Hydrogel Formulations For Ophthalmic Delivery

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Declaration

I, Athmar Dhahir Habeeb Al-shohani confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Acknowledgment

Undertaking this PhD has been a truly life-changing experience for me and my family and it would not have been possible to do without the support and guidance that I received from many people through my studies.

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Abstract

Going blind is incomprehensible and with an aging population the number of people with blinding disease is increasing. Glaucoma and age related macular degeneration (AMD) are two major causes of blindness affecting people as they age. The only proven treatment for glaucoma is lowering of the intraocular pressure (IOP) which is best done by surgically placing a channel from the anterior chamber to allow aqueous outflow to drain into the subconjunctival space. The drainage channel can be formed by the use of a glaucoma drainage device (GDD) or by glaucoma filtration surgery (GFS). Both GFS and the use of a GDD often fail over time because local fibrosis (scarring) in the subconjunctival space blocks aqueous outflow resulting in the increase of IOP and disease progression. It was hypothesised that a more biocompatible GDD could be fabricated from a hydrogel, and that the hydrogel material could be used to restrict aqueous outflow to control the IOP. Hydrogels are widely used in ophthalmic applications including contact lens and intraocular lens.

Since hydrogels are widely examined for use in drug delivery, it was also hypothesised that a hydrogel implant could be made for the subconjunctival space after GFS to stop tissue adhesion and to deliver locally an anti-fibrotic or anti-inflammatory drug to increase the chances for long-term surgical success. For AMD, the current treatment is intravitreal (IVT) injections of anti-VEGF antibodies approximately every 4-6 weeks. IVT injections are an invasive procedure and associated with some complications, but it is also becoming apparent that many healthcare systems around the world cannot cope with the increasing demands for IVT injections to treat AMD. To reduce the frequency for IVT injections, there is a need to develop formulations that allow a longer duration of action for therapeutic proteins in the back of the eye. Maintaining protein stability is a major challenge in formulation science and clinical use. It was further hypothesied that injectable hydrogels could also be used to formulate an antibody for IVT injection to display an extended residence time in the vitreous cavity.

Free radical polymerisations of 2-hydroxyethyl methacrylate (HEMA) and 2-methacryloyloxyethyl phosphoryl choline (MPC) in the presence of a cross-linker, poly(ethylene glycol diacrylate) (PEGDA) were conducted to prepare HEMA-MPC co-polymer hydrogel films. Both HEMA and MPC are widely used in ophthalmic hydrogel products and MPC is known to be exceptionally biocompatible, although it must only be used as a co-polymer to ensure there are suitable processing and mechanical properties in the resulting hydrogel. Different HEMA-MPC hydrogels

with increasing relative stoichiometries of MPC (0%-100% (w/w)) were prepared and characterised to determine if water flow through the gel was possible. Unfortunately the hydrogel films formed have low permeability (1.1×10^{-18} m^2 s^{-1} pas^{-1}) compared to the permeability required to control flow at a rate of 2 µL/min under 10-15 mmHg IOP, which is (6×10^{-14} m^2 s^{-1} pas^{-1}). Although the HEMA-MPC hydrogel films could not be used for flow control, they were further examined for use as potential implants for local tissue site drug delivery in subconjunctiva. HEMA-MPC hydrogels with 10% MPC were found to offer the best balance between water content, mechanical strength and drug loading and release that was required for the possible implantation drug loaded films derived from a range drugs (dexamethasone, pirfenidone and doxycycline).

The process used for drug loading of dexamethasone was optimised by using, methanol and the *in vitro* half-life of DEX was increased from 1.8 to 9.1 days with release being sustained for more than 3 weeks. There are other causes of subconjunctival scarring, in particular trachoma, which is the main cause of blindness due to infection. Doxycycline is thought to be a good candidate drug for treating patients after trachoma surgery because it has both anti-bacterial and anti-fibrotic properties. As a water-soluble drug, doxycycline release could not be sustained for more than 3 days, so the 10% MPC films were modified with the incorporation of β-cyclodextrin (β-CD) in an effort exploit the possible affinity of doxycycline with β-CD to prolong doxycycline release. Several methods were examined to introduce β-CD into the HEMA-MPC films including the formation of HEMA-MPC films with pendant β-CD, the embedding of β-CD cross-linked particles within the hydrogel network and formation of an interpenetrating network (IPN) of β-CD and HEMA-MPC. Unfortunately, the release profile of doxycycline was similar in the modified and non-modified HEMA-MPC hydrogels.

To evaluate hydrogels for use in IVT injections of antibodies, N-isopropylacylamide (NIPAAm) thermoresponsive hydrogels were evaluated. Three different macromolecular hydrophilic cross-linkers were evaluated; PEGDA, phosphorylcholine 3059 (PC 3059) and acrylated hyaluronic acid (Ac-HA). The prepared hydrogels were characterised regarding physical properties such as water content, water retention thermoresponsivness and protein release. The thermal responsiveness decreased with increasing cross-linker percentage. Modification in the type and percentage of cross-linker used allowed the preliminary screening of the different formulations. Hydrogel formulations made with 40 mg NIPAAm as monomer and 8 µL PEGDA, 20 mg PC3059 or 4 mg Ac-HA were able to sustain the release of antibodies for a month in a validated *in vitro* model of the eye.

Table of contents

List of Figures

List of Tables

Abbreviations

Ac-HA	Acrylated hyaluronic acid
AIBN	2,2-azobis(2-methylpropionitrile)
AGD	Ahmed glaucoma device
AMD	Age related macular degeneration
APS	Ammonium persulfate
β-CD	Beta cyclodextrin
BSA	Bovine serum albumin
CD	Cyclodextrin
Da	Dalton
DDS	Drug delivery system
DEX	Dexamethasone
DMF	Dimethylformamide
DOXy	Doxycycline hyclate
DSC	Differential scanning calorimetry
DW	Distilled water
ECM	Extracellular matrix
EWC	Equilibrium water content
GDD	Glaucoma drainage device
GFS	Glaucoma filtration surgery
GMA	Glycidyl methacrylate
HA	Hyaluronic acid
HEMA	2-hydroxyethyl methacrylate
HPLC	High performance liquid chromatography
IOL	Intraocular lenses
IOP	Intraocular pressure
IPN	Interpenetrating network
IVT	Intravitreal
kDa	kilodalton
LCST	Lower critical solution temperature
MMC	Mitomycin C
MPC	2-methacryloyloxyethyl phosphorylcholine
MW	Molecular weight
NaOH	Sodium hydroxide
NIPAAm	N-isopropyl acrylamide
PAA	Polyacrylic acid
PBS	Phosphate buffered saline
PC	Phosphorylcholine
PEGDA	Polyethylene glycol diacrylate
PEGDE	Polyethylene glycol diglycidyl ether

PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
RPM	Rotation per minute
SCL	Soft contact lenses
SEM	Scanning electron microscopy
SR	Swelling ratio
TEMED	*N,N,N′,N′*-Tetramethylethylenediamine
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
VPTT	Volume phase transition temperature
Wb	Bound water
Wf	Free water
WR	Water retention

Chapter 1. Introduction

Barriers to ophthalmic drug delivery: The big challenge

The aim of any final dosage form is to deliver the drug molecule in a therapeutic concentration to the site of action for an optimal period of time. Systemic drug distribution is often accompanied by side effects due to off-target effects (Short 2008; De Souza et al. 2010; Allen & Cullis 2004). Localised drug delivery to the site of action has the potential to minimise the amount of dose needed for efficacy. Systemic side effects can thus be minimised. Certain modes of administration allow for localised treatment, e.g. pulmonary, transdermal, nasal, rectal, vaginal, buccal and ophthalmic routes (Parikh et al. 2014; Jannin et al. 2014; Chai et al. 2013; Frank et al. 2014). Local drug delivery to the eye can in some cases be accomplished, but there are major anatomical, biological and physicochemical barriers that can limit efficacy (Ali & Byrne 2008; Patel et al. 2015).

Blinding eye disease has a huge impact on the quality of human life because vision impairment reduces people's ability to live and move independently (Skalicky et al. 2012; Habtamu et al. 2015; Uchino & Schaumberg 2013). Most blindness occurs as people age. With aging population increasing, the number of people diagnosed as blind or with visual impairment is expected to increase. It is estimated there are approximately 39 million blind people worldwide while there are approximately 246 million people suffering visual impairment that ranges from moderate to severe (Scott et al. 2016). People with blinding eye diseases and those with visual impairment require constant medical care, medication and social care to improve their quality of life. The medical costs and the loss of economic output have far-reaching social and financial impacts (Köberlein et al. 2013). Worldwide, the cost of health care and medication was estimated to be $ 3 trillion in 2010 and expected to increase to $ 3.5 trillion by 2020. In addition to health care costs, economic loss due to the prevalence of blinding eye diseases in working populations is also high (Gordois et al. 2012). It is estimated that by 2020, the economic loss due to blinding diseases in the working population will grow to $110 billion (Eckert et al. 2015).

Delivery of medicines is often needed at the back of the eye because the majority of blinding diseases result in damage to the retina and optic nerve. An understanding of the barriers associated with ophthalmic drug delivery requires an understanding of the main parts of the eye and the function of each part. The eye consists of two compartments; the anterior segment (which is the front of the eye) and constitutes 1/3 of the globe while the other 2/3 of the globe is the posterior

segment (which is the back of the eye). The eye is in direct contact with the environment and protected by the eyelids, tear film and the cornea (McCaa 1982).

The cornea is a transparent layer that covers the front of the eye (iris, which is the coloured part of the eye); it is highly innervated tissue with no blood supply. It refracts and transmits light to the lens and retina. It depends on the aqueous humour for nourishment and removal of waste products. The front surface of the cornea is covered with a tear film. The cornea consists broadly of three tissue layers each separated by a membrane (Molokhia et al. 2013). The cornea is a complex barrier for absorption of drugs into the eye. In addition to the cornea; tear turnover, nasolacrimal drainage and reflex blinking made topical administration of medicines using eye drop is only really appropriate to treat the periocular diseases (Figure 1:1) (Gaudana et al. 2010; Davies 2000).

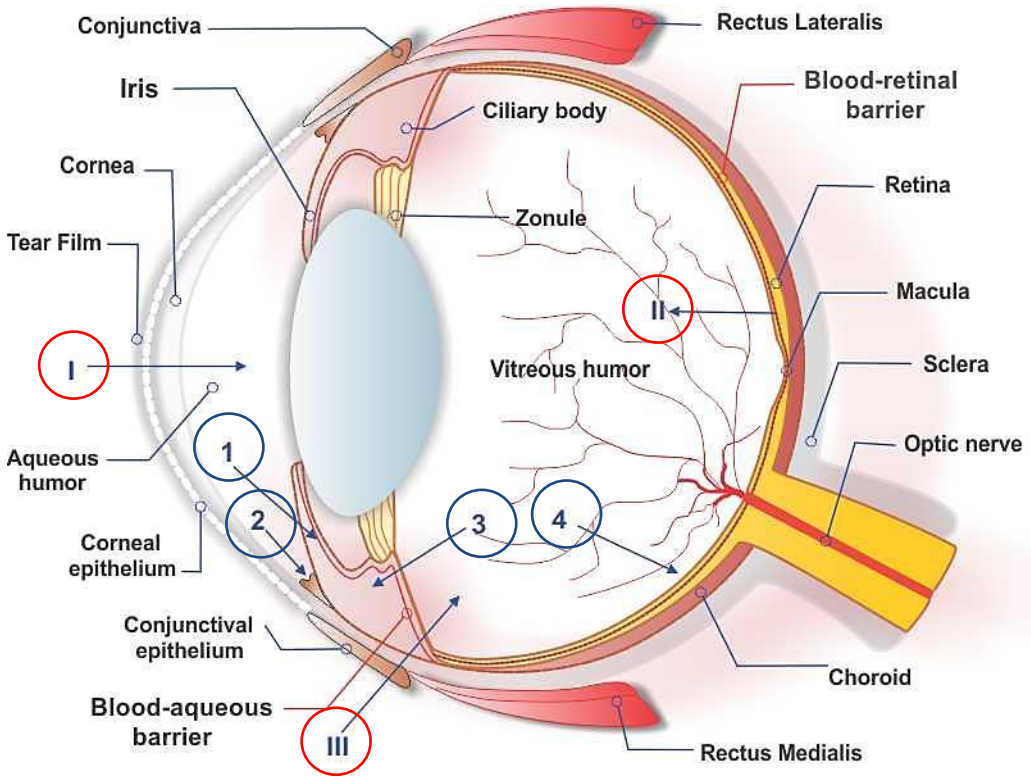


Figure 1:1 schematic illustration of the main parts of the anterior and posterior segments, barriers to ophthalmic drug delivery and routes of drug elimination from the vitreous. The location of ophthalmic barriers (encircled in red) are; I) the cornea and tear film; II) blood-retinal barriers; III) blood-aqueous barriers. Routes of elimination from the vitreous (encircled in blue) are; 1) venous blood flow after diffusing across the iris surface; 2) aqueous humour outflow; 3) diffusion into the anterior chamber (1, 2 and 3 are referred to diffusion through the blood-aqueous barriers); 4) diffusion through the blood–retinal barrier (Nakhlband & Barar 2011).

The cornea is connected to the sclera through the limbus region. The sclera is the white part of the eye that is tough in nature and is mainly comprised of collagen fibres. It preserves the shape of the eye. The sclera is covered by a mucous membrane called the conjunctiva (which covers the inside of the eyelid as well). The conjunctiva is a thin, transparent, mucous tissue that covers the front of the eye, except the cornea. It is enriched with blood and lymph vessels that help to nourish the ocular tissues. Its surface is covered by the mucus that helps to lubricate the eye ball during movement and impart wettability to the ocular surfaces (Willoughby et al. 2010; McCaa 1982).

The uvea comprises the iris, ciliary body, and the choroid plexus. The iris is the coloured part of the eye. The ciliary bodies are tissues located posterior to the iris and are responsible for the production of the aqueous humour and control vision by adjustment of the lens. The anterior chamber is the space between the iris and the cornea while posterior chamber is the space between the iris and the lens (posterior to the iris and anterior to the lens); both are occupied by the aqueous humour (Rawas-Qalaji & Williams 2012). The aqueous humour is a transparent fluid that fills the anterior and posterior chambers (Chowdhury et al. 2010).

The main parts of the posterior segment are vitreous humour, retina, choroid and optic nerve. The vitreous humour is a clear viscous gel that fills the posterior cavity of the eye. It is located between the lens and the retina. The vitreous helps to maintain the structure of the eye. The retina is a complex tissue that lies between the vitreous and sclera. The choroid is the vascularised tissue comprising the blood-retinal-barrier and separates the retina from the sclera. The blood-retinal barrier is considered the main key barrier for the absorption of drugs into the posterior segment after systemic administration (Figure 1:1) (Willoughby et al. 2010).

Topical drug delivery through eye drops is the common route for the treatment of anterior segment eye diseases due the non-invasive nature of the route; however for posterior drug delivery topical route is ineffective (Hughes et al. 2005). The amount of drug that can reach the posterior segment is below the therapeutic dose required. This is caused by drainage and loss of the drug through the nasolacrimal ducts and the inability of the drug to penetrate the cornea and blood-aqueous barriers of the eye in sufficient amount to induce therapeutic effect (Duvvuri et al. 2003; Moisseiev et al. 2014). Systemic administration is also ineffective most of the times due to blood-aqueous and blood-retinal barrier that restricts drug penetration to the vitreous. High oral doses will be required to allow

therapeutic level of the drug to be reached in ocular tissues which will increase dose related side effects (Hughes et al. 2005; Eljarrat-Binstock et al. 2010).

Over the years different routes for posterior drug delivery have been investigated such as transscleral, suprachoroidal, posterior subtenon, subconjunctival, subretinal and intravitreal (IVT) injection (Figure 1:2). The above routes offer the advantage of overcoming the barriers and side effects associated with topical and systemic routes such as low penetration and faster elimination from ocular surfaces (Kang-Mieler et al. 2016; Yamada & Olsen 2015; Geroski & Edelhauser 2001; Holz et al. 2014).

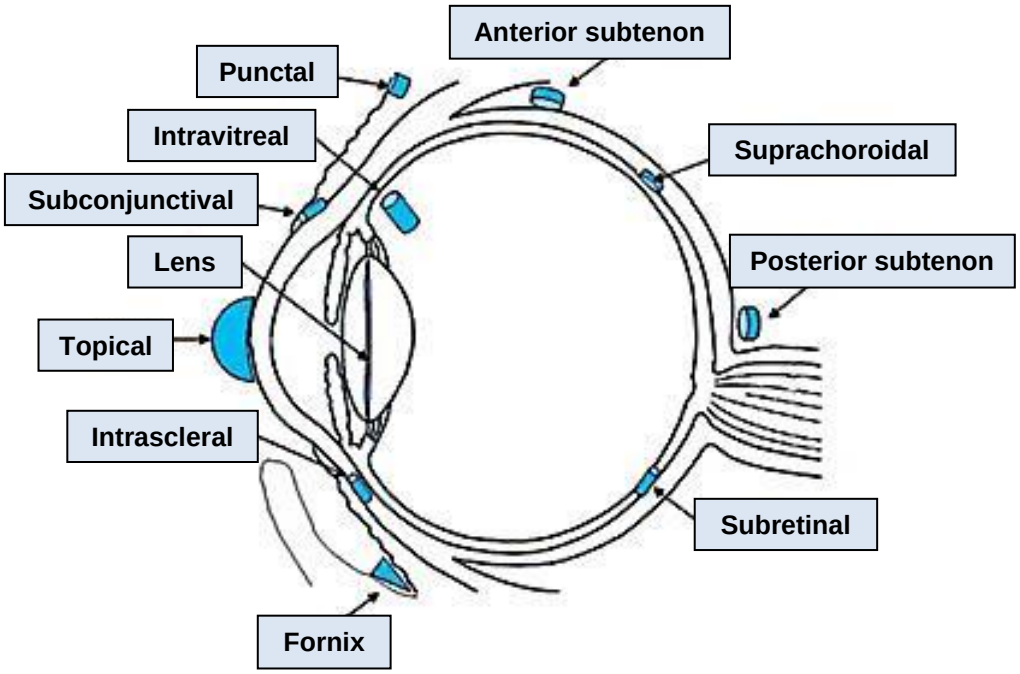


Figure 1:2 Different routes used and investigated for drug delivery to the anterior and posterior segments of the eye. The blue shapes represent the possible location for implantation of drug releasing implants or injections of the drug molecules for ophthalmic delivery. Topical route is mainly for anterior drug delivery and the other routes are for posterior drug delivery (Novack 2009).

Currently in the clinic, the delivery of large and reproducible drug doses into the posterior segment was only achievable using IVT route. The IVT route offers the advantage of high intraocular concentration for a longer period of time compared to other routes (Nomoto et al. 2009). For example, Wang *et al* reported that a favorable pharmacokinetics was observed with single IVT injection of anti-inflammatory drug ketorolac compared to suprachoroidal single injection in rabbits (Wang et al. 2012). The half-life in the retinal–choroid was (3.09 ± 0.13 hour) after IVT injection compared to (1.18 ± 0.27) hour after suprachoroidal Injection. The

mean residence time of the injected drug was also higher (4.04 ± 0.12 hour) after IVT injection compared to suprachoroidal Injection (1.58 ± 0.21 hour).

Intravitreal dosage forms

Drug molecules injected into the vitreous in the clinic are divided into two categories; high molecular weight proteins and small molecular weight molecules such as anti-inflammatory drugs (Sarao et al. 2014; Krohne et al. 2012; Magdelaine-Beuzelin et al. 2010). Injections and implants are the available dosage forms in the clinic through IVT route (Kuppermann & Loewenstein 2010).

IVT injections are widely used in the clinic compared to other routes. IVT injections are directly injected into the vitreous which assures the delivery of the accurate therapeutic dose into the site of action. However, IVT injected drugs have short half-life in the vitreous due to rapid elimination from the vitreous. Drug elimination is through two main routes; posterior and anterior elimination (Raghava et al. 2004; Shen et al. 2007). Drugs with large molecular weight and high water solubility, such as proteins, are eliminated by permeation through blood aqueous barriers to the anterior chamber followed by elimination through aqueous turnover and uveal blood flow. Hydrophobic drug molecules and small molecular weight drugs are eliminated through posterior route by permeation through blood retinal barriers (Figure 1:1) (Urtti 2006; Kidron et al. 2012). The short half-life of medications in the vitreous requires multiple injections which are often associated with complications such as inflammation, vitreous haemorrhage and retinal detachment in addition to the burden placed on patients financially and mentally (Sampat & Garg 2010; Falavarjani & Nguyen 2013).

Solid implants are used as drug delivery systems (DDSs) to increase drug efficacy in the posterior segment. It is hoped that with the development of IVT implants, patients do not have to endure frequent IVT injections. Intraocular implants are divided into two types; biodegradable, such as Ozurdex® (dexamethasone in poly(lactic-*co*-glycolic acid) PLGA), and non-biodegradable, such as Vitrasert® (ganciclovir with polyvinyl alcohol (PVA) and ethylene vinyl acetate), Retisert® (fluocinolone acetonide with silicone and PVA), Iluvien® (fluocinolone acetonide with polyimide and PVA) (Figure 1:3) (Haghjou et al. 2011; Bansal et al. 2016).

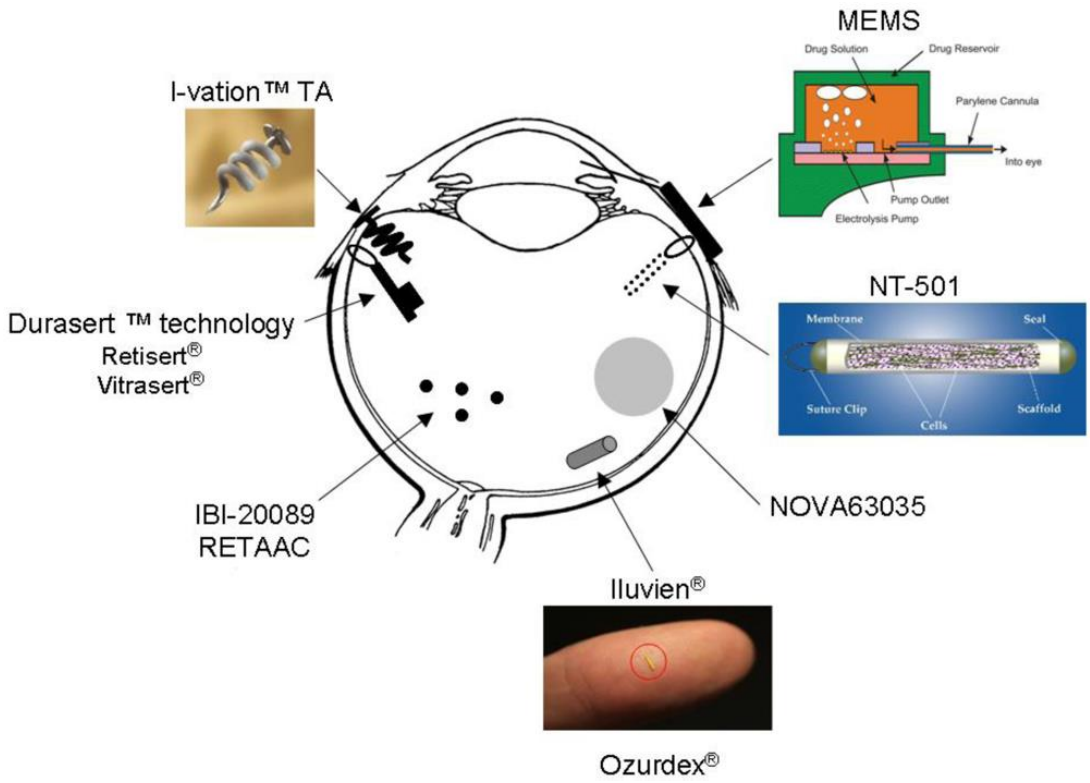


Figure 1:3 Examples of IVT implants and the possible position of the implants in the eye (Kuno & Fujii 2011).

