structures

passive ion-exchange observed for dexP loading has been observed previously, which suggests that the redox state of the polymer is not static. Therefore, a brief oxidation or reduction event cannot be expected to influence the release over several hours.

Figure 4.17: Drug permeation across PEDOT-coated cellulose membranes ‘as prepared’, reduced (Red) or oxidized (Ox) as well as across plain cellulose membranes (values represent average + SD, n=3).

The possibility of crack development with the application of CV has been briefly discussed in Section 3.5.5. Therefore, these PEDOT-coated cellulose membranes were examined after removal from the Franz-cell under a bench-top SEM to observe any structural changes in response to the reduction or oxidation stimuli as well as any other morphological changes during the drug permeation experiments. Figure 4.18a shows that there were no significant morphological changes in the membrane after the drug release study for the ‘as prepared’ PEDOT-coated cellulose membrane. However, minor cracks on the upper surface of the PEDOT-coated cellulose membranes after the drug permeation experiments were observed for both oxidised and reduced membranes (Figure 4.18b and c), which could explain the slightly faster permeation rate through these membranes when compared to ‘as prepared’. Therefore, it could be concluded that even though no morphological changes were observed, the intact PEDOT-coating over cellulose membranes still offered high permeability to dex and dexP, resulting in fast permeation of these drugs out of the sealed porous structures.
Figure 4.18: SEM image of a) ‘as prepared’, b) oxidised and c) reduced PEDOT-coated cellulose membranes after drug permeability studies. Minor cracks (as highlighted with white arrows) on the upper surface of ‘oxidised’ and ‘reduced’ PEDOT-coated cellulose membranes were observed.
Drug loading and release from PEDOT structures

In order to quantify the permeability of dexP through these membranes, the permeability coefficient was also calculated by Fick’s diffusion law [291]:

\[ P = \frac{J_s}{A \times \Delta C} \]

Where,

- \( J_s \) is the “steady state permeability flux” in mg/s
- \( P \) is the “permeability coefficient” in cm/s
- \( \Delta C \) is the difference in the concentration of permeate in mg/mL
- \( A \) is the area for permeability in cm\(^2\)

The steady state permeability flux was calculated from the data displayed in Figure 4.17, where the permeability of dexP has been plotted versus time. The area of permeation was calculated to be 1.77 cm\(^2\) (diameter: 1.5 cm). For cellulose, 55% of the drug permeated through during the first hour and the total amount of drug in the donor compartment was 140 mg (in 3.5 mL of 40 mg/mL). At the end of the permeability studies where there was no more concentration difference, the final concentration of drug in the donor and receiver compartment reached 9.15 mg/mL at 100% release. Therefore, 59 mg (to give a concentration of 5 mg/mL in the donor compartment) of dexP permeated through the plain cellulose membranes in 1 h compared to only 5.31 mg (to give a concentration of 0.45 mg/mL in the donor compartment) for ‘as prepared’ PEDOT-coated cellulose membranes (average permeation rate of 5% per h for ‘as prepared’), which gave a permeation flux of ~0.0163 mg/s for cellulose and 0.0014 mg/s for PEDOT-coated (five layers) cellulose. Thus, the permeability coefficient was calculated to be:

\[ P (Cellulose) = 2.22 \times 10^{-3} \text{ cm/s} \]

\[ P (PEDOT - coated cellulose membrane 'as prepared') = 0.09 \times 10^{-3} \text{ cm/s} \]

4.6 Conclusion

Drug loading of dexP via ion-exchange was successful, with higher active dexP loading achieved compared to passive dexP loading. The exchange of tosylate with dexP by passive and active dexP loading was further investigated in detail via XPS which showed a shift in the sulphur peak area from higher to lower levels of tosylate (sulfonate) after exchanging it with dexP. DexP release studies revealed no drug release upon the application of an oxidation stimulus and a burst of release upon the application of a reduction stimulus; however, release occurred faster and to a greater extent upon the application of a pulse stimulus. Porous VPP PEDOT structures showed a faster response in terms of drug release against a pulse stimulus than non-porous structures due to the higher surface area exposed to the release medium. Multi-day drug release studies showed a gradual loss in the amount of drug released with each stimulus application. This was possibly due to limited ion-exchange at the surface of the polymer, which was in agreement with previous studies. This issue would prevent such
Drug loading and release from PEDOT structures

an implant to be used clinically if reproducible and finely tuned release cannot be achieved. In addition, the amount of total drug loaded was also limited via ion-exchange. In an effort to increase the overall drug loading, porous VPP PEDOT structures were also explored for entrapment of neutrally charged dex into the pores. However, ~40% of the loaded drug was lost during the spin-coating process used for sealing. VPP PEDOT sealing layers were unable to hold the drug inside the pores; therefore, the permeability of PEDOT sealing layers was further investigated using PEDOT-coated cellulose membranes. The calculated permeability of PEDOT-coated cellulose membranes revealed that these membranes allow fast permeation of the drug, thus being highly permeable to dex or dexP. Therefore, other means need to be investigated to reduce the permeability of these sealing layers in order to eliminate the unwanted leakage of entrapped drugs.
CHAPTER 5: OCULAR IMPLANT PROTOTYPE

5.1 Introduction

Ocular implants available on the market or in the pipeline have been discussed in detail in Section 1.4. Non-biodegradable implants such as Vitrasert and Retisert, which provide sustained drug delivery to the posterior segment of the eye, are usually inserted through the pars-plana region and anchored to the sclera through a suture (with the exception of non-biodegradable Iluvien, which is injected directly into the vitreous) [27]. The physical dimensions of such implants depend upon the polymer composition, the method of fabrication, the amount and the density of drug loaded, and the duration of drug release. Vitrasert, the first FDA-approved implant (1996) for sustained drug delivery to the back of the eye, is 8 mm long, 2.5 m thick and 3.5 mm wide [292]. Retisert, which was developed later (approved by the FDA in 2005), is fabricated using the same platform technology but is smaller (5 mm long, 2 mm thick and 3 mm wide) [293]. Vitrasert contains 4.5 mg of ganciclovir, while Retisert is loaded with only 0.59 mg of fluocinolone due to the higher potency of this drug, which also enables Retisert a longer duration of action (up to 30 months) compared to Vitrasert (up to 8 months). As there have been no vision interferences reported for these implants, future implants intended to be placed in the same region of the eye could be fabricated with similar dimensions.

Currently available implants such as Vitrasert and Retisert are fabricated using non-biodegradable polymers (PVA, EVA, silicon or a combination of these polymers) through extrusion, compression/injection moulding, solvent casting or a combination of these techniques [294]. 3D printing, on the other hand, has recently gained popularity for the fabrication of implantable devices using either biodegradable or non-biodegradable polymers [294-296]. 3D printing offers the advantage of precise control over the fabrication process and has gained popularity as the ‘go-to’ technique for the quick fabrication of prototype devices over conventional techniques previously employed for device fabrication [297]. Polylactic acid (PLA) is currently the most popular biocompatible FDA-approved polymer to fabricate a solid device via 3D printing [298, 299]. This polymer could be employed to fabricate a biocompatible prototype device via 3D printing where CP structures are placed inside a solid implant casing for the purpose of stimuli-responsive drug delivery. However, for the purpose of an implant intended to be placed in the eye to release drug over an extended period of time, a non-biodegradable polymer (such as PVA, EVA or silicon) should be used where CP structures would stay inside the solid implant casing and would never be in contact with the surrounding tissue. Therefore, to develop and fabricate a prototype device here, PLA was chosen only for the purpose of this study as a model polymer due to its ease in fabrication via 3D printing. As presented in Chapter 4, leakiness and low levels of drug loading would limit the use of this implant for extended periods of time and thus
restricted the release studies from advancing into the \textit{ex-vivo} bovine eye model. Although the release profile may differ in bovine vitreous humour due to the various vitreous components (osmolality, proteins etc.), the implant casing was still designed and assembled with emphasis only to test the functionality of the implant assembly in synthetic medium and to observe any interference of the casing with the drug release pattern seen in Chapter 4:

\textbf{5.2 Aim and objectives}

The aim of the work presented in this chapter was to fabricate a prototype implant and evaluate its functionality. Specific objectives were:

- To design a prototype implant and 3D print the implant.
- To assemble the 3D-printed implant and assess the \textit{in-vitro} functionality of this assembly as a ‘proof of concept’ device.
- To observe dexP release from this implant upon the application of an \textit{in-situ} stimulus.