For non-biodegradable implants the main advantage is relatively better control over the release of the drug compared to biodegradable ones over prolonged period of time (Bansal et al. 2016). The implants are formulated as reservoir systems in which a pellet of drug is surrounded by a semipermeable polymeric membrane to control drug diffusion (with the exception of Iluvien® which is in matrix form). The main disadvantage is surgery which is required for implantation and removal of the empty implant and surgery is often associated with side effects such as inflammation and infection (Choonara et al. 2010). The only exception is Iluvien® which could be injected through 25G needle and remain in the vitreous when the entire drug loaded is released. Iluvien® was developed to deliver a very low dose of fluocinolone acetonide to the retina for up to 3 years as a treatment for diabetic macular edema (Kane et al. 2008). Non-biodegradable implants are often used for the treatment of chronic conditions in the eye (da Silva et al. 2010). For biodegradable implants the need of surgery for removal and application is eliminated; however they offer relatively less control over drug release compared to non-biodegradable implants and it is very difficult to retrieve the implant when side effects start to show (Bansal et al. 2016; da Silva et al. 2010).

Several new approaches for IVT implants have been investigated such as the use of refillable delivery systems and cell encapsulated technology (Chen 2015). Ciliary neurotrophic factor (CNTF) delivery through cell encapsulated technique has been investigated for the treatment of dry age related macular degeneration (AMD) and retinitis pigmentosa. The device is composed of sealed semipermeable membrane of polyethylene terephthalate surrounding human retinal pigment epitheliums cells (ARPE-19) genetically modified to secrete recombinant human CNTF (Kuno & Fujii 2011). Microelectromechanical systems (MEMS) are intraocular DDS that can be refilled with drug solution for long term therapy. The device consists of refillable drug reservoir with flexible canula. The device would be implanted in the subconjunctival space and allow the canula to be directed into the vitreous or the anterior chamber (Figure 1:3) (Bansal et al. 2016).

Clinically registered implants or implants under development for the posterior segment have been predominantly developed with steroids as the active drug agent. Current ocular implants that utilise steroids can provide a sustained release from months to over two years. The choice of steroids is due to the favorable properties of the drug molecules (Falavarjani 2009). Steroids inhibit inflammation and would be expected to minimise a foreign body response to an implant. Steroids are generally poorly water soluble, low MW actives that are stable within an implant. For the delivery of proteins Intravitreal (IVT) injections are the only available dosage form in the clinic (Hariprasad et al. 2006; Stewart et al. 2011).

Over 68% of the leading causes of blindness and severe visual impairment in older population are related to two diseases; glaucoma and AMD and the number is subjected to increase due to improvement of quality of life and diagnosis techniques in developed countries (Bunce & Wormald 2006). Understanding these two conditions will help in improving treatments available.

Glaucoma

Glaucoma is defined as a group of disorders that leads to optic nerve neuropathy. Glaucoma is one of the main causes of irreversible blindness in the world with more than 66 million people having high prevalence of glaucoma (Quigley & Broman, 2006; Jonas et al., 2014). There are several types of glaucoma but the main modifiable risk factor is the increase in the intraocular pressure (IOP) of the eye(Coleman & Miglior 2008). The IOP is controlled by a balance between the secretion and drainage of the aqueous humour, so the pressure inside the eye is maintained at normal level (around 12 mmHg). The aqueous humour consists of 98% water, electrolytes, ascorbic acid, amino acids, oxygen, carbon dioxide, low

amount of proteins and glutathione. It nourishes the lens and the cornea, removes waste products and stabilises the ocular structure (Brubaker 1982). The aqueous humour is secreted from the ciliary bodies, which are located behind the lens, into the posterior chamber and the aqueous flows into the anterior chamber through the pupil of the iris. The aqueous humour fills the posterior and anterior cavity of the eye and maintains the eye structure. The aqueous humour is in continuous production at a rate of approximately 2-2.5 µL/min. Aqueous drains from the eye into the blood circulation through two pathways. The first is through the trabecular meshwork, which is located in the limbal region (the limbal region is where the cornea and sclera meet). The trabecular meshwork is a spongy tissue that is converted into the schlemm's canal which is connected to the episcleral vein and venous circulation, this is called conventional pathway (Figure 1:4) (Goel et al. 2010).

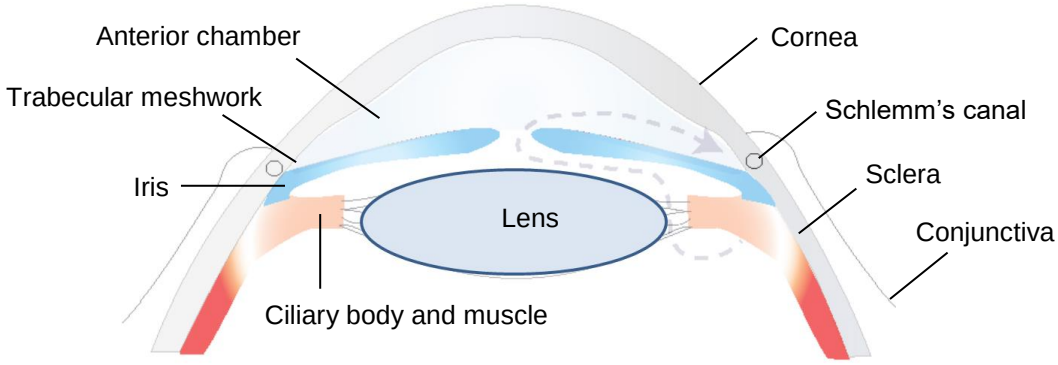


Figure 1:4 The release of aqueous humour from the ciliary body and its movement towards trabecular meshwork for drainage through conventional pathway. The aqueous humour is released from the ciliary muscles behind the lens and move to the anterior chamber followed by elimination through the trabecular meshwork and schlemm's canal (the dotted arrow). Adapted from (Goel et al. 2010).

The trabecular meshwork acts as a resistance barrier for the flow of the aqueous humour. A positive intraocular pressure in the eye is needed for the aqueous humour to drain from the eye. Drainage through the trabecular meshwork is considered the major pathway for aqueous drainage. The second drainage pathway is through the uveal meshwork into the ciliary muscles and out through the sclera. This is called non-conventional pathway (Siggers & Ethier 2012).

Problems with drainage of the aqueous humour from the conventional pathway, such as blockage of the trabecular meshwork, will cause an increase in pressure inside the eye (Tomarev 2001). Over time the elevated pressure affects

the retinal ganglionic cells, retinal cell axons and blood vessels, which lead to the death of the nerve ganglions and to optic nerve damage. The loss of vision does not happen instantly but gradually. First the peripheral vision is lost followed by deterioration in the central vision and blindness (Lee & Higginbotham 2005).

Current glaucoma treatments

Glaucoma treatments range from eye drops to surgery depending on the stage of the disease and the complications associated with each patient. Medication in the form of eye drops is often the first choice to control the elevated IOP (Babić 2015). Several drugs are available for the control and management of increased intraocular pressure. Beta-receptors play an important role in the regulation and the production of aqueous humour from the ciliary body. Blocking these receptors reduce the production of aqueous humour. Selective and non-selective beta-blockers (such as betaxolol) are used to treat glaucoma (Rait 1999). Carbonic anhydrase inhibitors also reduce the production of aqueous humour but by different mechanism. Carbonic anhydrase is an enzyme important in the production of the aqueous humour; therefore by inhibiting its effects, the rate of aqueous production is reduced (Supuran & Scozzafava 2000).

Prostaglandin analogues (such as latanoprost) increase the flow of the aqueous humour through the non-conventional pathway. Adrenergic agonists (such as epinephrine) are also used. They act by both reducing rate of production of aqueous humour and increase rate of flow through the trabecular meshwork through complex processes that involves the activation of both alpha and beta receptors (Sambhara & Aref 2014). Miotics (such as pilocarpine) are a class of drugs that act by improving the drainage efficiency of the aqueous humour through the trabecular meshwork. They cause contraction of the ciliary muscle, which leads to relaxation of the trabecular meshwork (Alward 1998).

Laser surgery is also used in certain cases. There are three types of laser surgery; the first is called argon laser trabeculoplasty. In this type of treatment the laser makes small burns (holes) in the trabecular meshwork to increase fluid drainage and open the blocked channels (Heijl et al. 2011). The second type of laser surgery is selective laser trabeculoplasty which selectively targets the pigmented cells of the trabecular meshwork, thus preserving the trabecular meshwork structure (Mahdy 2008). It is safer and there are fewer side effects compared to the argon laser trabeculoplasty (Kramer & Noecker 2001). The third type of laser therapy is called transscleral diode laser cyclophotocoagulation, which

destroys the tissues responsible for the formation of aqueous humour (Ansari & Gandhewar 2007).

If the progression of glaucoma is not halted, then a type of drainage surgery is required. The most widely used surgical procedure is known as trabeculectomy or glaucoma filtration surgery (GFS) (Chiselita 2001). This surgery involves creating a new channel to drain the aqueous humour into the subconjunctival space. A small space called the bleb is often formed to allow the aqueous humour to drain from the eyeball and be absorbed into the systemic circulation (Figure 1:5) (Yu et al. 2009). The GFS is a complex surgery and often associated with complications such as endophthalmitis, hypotony, bleb leakage, scarring and corneal complications (Watson et al. 1990). In patients where the risk of GFS complications is high or the patient underwent previous failed GFS, glaucoma drainage devices (GDDs) were introduced.

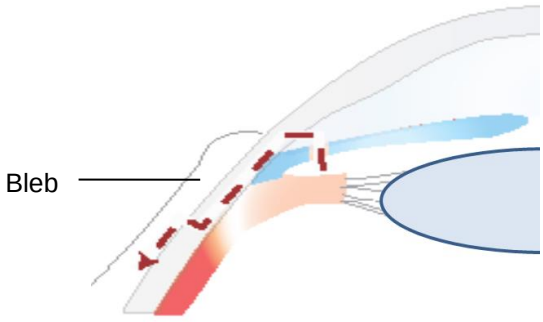


Figure 1:5 Glaucoma filtration surgery (GFS); the red arrow represent the alternative flow pathway created during the surgery where the fluid directed to the subconjunctival space.

GDDs are implants used to create an alternative aqueous drainage pathway from the anterior chamber. A new drainage channel is formed by the tube to direct the aqueous flow into the subconjunctival space (Gedde et al. 2007). Generally these devices are designed with a tube that is attached to a spacer plate. One end of the tube goes into the anterior chamber and the other end is attached to the plate, which is placed in the subconjunctival space (Figure 1:6) (Minckler et al. 1987). The tube is approximately 300 microns in diameter. This size of tube does not offer pressure control, so many GDDs had values designed to better control outflow and to provide pressure resistance. The spacer plate is designed to disrupt subconjunctival fibrosis that often occurs after surgery. The spacer plate serves to provide a drainage pathway for the aqueous after it drains from the eyeball (Patel & Pasquale 2010).

A fibrovascular capsule develops around the spacer and acts as the primary pressure resistance mechanism that controls the aqueous flow from the anterior

chamber. The inner layer of the capsule is collagen and the outer layer is vascularised tissue. The aqueous humour is directed from the anterior chamber to the capsule and cleared through the vascularised tissues to the systemic circulation (Dempster et al. 2011). The formation of the capsule varies between (4-6) weeks after implantation depending on the patient response (Mills et al. 1996).

Early stage pressure control is often achieved by temporarily suturing the tube until the capsule develops around the spacer. There is a need to develop other strategies for long term pressure control rather than rely on the formation of a fibrovascular capsule around the spacer plate since the foreign body reactions necessary for capsule formation can vary widely in patients.

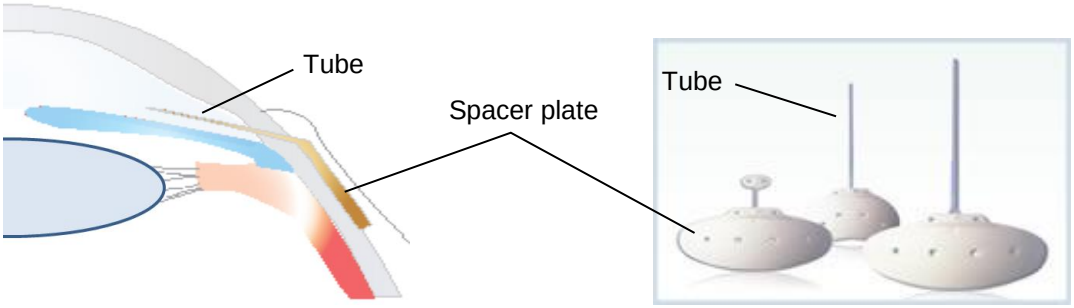


Figure 1:6 The position of the tube and plate of GDD in the eye. The tube is placed in the anterior chamber while the plate is placed in the subconjunctival space. The tube will direct the aqueous humour from the anterior chamber to the subconjunctival space.

The first glaucoma drainage device was developed by Molteno in 1969 (Molteno et al. 1976). The Molteno GDD had a tube without a valve. Unless considerable care is taken by sutering the tube, there is a lack of control over the IOP that can result in hypotony (very low IOP), which can result in the eye collapsing. The problems observed with non-valved devices led to the development of the valved GDD. The first valved GDD was introduced in 1976 by Krupin (Krupin, 1976), which was a unidirectional valve to provide resistance to the aqueous flow to prevent hypotony after the implantation of the device. When the IOP reaches (11-14) mmHg the valve opens. In 1993, the Ahmed glaucoma device (AGD) was introduced. The valve in the AGD is designed to open when the IOP is (8-12 mmHg). These devices have been developed over the years in different shapes and sizes (Figure 1:7 and Table 1:1) (Molteno et al. 1976; Schwartz et al. 2006; Wilson-Holt et al. 1992; Good & Kahook 2011; Nyska et al. 2003; Rivier et al. 2007; Knowlton 2009; Hueber et al. 2013; Acosta et al. 2006; Siegner et al. 1995; Ceballos et al. 2002; Ayyala, Zurakowski, et al. 1998).

Unfortunately many of the valved GDDs fail because a fibrovascular capsule still develops around the spacer plate resulting in IOP increases (Thieme et al.

2011). The only recourse for the patient is to have the site of surgery 'needled' where an ophthalmologist must try to break up the capsule to allow aqueous to flow. In severe cases another surgery is required where another GDD is implanted, but this strategy is not ideal since a non-functioning device often remains in the eye. Continued foreign body response occurs and the need to implant the second device in a suboptimal place in the eye all contribute to there being a greater chance for long term problems requiring considerable follow up to ensuring there is control of IOP.

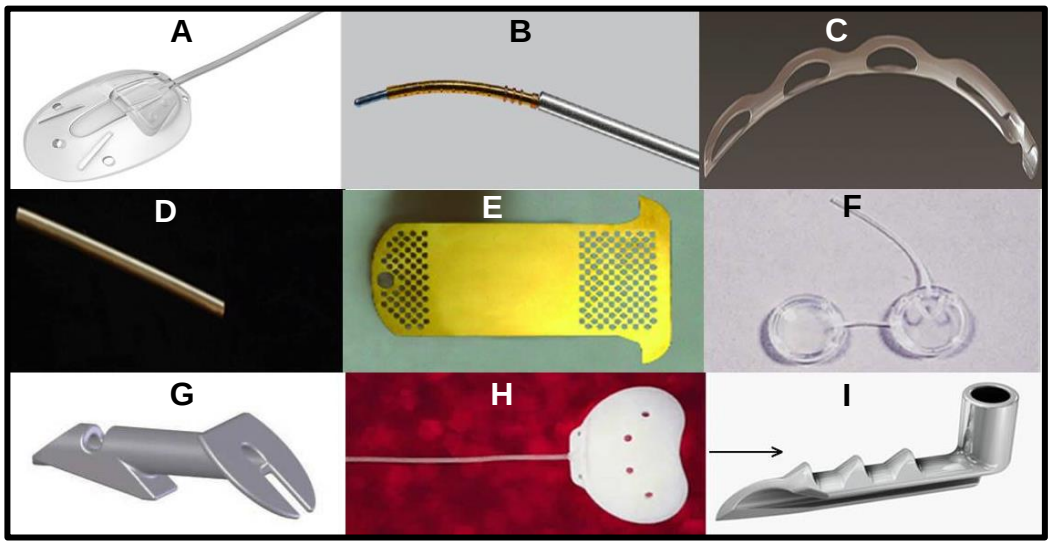


Figure 1:7 Different types of glaucoma drainage devices that are available in the clinic, A) Ahmed device, B) Cypass stent, C) Hydrus stent, D) AqueSys stent, E) Gold Micro Shunt, F) Double Molteno device and G) ExPress shunt device, H) Baerveldet device, I) Istent. All the devices have a tube and spacer to control the IOP while stents have no spacer and the inner diameter of the device itself is the resistant mechanism to control IOP. The choice of the device is based on the medical condition of the patient.

Table 1:1 Different GDDs and the description of each device. The table highlights the materials used for each device, the mechanism of control of pressure and the size of each device.

Device name	Description	Valved / Non- valved	Size of the device
Molteno single plate and double plate	Silicone tube attached to a single or double polypropylene end plate	Non-valved	134 mm^2 size of the single end plate and 268 mm^2 for the double end plate, 340 µm inner diameter of the tube
Baerveldet	Silicone tube attached to a barium impregnated silicone end plate	Non-valved	The end plate is of three sizes 250 mm^2, 350 mm^2 and 500mm^2, 300 µm inner diameter of the tube
ExPress R50	Stainless steel tube with no end plate with disc like flange at one end and spur like projection at the other end	Non-valved	3 mm long, 50 µm and 200 µm inner diameter of the tube available
Ahmed GV	Silicon tube connected to a polypropylene end plate	Valved	End plate is 185 mm^2, 300 µm inner diameter of the tube
Krupin	Silicone tube attached to a silicone end plate	Valved	End plate is 180 mm^2, 300 µm inner diameter of the tube
AqueSys	Cross-linked gelatin tube	Non-valved	6 mm long, 50 µm inner diameter, 150 µm outer diameter
MIDI-Arrow	Poly(styrene-b-isobutylene-b-styrene) [SIBS] tube	Non-valved	8.5 mm long, 70 µm inner diameter, 350 µm outer diameter
SOLX Gold shunt	24 carat gold	Non-valved	flat implant with numerous microtubular channels (5.2 mm long and 3.2 mm wide)
Schocket	Silicone	Non-valved	Width 4 mm (#20 band), 6 mm (#220 band), circumferential diameter 24 mm (at equator)
Optimed	PMMA	Valved	Width 10 mm, length 14 mm, plate thickness 1.3 mm
Istent	Titanium	Non-valved	Length 1 mm (body), 250 µm (snorkel), Length 360 µm
Hydrus	Nitinol	Non-valved	Length 15 mm
Cypass	Polyimide	Non-valved	Length 6.35 mm
Aquashunt	Polypropylene	Non-valved	Width 4 mm, length 10 mm, plate thickness 0.75 mm

Recently developed devices are based on different design concepts compared to the Molteno and Ahmed devices. These included the ExPress R50, Gold Micro Shunt, Istent, InnFocus and AqueSys (Table 1:1). The ExPress R50 is a stainless steel tube with no end plate and a disc like flange at one end and spur like projection at the other end. These modifications were introduced to prevent migration and extrusion of the tube. First it was placed underneath the conjunctiva (similar to the previous devices). Problems such as conjunctival erosion and hypotony were predominant so now it is placed under the sclera. Pressure resistence in the ExPress tube is achieved because the diameter of the tube is blocked to have an inner diameter of approximately 50 µm (Filippopoulos & Rhee 2008).

The Gold Micro Shunt is based on different approach which directs the aqueous humour to the suprachoroidal space (Hueber et al. 2013). The Istent is a (1 mm) long heparin coated titanium tube that is inserted surgically into the eye through the trabecular meshwork into the schlemm's canal. This creates a permanent opening in the trabecular meshwork to direct the aqueous humour directly to the schlemm's canal. AqueSys is a gelatin tube inserted into the eye through a specialised inserter without dissection to drain the aqueous humour from the anterior chamber to the subconjunctival space (Saheb & Ahmed 2012).

The use of a spacer with a tube may give better long term outcomes because aqueous can be channelled away from the drainage point from the eyeball. The formation of the fibrovascular capsule can vary widely, so there is an opportunity to develop a more biocompatible spacer. The choice of the biomaterial used for the synthesis of the spacer is important because it affects the initial inflammatory response to the implant (Ayyala 2000). Materials that are used for a GDD design should have biocompatibility, flexibility and the mechanical strength for implantation. The consideration of a more biocompatible spacer requires that there also be consideration for how outflow resistance will be achieved to control IOP.

Problems with current glaucoma treatments

As mentioned earlier glaucoma treatments are available in the form of eye drops, laser surgery, GDD and GFS. Most of the dosage forms available for glaucoma treatments are eye drops; however there are several barriers for topical drug delivery. First is the low efficacy because of the removal of the administered eye drops by tears and nasal drainage and poor penetration through the corneal barriers (Ghate & Edelhauser 2008). Since glaucoma is a chronic disease, cost and low patient adherence also play an important role in reducing the benefits of using

eye drops (Friedman et al. 2007; Rylander & Vold 2008). The eye drops need to be used several times a day which is inconvenient for patients and half of them discontinue the treatment or do not use the eye drops regularly (Tsai 2009; Sleath et al. 2006). The long term use of eye drops increases the sensitivity of the eye tissues and renders the eye more susceptible to inflammation and less responsive to treatment over time. This is mainly due to the chronic use of preservatives (such as benzalkonium chloride), which is a common component in eye drops (Noecker 2001).

Implantable drug delivery devices that can provide medication for prolonged time to overcome these problems have been investigated for years but they also offer a great challenge in terms of safety and prolong effect (Manickavasagam & Oyewumi 2013). Laser surgeries have short term and variable effect depending on the patient and still some of the patients need medication after the surgery. Irregular IOP after the surgery is noticed in many patients. This is due to the blockage of some of the newly formed channels (Wise 1987).

The main problem associated with current GDDs is lack of control on flow in the first few weeks before the fibrous capsule formation, which may cause hypotony (which is IOP lower than 5 mmHg). To overcome these problems certain surgical techniques are used, such as using external absorbable ligatures or internal removable suture stents to temporary control the aqueous flow over the first 4 weeks until the capsule is formed (Melamed & Fiore 1990; Molteno et al. 1986). Modifications in the design of the implant such as increasing surface area of the end plate and designing a subsidiary pressure ridge to reduce post-operative hypotony was also introduced but still no significant improvement were observed (Molteno 1990; Luong et al. 2014). Elevated IOP also may occur due to tube obstruction caused by excessive capsule formation and fibrosis around the tube and the end plate due to foreign body response to the material used for the device (Chaudhry et al. 2012). Modification in the devices was introduced but still no significant improvement observed (Luong et al. 2014).

Most of the available GDD devices are made of silicone or polypropylene. Stainless steel and gold are also used for tubes and drainage channel (Hueber et al. 2013; Rivier et al. 2007). Although these materials have been used for years they still produce inflammation, irritation and scarring due to foreign body response (Ayyala 2000). Recently other materials have been used such as cross-linked gelatin and poly (styrene-b-isobutylene-b-styrene) [SIBS] (Pinchuk et al. 2008). There is still a need for the development of a biocompatible material that could be used in a GDD and produce less inflammation and less fibrous capsule formation

(Knowlton 2009; Acosta et al. 2006). In general devices available in the clinic now lack the consistency to control the IOP in patients (Chaudhry et al. 2012).

The major problem with GFS is the wound healing. During the wound healing process inflammation, collagen deposition and scarring occur at the surgical site. This may cause the closure of the newly formed channel and failure of the operation (Skuta & Parrish 1987). A scar is a fibrous tissue that replaces a damaged tissue and considered as the last part of the wound healing process and could also be a result of pathological condition such as myocardial infarction and trachoma (Gurtner et al. 2008). Trachoma is main cause of blindness due to infection. Inflammation and fibrosis cause the eyelid to contract and the eyelashes to turn inward, so that blinking causes damage to the cornea resulting in blindness.

While a scar in the skin is not usually a function problem, a scar in the eye tissues often causes a loss of function resulting in blindness (Hsu et al. 2000; Tovell 2011; Bowman 1999). Most blinding conditions are characterised by fibrosis. Wound healing is broadly characterised by four phases which are the i) coagulative phase, (ii) inflammatory phase, (iii) proliferation and tissue repair (iv) remodelling (scar formation) phase. Although these phases are distinct from each other, they overlap during the healing process which can last for a long time (Figure 1:8) (Gurtner et al. 2008).

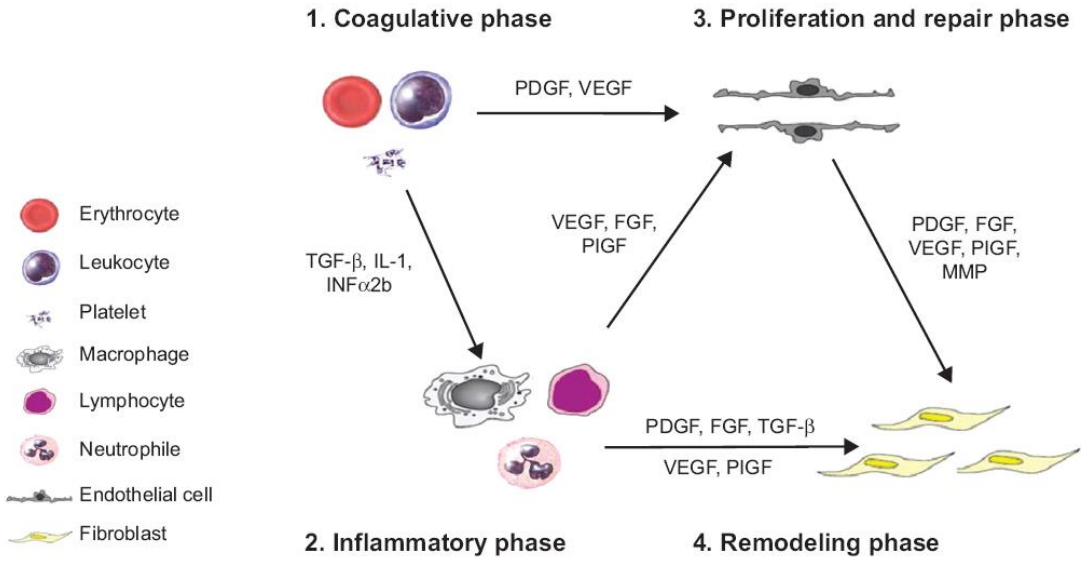


Figure 1:8 The wound healing process is recognised by four phases which are i) coagulative phase, (ii) inflammatory phase, (iii) proliferation and tissue repair (iii) remodelling (scar formation) phase. These phases overlap with each other during the healing process (Van Bergen et al. 2014).

During coagulation there will be the release of inflammatory mediators causing inflammation to occur. The inflammatory response involves several inflammatory mediators secreted from immune cells, e.g. macrophages and neutrophils. Inflammation plays an important role in regulation of the healing process during the first week after surgery (Eming et al. 2007). Proliferation starts in parallel with the inflammation phase, and includes epithelialisation, angiogenesis and the formation of a temporary extracellular matrix ECM. Proliferation will result in the activation of fibroblasts that will cause wound contraction to close the exposed area of the wound. The final phase includes tissue remodelling and scar formation. This process could start as early as 8 days after surgery and continue for months. The main cell responsible for this phase is the fibroblast. Targeting any of these phases will in principle modulate wound healing to potentially mediate scar formation (Witte & Barbul 1997).

Several approaches and medications have been investigated to modulate wound healing and to achieve a greater chance for bleb survival after GFS (Seibold et al. 2012). The most widely used medicines are the off label use of mitomycin C (MMC) and 5-fluorouracil during GFS or GDD implantation. Both of these medicines are cytotoxic agents used to treat malignant disease. The mechanism of action of both medications is by inhibiting DNA synthesis, which leads to cell apoptosis, including fibroblasts. Fibroblasts play an important role in collagen synthesis and fibrosis.

During glaucoma surgery a sponge soaked with MMC is placed in the subconjunctival space at the site of surgery for 5 minutes. The MMC will prevent excessive scarring by reducing cell growth and division during the healing process. Although the success of GFS was significantly improved by the treatment with MMC, the risk of side effects such as infection was also increased due to the toxicity of these drugs (Wu et al. 2013; Khaw et al. 2012). Topical corticosteroids such as dexamethasone and prednisolone have been reported to effectively improve the outcomes of GFS by supressing the inflammation process. In the clinic topical eye drops of corticosteroids are prescribed after surgery. Dexamethasone drops are used between 2-4 weeks after surgery.

Other strategies have been investigated over the years to modulate the scarring process. These include targeting one or more of the overlapping wound healing phases and the mediators involved in the phases (Lockwood et al. 2013; Georgoulas et al. 2008; Schlunck et al. 2016; Masoumpour et al. 2016). A wide range and number of drugs have been evaluated preclinically for subconjunctival use after glaucoma surgery including ilomastat, pirfenidone, doxycycline and anti-

vascular endothelial growth factor (VEGF) proteins in different dosage forms; however none are yet available in the clinic (Jung & Park 2016; Cui et al. 2008; Wu et al. 2013; Fakhraie et al. 2016; Martorana et al. 2015).

Age related macular degeneration

In England and Wales approximately 57% of blindness is related to age related macular degeneration (AMD) because of the aging population. Approximately 9% of global blindness is caused by AMD (Bunce & Wormald, 2006; Jonas et al., 2014). With age, the macula (which is the part of the retina responsible for central vision) degenerates and damaged due to local angiogenesis that damages the retinal tissue. Localised inflammation and fibrosis also occurs and the tissue damage cause loss of central vision. Often a patient has blurred vision and an inability to clearly see objects in detail.

There are two types of AMD; dry and wet AMD. Dry AMD is characterised by deposition of yellow debris comprised of lipids and fatty proteins (drusen) under the retina causing damage to the macula (Coleman et al. 2008). Wet AMD is characterised by excessive growth of abnormal blood vessels underneath the macula. These new blood vessels will leak blood and fluids in the area surrounding the retina and damage the macula (Figure 1:9). While dry AMD slowly progresses and take years to develop, wet AMD, if untreated, may end with severe vision loss within days (Kulkarni & Kuppermann 2005). This growth of blood vessels is driven by growth factors including vascular endothelial growth factor (VEGF). Overexpression of VEGF is observed in wet AMD (Coleman et al. 2008).

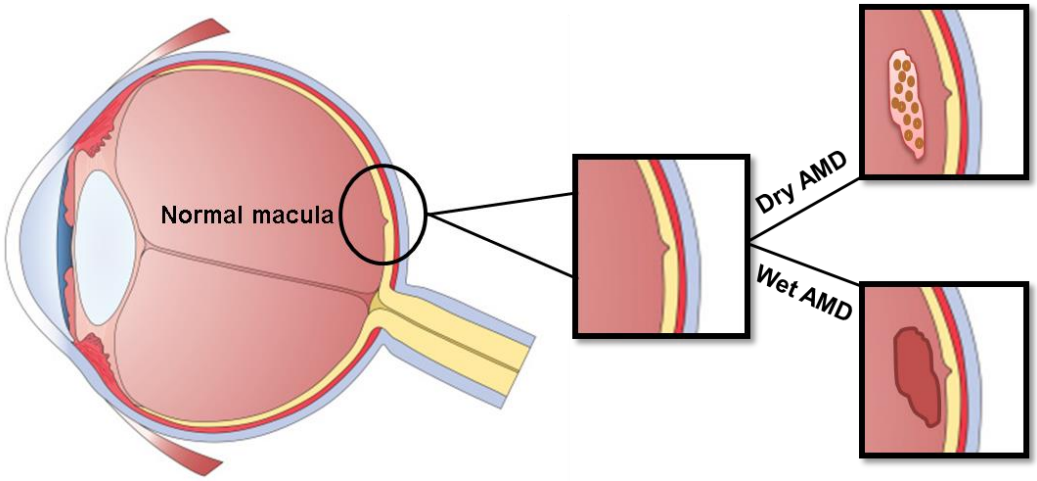


Figure 1:9 Normal macula and diseased one (wet and dry AMD). In dry AMD deposition of yellow debris is observed. In wet AMD excessive growth of abnormal blood vessels underneath the macula is observed. Adapted from (Patel 2016).

There is no available treatment for patients with dry AMD. Patients with wet AMD were previously treated by laser surgery (photodynamic therapy), but first line treatment is the intravitreal (IVT) injection of anti-VEGF antibodies. Photodynamic therapy can be used in an effort to halt the progression of the disease by destroying the abnormal blood vessels. The treatment involves the intravenous infusion of photosensitising agent (verteporfin) followed by laser activation of the drug. Unfortunately there is a high recurrence of angiogenesis (50%) and restoration of vision is limited after the photodynamic therapy (Lim et al. 2012; Yonekawa et al. 2015).

Currently the most widely used treatment is the IVT injection of anti-VEGF antibody based medicines Lucentis (ranibizumab; Genentech) and Eylea (aflibercept; Regeneron). These medicines are approved around the world for the treatment of wet AMD and are now widely used in the clinic (Meadows & Hurwitz 2012). Avastin (bevacizumab; Genentech) is a full antibody that targets VEGF and is licensed to treat angiogenesis in malignant disease. Bevacizumab is widely used unlicensed to treat AMD because it is much cheaper per dose than either ranibizumab or aflibercept (Avery et al. 2006). An early anti-VEGF drug derived from a poly(ethylene glycol) conjugated aptamer called Macugen (pegaptanib; Valeant Ophthalmics) was the first anti-VEGF drug to market, but is not widely used since the antibody based drugs have been approved. Since AMD is a chronic condition, it is often necessary to treat patients for many years. Ranibizumab is labelled for monthly administration and aflibercept is administered about every 6-8 weeks. Repeat IVT injections can be associated with several problems such as infections, retinal detachment and poor patient compliance (van Wijngaarden et al. 2005). The frequency of IVT injections is also costly. There is a need to determine if it is possible to increase the duration of action for these antibodies based medicines so that the frequency of IVT injections can be decreased.

Hydrogels a possible solution to current treatment problems

Glaucoma and AMD are considered as the main causes of preventable blindness worldwide. For each condition different treatments are available; however all the current treatments have limitations and problems.

GDDs were considered as a second line treatment in glaucoma and now they have been introduced as a first line treatment (Ayyala, Pieroth, et al. 1998). The main problem associated with GDDs is the lack of control on flow in the first few weeks before the fibrous capsule formation, which may cause hypotony. The other problem is foreign body response to the material used for the device

manufacture which often causes too much scarring around the device resulting in the failure of the device to control pressure (Chaudhry et al. 2012).

Implantable devices generally have sub-optimal biocompatibility with an associated foreign body response (Anderson 2001). When an implant is placed in the human body the initial inflammatory reactions often result in the formation of a fibrous capsule to encapsulate the implant. In drug delivery, encapsulation can completely ruin the release profile of the drug from the implant. In the case of GDDs, encapsulation often results in surgical failure by an increase in IOP that allows progression of the glaucoma. The foreign body response is varied based on many factors related to the material that is to fabricate the implant and the physical properties of the implant itself (e.g. surface smoothness, lack of sharp edges) (Onuki et al. 2008).

To overcome the problem of foreign body response, implants can potentially be coated with a biocompatible polymer (e.g. coronary stents). It is also possible to fabricate an implant from a biocompatible material (e.g. intraocular lens). Yu *et al* reported that coating a polyurethane glucose sensor with poly (hydroxyethyl methacrylate-2,3-dihydroxypropyl methacrylate-N-vinyl-2-pyrrolidone) hydrogels reduce the inflammatory response around the implant. The glucose sensor was subcutaneously implanted in rats for 28 days. There was a thin fibrous capsule and less inflammatory cells surrounding the sensor after 28 days implantation compared to non coted sensors (Yu et al. 2008).

An ideal GDD will (i) have minimal foreign body response, (ii) be able to control the IOP over a wide range of patients for a prolonged period of time, (iii) be easily implanted with minimum incision and (iv) be affordable for patients around the world. A GDD made from hydrogels may offer a better solution to the problems associated with the current devices available in the clinic. Hydrogels are known to be biocompatible materials that may elicit a decreased fibroblast reaction commonly seen with other materials (Poppas et al. 2016). Reduction in the inflammatory response will minimise scarring in the conjunctiva around the spacer plate if coated or fabricated from a hydrogel.

Hydrogels are also polymer networks where the polymer chains are cross-linked by covalent bonds or non-covalent associations. Cross-linking the polymer chains can render them to be a permeable network capable to transfer molecules such as oxygen and loaded drugs through the entangled polymer chain structure (Brennan et al. 1987). A GDD made from a hydrogel could control the IOP by restricting the flow of the aqueous humour at a constant rate from the anterior chamber. For example using an existing tube, it might be possible to allow outflow

into a spacer fabricated from a hydrogel. Depending on the character of the hydrogel (e.g. cross-link density, polymer molecular weight, water association properties), it might be possible to restrict aqueous flow through to control IOP.

Hydrogels could also be used to improve the outcome of GFS which is jeopardised by the excessive scarring after the surgery. A GDD could be made entirely from a hydrogel or a hydrogel could be used to fabricate a spacer connected to a tube (Figure 1:10). A drug could also be loaded into the device for the synthesis of GDD that have the ability to release drugs (combination device) to further decrease the foreign body response. The use of a hydrogel film made with biocompatible polymers for the delivery of anti-scarring or anti-inflammatory agents for subconjunctival implantation after GFS could also be considered as a way of improving success rate of GFS by reducing inflammation and scarring associated with GFS. A hydrogel film could also help to mechanically separate and prevent adhesion between the conjunctiva and episcleral surface after GFS (Figure 1:10).

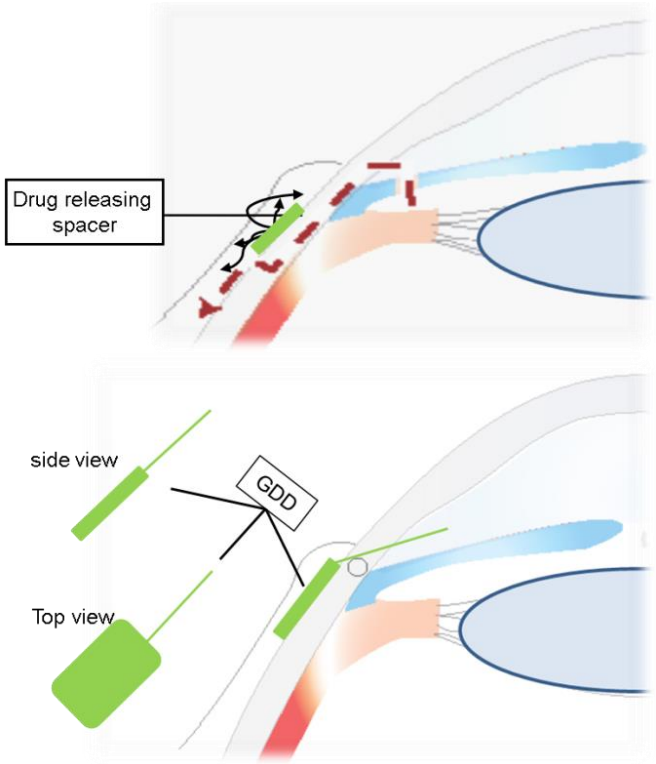


Figure 1:10 The position of a hydrogel GDD and releasing spacer (green) after GFS. For a GDD one end of the tube will be in the anterior chamber and the other end attached to a hydrogel plate placed in the subconjunctival space. For the spacer the drug will be released to the surrounding area of the implant in GFS (black arrows).

For the treatment of wet AMD, longer, more extended release formulations of anti-VEGF antibodies are required to reduce the frequency of injections. Extended release in the vitreous body could potentially be achieved either by formulating the protein into a solid implant or into a highly concentrated form

(Stewart 2016; Agarwal & Rupenthal 2013; Li et al. 2012; Rauck et al. 2013). Formulating a protein into a solid implant is challenging due to protein instability and the sensitivity of these molecules to aggregate and misfold (Mitragotri et al. 2014; Jiskoot et al. 2012). It would be also difficult to maintain the activity of the protein throughout device fabrication and so it is not surprising that no implants of therapeutic protein have been approved for clinical use in the eye or elsewhere in the body (Frokjaer & Otzen 2005).

Injectable hydrogels could be used to overcome the problems associated with solid implants. They have long been examined to encapsulate drugs for sustained local release. Due to the hydrophilic nature of the hydrogels they could help in preserving the activity of the protein while sustaining its release through the formation of semisolid implant. In order to explore the potential of hydrogels as delivery systems in the eye general knowledge on hydrogels is required. Understanding the unique properties, types, and the potential issues of hydrogels in drug delivery is important.

What are hydrogels

Hydrogels are three dimensional polymeric cross-linked networks that have the ability to absorb hundred to thousand times the dry weight of the hydrogels of water in the dry state and swell without dissolving or changing chemical structure (the swelling behaviour is a reversible process) (Figure 1:11). Hydrogels can be soft to display similar mechanical properties to tissues and extracellular matrix (they contain large amount of water and highly flexible). Hydrogels preserve the three dimensional shape upon swelling due to the presence of cross-linking points (junction points) (Buwalda et al. 2014). These points are physical or chemical cross-linking points that will form a meshwork structure which will prevent complete solubilisation of the polymer matrix.

The cross-links in hydrogels can either be covalent or non-covalent (e.g. ionic, metal chelated). Covalently cross-linked hydrogels can be prepared during polymerisation by the addition of cross-link monomers, which possess more than one polymerisable function. An example of covalently cross-linked hydrogels are those used in contact lenses. Many contact lenses are made using 2-hydroxyethyl methacrylate (HEMA). HEMA monomers are acrylates which undergo free-radical polymerisation. HEMA can be covalently cross-linked using a diacrylate (e.g. ethylene glycol dimethacrylate) (Han et al. 2009). Schmedlen *et al* described another chemical hydrogel using poly(vinyl alcohol) (PVA) prepared by photopolymerisation as tissue engineering scaffolds. The PVA was modified by

introducing photosensitive group into the PVA structure and mixed with photoinitiator. Once the mixture exposed to long wavelength a hydrogel is formed (Schmedlen et al. 2002).

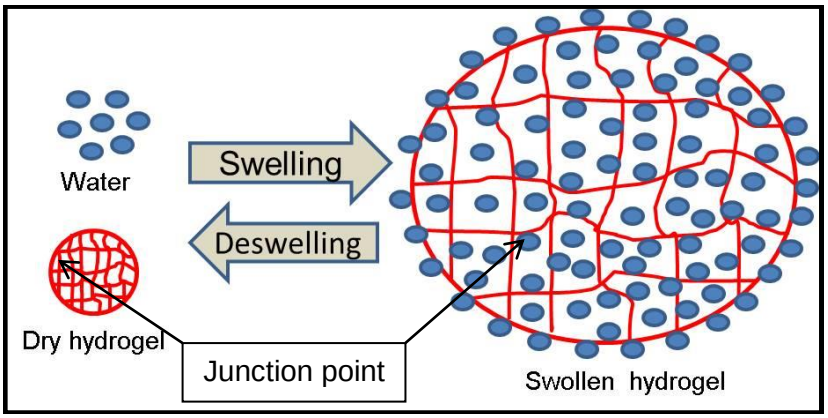


Figure 1:11 Swelling and deswelling behaviour of hydrogels. Dry hydrogel once placed in water swollen due to the formation of hydrogen bonds. The hydrogel increases in size several times. Once the water is removed the hydrogel returns to its original size and shape.

Hydrogels that are cross-linked by non-covalent interactions are sometime referred to as physical hydrogels. Non-covalent interactions between the polymer chains that can occur to give a material with hydrogel-like properties include ionic, hydrophobic, entanglement, hydrogen or van der Waals bonds. PVA hydrogels can be formed by physical cross-linking through freeze/thaw cycles. These cycles will help to generate intermolecular hydrogen bonds between the hydroxyl groups in the polymer chains and formation of the hydrogel (Stasko et al. 2009). Alginate hydrogels have been synthesised by physical cross-links through the formation of an ionic bond between alginates and multivalent cations (e.g. Ca^{+2}) (Bruchet & Melman 2015).

In both covalent and non-covalent hydrogels these bonds can be called junction points. The number of junction points is also referred to as the degree of cross-linking or the cross-linking density determines the many physical and mechanical properties of the hydrogel. The amount of polymer chain entanglement and the internal volume of the hydrogel, often referred as the hydrogel pore size can often be correlated to the cross-link density which is usually determined by the percentage of cross-linker used during the polymerisation reaction. Generally the higher formed and the cross-link density, the smaller the pore size or less permeable will be the hydrogel (Jadhav et al. 2012). Often the cross-link density is varied in an effort to vary hydrogel properties. For example, alginate hydrogels with different pore sizes were synthesised by varying the percentage of Ca^{+2} (cross-linking agent). An increase of Ca^{+2} concentration from 36 mM to 72 mM cause a reduction in the pore size from 247 µm to 52 µm. A further increase of Ca^{+2}

concentration to144 mM resulted in a decrease in pore size to 30 μm (Jang et al. 2014). Another factor that could be affected by the degree of cross-linking is the rheological and flow properties of the prepared hydrogels. Generally hydrogels made with a relatively low cross-linking density may be partially soluble and injectable. In contrast hydrogels with a higher cross-linking density would require implantation as if being a device.

Types of hydrogels

Hydrogels were first described in 1960 by Wichterle and have since been extensively investigated. Many types of hydrogels have now been described (Hoffman 2001). Hydrogel classification can be done by considering fabrication (e.g. cross-linker/monomer composition and process method) and by considering the intended use (e.g. degradable/non-biodegradable, stimuli responsive, drug release/tissue regeneration).

As mentioned previously hydrogels are cross-linked polymers. Based on the starting polymer hydrogels can be classified as natural, semisynthetic and synthetic. Natural polymers such as polysaccharides, cellulose, starch, chitin, gelatin and hyaluronic acid (HA) are widely used for food and pharmaceutical applications (Shanmugam et al. 2005). These natural polymers are often used in the manufacture of different dosage forms (such as tablets, micro and nanoparticles, films, beads) being used as binders, fillers, stabilisers, thickening agents and disintegrators. For example, gelatin is used in food, tablet coating and hard and soft gelatin capsule synthesis. Hyaluronic acid (in the form of sodium hyaluronate) is used as in eye drops to treat dry eye (Shanmugam et al. 2005). Hydrogels made from common natural polymers already used in healthcare applications tend to be more biocompatible, available for sourcing, safe and can have minimal (if no) side effects. However; many of these polymers are found to suitable lack mechanical properties, are subjected to batch to batch variability and may cause immunogenic reaction in parenteral applications (Vishakha Kulkarni, Kishor Butte 2012) .

Semisynthetic and synthetic polymers are also widely examined, sometimes in an effort to overcome the limitations of natural polymers. Semisynthetic polymers are derived by the modification of natural polymers. The resulting hydrogels can display more tunable mechanical properties (Zhu & Marchant 2011). An example is chitosan, which is the deacetylated derivative of natural polymer chitin. While chitin is poorly soluble in both aqueous and organic solvents, the deacetylated form of

chitin (chitosan) has been widely investigated and is much more commonly used in drug delivery due to its improved solubility (Dash et al. 2011).

The use of synthetic polymers offers the potential to further control the properties such as pore size, degradation time and mechanical strength of hydrogels (Ahmad et al. 2012). Many synthetic hydrogels are prepared by the free radical polymerisation of acrylate and methacrylate derived monomers. Examples of widely used monomers to makes synthetic polymers include derivatives of phosphoryl choline (PC) and acrylic acid (especially HEMA), and PVA and poly (N-vinyl pyrrolidone) (Gong et al. 2012; Kim et al. 2015).

Hybrid hydrogels synthesised from a mixture of synthetic and natural polymers can possess the advantages of both. Nguyen *et al* reported the synthesis of polyvinyl alcohol (PVA)-gelatin hydrogel for bone regeneration (Nguyen et al. 2016). Although PVA has low toxicity and good mechanical strength for implantation, it is limited for allowing proliferation and growth of cells. By combining PVA with gelatin, the hydrogel has 32% increased bone formation after 15 weeks compared to control.

Hydrogels can further be classified based on how they are prepared. The polymer chains can be homopolymers (derived from a single monomer), copolymers, block copolymer and interpenetrating networks (IPN). An IPN is the cross-linking of a polymer chain in the presence of another polymer (semi-IPN) or another monomer mixture (IPN) (Figure 1:12) (Dragan 2014). The two polymer chains are entangled with each other without chemical cross-linking between them (Aminabhavi et al. 2015).

For the synthesis of IPN two monomer mixtures are used. They are either polymerised together in the same time at the same pot (*in situ*) or sequentially polymerised. In both methods the two monomer systems should not undergo polymerisation with each other. In sequential polymerisation the first monomer mixture is polymerised. Following the polymerisation the mixture is impregnated in the other monomer mixture and polymerised again allowing the formation of entangled matrix of two different hydrogels. For *in situ* IPN synthesis the two cross-linkers used for one monomer mixture should not interact with the other monomer mixture (Aminabhavi et al. 2015). Park & Kim reported that a IPN made from glycol chitosan and PVA will have higher mechanical strength compared to glycol chitosan hydrogels alone (Park & Kim 2006).