\textbf{5.3 Materials}

PLA was kindly donated by Clariant Inc., NZ. 3D printing was carried out on a UP Plus 3D printer purchased from 3D Printing Systems, NZ. All other materials have already been described in Chapter 3 (Section 3.3). Water repellent adhesive (Selleys Inc., NZ) was purchased from a local hardware store.

\textbf{5.4 Methods}

\textbf{5.4.1 Implant design}

AutoCAD (Autodesk Inc., USA) was used to design the prototype implant for \textit{in-vitro} studies (Figure 5.1). The dimensions of this prototype implant were adopted from Vitrasert and Retisert. However, for the purpose of the current \textit{in-vitro} and any future animal \textit{ex-vivo} release studies (out of the scope of this PhD work); these dimensions were adjusted accordingly due to the size differences between the human and the bovine eye (the \textit{ex-vivo} model selected for future studies). This prototype implant was 8 mm long, 3.5 mm wide and 4 mm thick.
5.4.2 Drug loading

Template-based PEDOT porous structures were fabricated as described in Section 3.4.2.1 and placed on a stainless-steel sheet. Drug loading was performed in 0.1 M dexP aqueous solution via active dexP loading only, according to the methodology described in Section 4.4.4.1. Drug-loaded porous VPP PEDOT-containing stainless-steel sheet was cut into pieces to fit the inner dimension of the 3D-printed prototype implant. Each piece was 7 mm long and 2.5 mm wide (area: 0.175 cm²).

5.4.3 Implant assembly

The prototype implant consisted of two equal halves. For each half, a 1 mm diameter hole was drilled in the centre in order to insert a wire for connecting the working and the counter electrodes. Two drug-loaded porous PEDOT-containing stainless-steel sheet pieces were placed inside each half with one as a working electrode and the other one as a counter electrode. The two halves were then joined together using water repellent adhesive to keep the assembly intact within the release medium (PBS). A connection was made between the electrodes and the stainless-steel sheet by inserting a wire from the outside through the hole in the centre of the implant casing to make contact. This hole was later covered with the water repellent adhesive to fix the wires and avoid any release medium entering through the hole. The entire implant assembly process is presented in Figure 5.2.

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**Figure 5.1:** AutoCAD design of the implant prototype a) one-half and b) both halves joined together.

**Figure 5.2:** Schematic diagram of prototype implant assembly: a) one-half of the 3D-printed implant, b) fitted with the drug-loaded porous VPP PEDOT structure on the stainless-steel sheet, c) both halves of the implant joined together with the square opening allowing release medium to reach the drug-loaded
PEDOT structures and d) connections were established between the stainless-steel sheet pieces and the working and counter electrode wires.

### 5.4.4 Drug release from prototype implant

Drug release was performed in a three-electrode, one-compartment setup where active dexP-loaded porous VPP PEDOT films were used as the working and the counter electrodes and Ag/AgCl was used as a reference electrode. Assembled implants were put inside 50-mL Falcon tubes containing 20 mL of 0.01 M PBS (pH 7.4) (Figure 5.3a). Although the volume of vitreous humour in the human eye is 4-5 mL, the volume of synthetic medium used here was selected in accordance with the volume of bovine eye vitreous humour (~ 20 mL) as this implant would initially be assessed in an ex-vivo bovine eye setting. The tubes were then placed in a ThermoMixer operating at 300 rpm and 37°C (Figure 5.3b). Release samples (200 µL) were taken every 5 min over a period of 1 h. A pulse stimulus of ±1 V at 0.5 Hz was applied in-situ for the last 30 min of the release study via a Bio-Logic VMP-300 model multichannel potentiostat equipped with EC-Lab® software (Bio-Logic Science Instruments Pvt. Ltd., France).