For semi-IPN synthesis a polymer and a monomer mixture are used. The monomer mixture is polymerised in the presence of the polymer. Mixing two different polymers is often difficult to achieve, so a semi-IPN offers the chance to for

two polymers to be more intimately entangled. A cross-linked IPN hydrogel often has better mechanical properties, e.g. greater strength than non-IPN hydrogels. IPN hydrogels can be prepared in one pot (*in situ*) or the monomer mixture cross-linked first then impregnated in the polymer solution (which is called sequential synthesis) (Samanta & Ray 2014). Mahdavinia *et al* reported that when chitosan is polymerised around polyacrylic acid (PAA) to form semi-IPN hydrogels the swelling behaviour of the synthesised hydrogel was affected by the degree of cross-linking of chitosan. The swelling ratio of the semi-IPN hydrogel increased in acidic pH and decreased in neutral pH with increasing chitosan ratio (Mahdavinia et al. 2008).

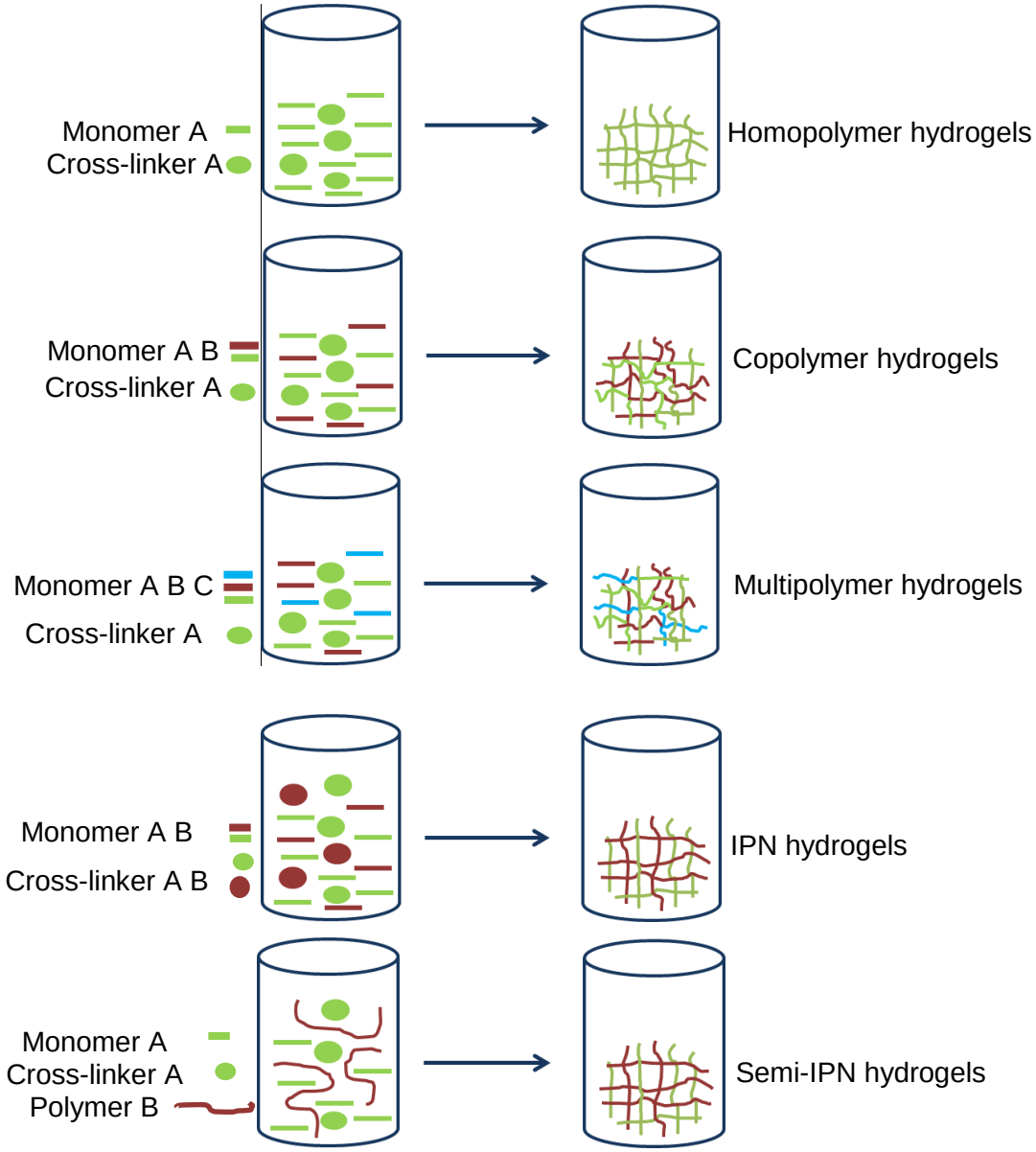


Figure 1:12 Classification of hydrogels based on hydrogel preparation method. They are divided into homopolymer, copolymer, multipolymer, full IPN and semi-IPN hydrogels.

Hydrogels can also be classified into Non-biodegradable and biodegradable. Non-biodegradable hydrogels are often covalently cross-linked hydrogels, and a well-known example is soft contact lenses (López-Alemany et al. 2002). The resistance of some hydrogels to degradation made them good candidates for some applications in tissue replacement, coating of medical devices and wound healing dressings (Bach et al. 2013; Butruk et al. 2012). Ma *et al* reported that a non-biodegradable hydrogel synthesised using PVA and PVP polymers could be used as articular cartilage replacement (Ma et al. 2009).

Degradable or resorbable hydrogels are designed to clear from the human body after a specified period of time (Stauffer & Peppast 1992). The degradation products must be biocompatible and non-toxic. There are examples of hydrogels that have been designed to clear either within days or months (Lawyer et al. 2012; Bhattarai et al. 2010; Lee et al. 2015). The degradable element can be either in the polymer main chain or within the cross-links of a hydrogel. For example Bryant and Anseth reported that the degradation behaviour of a poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) diacrylate cross-linker could be modified using poly (ethylene glycol) dimethacrylate (PEGDM) to support the growth of chondrocytes and the formation of extracellular matrix that is similar to human tissues (Bryant & Anseth 2003).

Hydrogels can also be classified into non-responsive (conventional hydrogels) and stimuli responsive (smart hydrogels). Non-responsive hydrogels once swollen will remain in the same shape and size with changing conditions such as pH, temperature and ionic strength. This type of hydrogel is often used for tissue replacement applications and coating of medical devices (Gonzalez & Alvarez 2014). PVA hydrogels have been widely investigated for the repair and replacement of articular cartilage because of similar physical properties (e.g strength, and frictional and lubricating behaviour to articular cartilage) (Spiller et al. 2011).

Stimuli responsive hydrogels change conformation with changes in surroundings (e.g. pH and ionic strength). The physical and mechanical properties of stimuli responsive hydrogels can be changed, including swelling and pore size when there is a change in the local environment surrounding the hydrogel. Change in hydrogel properties can be reversible once the stimulus is removed. In addition to changes in pH and ionic strength, other stimuli include changes in temperature, electricity, magnetic field, pressure, and biological changes such as enzyme, antibody, and glucose concentrations (Figure 1:13) (Soppimath et al. 2002).

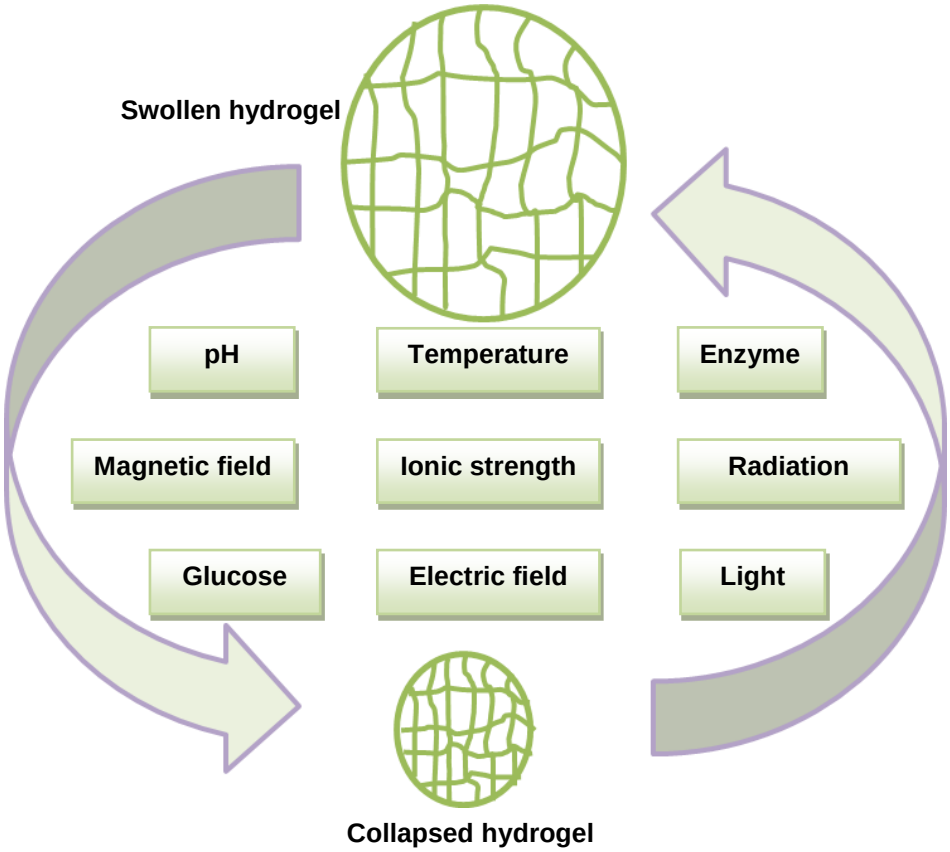


Figure 1:13 Factors affecting the swelling and collapsing process of smart hydrogels. The swelling and deswelling is reversible and depending on the stimuli such as pH, temperature, radiation and ionic strength.

Stimuli responsive hydrogels have been examined to develop gels that undergo conformational change, such as collapsing after injection to form a semi-solid depot to prolong the release of drug from the hydrogel. Known as *in situ* forming gels, these collapsing hydrogels have been evaluated in many applications, e.g. eye drops (Kumar et al. 2013). Stimuli responsive hydrogels that change properties at a pH have widely investigated due to the different range of pH values in body. Polymers with basic groups, such as poly (2-vinylpyridine), and with acidic groups, such as polyacrylic acid (PAA) have been examined (Li et al. 2016; Sensitif et al. 2015).

Hydrogels that collapse at a specific temperature are known as thermo-responsive hydrogels. Much effort has focused on hydrogels that are swollen at ambient temperature but which collapse at physiological temperature entrapping the drug inside and slowing its release. The gel transition can be fast, within seconds once the transition temperature is reached (Lue et al. 2011). The transition temperature for hydrogels that collapse is called the low critical solution temperature (LCST). LCST is the temperature below which a polymer or hydrogel exists in an extended form. When the LCST is reached, the polymer conformation

changes as the polymer chain interactions increase to expel water (and entrapped drug) with the polymer collapsing to a more globular form (Figure 1:14) (Patil et al. 2013).

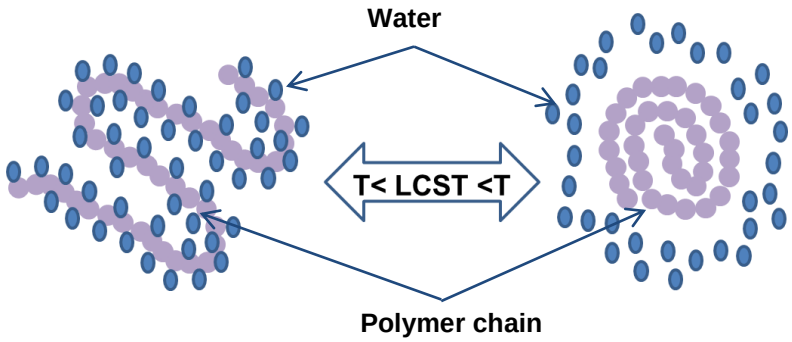


Figure 1:14 The effect of temperature on the conformation of PNIPAAm polymer in water solution. Below LCST the polymer chain is relaxed and extended. Above LCST the polymer becomes insoluble in water and collapse (the process is reversible).

At temperatures below the LCST, the hydrogel is swollen with water with hydrogen bonds between the polymer chains and water. Once the temperature increases above the LCST, the hydrophobic interactions between the polymer chains begins to predominate over the hydrophilic non-covalent interactions the polymer chains have with water. This causes the polymer to collapse becoming insoluble in water. The term volume phase transition temperature (VPTT) is also used to describe the temperature at which the hydrogel undergoes a transition from a completely swollen form to a solid form (deswelled) (Chang et al., 2015; Constantin, Cristea, Ascenzi, & Fundueanu, 2011).

Natural polymers that could be used for the synthesis of thermoresponsive hydrogels are cellulose, chitosan and gelatin. Upon deswelling drug molecules could be entrapped in the hydrogel matrix and slow release of the drug could be achieved. The most widely investigated synthetic polymer for thermoresponsive hydrogel formulation is poly (N- isopropylacylamide) (PNIPAAm). It has an LCST of approximately 32^{o}C (Stile et al. 1999; Lue et al. 2011; Schild 1992).

Hydrogel synthesis

Hydrophilic polymers tend to be used in biomedical studies because they can interact with water through common hydrophilic groups such as –OH, -CONH, -CONH2, -COOH and –SO3H. Hydrogels prepared from hydrophilic polymers are soft and often lack mechanical strength. Hydrophobic polymers cannot be used alone for hydrogels that are destined to be used in an aqueous application. Hydrophobic polymers are either copolymerised with hydrophilic ones or a

hydrophilic group applied onto the polymer structure to modify the polymer properties and improve their interaction with water (Volkmer et al. 2013).

Several chemical and physical cross-linking methods are used for hydrogel synthesis depending on the properties of the cross-linking bonds required (such as covalent or ionic bonds) but the most widely used method is free radical polymerisation (Hennink & Nostrum, 2002; Brazel & Peppas, 1995). In this process a mixture of monomers, cross-linker and initiator is used. Decomposition of the initiator causes the polymerisation by free radical addition to occur. Variation in the amount of monomers, cross-linker, initiator and polymerisation conditions will result in the formation of different hydrogels (Gelfi & Righetti 1981). Chung *et al* reported the synthesis of dextran-hydroxyethyl methacrylate microspheres and macrohydrogels with different mechanical properties was obtained when the preparation conditions modified. When the initiator concentration increased the gelation time reduced and the mechanical strength of the spheres increased (Chung et al. 2005).

There are several types of polymers that could be used for non-biodegradable hydrogel synthesis in ophthalmic applications. Among the groups of polymers that are of interest, widely investigated and used for ophthalmic applications are HEMA and MPC.

2-hydroxyethyl methacrylate (HEMA)

Since their first discovery in 1960 by Wichterle and Lim, HEMA hydrogels have become the corner stone for hydrogel development. Hydrogels made from HEMA have been investigated for many years for different biomedical and pharmaceutical applications such as contact lenses, drug delivery and implantation (Tomar, Tomar, Gulati, & Nagaich, 2012; Stirbu et al., 2011). HEMA is from the family of acrylate polymers in which several other polymers are also available and used such as poly (methyl methacrylate) (PMMA), which is used for IOL synthesis (Figure 1:15).

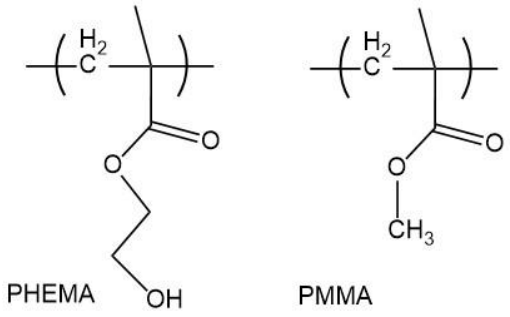


Figure 1:15 Chemical structure of poly (hydroxyethyl methacrylate) (PHEMA) and poly (methyl methacrylate) (PMMA).

Monomers used in hydrogel synthesis are generally toxic while the resulting polymers are not toxic. As a polymer, HEMA, being a polyester is not soluble in water in nature but when cross-linked into a hydrogel the gel matrix swells in water or biological fluids. The swelling behaviour is related to the hydrophilic hydroxylethyl pendant groups available along the polyHEMA main chain that are capable of forming hydrogen bonds with water. HEMA hydrogels are known to be biocompatible. This is due to the reduction of protein adsorption onto polyHEMA surfaces and improved biocompatibility with blood cells. HEMA has been used for the synthesis of soft contact lenses, ophthalmic implants such as Esnoper-V2000® and Esnoper clip®. These implants are investigated to improve the outcome of deep scleretomy surgery for glaucoma patients. HEMA has also been modified by copolymerisation with other polymers for hydrogel synthesis to improve their water uptake, mechanical properties and modify their drug releasing properties when used for drug delivery (dos Santos et al. 2008; Goda & Ishihara 2006).

2-methacryloyloxyethyl phosphoryl choline (MPC)

Zwitterionic polymers are biocompatible materials that are used as coating material or for the synthesis of biocompatible implants (L. Zhang et al. 2013). Zwitterionic means the overall charge of the surface of the molecule is zero. Hydrogels prepared from zwitterionic polymers are considered more biocompatible than other types of hydrogels. One of the most widely investigated zwitterionic polymers is phosphorylcholine polymers (PC). PC polymers have zwitterionic pendant groups that are similar in structure to head group of PC lipids in the cell membrane bilayer (Figure 1:16) (Bretscher & Raff 1975).

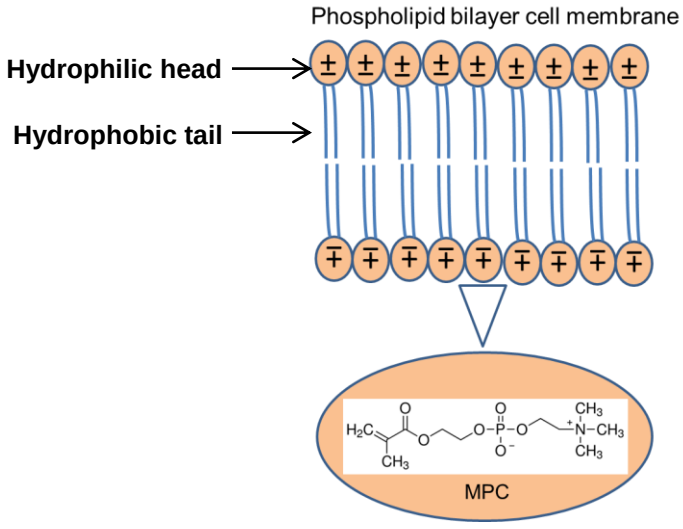


Figure 1:16 The resemblances of 2-methacryloyloxyethyl phosphoryl choline (MPC) to the phospholipid bilayer comprising the cell membrane.

The zwitterionic charge is thought to be a key property which renders PC polymers to be biocompatible with body tissues and results in decreased protein adsorption and foreign body response (Lewis et al. 2002; Schlenoff 2014). The neutral charge on the PC group helps to reduce the electrostatic charge on the surface of the polymer chains which will reduce interactions between proteins and the polymer. The highly polar nature of the PC polymer head results in strong hydration of the PC group. A layer of water molecules will surround the PC polymer surfaces making it difficult for proteins and cells to bind irreversibly to a PC surface (Jiang & Cao 2010). It is this antifouling nature that PC can impart on biomaterials to improve biocompatibility of the materials. They also have excellent blood biocompatibility (Ishihara, Aragaki, Ueda, Watenabe, & Nakabayashi, 1990; Andrew L. Lewis, 2000).

Several PC based polymers are clinically available and widely used in many applications including coating of heart stents, blood filtration and processing devices and contact lenses. Vertellus Biomaterials specialises in the synthesis of PC based polymers (http://www.pcbiomaterials.com/). The nomenclatures of the PC polymers that are commonly used clinically are PC-1059, PC-1036 and PC-1015. Each product number represents a copolymer derived from a different formulation in which 2-methacryloyloxyethyl phosphoryl choline (MPC) is always included (Lewis 2006). For example PC-1036 is a cross-linked co polymer of MPC, laurylmethacrylate, 2-hydroxypropyl methacrylate and trimethoxysilyl propyl methacrylate and used for coating of BiodivYsio™ heart stent (Goreish et al. 2004; Iwasaki & Ishihara 2005).

Ophthalmic implants made of HEMA alone and devices coated with PC polymers are available in the clinic. Ophthalmic implants made of HEMA such as Esnoper-V2000® which is used in deep sclerotomy surgery of the eye (Stirbu et al. 2011). In the year 2000 FDA has approved the use of BiodivYsio™ AS PC coated heart stent. This device is a small stainless steel wire mesh stent coated with phosphorylcholine (PC). This implant is to be implanted permanently in the blocked coronary arteries to improve blood flow. PC was used as a coating to improve the biocompatibility of the metal stent (Butany et al. 2005). Combining both HEMA and MPC in an implant will have the potential of higher biocompatibility and less foreign body response compared to using each alone.

N-isopropylacrylamide (NIPAAm)

The most widely used and investigated synthetic polymer for thermoresponsive hydrogel formulation is poly (N- isopropylacylamide) (PNIPAAm). It is a water

soluble polymer with an LCST of approximately 32^{o}C (Stile et al. 1999; Lue et al. 2011; Schild 1992). PNIPAAm has gain popularity due to its ability to copolymerise with other monomers. Copolymerisation strategies provide a means to engineer a hydrogel with varying VPTT and properties (e.g. hydrophilicity). When copolymerised with hydrophilic polymers, the VPTT of PNIPAAm often increases (Villa et al. 2014; Alexander et al. 2014; Lee & Lu 2013). This is an advantage in formulating thermoresponsive hydrogels for pharmaceutical applications as the transition temperature can be closer to physiological body temperature. Also when polymerised with pH sensitive polymers a hydrogel with dual activity can be prepared (Zhang et al. 2007; Gao et al. 2013). When NIPAAm and chitosan were polymerised and used for the synthesis of a thermosensitive *in situ* hydrogel for the delivery of timolol maleate as eye drops, there was a two-fold increase in the maximum concentration of timolol maleate when compared with conventional eye drops in the rabbit eye. The control of IOP was also improved by using the *in situ* hydrogel (Cao et al. 2007).

The characteristics and physical properties of NIPAAm hydrogels are often affected by the co-monomer and cross-linker used to fabricate the hydrogel. The thermal responsiveness of NIPAAm hydrogels is controlled by a balance between hydrophilic and hydrophobic interactions between the polymer chains and the surrounding medium (Stewart et al. 2011; Gan et al. 2016). This balance is affected by the type and percentage of cross-linker used. Lee and Lin reported that the swelling behaviour of NIPAAm-co-(ethylene glycol) methylether acrylate (NIPAAM-co-PEGMEA) hydrogels was affected by the cross-linker that was used. When tetraethylene glycol diacrylate (TEGDA) was used as the cross-linker, the swelling ratio (SR) was 23 compared to 7 for *N,N*-methylene bisacrylamide (NMBA) and 6 for ethylene glycol dimethacrylate (EGDMA) when they were used as cross-linkers in the same molar ratio. Lee and Lin also reported that the SR of NIPAAm hydrogels decreased with increasing the molar ratio of TEGDA as cross-linker. The reported SR was 23, 20 and 11 for 5 mol%, 10 mol% and 15 mol% respectively (Lee & Lin 2006).

In case of proteins, the use of a hydrophilic cross-linker could create a hydrophilic environment around the loaded protein that helps to preserve its stability and activity during formulation and use.

Hydrogels in ophthalmic applications

Hydrogel have been examined in applications such as tissue engineering, artificial replacement of organs, coating of implantable devices, drug delivery, gene delivery, scaffolding and wound dressings (Du et al. 2012; Vashist et al. 2014; Hoffman 2012). One of the major successes for the use of hydrogels is as wound dressings. Hydrogel wound dressings can absorb exudates from wounds are useful to treat necrotic wounds. Examples of the available hydrogel wound dressings in the clinic are Intrasite®, Neoheal®, Purilon® and Aquaflo™ (Calo & Khutoryanskiy 2015).