![Figure 5.3: a) The implant placed inside a 50-mL Falcon tube filled with 20 mL of PBS and b) the entire drug release assembly within the ThermoMixer operating at 300 rpm and 37°C.](image)

### 5.5 Results and discussion

The purpose of the in-vitro release study was to evaluate the implant with dexP-loaded porous VPP PEDOT structures inserted inside the implant and to observe the in-situ stimulation of the implant assembly. PLA, the 3D-printable polymer used to fabricate the prototype implant casing is biodegradable; however, for the purpose of this in-vitro evaluation, this polymer is insoluble in water. Furthermore, during the later stages of implant development, a non-biodegradable biocompatible
polymer (such as PVA or EVA) should be utilised for implant fabrication via conventional techniques for further *ex-vivo* and *in-vivo* evaluations (out of the scope of this PhD work). As discussed in Section 4.5.3.5, dexP loading estimated via ion-exchange was 140 ± 17 µg for a VPP PEDOT film with an area of 3.75 cm². Therefore, the anticipated dexP loading for porous VPP PEDOT structures placed inside the prototype implant was calculated to be 13 ± 1.6 µg (6.5 ± 0.8 µg for each of the two pieces with an individual area of 0.175 cm²), assuming a uniform distribution of ion-exchange loaded dexP within these films. The application of a 30 min *in-situ* stimulus was carried out to release all of the loaded drug.

Result showed that the total amount of drug released from the implant was indeed ~13 µg, as calculated. Furthermore, all of the loaded drug was released within 15 min after the application of the *in-situ* stimulus, similar to porous VPP PEDOT films described in Section 4.5.3.4. Figure 5.4 shows the drug release from these prototype implants, where only 2.1 ± 1.3 µg was released within the first 30 min. After the application of a stimulus, a total of 13.4 ± 1.2 µg was released by the 45-min time point; however, there was no further drug release from these prototype implants up to 60 min. These results aligned well with the estimated active drug loading via ion-exchange; furthermore, they also suggest that the distribution of dexP dopant loaded via ion-exchange was uniform throughout the films. The release pattern observed here upon the application of the *in-situ* pulse stimulus correlated well with the release pattern observed for porous VPP PEDOT (Section 4.5.3.4). However, here it took slightly longer for all of the loaded drug to be released, which could be attributed to the fact that these porous VPP PEDOT structures were placed inside the implant casing. When compared to the *in-situ* dexP release setup described in Section 4.4.4.3, the drug released from the prototype implant may take longer to distribute, from the square opening on the side of the implant, throughout the entire release medium when the release samples were collected.

![Graph showing drug release](image)

Figure 5.4: Drug release from active dexP-loaded porous VPP PEDOT films placed inside the prototype implant where the application of a continuous pulse (±1 V at 0.5 Hz) for 30 min released all of the loaded drug (values represent average + SD, n=3).
These release results revealed that the amount of drug loading achieved via active ion-exchange was insufficient for the long-term treatment of chronic posterior eye conditions. When compared to Ozurdex, which contains 700 µg of dex, the drug loading achieved here was only ~13 µg. Ozurdex is a biodegradable implant; therefore, excessive drug dumping has been observed at the later stages of the implant degradation [51]. However, the dexP loading achieved here would still be insufficient to provide dexP release within the therapeutic range for up to six months. Based on the pharmacokinetic study performed for the Ozurdex implant [72], 13 µg of dexP loading achieved here for the prototype implant would only provide therapeutic drug concentrations for up to a week. Therefore, physical entrapment should be further explored as it may enhance the drug loading for the area of these films (two pieces each with an area of 0.175 cm²) up to 0.8 mg (in the case of dexP) or 0.25 mg (in the case of dex). These calculations are based on the drug loading achieved via physical entrapment as described in Section 4.4.5 for a 3.75 cm² surface area of the film. When compared to Ozurdex, this amount of drug loading from a non-biodegradable implant where drug release is tuned by the inserted PEDOT structure should be able to provide drug release within the therapeutic range for up to six months. However, further research needs to explore ways for efficient sealing of these porous structures.