Hydrogels are also used as sealants during surgery. Duraseal® is used as a Spine Sealant System in spinal surgery. This product consists of two solutions (polyethylene glycol (PEG) ester and trilysine amine) that undergo reaction during injection to form a hydrogel (Annabi et al. 2015). Spaceoar® is another hydrogel and is used to reduce rectal injury in men receiving prostate cancer radiation therapy (RT) by acting as a spacer pushing the rectum away from the prostate. Spaceoar® consists primarily of water and PEG. Upon injection of the liquid precursors the hydrogel solidifies without producing measurable heat. The hydrogel maintains space during radiation therapy and then gradually liquefies, and is cleared from the body (Pinkawa et al. 2011). These hydrogels are non-medicated hydrogels.

Medicated hydrogels are also available in the clinic. For example, Supprelin® LA is a subcutaneous implant for the treatment of children with central precocious puberty. This product consists of 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, trimethylolpropane trimethacrylate, benzoin methyl ether, perkadox-16, triton X-100 hydrogel incorporating 50 mg of histrelin acetate, which is delivered over 12 months (du Toit et al. 2016). Another hydrogel product is Atridox® which is a subgingival controlled-release formulation of doxycycline for infection. This system consists of two solutions; Atrigel® polymeric formulation of poly(DL-lactide) dissolved in N-methyl-2-pyrrolidone and contains doxycycline hyclate. Upon contact with the crevicular fluid, the liquid product solidifies into a gel and then allows for controlled release of drug for a period of 7 days (Gad et al. 2008). In ophthalmic applications hydrogels are widely used as contact lenses and implants. They are also investigated as drug delivery systems and vitreous substitute (Figure 1:17).

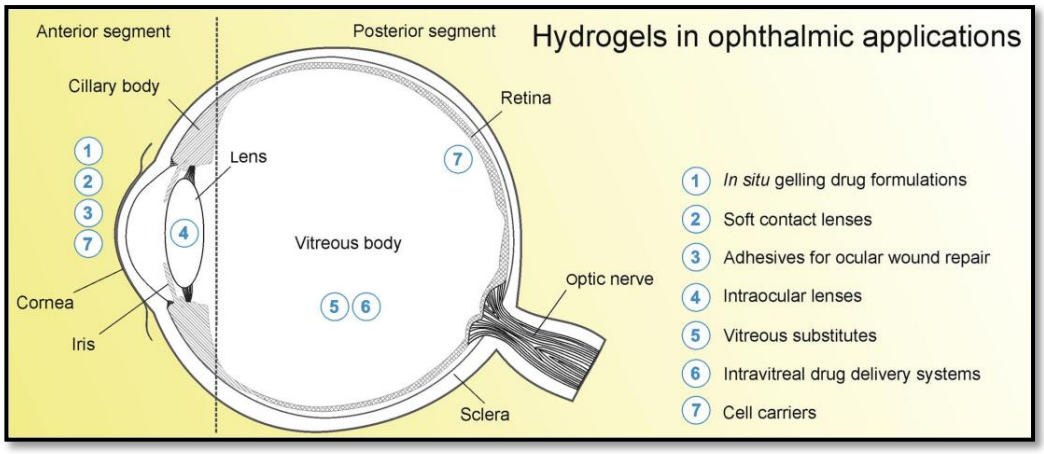


Figure 1:17 Different forms of hydrogels and the possible application sites of the hydrogels in the eye for ophthalmic drug delivery (Kirchhof et al. 2015).

In ophthalmic applications the most successful area for hydrogel products is soft contact lenses (SCL). Contact lenses are widely used for vision correction and cosmetic purposes. Some contact lenses are used for medical purposes such as bandage contact lenses and intraocular lenses (IOL) (Lai 2013). The first SCL was synthesised from poly (2-hydroxyethyl methacrylate) (pHEMA) hydrogel. Since then several polymers have been developed and used for contact lens including methacrylic acid and N-vinylpyrrolidone (Nicolson & Vogt 2001).

An important development was the introduction of silicone hydrogel contact lenses. The silicone in the contact lens structure offers the advantage of higher oxygen permeability compared to SCL which allowed extended wear overnight and for several days (González-Méijome et al. 2006). Bandage contact lenses are used to protect the cornea after injury or surgery and allow it to heal properly (Fraunfelder & Cabezas 2011). Contact lenses have been thoroughly investigated as ocular delivery systems (Christie, 1999; Gupta & Aqil, 2012; Maulvi, Soni, & Shah, 2016). Kim *et al* reported the potential extended release of timolol and dexamethasone for more than 4 weeks from silicone hydrogel contact lens depending on the monomer mixture used for the synthesis of the lenses and the loading method used (Kim et al. 2008).

SCLs have also been examined as a platform for the delivery of other dosage forms such as liposomes, micro and nanoparticles. Gulsen *et al* reported the dispersion of a lidocaine loaded dimyristoyl phosphatidylcholine liposomes into a HEMA contact lens. The release of the drug was sustained to up to 7 days with 65% of the loaded drug being released during this period (Gulsen et al. 2005).

Ophthalmic hydrogel implants have also been used for several clinical applications. IOLs are used in cataract surgery. Cataract is the opacification of the

natural eye lens and can be treated by surgery. The surgery involves the removal of the natural lens and replacing it with an IOL (Hollick et al. 1999). IOLs generally are made from acrylic polymers such as polymethylmethacrylate (PMMA) and pHEMA. The AcrySof® IOL is made of copolymer of phenylethyl acrylate and phenylethyl methacrylate.

The flexibility of the hydrogel materials allowed the synthesis of foldable IOLs that can be implanted with minimum incision (Bozukova et al. 2010). Hydrogels are also used as sealant of corneal incisions when IOL implanted after cataract surgery. Resure® sealant (which has similar composition with Duraseal) is currently available in the clinic (Masket et al. 2014). In addition to contact lenses, HEMA was used for the synthesis of ophthalmic implants. Esnoper® (HEMA Implant) is used as a spacer in deep sclerotomy which is an operation used for the treatment of glaucoma patients (Stirbu et al. 2011). Hydrogels are also investigated as a biomaterial for vitreous substitution in the posterior segment. Giannetti et al demonstrated the possibility of using hydrogels synthesised from PVA and tri-sodium trimetaphosphate as a substitute for the vitreous humour (Parikh et al. 2014). Chang *et al* also reported the synthesis of *in situ* hydrogel made from zwitterionic sulfobetaine methacrylamide and acryloyl cystamine monomers as substitute. These gels demonstrates excellent biocompatibility when *in vivo* studies were performed in rabbits (J. Chang et al. 2015).

Hydrogels have been widely investigated as potential drug delivery systems for different ophthalmic applications. Hydrogels were investigated to prolong the corneal contact time of eye drops in efforts to improve bioavailability and patient compliance. An *in situ* hydrogel that is applied as a solution that then forms a gel led to the development of Timpotic XE®, which is a gel forming solution of timolol maleate that is used to treat glaucoma. The formulation is present in a solution form of timolol maleate and anionic gellan gum polymer. Once instilled into the eye, the aqueous gellan gum solution starts to gel by forming a complex with the cations in the tear film and prolong the contact time of the drug with ocular tissues. It is used once daily compared to the traditional timolol eye drops, which are used twice a day (Khare et al. 2015; Shedden et al. 2001).

Injectable thermoresponsive hydrogels were widely investigated as IVT injections to sustain the release of therapeutic proteins in the posterior segment of the eye. Xie *et al* reported the sustained release of Avastin for up to 14 days *in vitro* when a poly(lactic-co-glycolic acid)-poly(ethylene glycol)-poly(lactic-co-glycolic acid) (PLGA-PEG-PLGA) hydrogel was injected in rats. This formulation was compared to Avastin injection which lasts only 3 days (Xie et al. 2015). In spite of the

extensive research in the area of hydrogels in ophthalmic drug delivery only Timpotic XE® is clinically available.

Hydrogels and water

Hydrogels have many advantages as implantable materials because they are biocompatible and the ability of hydrogels to load and release drugs. These two characteristics are highly affected by the amount, type and distribution of water inside the hydrogel (Shi et al. 2012). The relationship between water and hydrogels has long been the focus of researchers and has been investigated for many years (Lee et al. 1975; Jhon & Andrade 1973). The water inside hydrogels can be thought to have two main functions; (i) control the transport of materials (nutrients, cells, drugs) in and out of the hydrogel and (ii) to provide flexibility and an environment similar to body tissues.

The water in hydrogels is often classified as free (bulk) and bound water, which is non-covalently associated to the polymer within the hydrogel. Bound water is further classified into slightly and tightly bound water (Li et al. 2005). When a dry hydrogel device (e.g. film) is placed in an aqueous environment the water binds initially to the polar, hydrophilic groups through tight hydrogen bonding leading to what is called (primary bound water) and the matrix starts to swell. Once the gel matrix swells, hydrophobic groups will be exposed to water. Extra water entering the gel will interact with the hydrophobic groups forming (secondary bound water); both are called total bound water (Hoffman 2012; Hatakeyama & Hatakeyama 1998). The polymeric matrix continues to absorb water until it reaches equilibrium and complete swelling is observed. This extra water will fill the pores and spaces within the hydrogel, and once equilibrium is reached, this water is called free or bulk water (Figure 1:18) (Wang & Gunasekaran 2006). The total water in a hydrogel is the sum of free and bound water. Sometimes water in hydrogels is classified according to its ability to freeze. Non-freezable water is a term used to describe water molecules that are tightly bound to the polymer matrix. The bulk and loosely bound water is called freezable water (Tranoudis & Efron 2004).

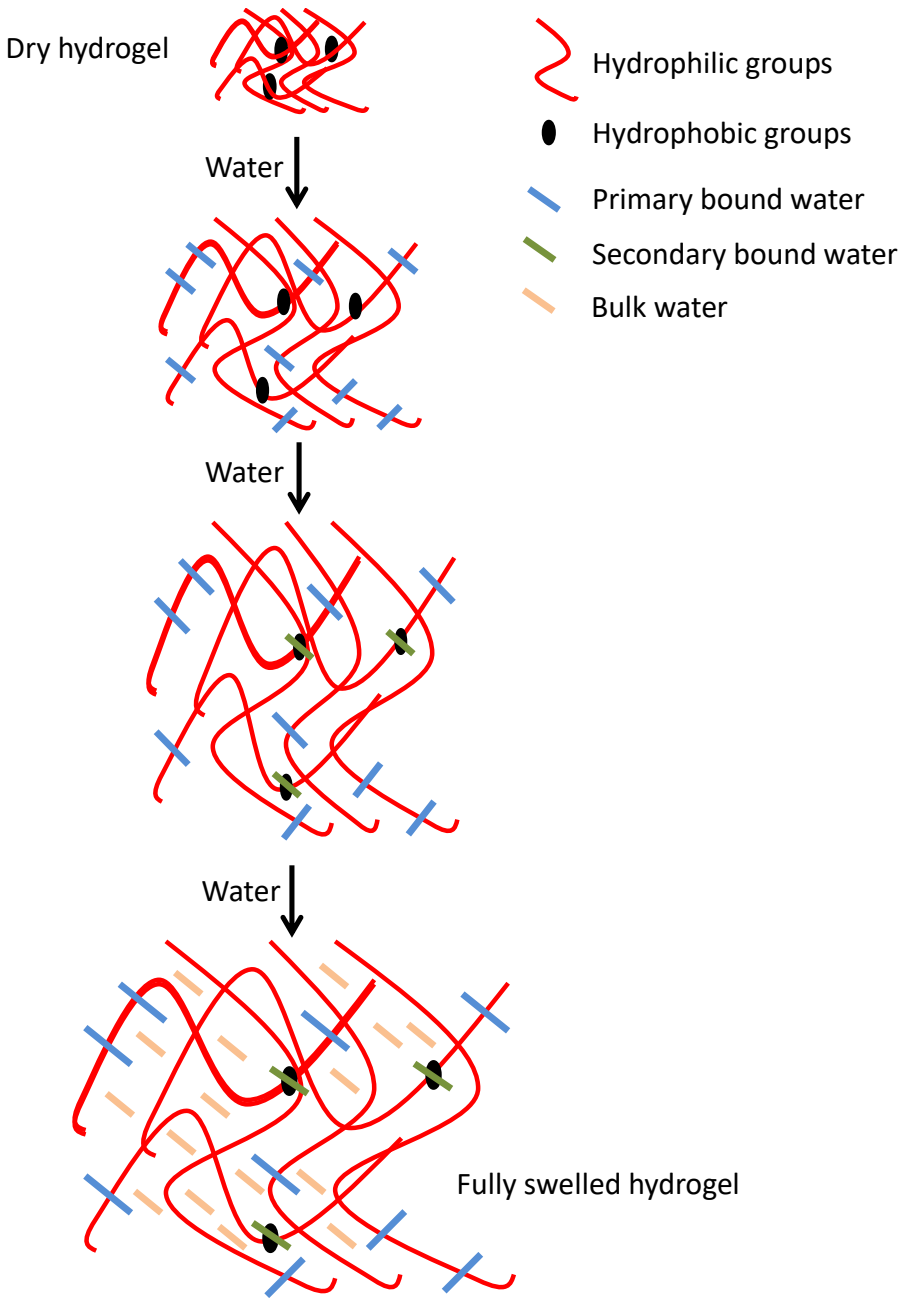


Figure 1:18 Interactions of water with hydrogels. First the water binds only to hydrophilic groups forming the primary bound water. Extra water binds with hydrophobic groups and form secondary bound water. Excess water fills the voids in the hydrogel matrix and called bulk water.

The amount of water at the polymer surfaces can play an important role in biocompatibility (platelet and protein adhesion) and drug release from hydrogels (He et al. 2008). Higher water content often results in a softer hydrogel that has less effect on the surrounding tissues (less inflammation and foreign body response) (Morais et al. 2010). The presence of water also reduces the interaction between polymer and protein, and typically reduces protein adsorption on the surface of hydrogel device leading to increased biocompatibility. Proteins display less conformational change in the presence of associated water around a hydrated

surface and the adsorption of a protein to the hydrogel surface can be reversible (Ishihara et al. 1998).

In general when the total water content increased the biocompatibility of a hydrogel matrix increases as well. Researchers were in disagreement on which type of water responsible for biocompatibility; free, slightly bound or bound water. Shi *et al* reported that free or bulk water is largely responsible for the improvement in biocompatibility (Shi et al. 2012). Tanaka and Mochizuki reported that the slightly bound water on the polymer surface is the type of water responsible for the reduction in protein adsorption while He *et al* suggested that bound water is the key player (He et al. 2008; Tanaka & Mochizuki 2010).

Limitation of hydrogels

While hydrogel derived devices (e.g. SCL and IOLs) are available in the clinic, the number of hydrogel based products used for drug delivery system is limited. One challenge is the large amount of water that is usually associated within most biomedical hydrogels. Dissolved substrates tend to rapidly diffuse throughout hydrogel. The drug is usually loaded by two main methods; soaking (drug imbibition) and *in situ* loading (during polymerisation) (Figure 1:19) (Kim et al. 1992). Unfortunately release of the drug is often too rapid once the drug-loaded hydrogel is placed in a drug free environment.

In situ loading involves the polymerisation of the hydrogel matrix in the presence of the drug. This method of drug loading is limited by the requirement to remove leachable monomer components after polymerisation. Removal of toxic leachables will be accompanied by loss of the drug which is of special concern for small molecules especially hydrophilic drugs. In situ loading may better entrap large molecular weight drugs such as proteins within the hydrogel matrix. It is difficult for a protein to mix within a hydrogel. Large molecules do not effectively mix, which is nearly impossible when one of the macromolecules is a cross-linked network. Another disadvantage is the polymerisation conditions may result in side reactions with the drug. To avoid these problems soaking methods are often used.

In the soaking method the hydrogel matrix is prepared in advance and the xerogel (which is the fully dried hydrogel) is allowed to hydrate in the drug solution allowing the drug to be absorbed within the swollen hydrogel (Figure 1:19). The amount of drug absorbed depends on the solubility of the drug, drug conformation, molecular weight, interaction with the hydrogel, concentration of the drug in the loading solution and the degree of swelling of the hydrogel in the loading solution (Maulvi et al. 2014).

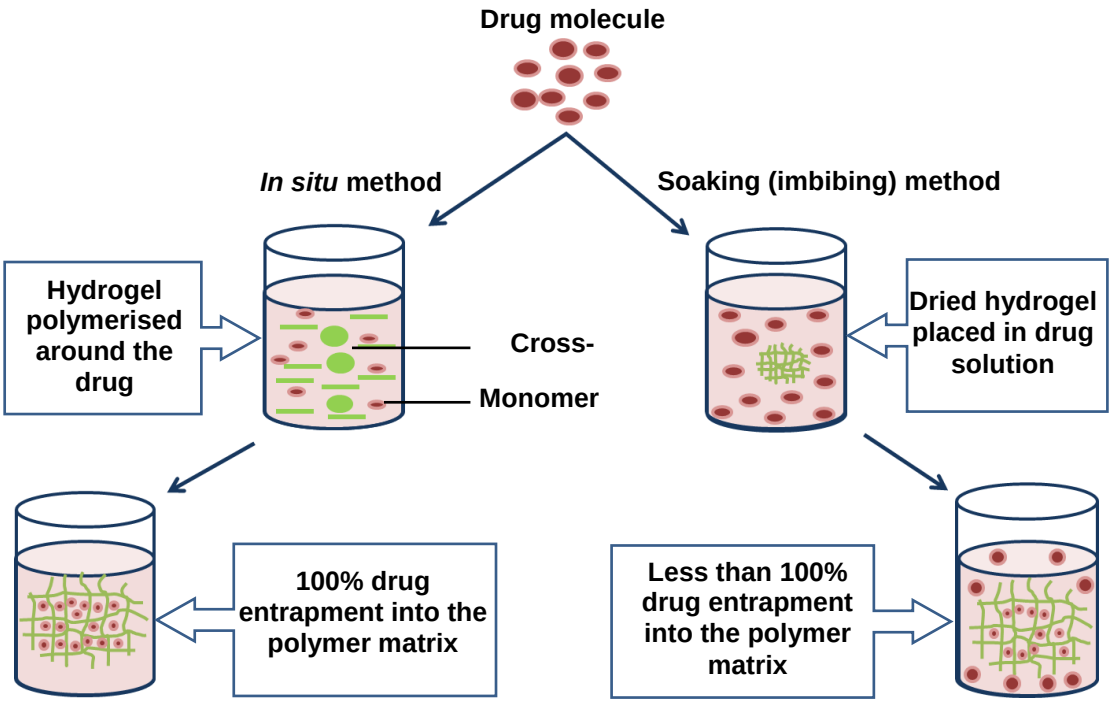


Figure 1:19 Drug loading method for hydrogels; soaking method and *in situ* method. In soaking method a dried hydrogel is incubated in the drug solution until equilibrium is reached between the hydrogel and the solution (a fraction of the drug will be entrapped). In *in situ* method the monomer and/or cross-linker polymerised in the presence of the drug (100% entrapment of the drug).

The large amount of water in hydrogels facilitates the loading of low molecular weight hydrophilic drugs, but subsequent release is often too fast and is accompanied by a burst effect. In most cases the burst release is problematic especially with narrow therapeutic index drugs and may cause toxicity to the tissues surrounding the hydrogel. Drugs with low water solubility can be released slowly from hydrogels depending on the drug solubility; however drug loading efficiency is low because of the limited drug solubility in water which makes it difficult to load therapeutic doses (Kirchhof et al. 2015). Although extensive research has been conducted over the years to address such problems there is limited number of drug releasing hydrogels in the clinic to this day (e.g. Supprelin® LA, Atridox®, Timpotic XE®).

Hydrogels with higher water content also tend to lack mechanical strength and may be subjected to rupture during implantation (Calvert 2009). Sterilisation is also a problem with implantable hydrogels as they may be subjected to chemical or physical changes under the influence of thermal, chemical or radiation sterilisation (Kanjickal et al. 2008; Stoppel et al. 2014).

Hypothesis and aims

Hydrogels are potentially good candidate materials for continued development in ophthalmic applications. Hydrogels may be exploited in ocular drug delivery and as ocular implants to offer possible solutions to improve the treatment of blinding eye diseases that affects most of the aging populations around the world (glaucoma and AMD). As hydrogels are used as lens materials for both contact and intraocular lens, the work in this thesis set out to examine the use of hydrogels in two different parts of the eye: (i) subconjunctiva and (ii) vitreous cavity.

Hydrogels can be made to associate with a large amount of water, so they are soft and may better match tissue properties for a hydrogel based implant. Athough hydrogels have limitations (e.g. easy to tear), they may be more biocompatible (low foreign body response) than currently used implant materials (e.g. silicone). It was hypothesised that a GDD made using a hydrogel could be used within the subconjunctival space to restrict aqueous outflow to control pressure so that the IOP could be maintained at 10 mm Hg.

Use of a hydrogel in a GDD could reduce complications due to biocompatibility that are associated with current GDDs. Since HEMA and MPC are used in widely used ophthalmic devices (e.g. contact lens), these materials would be used to fabricate hydrogel films to be examined for use as a possible GDD. The initial aim was that by modifying the water content of the hydrogel the permeability of the hydrogel could be controlled to allow the constant outflow of the aqueous humour at a rate of 2 µL/min. It was thought by modification of the molecular porosity of a hydrogel, that there would be an opportunity to develop a material that could control the rate of diffusion of aqueous humour and control IOP in a more effective way than is currently possible.

It is also hoped that a hydrogel based GDD could be folded and used in such a way that only a small incision in the eye would be required for implantation. If this can be achieved, it is possible that a device can be developed that can be implanted requiring less surgical skill. It was first a necessary aim to determine the hydraulic conductivity and permeability of hydrogel films to determine if they could in fact be used to control the aqueous outflow.

Should the HEMA-MPC hydrogels not be able to control the aqueous outflow, then it was hypothesised that a HEMA-MPC hydrogel film could also be used as a drug releasing spacer to reduce subconjunctival scarring to improve the success rate of GFS. The hydrogel film would act as drug delivery system of an anti-inflammatory or anti-scarring agent and at the same time could also act as a spacer

that could mechanically separate the conjunctival tissue from the sclera to prevent adhesion due to localised scarring.

Since the treatment of AMD utilises antibody based drugs that are administered by IVT injections, hydrogels were also evaluated for IVT injection into the vitreous cavity. It was hypothesised that it could be possible to utilise a hydrogel to slow the clearance of a protein-based medicine from the vitreous cavity. The challenge was to develop a strategy to both mix the protein within the hydrogel and to determine if the presence of the hydrogel delayed protein clearance. Thermoresponsive hydrogels made from NIPAAm using a hydrophilic cross-linker were examined to determine if the clearance time for proteins from the vitreous cavity could be increased while maintaining the activity and stability of the protein.

The aim of this work is the fabrication of HEMA-MPC hydrogels as GDD. The fabricated hydrogels will be studied and compared regarding water permeability as a mean to control aqueous outflow. The hydrogels will also be examined regarding total water content, water distribution and swelling in different solvents. The promising formulation will be loaded with anti-inflammatory drug, dexamethasone, for the formulation of combination device or drug releasing subconjunctival spacer.

The aim is also to formulate stimuli responsive NIPAAm hydrogel for protein delivery to the vitreous. NIPAAm thermoresponsive hydrogels with different types of cross-linker will be prepared. The cross-linker percentage and type will be varied and the effect of formulation variation will be studied regarding VPTT, swelling ratio, water retention percent and protein loading and release.

Chapter 2. Materials and methods

Materials

Chemicals and solvents used are listed in Table 2:1. Reagents were all used as received without further purification. Spectrophotometric and instruments used for characterisation are all described in the methods section below.

Table 2:1 List of chemicals and solvent used.

Material	Cat no	supplier
2-hydroxyethyl methacrylate (HEMA) 130.14 g/mole Density 1.073 g/mL	128635	Sigma-Aldrich Chemicals (UK)
Polyethylene glycol diacrylate-Mn 700 (PEGDA) Density 1.12 g/mL	455008	Sigma-Aldrich Chemicals (UK)
2,2-azobis(2-methylpropionitrile) (AIBN) (MW 164.21 g/mole)	441090	Sigma-Aldrich Chemicals (UK)
2-methacryloyloxyethyl phosphoryl choline (MPC) (MW 295.27 g/mole)	88J4253P	Vertellus biomaterials (UK)
Pirfenidone (MW 185.2 g/mole)	13986	Cayman chemical company (UK)
Acetonitrile HPLC grade	75-05-8	Fisher Scientific Ltd (UK)
Trifluoroacetic acid	T6508	Sigma-Aldrich Chemicals (UK)
Methanol HPLC grade	M/4056/17	Fisher Scientific Ltd (UK)
Phosphate buffered saline tablets PBS	188912014	ThermoFisher Scientific Ltd (UK)
Dexamethasone (MW 392.46 g/mole)	D4902	Sigma-Aldrich Chemicals (UK)
Pirfenidone	13986	
Doxycycline hyclate (MW 512.94 g/mole)	D9891	Sigma-Aldrich Chemicals (UK)
Beta-cyclodextrin (βCD) (MW 1134.98 g/mole)	W402826	Sigma-Aldrich Chemicals (UK)
Glycidyl methacrylate (GMA) (MW 142.15 g/mole) density (1.042 g/mL)	151238	Sigma-Aldrich Chemicals (UK)
Dimethylformamide MW 80.14 Density 1.03 g/mL	227056	Sigma-Aldrich Chemicals (UK)
Sodium chloride	7647-14-5	Fisher Scientific Ltd (UK)
Sodium hydroxide	1310-73-2	Fisher Scientific Ltd (UK)

Polyethylene glycole diglycidyle ether (Mn 500) Density (1.1 g/mL)	475696	Sigma-Aldrich Chemicals (UK)
N-isopropylacrylamide 97% (MW 113.16 g/mole)	415324	Sigma-Aldrich Chemicals (UK)
PC 3059	Gift from Vertellus biomaterials (UK)	
Ammonium persulfate (APS) (MW 228.2 g/mole)	A3678	Sigma-Aldrich Chemicals (UK)
N,N,N′,N′-Tetramethylethylenediamine (TEMED) ~99% (MW 116.2 g/mol) density (0.775 g/mL)	T9281	Sigma-Aldrich Chemicals (UK)
Bevacizumab (Avastin® 25 mg/mL)	(Genentech, South San Francisco, California) were supplied from the left over syringes and purchased from Moorfields Eye Hospital	
Acrylated-HA (~50 KD)	Synthesised in our lab	
Infliximab	Vial of 10 mg/mL for Iv infusion from HEALTHCARE CELLTRON™	

Methods

Fabrication of HEMA-MPC hydrogel films

HEMA-MPC hydrogel films were fabricated according to compositions described in (Table 2:2) by thermally induced free radical polymerisation. MPC monomer was weighed and dissolved in HEMA monomer in a 14 mL glass tube with a magnetic stirrer to prepare the monomer mixture solution. When a clear mixture was visually observed, the cross-linker, poly(ethylene glycol diacrylate) (PEGDA) and the initiator, 2,2-azobis(2-methylpropionitrile) (AIBN) were added to the monomer solution and mixed until a clear mixture was observed using a magnetic stirrer. A suba-seal was attached to the lid of the glass tube and an outlet needle (yellow) was placed at the top of the tube. Argon was passed through the long needle directly into the reaction mixture and degassed for 5 minutes in an effort to displace dissolved oxygen, which can inhibit the polymerisation reaction. The reaction mixture was then injected into a polypropylene mould using 23G needle. Care must be taken during injection to avoid air bubbles inside the mould.

After injection of the polymerisation mixture, the mould was placed flat in the oven at 70^{o}C for 6 hours to polymerise. After 6 hours the polymeric xerogel was formed. The term xerogel is used to describe a hydrogel in a completely dried, unhydrated state. The xerogel was then removed from the mould and immersed in

(50 mL) of water for hydration and to remove leachable molecules including unreacted monomer and oligomeric species. The water was changed twice daily and the washing water was scanned with UV visible spectrophotometer over a range of 400-200 nm to detect any unreacted monomers. The washing of the hydrogel continued for several days. The hydrogels were considered clean when no signals were detected by UV from the water used to wash the films. Typically for a hydrogel film with the dimensions of 30 × 80 mm and 1 mm thickness 4 days are required to complete the washing process. The hydrogels were then stored in plastic containers in (50 mL) of water to be used later. The storage water was changed every week to avoid mold growth. Three different batches of films were prepared for each monomer-cross-linker formulation.

Table 2:2 Compositions of different HEMA-MPC hydrogel films prepared by free radical polymerisation. The MPC weighed and dissolved in HEMA with PEGDA and AIBN. For the preparation of 100% MPC formulation the MPC was dissolved in 1 mL DW.

Code	HEMA (g) (mmole)	MPC (g) (mmole)	PEGDA (µL) (0.076 mmole)	AIBN (g) (0.45 mmole)	~MPC% (w/w)
S1	9.87 (75.84)	0	50	0.074	0
S2	9.45 (72.61)	0.42 (1.42)	50	0.074	5
S3	8.95 (68.77)	0.92 (3.12)	50	0.074	10
S4	8.45 (64.93)	1.42 (4.81)	50	0.074	15
S5	7.95 (61.09)	1.92 (6.5)	50	0.074	20
S6	6.95 (53.4)	2.92 (9.89)	50	0.074	30
S7	4.93 (37.88)	4.94 (16.73)	50	0.074	50
S8	0	1.0 (3.39)	50	0.074	100

Abbreviation: 2-hydroxyethyl methacrylate (HEMA), polyethylene glycol dimethacrylate-700 Da (PEGDA), 2,2-azobis(2-methylpropionitrile) (AIBN), 2-methacryloyloxyethyl phosphoryl choline (MPC).

The polypropylene and silicone moulds were prepared and assembled as shown. (Figure 2:1). The dimensions were 40 × 90 mm and 30 × 80 mm for the polypropylene and silicone sheets respectively. The thickness of the silicone sheet used was 1 mm. Both polypropylene and silicone sheets were first sonicated with isopropanol for 10 minutes for cleaning followed by drying in the oven at 50^{o}C for 30 minutes. After assembling the mould the polymer mixture was injected slowly and carefully starting from the edges to avoid air bubbles

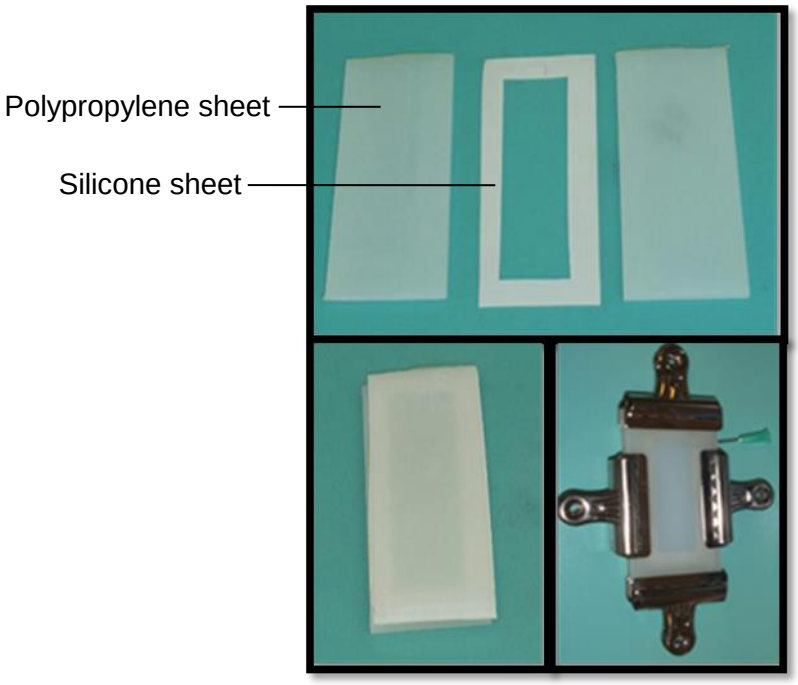


Figure 2:1 The mould used for the hydrogel fabrication before and after assembly. The mould consists of two polypropylene sheets and one silicone sheet sandwiched between them.

Characterisation of the hydrogel films

General appearance

Once removed from the oven, the HEMA-MPC films were characterised regarding their general appearance. The thickness of the dried films (xerogel) and the resulting hydrated gels were measured using a caliper and the values recorded. The effect of MPC on the general appearance and thickness of the gels was noted and recorded.

Equilibrium water content percent measurements (EWC%)

The term equilibrium water content percent (EWC%) refers to the maximum percentage of water absorbed by a xerogel to reach full hydration. To calculate the EWC% a hydrogel discs of 1 cm in diameter were cut from fully hydrated hydrogel films and weighed which is considered as the weight of the disc in equilibrium with water (We). The discs were then fully dried by placing them in vacuum oven at 70^{o}C until they reached constant weight (Wd). Equation 1 was used for the calculation of EWC% (Rohindra et al. 2004).

$$\boldsymbol{EWC}\ (\%) = \left(\frac{\boldsymbol{We-Wd}}{\boldsymbol{We}}\right) \times \mathbf{100} \qquad (1)$$

Swelling ratio (SR) measurements in different solvents and solvent mixtures

The term swelling ratio (SR) refers to the ratio between the weight of solvent absorbed by the hydrogel and the dry weight of the gel. It gives an indication to the increase in size of the dry xerogel when fully hydrated. To calculate the SR a hydrogel discs of 1 cm in diameter were cut from fully hydrated hydrogel film and weighed. The discs were then fully dried by placing them in vacuum oven at 70^{o}C until they reached a constant weight (Wd). To measure the SR in different solvents each dry discs was placed in 5 mL of water, methanol, ethanol, water: methanol (1:1) or water: ethanol (1:1) at 25^{o}C. The SR was measured in different solvent systems to allow loading of a wide range of drugs with different solubilities. The discs were left in the solvents for 48 hours to ensure complete hydration. After 48 hours the discs removed from the solvents; the surfaces of the discs were wiped carefully to remove extra solvents and weighed at equilibrium (We). Equation 2 was used for the calculations.

$$\boldsymbol{SR} = \left(\frac{\boldsymbol{We - Wd}}{\boldsymbol{Wd}}\right) \quad (2)$$

Measurement of hydraulic conductivity (L) and permeability (K)

The permeability measurement for each sample was calculated using a dynamic method which involves placing the sample into a flow chamber and allowing water to pass from the fluid reservoir to the flow chamber. The system used was an established system by Dr Ali Hussain in UCL Institute of Ophthalmology for measuring hydraulic conductivity and permeability of bruch's membrane of the eye. The flow chamber and the reservoir were connected using a three-way valve. One opening of the three-way valve was attached to the flow chamber and another opening was attached to the fluid reservoir through a tube. The third opening of the value remained closed and was used to remove air bubbles if any were entrapped in the tube. The fluid reservoir is a vertical capillary tube that was filled with water and which served as a manometer. The flow chamber consisted of a glass plate and a capping plate. The capping plate can be fixed on the glass plate with screws. The glass and capping plate had a small circular indentation with a radius of (3 mm) for sample placement. The sample places were on the circular indentation of the glass plate with the capping plate placed on top and screwed to seal the flow chamber. Two different flow chambers were used. In the first, the capping plate was open at top of the sample (open system) while in the other chamber, the capping plate had no opening (closed system) (Figure 2:2).

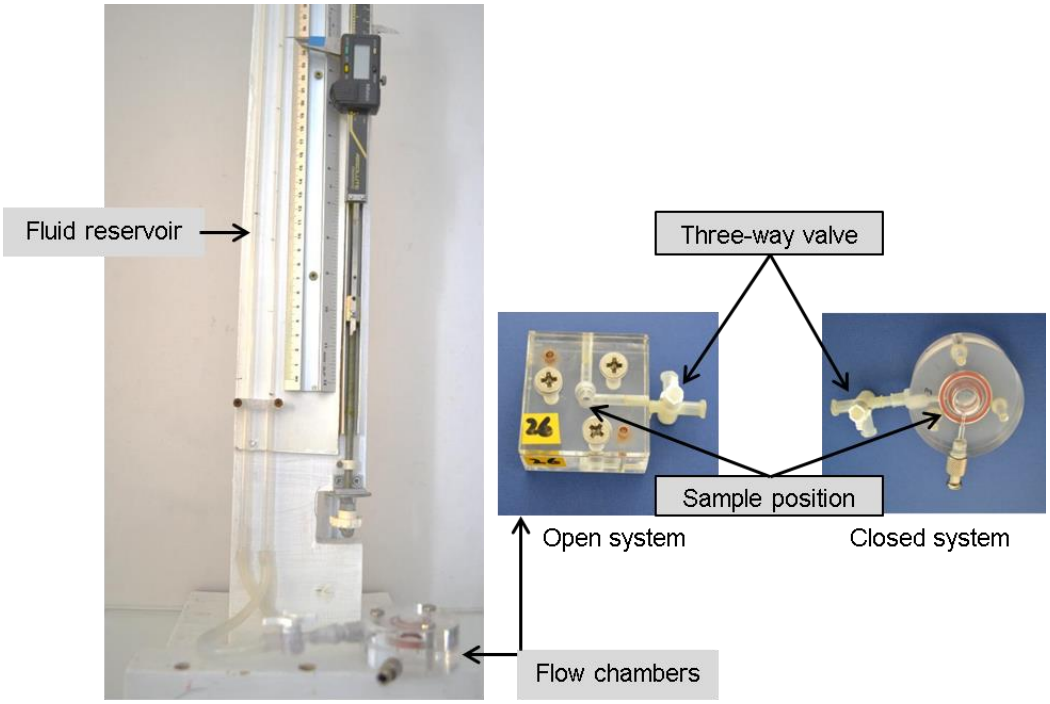


Figure 2:2 The system used for measurement of hydraulic conductivity where we can see the fluid reservoir and the flow chambers. The position of the sample and the three way valve that is used to control the flow of water to the sample is highlighted.

Once the apparatus was assembled and the sample was fixed, it was necessary to avoid air bubbles in the tubes and chamber. A (4 × 4 mm) sample was cut from each fully hydrated film because the sample size should be bigger than the radius of the opening in the middle of the chamber (3 mm) to allow the film to be fixed and ensures no water can escape from the edges of the film. Once the sample was fixed to the chamber, water was allowed to run through the system from the fluid reservoir to the flow chamber. The water moved down the column to the flow chamber through the tube and three way valve. The height of the fluid in the column decreases with time. The water levels were recorded at different time points over a period of 24 h. The values of the height of water verses time were plotted and the gradient was used for calculating the hydraulic conductivity (L) using Equation 3:

$$\boldsymbol{L = -\frac{r^2}{a^2\,\mathrm{p}\,g}\;G} \qquad (3)$$

Where (L) is the hydraulic conductivity, (r) is the radius of the water column, (a) is the radius of the sample, (ρ) is the density of water and (g) is the gravitational constant (9.81 m/sec^2).

The hydraulic conductivity value (L) was used to calculate the permeability (K) using Equation 4 where (T) is the thickness of the film. An example of the calculation used can be seen in the appendix

$$\boldsymbol{K = L \times T} \tag{4}$$

Preparation of 10% MPC hydrogels to contain a pouch

Two approaches were used to attempt to create a pouch in a HEMA-MPC hydrogel film; ablation using a femtosecond laser and polymerisation around soluble powder as a positive mould. A femtosecond laser was used to try to create a pouch in HEMA hydrogel films with 15% MPC. The femtosecond laser that is used for cataract surgery in Moorfields Eye Hospital was used for the creation of a pouch in the hydrogel. The hydrogel was fully hydrated when the laser beam was directed to create a pouch.

A phosphate buffer solution (PBS) tablet was also used to try to fabricate a pouch within a HEMA-MPC hydrogel film. The PBS tablet was crushed using mortar and pestle into large particles. The monomer mixture of 10% MPC was used to fill half of the mould used for the fabrication of HEMA-MPC hydrogels. The crushed PBS tablet was spread in the liquid monomer mixture. The mould was then filled with the monomer mixture. Care must be taken to make sure that all the PBS particles were covered in the monomer mixture. After polymerisation a film was formed and placed in DW for hydration and washing of unreacted monomers.

Distribution of water inside the hydrogel, free to bound water ratio

Differential scanning calorimetry (DSC) is a technique that can be used to measure the free to bound water ratio of hydrogels. The principle is that only free water and slightly bounded water are frozen so the endotherm obtained from DSC represents the amount of frozen water only. Equation 5 assumes that the heat of fusion of freezable water in hydrogels is the same as ice. The amount of bound water is the difference between the total water content and freezable water. The melting enthalpies achieved from DSC were used to calculate the bound to free water ratio as in Equation 5 ,where (Wb) is the amount of bound water, ($Wf_{)}$ is the amount of free water, (Wfb) is the amount of lightly bound water, (Q_{endo}) is the melting enthalpies derived from the DSC chart and (Qf) is the melting enthalpies of free water which is the same as ice 79.9 cal/g (Rohindra et al. 2004).

$$W_b(\%) = EWC\% - (W_f + W_{fb}) \times 100,$$

$$W_b(\%) = EWC\% - (Q_{endo}/Q_f) \times 100, \quad (5)$$

Experiments were performed on DSC Q2000 (TA instruments, Waters, LLC) using a rate of 3°C/min from -40 to 20°C. Typical sample size range from 4-10 mg. Calibration with indium (T_m = 156.6, ΔH_f =28.71 J/g) was performed according to the manufacturer instructions. Nitrogen was used as a purge gas with a flow rate of 50 mL/min for all the experiments. TA zero aluminium hermetic pans and lids were used. Data were analysed using TA Instruments Universal Analysis 2000.

Swelling and deswelling kinetic studies

The swelling and deswelling of the HEMA-MPC hydrogel films were measured by measuring the change in SR with time using a gravimetric method. For the swelling measurement, a fully dried hydrogel disc was weighed (Wd) and immersed in 5 mL PBS, pH 7.4 at 25^{o}C. The disc was removed from PBS at predetermined time intervals (every 10 minutes for the first hour followed by every hour for 12 hours) and weighed (We). When removed the surface of the disc carefully wiped to remove any extra liquid. The SR for each time point was calculated as in Equation 2.

For deswelling measurements a fully dried disc was weighed (Wd) and allowed to swell completely in PBS, pH 7.4 for 24 h at 25^{o}C. The fully hydrated disc was removed from hydration solution and weighed (We) then placed on the bench at ambient temperature. After removing from solution the weight of the disc was measured at different time intervals and the SR was calculated at each time point using Equation 2.

Mechanical testing using Instron

An Instron Universal testing Instrument based in UCL School of Pharmacy (Model 5567, Instron Ltd, Norwood, USA) was used to measure the mechanical properties of the hydrogel films. Films were cut into a 'dog bone' shape using a dog bone shape punch. The films were cut in dog bone shape to avoid having a break in the area being gripped and they were cut from fully hydrated films. The dimensions of each sample were 15.5 mm length, 3.6 mm width and 1 mm thickness. The samples were put between the clamps of the machine and were pulled apart at a rate of 10 mm/min and 100 N static load (2 Kg). The sliding of the films in the clamps was prevented by covering the surface of the clamps with a piece of wetted

cloth. Samples were sprayed with water as they were mounted on the grips prior to testing to ensure they remain fully hydrated; they were also sprayed every 30 seconds during testing to ensure they remained fully hydrated. The cut-off point was at failure when the film was completely separated into two pieces. The tensile modulus of elasticity (represented by Young's modulus) for each sample was determined as the slope of the linear part of the stress–strain curves. The data were analysed using Bluehill software 2 (version 6).

Scanning electron microscopy SEM

Quanta ™ Scanning Electron Microscopy (FEI Quanta200 FEGESEM, Eindhoven, The Netherlands) which is maintained at the UCL School of Pharmacy was used for the examination of the inner structure of the hydrogels and their pore size. The hydrogel samples were freeze-dried prior to SEM analysis. The fully hydrated gels were frozen at -40^{o}C then dried using VIRTIS- Advantage freeze-dryer for three days. The dried samples were cut and adhered onto aluminium SEM stubs using carbon-coated double-sided tape. In order to make them electrically conductive, they were sputter coated with gold prior to imaging.

Drug loading into hydrogel films

The drug soaking method was used for the loading of 0%, 10% and 30% MPC hydrogels. Discs of 1 mm thickness and 1 cm in diameter were cut from fully hydrated films. The discs were then fully dried to remove all water at 70^{o}C in vacuum for 24 hours. The fully dried discs were then soaked in drug solution for 24 hours. After incubation the discs were removed carefully from the drug solution and placed in 3 mL DW for 30 seconds to remove extra drug presumably adsorbed to the surface of the films and dried at ambient temperature under vacuum for 24 hours and stored to be used for release studies. The amount of drug loaded for each disc was calculated as the difference in UV absorbance reading between the starting solution and the solution left after loading. The loading efficiency was also calculated. The loading efficiency is a representation of the percentage of drug loaded from the actual amount placed in the loading solution. It can be calculated using Equation 6.

$$\textbf{Loading efficiency} = \left(\frac{\textbf{amount loaded}}{\textbf{actual amount in loading solution}} \right) \times \mathbf{100} \qquad (6)$$

Two different solutions were used for pirfenidone loading, the first was (1 mg/mL, 1 mL) in water and the second was (1 mg/mL, 1 mL) in water:ethanol (1:1). For dexamethasone (DEX), based on its solubility (DEX is poorly soluble in water), two different solvents were used as loading solutions which are water: ethanol (1:1) and methanol. When water:ethanol mixture was used as a solvent, the concentration of DEX in the loading solution was (1 mg/mL, 1 mL). DEX concentration was (15 mg/mL) when methanol was used. In the first method once removed from the incubation solution the discs were placed in 4 mL of ethanol for 1 minute to remove any drug attached to the surface of the discs followed by drying under vacuum for 24 hours. In the second method the discs were placed after removal from incubation solution in 2 mL of water for 4 hours with mild agitation followed by drying under vacuum for 24 hours. The amount of drug lost in the washing was quantified using HPLC.

For doxycycline hyclate (DOXy) loading, aqueous solutions with different concentrations (6 mg/mL, 15 mg/mL and 30 mg/ml, 1 mL) in PBS were used as incubation solutions. DOXy was also loaded from non-aqueous solutions using different solvents and solvent combinations which are water, ethanol, methanol, water: ethanol (1:1) and water: methanol (1:1) ratios at a concentration of (15 mg/mL, 1 mL). The loaded discs were washed similar to pirfenidone loaded discs.

Release of the drug from the hydrogel films

The intended use of the hydrogels films is in the subconjunctival space. To determine the release profile a flow rig model was used. This model mimics the bleb formed in GFS. A similar aqueous flow rate to that in the subconjunctival space was maintained in the rig using a pharmaceutical dispensing pump set to dispense 2 µL of PBS per minute. The rig had a capacity of 400 µL (Figure 2:3). A fully dried loaded disc was placed in each rig. The rig had both input and output tubes. The input tube was connected to a multi-channel dispenser (ISMATEC pump, Switzerland) to pump PBS buffer (pH 7.5) at a flow rate of 2 µL per minute. The output tube was used for collection of samples. Samples were collected at various time points and quantified using HPLC. The flow rigs were immersed in a preheated oil bath adjusted to 37°C.