5.6 Conclusion
An implant prototype was successfully designed, fabricated and assembled for in-vitro evaluation. This implant assembly released the loaded dexP upon the application of an in-situ pulse stimulus, with the amount of drug released aligning well with the anticipated drug loading. Furthermore, drug-loaded films could be employed as the working and the counter electrodes to maximise drug loading and enhance the use of space inside the implant assembly. The implant prototype presented here exhibited good potential to be further employed for ex-vivo evaluations; however, the drug loading should be enhanced by physical entrapment (up to 0.8 mg for dexP or 0.25 mg for dex) and efficient sealing in order to achieve sufficient drug loading for long-term use of this implant. Furthermore, the reproducibility, i.e. a defined amount of drug released against the application of each stimulus to achieve long-term clinical outcomes, is critical for further evaluation and development of this implantable system.
CHAPTER 6: THESIS SUMMARY, FUTURE OUTLOOK AND THESIS CONCLUSION

6.1 Thesis summary

Efficient drug delivery to the posterior segment of the eye for the long-term treatment of chronic conditions presents many challenges and opportunities for formulation scientists [5, 300]. Ocular implants have become an attractive area of research due to these challenges and thus offer further opportunities for the development of innovative technologies to meet long-term drug delivery needs for treating posterior-segment diseases [27, 88, 122]. Implants where drug release can be initiated, terminated, or modified following the application of a stimulus have gained attention as promising drug delivery systems [27, 301]. Such stimuli-responsive implants offer the advantage of tailoring release profiles according to the patient's disease condition, dosage requirements and vulnerability to side-effects. CP are biocompatible organic materials that are able to tune the drug release in response to an electrical stimulus [26, 27, 148, 150, 152, 154, 155]. PEDOT, a robust CP, has exhibited good biocompatibility, reproducibility and long-term in-vivo stability. It has previously been explored as a potential candidate for long-term electrical stimuli-responsive regional drug delivery [176, 231, 252]. This polymer was explored to fabricate an implantable system for on-demand ocular drug delivery during this PhD.

Dex is a potent corticosteroid and dexP is its phosphate salt. Both forms have an established history in treating a number of posterior eye conditions including age-related macular degeneration, diabetic retinopathy and uveitis [78, 79]. Due to its high potency, a relatively low dose is needed in order to achieve a therapeutic response when compared to less potent corticosteroids [81, 82]. However, dex and dexP possess very different physicochemical and pharmacokinetic properties which could be exploited for tailored release [75]; therefore, both of these variants have been explored during this PhD, with dex being the ‘hydrophobic drug’ and dexP being the ‘hydrophilic drug’. Reports for the separation and simultaneous quantification of a hydrophobic drug and its hydrophilic salt using HPLC are scarce. Therefore, an HPLC method was developed and validated in order to be used for the quantification of these drugs during release studies. This method was further employed to evaluate the degradation of these drugs under various stress conditions and successfully separate these drugs from the degraded
products. This was performed to eliminate quantification interferences from any degradation products produced during the fabrication of DDS as well as during the long-term release in human body. Furthermore, the simultaneous extraction and quantification of dex and dexP from vitreous humour was also carried out, which could be useful for quantification during the future ex-vivo studies.