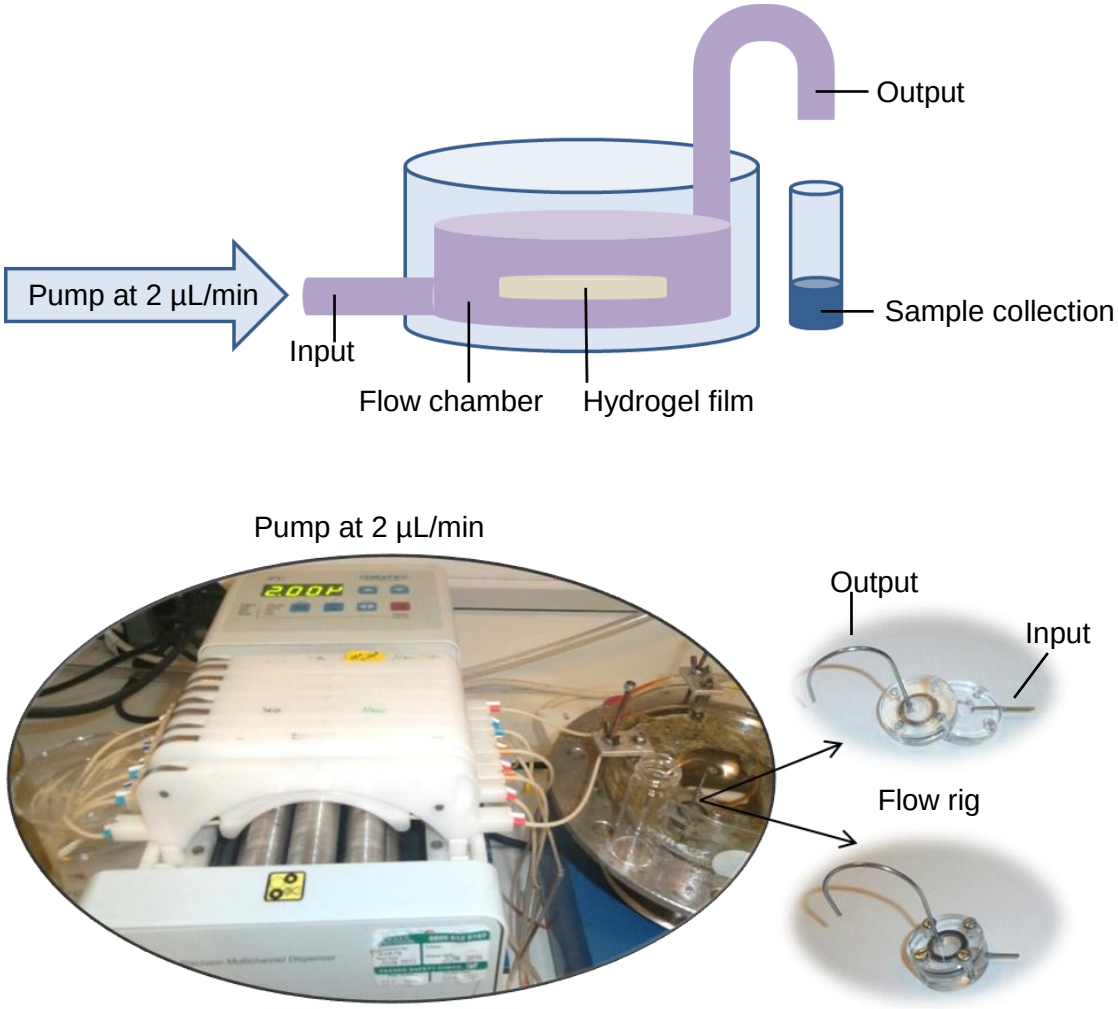


Figure 2:3 Schematic representation of the flow rig used for the release studies of drugs loaded into hydrogel films with a picture of the peristaltic pump and the flow rig used.

HPLC and UV methods for drug quantification

Pirfenidone samples were analysed by a HPLC system Agilent 1200 series (Agilent, Wokingham, Berkshire, UK) equipped with Chemstation software (Agilent, Wokingham, Berkshire, UK). The stationary phase was a Synergi 4u Hydro- RP 80 A (150 x 4.6 mm, 4 micron) column (Phenomenex Co., California, USA) kept at 40^{o}C. The mobile phase was composed of 50% (v/v) acetonitrile in water. The mobile phase flow rate was 1 mL/min, the injection volume was 20 µL and the detection wavelength 315 nm. The retention time for pirfenidone was 3.2 min. The correlation coefficient of the calibration curve was R^2: 0.999 for a concentration range of (100-3.125 µg/mL), indicating acceptable linearity. The samples for the calibration curve were made using PBS, pH 7.4 as solvent.

Analysis of pirfenidone by UV spectroscopy was performed using Hitachi U-2800A spectrometer. The detection wavelength was 310 nm and the correlation

coefficient of the calibration curve was R^2: 0.999 for a concentration range of (25-1.56 µg/mL), indicating acceptable linearity. The samples for the calibration curve were made using water as solvent.

The same HPLC system and column used for pirfenidone detection was used for DEX and DOXy detection. For DEX the mobile phase composed of acetonitrile and aqueous trifluoroacetic acid solution (0.1% v/v) at 40/60 volumetric ratio. The mobile phase flow rate was 1 mL/min, the injection volume was 10 µL and the detection wavelength 240 nm. The retention time for DEX was 4.8 min. The correlation coefficient of the calibration curve was R^2: 1 for a concentration range of 100-3.125 µg/mL, indicating acceptable linearity.

For the detection of DOXy HPLC gradient method was used. The mobile phase composed of acetonitrile and aqueous trifluoroacetic acid (TFA) solution (0.1% v/v) at 30/70 volumetric ratio at the start. The gradient used shown in (Table 2:3). The mobile phase flow rate was 1 mL/min, the injection volume was 10 µL and the detection wavelength 347 nm. The retention time was 4.8 min. The correlation coefficient of the calibration curve was R^2: 0.998 for a concentration range of 250-6.25 µg/mL, indicating acceptable linearity.

Table 2:3 Gradient method for the detection of DOXy using HPLC.

Time minutes	Acetonitrile%	(0.1% TFA)%
0	30	70
12	80	20
12.1	30	70
15	30	70

Analysis of DOXy in by UV spectroscopy was performed using Hitachi U-2800A spectrometer. The detection wavelength was 350 nm and the correlation coefficient of the calibration curve was R^2: 0.999 for a concentration range of 50-3.125 µg/mL, indicating acceptable linearity.

Modifications of the films to extend the half-life of water soluble drugs

Use β-CD as affinity barrier in the 10% MPC films

β-Cyclodextrin (β-CD) was used as affinity complexing agent to create an affinity barrier that may potentially slow the release of loaded drugs in 10% MPC films. Three different methods were used to incorporate β-CD into 10% MPC films. Before incorporating β-CD into the hydrogel film, the ability for β-CD to form a complex DOXy was examined. For the preparation of the complex equal molar weights of β-CD and DOXy were mixed and dissolved in 5 mL of DW. After mixing, the mixture

was sonicated for 1 hour and placed in freeze dryer for three days. For the preparation of physical mixture, equal molar weights of β-CD and DOXy were mixed. DSC analysis was performed on the β-CD, DOXy, physical mixture and inclusion complex of both. The melting enthalpies from the DSC graphs were compared. Experiments were performed on DSC using a rate of 10°C/min from 30 to 280°C.

Preparation of 10% MPC films with pendant β-CD

One of the widely used methods for pending CD into a hydrogel is the use of glycidyl methacrylate monomer (GMA). 10% MPC films were prepared with different percentages of GMA. The 10% MPC monomer mixture was prepared as described previously. To each 1 mL of the 10% monomer mixture different amounts of GMA (1%, 2.5%, 5% and 9% (w/v)) was added and allowed to mix until a clear solution was formed. After mixing the mixture placed in mould to polymerise at 70^{o}C for 6 hours. After polymerisation the films left to hydrate in water. After hydration for 24 hours discs with 1 cm in diameter were cut and each disc was placed in (6 mL) activation solution to activate the GMA and conjugate β-CD to it. The activation solution consists of dimethylformamide DMF: 0.5 M NaCl (1:1) that contains 0.08 M β-CD and 0.18 g NaOH. The discs were incubated in the activation mixture at 80^{o}C for 24 hr. After incubation the discs were washed for 3 days with water to remove any residual DMF and dried under vacuum to be used later for drug loading.

Another method used to incorporate β-CD is *in situ* polymerisation of 10% MPC monomer solution and 20% (w/v) β-CD solution in 0.2 M NaOH in the presence of GMA. The two monomer solutions were prepared separately and mixed in different volume ratios with different amounts of GMA (Table 2:4). The formulations were then the injected into the mould and placed in the oven for polymerisation at 80^{o}C for 6 hours. After polymerisation the xerogels formed were removed and examined.

Table 2:4 Different formulations for *in situ* hydrogel synthesis using 10% MPC monomer solution and 10% w/v β-CD solution in 0.2 M NaOH in the presence of GMA.

Formulation No	10% MPC (mL)	20% (w/v) β-CD (mL)	GMA (g)
1	1	1	0.5
2	1	1	0.2
3	1	1	0.1
4	1	0.5	0.5
5	1	0.5	0.2
6	1	0.5	0.1

Abbreviations: Glycidyl methacrylate (GMA), 2-methacryloyloxyethyl phosphoryl choline (MPC), Beta-cyclodextrin (β-CD).

Preparation and embedding of β-CD cross-linked particles in 10% MPC films

β-CD hydrogels were synthesised by a step polymerisation reaction. β-CD powder (1.5 g) was dissolved in 0.2 M NaOH (10 mL) solution at a concentration of (15% w/v). The β-CD solution vortexed for 1 minute and poly(ethylene glycol)diglycidyl ether (PEGDE) was added as cross-linker in volume ratios of (1:1, 1:2, 2:1) with β-CD solution. The mixture then vortexed for another minute and placed in glass vial in the incubator at 50^{o}C for 12 hours at 150 RPM. The hydrogel was then removed from the incubator and allowed to cool for 1 hr then placed in 20 mL of 0.5 M hydrochloric acid (HCl) for 1 hr to stop the reaction followed by soaking the hydrogel in water for 24 hours. The resulting hydrogel in the hydrated form was then crushed first using a mortar and pestle then passed through a 40 mesh sieves (500 µm in diameter) to create uniform particles. The particles were then freeze dried for three days to remove any residual water and stored in a tight container ready to use.

To embed the β-CD particles in 10% MPC films a 10% MPC monomer solution with cross-linker and initiator was prepared. The freeze dried particles were then suspended in the monomer solution at a concentration of 1% and 2% (w/v), injected into polypropylene mould and placed in the oven to polymerise at 70^{o}C for 6 hours. After polymerisation the xerogel was washed to remove any unreacted monomers. Discs with 1 cm in diameter were cut from the hydrated hydrogel, dried under vacuum and stored to be used for drug loading.

Formulation of IPN of 10% MPC and cross-linked β-CD

To formulate an interpenetrating network (IPN) of HEMA-MPC film with cross-linked β-CD hydrogel, the two monomer mixtures were mixed together and polymerised at the same time in the same pot (*in situ*). To ensure that each monomer system underwent separate, orthogonal polymerisation reactions as much as possible, the HEMA-MPC hydrogel formulation was prepared using PEGDE as cross-linker. The 10% MPC monomer solution was prepared and the 50 µL of PEGDA was replaced with 50 µL PEGDE. The monomer mixture was injected into a mould and placed in the oven at 70^{o}C for 6 hours. β-CD monomer mixture was also prepared by replacing 50% (v/v) of PEGDE with 50% (v/v) PEGDA in the monomer mixture. The monomer mixture was placed in a glass vial and incubated at 50^{o}C for 12 hours at 150 RPM. A monomer solution of 10% MPC with PEGDA as cross-linker was prepared and incubated at 50^{o}C for 12 hours at 150 RPM.

For the preparation of the IPN a monomer solution of 10% MPC and 89.5% (w/w) HEMA and a β-CD monomer solution with different percentages of β-CD in

0.2 M NaOH (15%, 30% and 50% w/v) were prepared. To the β-CD monomer solution PEGDE as cross-linker was added at a concentration of 50% (v/v). The two monomer mixtures (10% MPC and β-CD) were mixed in 1:1 volume ratio and vortexed for 1.0 minute. The mixture was injected into a mould and placed at the incubator at 50^{o}C for 12 hours at 150 RPM. After polymerisation the xerogel was removed and washed with DW. Another two IPN formulations were also prepared. β-CD monomer solution made with 30% (w/v) β-CD and 50% (v/v) PEGDE was mixed in two different ratios with 10% MPC monomer solution (1:2, 2:1 30% (w/v) β-CD:10% MPC). The mixtures were then injected into a polypropylene mould and placed in the incubator at 50^{o}C for 12 h at 150 RPM to for a hydrogel film.

Another method to prepare an IPN is by sequential polymerisation where one monomer is polymerised in the presence of the other monomer mixture followed by polymerisation of the second monomer mixture. First a 10% MPC monomer mixture was prepared by replacing AIBN with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine/ammonium persulfate (TEMED/APS) as initiator system. The 10% MPC and 89.5% (w/w) HEMA monomer mixture was prepared by replacing AIBN with 4 mg APS. β-CD monomer solution with 30% (w/v) β-CD and 50% (v/v) PEGDE was also prepared. The two monomer mixtures were mixed in equal volumes (1 mL each) thoroughly. After mixing 30 µL of TEMED was added to the mixture to start the polymerisation process of HEMA-MPC hydrogels. The mixture was quickly injected into the polypropylene mould and allowed to polymerise for 30 minutes at the bench. After 30 minutes the mould was placed in the incubator at 50^{o}C for 12 h at 150 RPM to start the polymerisation process of β-CD. In both methods the Xerogel was washed to remove unreacted monomers. Discs with 1 cm in diameter were cut from the hydrogel, dried under vacuum and stored to be used for drug loading.

Preparation of thermoresponsive NIPAAm hydrogels with and without protein

Different NIPAAm hydrogels with varying amounts of PEGDA as cross-linker were synthesised by free radical polymerisation. For the preparation of empty hydrogel (i.e without protein), NIPAAm monomers (40 mg which is equivalent to 0.35 M) were weighed and dissolved in deionised water (1 mL) at room temperature (~25°C). The contents were stirred until a clear solution was visually observed. Different amounts of PEGDA (2, 4, 8, 12 and 15 µL which are equivalents to 3.2, 6.4, 13, 19 and 24 mM respectively) were measured and added to the above solution and thoroughly mixed. To each mixture APS (initiator) was weighed (4 mg which is equivalent to 17.5 mM) and added to the solution. After mixing for 5

minutes, (20 µL which is equivalent to 0.13 M) TEMED (accelerator) was added to each formulation and vortexed for ~10 seconds. The monomer solution started to gel within one minute of the addition of TEMED and the entire system was left to polymerise in the fridge at 4^{o}C for 24 hours. The hydrogels were then washed 5 times (15 minutes each time with 4 mL of PBS) in a total volume of 20 mL (Drapala et al. 2011).

For the preparation of NIPAAm hydrogel with MPC as a monomer, the NIPAAm monomer mixture was prepared as described previously. To 1 mL of the mixture two different amounts of MPC were added (1 mg and 5 mg). The MPC was dissolved in the monomer mixture and the hydrogel polymerised as described previously.

The same method used for synthesis of PEGDA cross-linked hydrogels was used for the synthesis of phosphoryl choline (PC) and acrylated hyaluronic acid (Ac-HA) cross-linked hydrogels. Different NIPAAm hydrogels with different percentages of the biocompatible PC 3059 as cross-linker (9, 15, 20, 25 and 30 mg which represents 13%, 20%, 25%, 30% and 33.5% (w/w) respectively) were prepared. For the preparation of Ac-HA hydrogels PC was replaced with different amounts of Ac-HA (2, 4, 7 and 10 mg which represents 3.2%, 6.3%, 10.5% and 14.4% (w/w) respectively).

The proteins used for hydrogel loading and release were bevacizumab and infliximab, both have a molecular weight of 150 kDa. For the loading of bevacizumab using imbibing method, NIPAAm hydrogel with 8 µL cross-linker was prepared and freeze dried for 3 days. The dried hydrogel was incubated in 1 mL of bevacizumab solution (12.5 mg/mL in PBS) for 48 hr at 4^{o}C. After incubation, the hydrogel was removed from the incubation solution and then rinsed gently 5 times, 4 mL each time in PBS; each wash cycle was 15 minutes long. Each wash fraction was analysed by HPLC to calculate how much of the protein was lost during the wash. The drug loading was the difference between the starting solution and the amount lost during washing

For the preparation of bevacizumab or infliximab loaded hydrogels using the *in situ* method the NIPAAm monomers were dissolved in (1 mL) of protein solution (25 mg/mL from vial for bevacizumab) and (9.4 mg/mL from vial for infliximab) prepared in the same way as the unloaded hydrogels. After polymerisation, the protein loaded hydrogels were rinsed gently similar to empty hydrogels washing. Each wash fraction was analysed by HPLC to calculate how much protein was lost during the wash. The work reported for Ac-HA was in collaboration with Mr Abdullah Abubakre.

Characterisation of NIPAAm hydrogels

The characterisation was studied for both empty and protein loaded hydrogels.

Volume phase transition temperature (VPTT) and injectability

The VPTT was measured using differential scanning calorimeter (DSC). The fully swollen samples were placed in DSC and the temperature increased from 20 to 50°C at a rate of 2°C/min. Calibration with Indium (Tm = 156.6, ∆Hf =28.71 J/g) was performed according to the manufacturer instructions. Nitrogen was used as the purge gas with a flow rate of 40 mL/min for all the experiments. The onset temperature of the DSC endothermic peak was considered as the VPTT. A 2 mL syringe with 23G needle was used to determine the injectability of the hydrogels prepared. Injectability was qualitatively determined based on the ease of injection of the prepared hydrogels.

Swelling ratio (water content) of the hydrogels

The same method that was used to measure the SR of MPC hydrogel films was used to measure the SR of NIPAAm hydrogels in water. The SR was calculated at three different temperatures 25^{o}C, 37^{o}C and 48^{o}C.

Percentage water retention percent (deswelling) of the hydrogels

The percentage of water retention (WR%) was measured using a gravimetric method at 48^{o}C. Hydrogel samples were freeze-dried for 3 days and weighed (Wd) then fully hydrated at room temperature (~25^{o}C) in (10 mL) deionised water for 48 hours. The fully hydrated samples were weighed at equilibrium (We) and quickly transferred to pre-heated water at 48^{o}C in the incubator. The samples were weighed at predetermined time intervals (Wt) (1, 3, 5, 10, 20, 30, 40, 60 and 120 minutes). Before each measure the sample was removed from incubator, quickly weighed and returned back to the incubator. Water retention was calculated for each time point using Equation 7.

$$\mathbf{WR\%} = \mathbf{100} \times \left[\frac{\mathbf{Wt-Wd}}{\mathbf{We}}\right] \quad (7)$$

In vitro release of protein from injectable NIPAAm hydrogels

The NIPAAm hydrogels are intended to be injected in the posterior segment (back of the eye) for protein delivery. To mimic the flow and conditions in the posterior

segment an *in vitro* PK-Eye model was used for release studies. This model is a prototype known as the pharmacokinetic eye (PK-Eye) and is a 2-compartment model of the eye that is fabricated from polyacrylate with an anterior cavity (200 µL) and a posterior (4.2 mL) cavity integrated within. A washer with a Visking membrane of a molecular weight cut off (MWCO) of 12-14 kDa separates the two segments. The inlet port was connected to the same peristaltic pump used for the previous release studies and the flow rate was adjusted to (2 µL/min). One outlet port is present in the anterior segment of the model for continuous sampling and an injection port is present in each segment with a diameter of 2-3 mm to allow administration of the desired formulation into the model (Figure 2:4) (Awwad et al. 2015). Prior to starting each experiment, the PK-Eye model was unscrewed and the washer with a fresh Visking membrane was adjusted inside the model. The PK-Eye models were assembled together with PBS, pH 7.4. The models were immersed in a preheated oil bath adjusted to 37°C for 2 hours to equilibrate prior to the release studies. Temperature was maintained at 37^{o}C using a probe connected to a hotplate heater.

After equilibrating the models, bevacizumab solution and hydrogel were injected *via* the injection port in the posterior cavity of the PK-Eye. Samples were collected from the outlet port of the anterior segment and quantified by HPLC. Bevacizumab samples were analysed by HPLC system Agilent 1200 series (Agilent, Wokingham, Berkshire, UK) equipped with Chemstation software (Agilent, Wokingham, Berkshire, UK). The stationary phase was an Agilent Zorbax GF-250 column kept at 25^{o}C. The mobile phase was PBS, pH 7.4 buffer previously purged with argon and sonicated. The mobile phase flow rate was 1 mL/min, the injection volume was 100 µL and the detection wavelength 280 nm. The retention time was 2.3 min. The correlation coefficient of the calibration curve was R^2: 0.996 for a concentration range of (1000-0.97 µg/mL), indicating acceptable linearity. The work reported for release was in collaboration with Dr Sahar Awwad.

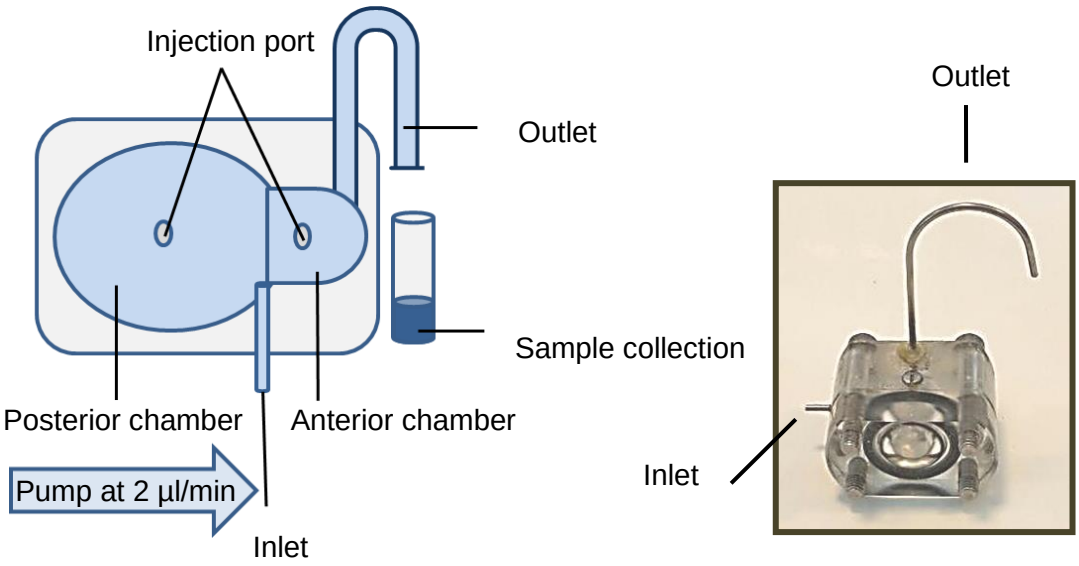


Figure 2:4 Schematic representation and a picture of PK-Eye release model used for studying the release of protein from NIPAAm hydrogels. The peristaltic pump used with eye flow rig is used with PK-Eye model.

Data analysis

All results are presented as the mean and standard deviation (± SD) of three samples unless stated otherwise and data were plotted using OriginPro 9.1. Half-lives was calculated according to the best fitting model in OriginPro. First-order kinetic rate constants (*k*) were derived from the monoexponential curve and half-lives were calculated using the equation: 0.693/*k*. The analysis of variance (one-way and repeated measure ANOVA) with Tukey's post hoc test was carried out to evaluate differences between the experimental data (mean values) using OriginPro 9.1 (software, Origin lab cooperation, USA) and IBM SPSS statistics 23. Probability values less than 0.05 ($p<0.05$) were considered as indicative of statistically significant differences.

Chapter 3. HEMA-MPC hydrogels for the subconjunctiva

There is an estimated 60.5 million people (2010 figures) that are suffering from glaucoma. The number of people suffering from this blinding disease is estimated to increase to 111.8 million by 2040 (Tham et al. 2014; Quigley & Broman 2006). Current treatments available in the clinic or under development have limitations in terms of properly controlling of IOP with current GDDs and lack of anti-scarring drug delivery dosage forms to improve success rate of GFS.

The main problem associated with current GDDs is the limited ability to control pressure in an effective way for a long enough period of time and with consistency among patients. The current materials that are used, such as silicone and polypropylene, cause excessive foreign body response that may affects the function of the GDD in controlling flow. There is a need to develop implantable devices for use in the subconjunctival space that do not elicit a strong foreign body response. Although the use of metals can reduced foreign body response, such as gold and stainless steel, variability in response among patients is still a problem. With current GDDs, medication is also needed at least during the first few weeks after implantation.

One of the new approaches investigated to improve the outcome of current GDDs is to coat them with biocompatible materials and in some cases loading the coat with anti-scarring for the fabrication of combination device (Gökce et al. 1996; Lee et al. 2014; Hovakimyan et al. 2015; Siewert et al. 2012). This strategy is used to make coronary stents that are now widely used. With better control of scarring there is a chance for better control of IOP. Current GDDs fail by the formation of an intractable, fibrous capsule that might be more thin and permeable with a more biocompatible material.

Ponnusamy *et al* reported that a coat made of PLGA and loaded with mitomycin C and 5-FU has the ability to reduce sacrring after GDD implantation (Ponnusamy et al. 2013). Sahiner *et al* reported that attaching HEMA loaded mitomycin C into the lower part of Ahmed GDD will improve the outcome of the device (Sahiner et al. 2009). When the modified GDD was implanted in rabbit eyes the fibrous capsule formed was significantly thinner compared to the non-modified device. Even with the use of a biocompatible polymer such as SIBS for the synthesis of GDD there is still a need to use MMC to improve the success of the GDD (Pinchuk et al. 2015).

The focus of the current research was for the development of biomaterials, coatings and the use of anti-scarring agent with current GDDs to improve the

success rate of GDDs in controlling IOP in a more effective way; however, new methods to modify aqueous outflow through GDDs to control IOP were less investigated. There is still a gap to be filled with current GDDs that are available in the clinic. Developing a combination device that is able to control flow with minimum, if no, inflammation and release medication at the same time is essential.