Fabrication of PEDOT structures with high porosity is desired in order to maximise the surface area available for drug loading, as it provides space to physically entrap the drug. This was attempted via ECP and VPP; however, ECP fabrication of porous structures was unsuccessful due to hydrolysis of the electrolyte solution. Therefore, VPP was selected and used as the main polymerisation technique to fabricate highly porous and non-porous PEDOT films. XPS surface elemental analysis was performed to compare ECP PEDOT with non-porous VPP PEDOT. It showed that the chemical composition of non-porous VPP PEDOT was comparable to ECP PEDOT; however, limited penetration of this technique restricted the use to non-porous VPP PEDOT structures. Non-porous and porous VPP PEDOT structures were then further characterised and compared using a variety of techniques. The growth of VPP PEDOT over a PS template was evaluated by AFM, which revealed that the polymer film sinks into the oxidation solution, suggesting that PEDOT films are being imprinted by the deposited template. SEM images revealed that highly porous PEDOT structures were obtained by this fabrication method, indicating the formation of pores. CV was performed to determine the electrochemical behaviour of these structures and revealed that porous PEDOT structures offer a larger electrochemically active surface area, almost three times more than non-porous PEDOT structures. CV also confirmed the reproducible electroactive nature of non-porous and porous VPP PEDOT. EIS was performed in order to determine the resistance offered by these structures; it predicted that porous VPP PEDOT structures offer less impedance than non-porous VPP PEDOT and would thus be more suitable for the final implant application due to lower power requirements for stimulation via wireless electricity. An MTT assay was performed to assess the biocompatibility of release extract from VPP PEDOT structures and confirmed that there was no significant cytotoxicity in the absence or presence of any stimulation. In addition, to mimic PEDOT sealing layers, PEDOT-coated cellulose membranes were also successfully fabricated by coating PEDOT over cellulose membranes and subjected to further characterisation. SEM images revealed that these membranes had a rough and inhomogeneous surface morphology due to the dip-coating process. CV revealed the formation of cracks on the surface of these membranes due to the actuation of PEDOT during oxidation or reduction. An MTT assay confirmed that there was no significant difference in the cell viability after the incubation of retinal cells with PBS subjected to PEDOT-coated cellulose membranes (non-stimulated and stimulated) when compared to control PBS.

Drug loading and release was achieved either via ion-exchange for non-porous and porous VPP PEDOT films or via physical entrapment for porous VPP PEDOT structures only. Drug permeation across PEDOT-coated cellulose membranes was also studied using Franz-cells. Drug loading via ion-exchange was only performed using dexP as it is an anionic drug that can be exchanged against the anionic dopant tosylate. Active dexP loading via ion-exchange was achieved using CV, with a saturated level of drug loading confirmed by CV stabilisation. Passive dexP loading was also carried out by simply immersing PEDOT films in dexP solution. DexP loading was confirmed by the XPS surface elemental analysis. Sulphur peak analysis was performed to observe any changes between
blank, passive and active dexP loaded PEDOT (again, XPS was only performed for non-porous VPP samples). A clear shift in the sulphur peaks was observed after the deconvolution of sulphur peaks for these samples, confirming that tosylate (previously incorporated dopant) had been replaced by dexP. DexP release was initially carried out by CV. However, a pulse stimulus was later explored due to its suitability for the final implant application. The effect of the redox state was studied for the active dexP-loaded samples (as maximum drug loading was anticipated by this method). It revealed that oxidation was able to hold dexP by preventing its release out of the polymer. Reduction, on the other hand, expelled dexP molecules out of the polymer due to electrostatic repulsion; however, it only concerned dexP molecules that were on or near the surface of the polymer. This was determined by the difference in the amount of dexP released from porous and non-porous VPP PEDOT films as well as the duration it took for dexP molecules to diffuse out of the polymer and into the release medium. Furthermore, the application of a pulse stimulus was able to achieve maximal drug release within 15 min. The pattern of drug release was slightly different for porous and non-porous VPP PEDOT films, indicating that a higher surface area resulted in a more rapid release of dexP. Statistical analysis revealed that there were significant differences in the amounts of drug released with an oxidation, reduction or pulse stimulus for porous as well as non-porous VPP PEDOT samples. A pulse stimulus for 17 min was selected and applied to provide maximal dexP release in order to evaluate the drug loading among different samples. Significantly higher levels of ion-exchanged drug loading were achieved via active when compared to passive drug loading, while there was no significant difference for passive or active drug loading between porous VPP PEDOT and non-porous VPP PEDOT. A multi-day release study of the actively drug-loaded VPP PEDOT (non-porous and porous) samples showed a gradual decrease in the amounts of drug released with each stimulus (a 2-min pulse stimulus) due to the subsequent loading and release of counter-ions present in the release medium after the initial release stimulus. This aligned well with the previously reported studies and initial CV findings during release [206]. However, this lack of reproducibility needs to be overcome for efficient long-term use of this implant.

Physical entrapment was attempted for both dex (hydrophobic) and dexP (hydrophilic) in order to study their entrapment into and release out of the pores. However, some drug loss was observed during the spin-coating when an additional PEDOT sealing layer was polymerised on top of the pores. Furthermore, the PEDOT sealing layer displayed high permeability towards both drugs, resulting in the entire loaded drug being released within 1 h. Therefore, PEDOT-coated cellulose membranes were further analysed with regards to their permeability behaviour. Drug permeation studies showed that PEDOT-coated cellulose membranes were highly permeable, with calculations using Fick’s law of diffusion revealing the permeability to be too high to be used as a sealing layer. In comparison, Vitrasert, which is an FDA-approved implant available on the market, uses a PVA/silicon combination to seal the loaded drug (0.59 mg of flucinolone acetonide). This sealing layer only allows a maximum drug release of 0.6 µg/day, compared here to 2.68 mg of dex or 8.6 mg of dexP released within 1 h.

Finally, a prototype implant was designed using AutoCAD, for the purpose of in-vitro. The dimensions of this implant were adopted from Vitrasert and Retisert; two FDA-approved ocular implants already in use for long-term drug delivery to the posterior segment of the eye. However, the dimensions were later modified to suit any future ex-vivo studies in bovine eyes due to their larger dimensions when compared to human eyes. The prototype implant casing was fabricated via 3D printing using PLA,
which is a biocompatible FDA-approved thermoplastic polymer. It was then assembled and tested for the release of active dexP-loaded porous VPP PEDOT structures only. *In-vitro* drug release results correlated well with the estimated drug loading and the anticipated dexP release pattern. However, the amount of drug loading achieved via ion-exchange was deemed insufficient for long-term clinical applications; therefore, physical entrapment may be the only way to improve drug loading. Nevertheless, more efficient ways to seal the pores need to be explored to prevent leakage of drug out of the pores.

## 6.2 Future outlook

The work performed over the course of this PhD has resulted in the successful fabrication of template-based porous VPP PEDOT structures; this has never been achieved before. In addition, this work has provided a useful understanding of the ion-exchange mechanism for passive and active drug loading as well as for non-stimulated and stimulated drug release using an anionic drug (dexP). Furthermore, this work evaluated the possibility of increasing drug loading for the porous VPP PEDOT structures by physically adding drug into the pores and then sealing these pores with non-porous VPP PEDOT layers. However, the high permeability of these sealing layers limited any further evaluations. A prototype implant was also fabricated and characterised, but was limited to only one *in-vitro* release study to evaluate the functionality of the implant assembly. Following on from the findings here, a number of parameters could be optimised and improved in order to fabricate an implantable system that could be evaluated in further *ex-vivo* and *in-vivo* studies to achieve clinically relevant on-demand ocular drug delivery of these corticosteroids. An ideal system would be able to have enough drug loading which could last for at least six months and where drug release could be successfully and reproducibly tuned in response to the application of an electrical stimulus. Some of suggestions to achieve such a system are:

- **Improve the sealing of drug loaded porous structures;** investigations should look into the fabrication of such sealing layers where minimal drug permeates across in the absence of a stimulus and where there is a surge in the amount of drug released when a stimulus is applied. This could be achieved by exploring different polymers such as PVA/silicon (already used in Vitrasert) for sealing purposes where the chosen polymer exhibits relatively low permeability towards the physically entrapped drugs. However, drug release from such implants would then be controlled by the permeability of by such sealing layer and the surge in the amount of drug being released out of conducting polymer reservoir upon the application of a stimulus.

- **Investigate the actuation of porous and non-porous VPP PEDOT structures upon the application of a stimulus;** studies could look at the formation of cracks when PEDOT-coated cellulose membranes are stimulated and find various ways to eliminate the formation of these cracks by matching the tensile strength of the substrate and the PEDOT coating layer. Furthermore, the fabrication of such implants could also be investigated where actuation of
PEDOT is used as a mean to push drug out of a reservoir to achieve stimuli-responsive drug delivery (similar to the Replenish drug delivery system discussed in Section 1.5.2).

- Investigate the prototype implant further for wireless electricity integration and determine power requirements in order to stimulate drug-loaded PEDOT structures; such investigations should further improve the functionality of the implant by calibrating the amount of drug released as a function of stimulus applied and achieve reproducible drug release. In addition, integration with the wireless electricity would also be required to advance the implant into ex-vivo and in-vivo studies.

Findings of this PhD could also be employed to:

- Explore porous VPP PEDOT structures as biosensors; these structures exhibited higher surface area and a faster response upon application of a stimulus, which is highly sort after in biosensors; therefore, such investigations could look into utilising these structures as potential artificial neurons where a change in ionic concentrations would produce an electrical response.

### 6.3 Thesis conclusion

From all of the work presented in this thesis, it can be concluded that non-porous and porous VPP PEDOT structures were successfully fabricated and evaluated for the purpose of electrical stimuli-responsive drug delivery. In particular, the work carried out during this PhD achieved the following:

- Develop an HPLC method for the simultaneous recovery and quantification of dex and its phosphate salt dexP from the release medium and the vitreous humour. This method also investigated the degradation of these drugs under various stress conditions and was also able to separate these drugs from the degraded products.

- Fabricate template-based porous PEDOT structures via VPP. The formation of these structures depends upon the rate of polymerisation at the interface of the monomers (in the gas phase) and the oxidant solution (in the liquid phase), as well as the rate at which the formed polymer film 'sinks' through the oxidant solution onto the template to get imprinted.

- Explore ion-exchange to load anionic drugs in the absence (passive) or presence (active) of a stimulus. The surface area plays an important role in the case of passive drug loading. However, the amount of drug loading achieved here (~13 µg for the prototype implant) was insufficient to be further employed for the purpose of long-term stimuli-responsive drug delivery
Thesis summary, future outlook and thesis conclusion

in comparison to Ozurdex (loaded with 700 µg of dex to produce a therapeutic effect in a
disease state for up to six months).

- Investigate the release of an anionic drug molecule loaded as a dopant upon the application of
  a stimulus. It depends on the type and duration of the stimulus applied as well as the surface
  area of the polymer. A pulse stimulus applied for at least 15 min was able to provide complete
  drug release with a faster release pattern observed for porous PEDOT films. For the purpose
  of final implant application, porous structures would be more suitable to achieve a rapid
  response against the applied stimulus.

- Attempt to load additional drug by physically entrapping concentrated drug solution into the
  pores of porous PEDOT films. Drug loading could be enhanced (up to 800 µg of dexP for the
  prototype implant when compared to 700 µg of dex for Ozurdex) to achieve long-term stimuli-
  responsive drug delivery. However, inefficient sealing led to rapid leakage of the physically
  loaded drugs.

- Fabricate PEDOT-coated cellulose membranes via VPP. It made these membranes less
  permeable in comparison to plain cellulose membranes; however, the permeability was still too
  high towards the loaded drugs for the final implant application.

- Design a prototype implant via AutoCAD and fabricate this implant casing via 3D printing. The
  prototype implant assembly was functional to study in-vitro stimuli-responsive drug release
  upon in-situ application of a pulse stimulus; however, the amount of drug loaded was deemed
  too low for clinically relevant ex-vivo studies.
REFERENCES


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A bidirectional silicon micropump.


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