Hydrogel biomaterials are usually considered to have a reduced propensity to cause a foreign body response (L. Zhang et al. 2013). As networks, hydrogels are also considered to be porous polymeric materials that are permeable at least to gases as in contact lens and for water transport in the context of drug delivery (López-Alemany et al. 2002; Gemeinhart et al. 2000). Water flow through hydrogel contact lenses has been reported (Monticelli et al. 2005). Pishko *et al* suggested that contact lenses have hydraulic permeability and allow water to flow through them under low pressure (Pishko et al. 2007). They reported that the permeability of HEMA-methacrylic acid contact lens with 686 µm thickness under <3 KPa was 1.80 x 10^{-14} m^4/N s.

Based on favourable biocompatibility and potential water flow characteristics, hydrogels may be considered as materials for the development of new GDDs or drug eluting implants within the subconjunctival space. It might be possible to use a hydrogel material to control water flow from the anterior chamber through a tube into a hydrogel spacer in the subconjunctival space. Such a GDD would potentially not require surgical manipulation to adjust water flow; hence pressure control could be achieved in a new way that might be easier to maintain clinically.

A hydrogel spacer would be expected to have a negligible foreign body response resulting in minimal scarring. Considering the current clinical use of biomaterials, it is possible that a drug could be incorporated into the spacer to mediate the healing response after implantation. A biocompatible ophthalmic implant with extended delivery of an anti-inflammatory or anti-fibrotic medication could reduce the foreign body response. A long acting form of a less toxic agent than mitomycin C would potentially be beneficial to modulate the wound healing process after surgery over extended time periods. One of the more difficult aspects of glaucoma surgeries is the administration of toxic medicines such as mitomycin C. One reason for the use of mitomycin C is that it is so toxic, thus killing the activated fibroblasts at the site of surgery. However, exposure to the drug is short. What is needed are less toxic drugs with longer local tissue exposure times.

Hydrogels when used as biomaterial are generally fabricated from hydrophilic polymers. The fabrication conditions, monomer content and type, percentage and type of cross-linker used and initiator concentration all play important roles in

determining the final characteristics of the prepared hydrogels (Ahmed 2015; Bajpai & Singh 2006). Chavda *et al* reported that the characteristics of a superporous hydrogel were affected by the percentage of cross-linker used (Chavda & Patel 2011). When the percentage of cross-linker increased from 7.37% to 14.36% the density increased from 0.63 g/cc to 0.85 g/cc and the percentage porosity was reduced from 79.23% to 17.87%. McElroy *et al* reported the formation of hydrogel contact lenses with different properties when the intiator percentage was increased (McElroy et al. 2014). When the percentage of diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide (the initiator) was increased from 0.5% to 0.75% the percentage gel fraction increased from 90.35% to 93.68% and the water content percent reduced from 43.87% to 35.44%.

As discussed previously two monomers of particular interests are HEMA and MPC. Combining both HEMA and MPC in an implant will have the potential of higher biocompatibility and less foreign body response compared to using HEMA alone. Homopolymers derived from MPC are brittle and difficult to fabricate into devices, however small amounts of MPC in co-polymers are sufficient to impart biocompatibility. Abraham *et al* reported that HEMA hydrogels made with 5-10 mol% MPC have 64% less protein adsorption compared to HEMA hydrogels alone (Abraham et al. 2005). Contact lenses made from cross-linked HEMA and MPC are widely available and have higher biocompatibility and less biofilm formation in the eye compared to HEMA contact lenses (Selan et al. 2009; Goda & Ishihara 2006; Huang et al. 2007).

When fabricating a hydrogel the relative proportion of the monomers used (in this case HEMA and MPC) will have an effect on the properties of the final hydrogel (Hiratani & Alvarez-Lorenzo 2004). Properties such as hydrogel morphology, water flow characteristics, swelling behaviour, water content, free to bound water ratio, mechanical strength, drug loading and drug release will be affected by both hydrogel fabrication process and composition differences (Wu et al. 2009). It is thought that water content can be controlled so that a hydrogel can be fabricated that has the ability to control aqueous flow. Refojo *et al* reported that the permeability of HEMA membranes with 53.8% water content is 1.05×10^{-17} cm^2/sec.dyne which is higher compared to the permeability of membranes with 38.7% water content which is 8.4×10^{-18} cm^2/sec.dyne (Refojo 1965).

Medication is required with both GFS and GDDs and hydrogels are often investigated as drug delivery systems. If a GDD was fabricated from hydrogel, it could also be loaded with anti-inflammatory or anti-scarring agents to be used as combination device. In the clinic topical eye drops of corticosteroids are prescribed

after GDD implantation or GFS. DEX drops are used between 2-4 weeks after surgery. However, the use of eye drops has many disadvantages such as patient adherence to the treatment, irregular dosing and irritation to the already inflamed eye tissues due to the presence of preservatives. The disadvantages of eye drops may cause discomfort to the patient, reduce the therapeutic efficacy of the dose and the success rate of the surgery. If the hydrogel GDD was loaded with DEX it would eliminate the need to use DEX eye drops after implantation and reduce the side effects associated with eye drops.

The same hydrogel material used for GDD could be used as a spacer after GFS to modulate wound healing process and reduce scarring for patients not able or who do not need to have a GDD. For example in corneal injury, it is sometimes necessary to inject a medicine into the subconjunctival space in an effort to control inflammation and angiogenesis. A drug loaded implant could be beneficial for these patients to ensure that a therapeutic level of drug is available while the cornea recovers from injury.

Hypothesis and aims

The flow control mechanisms used in current GDDs have limitations for controlling the IOP in glaucoma patients. Hydrogels are potentially good candidate materials for the development of new GDDs and other subconjunctival implants. Compared to polypropylene and silicone which is used in current GDDs, hydrogels may display better biocompatibility, so would elicit less foreign body response and inflammation. Additionally hydrogels would be expected to be permeable to water and have potential to extend the local residence time of a drug subconjunctival space. The GDD should have the following properties; i) mechanical strength to withstand implantation; ii) resist water flow enough through the hydrogel film to regulate the intraocular pressure; iii) higher free to bound water ratio to improve biocompatibility; iv) the ability to load and release drugs for local action in the subconjunctival space.

It was hypothesised that water can flow through a hydrogel matrix to control aqueous outflow from the eye. Aqueous flow could be controlled through the hydrogel by controlling the hydrogel permeability through formulation changes. By modifying the water content and water distribution, the permeability of a hydrogel could be modified to allow flow of aqueous humour through the hydrogel at a rate of 2 µL/min (Figure 3:1).

Controlling the flow at the rate of 2 µL/min may help in controlling the IOP in a more effective way. It is also hoped that the hydrogel could be developed that can be folded and used in such a way that only a small incision in the eye will be

required for implantation. If folding can be achieved, it is possible that a device can be developed that can be implanted requiring less surgical skill in a faster procedure that could be conducted in any ophthalmology clinic. Anti-inflammatory and anti-scarring agents could also be loaded into the hydrogel formulating a combination device. The hydrogel could also be used as a drug releasing spacer in the subconjunctival space to reduce scarring after GFS. The spacer could also mechanically separate the conjunctival and scleral tissues to prevent adhesion and local fibrosis after surgery. The suggested dimensions of both the device and spacer are 1 cm in diameter (the surface area will be 78.5 cm^2) and 1 mm thickness.

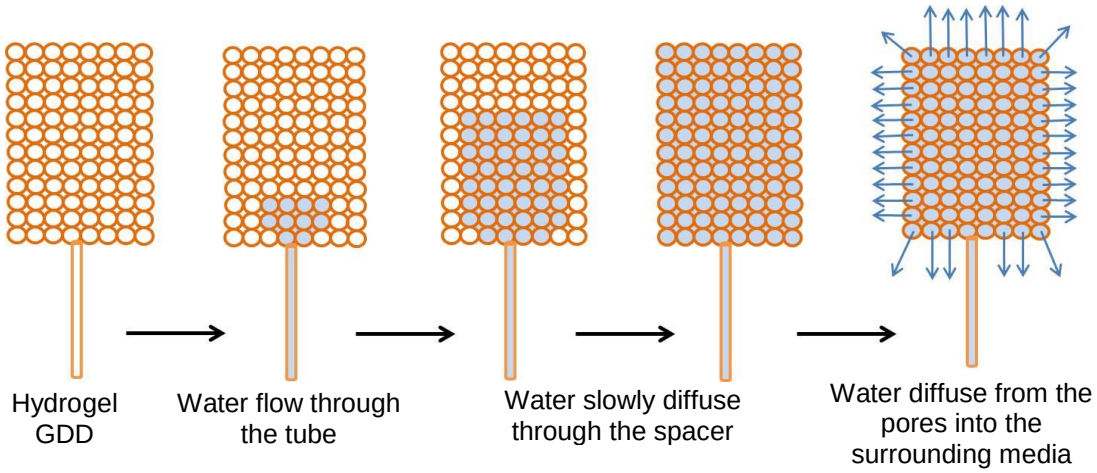


Figure 3:1 A suggested mechanism to control flow in GDD through hydrogels directly through diffusion into the hydrogel matrix. The aqueous will pass through the tube into the hydrogel matrix and when fully hydrated the aqueous will slowly diffuse at a slow rate through the hydrogel matrix to the surrounding subconjunctival space.

Any modification in the monomer type, amount and cross-linker will have an impact on the properties of the fabricated hydrogel. The aim of work described in this chapter was to screen different formulations of biocompatible polymers (HEMA and MPC) to determine if a hydrogel based GDD is feasible. Water permeability and flow through the different formulations will be measured. Other characteristics of the fabricated hydrogels such as water content, water distribution, swelling behaviour, mechanical strength, morphology and the ability to load and release medication were also studied. The effect of the percentage of MPC incorporation on the above mentioned properties is also discussed to evaluate what might be the best formulation to be used to sustain the release of DEX within the subconjunctival space.

Results and discussion

Effect of MPC content on general appearance of the prepared films

The general properties of hydrogels are widely affected by the composition of the hydrogel. There is a need to understand the correlation between formulation and properties, in particular permeability and water content. Permeability and water content are important in developing GDD that control aqueous flow through the device. A range of hydrogel films were formulated with HEMA and increasing MPC stoichiometries ranging from 0% to 100% MPC. The monomers were cross-linked using PEGDA as cross-linker and AIBN as initiator by free radical polymerisation (Figure 3:2). MPC was used due to its zwitterionic nature which is responsible for its high biocompatibility. MPC is also a hydrophilic molecule that attracts large amount of water compared to HEMA which could modulate the water content and distribution of water within the hydrogel matrix.

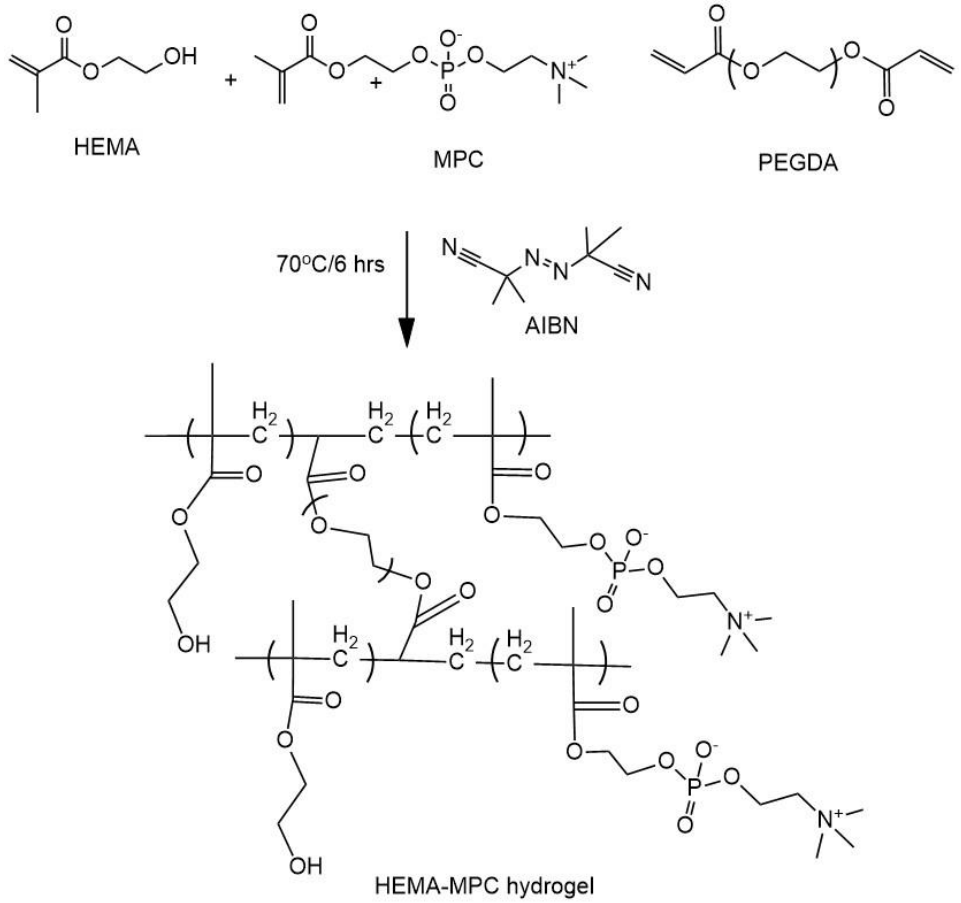


Figure 3:2 Chemical synthesis of HEMA-MPC hydrogel films by free radical polymerisation. The two monomers (HEMA and MPC) were mixed with cross-linker PEGDA to form clear solution. The initiator AIBN then added and placed in the oven at 70^{o}C for 6 hours.

The percentage of MPC was increased in increments from 0-100% in the formulation to study the effect of MPC percentage on the properties of the prepared hydrogels. The term “Xerogel” is used to describe hydrogels in the fully dried state. Formulations labelled S1 to S7 (which represents 0% to 50% MPC) were hard and glassy in xerogel form. The hydrogel comprised of 100% MPC (S8) was jelly like in consistency and no hard film was formed (Figure 3:3).

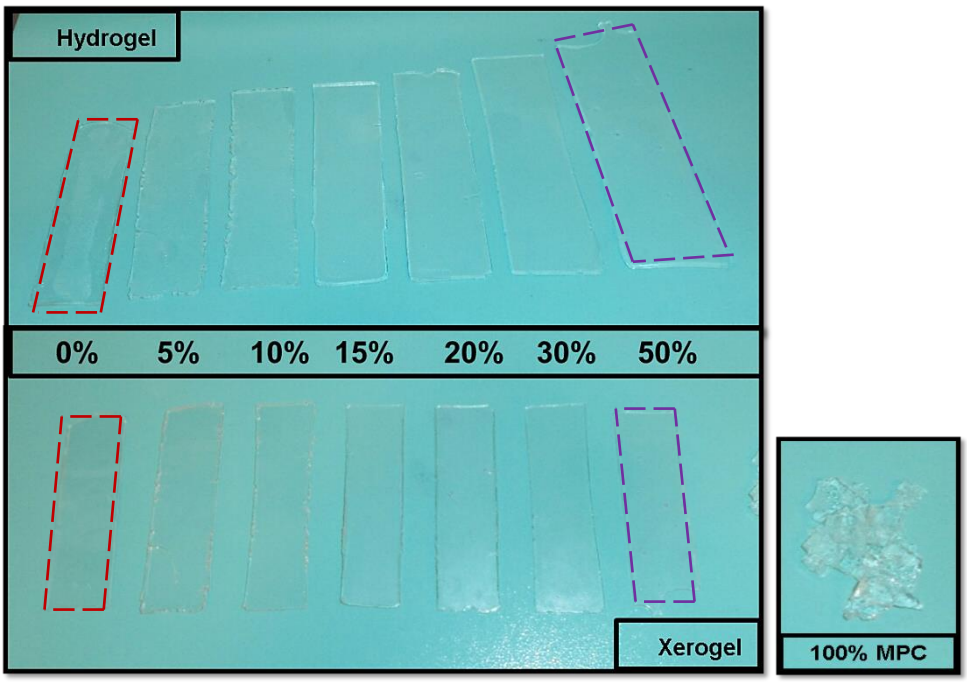


Figure 3:3 Formulations S1 to S8 (which represents 0% to 100% MPC) as xerogel and in fully hydrated state (hydrogel). At xerogel all the hydrogels, except 100% MPC, have the same dimensions. However, as hydrogels the dimensions (width, length and thickness) increased with increasing MPC percentage.

Before hydration the thickness of all the xerogel films (S1 to S7) was 1.1 ± 0.1 mm. The films were cleaned after removing the moulds and left to hydrate. When fully hydrated, formulations from S1 to S7 remained intact in film shape; however 50% MPC film (S7) were very fragile and could be easily broken (Figure 1:2). The thickness of the hydrated hydrogel films increased with increasing MPC content. (Table 3:1).

Table 3:1 Thickness of fully hydrated hydrogel films. The thickness of the hydrogel films increased with increasing MPC content.

MPC%	0%	5%	10%	15%	20%	30%	50%
Thickness mm	1.13 ± 0.01	1.15 ± 0.01	1.19 ± 0.01	1.30 ± 0.02	1.35 ± 0.01	1.50 ± 0.01	1.80 ± 0.02

Effect of MPC on film morphology

Scanning electron microscopy (SEM) was used to examine the effect of MPC% on the morphology of the prepared films. The samples were freeze-dried prior to examination. Films with 0%, 10% and 30% MPC were analysed. The size and number of pores increased with increasing MPC% (Figure 3:4).

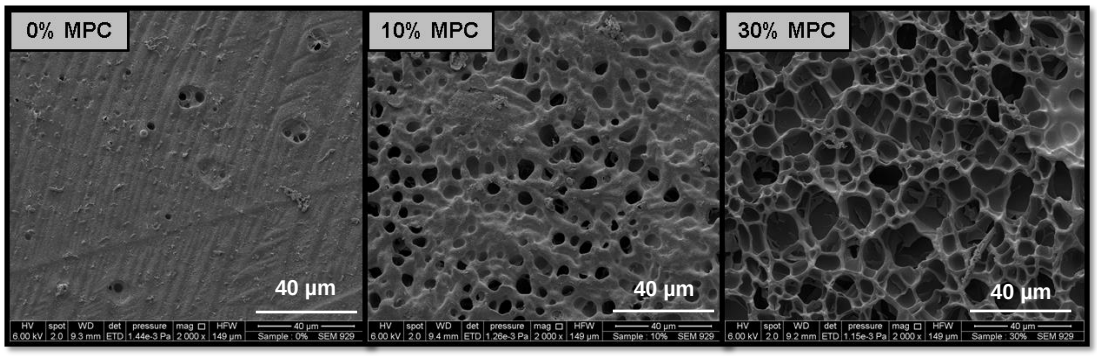


Figure 3:4 The SEM images of 0%, 10% and 30% MPC films. The size and number of pores increased with increasing MPC percentage. The scale bar is 40 µm.

It is thought that flow through the hydrogel could be adjusted at a desired rate by adjusting the porosity of the hydrogel. It was also thought that hydrogels with larger pores would allow more water to pass through and a higher percent of free water to be entrapped inside the films. Hydrogels with a larger pore size may allow entrapped materials (e.g. drugs) to be released quickly from the films. Formation of hydrogels with different pore sizes is possible by modifying MPC percentage.

GDD flow design

Aqueous humour in the eye is secreted and released at a rate of 2 µL/min. The balance between secretion rate and elimination rate control the pressure of the eye in the anterior chamber at around 15 mmHg. When the balance is disturbed by blocking the release, the pressure starts to build in the eye. GFS and GDD offer an alternative pathway for the flow of the aqueous humour to maintain the pressure around 12 mmHg. The main problem associated with GDDs is lack of control on flow and individual patient variability. As discussed previously the two mechanisms for control flow through current GDD with spacers are pressure controlled valve, and diffusion through fibrovascular capsule around the spacer. We are suggesting a new mechanism to control flow which is based on the permeability of hydrogels. It was thought that the aqueous humour could be adjusted to flow at a rate of 2 µL/min under pressure through the hydrogel pores.

Measurement of hydrogel hydraulic conductivity (L) and permeability (K)

To explore the possibility of using the hydrogel matrix for flow control, hydraulic conductivity (L) and permeability of the hydrogel films were studied. Hydraulic conductivity (L) is a term used to describe the fluid transport characteristics of a given membrane. It is the rate of fluid transported (m^3/sec) per unit area of the membrane (m^2) divided by the pressure (Pascal) that causes fluid flow and has units of m/sec/Pa. Since the rate of fluid transport is dependent on the thickness of the membrane, multiplying (L) by the thickness (T) gives the permeability (K) of the membrane. Thus permeability reflects the transport characteristics of the material comprising the membrane whereas the hydraulic conductivity describes the transport characteristics of a membrane of a given thickness.

Hydraulic conductivities are usually measured using dynamic approach or fixed flow approach. In the dynamic method, a pressure head is applied to the membrane to instigate flow and the fall in pressure is followed as a function of time. Hydraulic conductivity is then determined mathematically as described in the appendix. This method has been used to determine the hydraulic conductivity of human Bruch's membrane (Moore et al. 1995). In the fixed flow approach the flow of water is fixed at certain rate and the elevation in pressure is measured. Pressure is recorded at different time intervals and the change in pressure used to calculate the permeability. The permeability measurement for each sample was calculated using dynamic approach. The ideal permeability of a hydrogel film of 1 mm thickness that will allow the flow of aqueous humour at a rate of (2 µL/min) while maintaining the IOP at 10 mmHg was calculated to be (6×10^{-14} m^2 s^{-1} pas^{-1}). The permeability of 5%, 10%, 15% and 20% MPC films were measured (Table 3:2).

Table 3:2 The permeability of different hydrogel samples using dynamic approach.

Sample name	5% MPC	10% MPC	15% MPC	20% MPC
Permeability m^2 s^{-1} pas^{-1}	$1.6x10^{-14}$ ± 0.2	5.1×10^{-14} ± 0.9	$8.1x10^{-14}$ ± 0.8	$8.4x10^{-14}$ ± 1.2

The results demonstrated that all the films were permeable at relatively similar rate which was unexpected and drew our attention to the problems encountered during the actual setting up of the experiment. During the experiment several problems arose with using the flow chamber with opening on the capping plate (open system). One of the problems was that these flow chambers are designed to measure hydraulic conductivity in human Bruch's membrane. Bruch's membrane is a few nanometres in diameter and once placed in the flow chamber the upper lid should fit into the chamber and seal the edges completely. The

hydrogel films thickness was significantly larger at 1 mm, which made it hard to completely seal the chamber as extra pressure may break the film around the edge and allow water to pass.

To overcome these problems and confirm that the results obtained are a true reflection of permeability a new chamber with a closed system was designed. The new chamber has a top that is completely sealable and an indentation allowing a better fit of the sample. Hydrogel films with 10% MPC was chosen for the comparison between the new and the old flow chamber. When the new chamber used the permeability of 10% MPC films measured reduced sharply to a value of ($1.1\times10^{-18} \pm 0.7$ m^2 s^{-1} pas^{-1}) which indicates that the previous measurement was due to evaporation from the incomplete closed edges of the open system chambers. The permeability measured is low and shows that the aqueous humour does not pass through the hydrogel films at low pressure. Therefore controlling the porosity of the hydrogel cannot be used as the pressure controlling mechanism in GDD.

Creation of a pouch in a hydrogel film

Permeability through the hydrogel films was very low. A suggested mechanism to increase the permeability is to create a pouch inside the hydrogel matrix. The pouch may exert extra pressure from inside the hydrogel to speed the flow rate through the hydrogel surfaces (Figure 3:5). The pouch will be deflated at low pressure and when the pressure increases, due to accumulation of aqueous inside the pouch, the pouch will expend (similar principle to balloons) and flow. Two approaches have been investigated to create a pouch; femtosecond laser and polymerising around a soluble matrix.

Femtosecond lasers are used in cataract surgery. Cataracts are cloudy lens in the human eye and are treated by lens removal and replacement with a synthetic intraocular lens (IOL). In the surgery, an incision is created where the cornea and sclera meets to remove and insert the new IOL. Precise incisions could be produced by the use of femtosecond laser machines. The laser eliminates variability due to surgical skills and produces incisions without damage to surrounding tissues (Feizi 2011; Nagy 2014). Because of the precise incision capabilities of the laser without damaging the surroundings, it was thought that a pouch could be created inside a hydrogel without damaging the overall hydrogel matrix. The laser will be able to cut a full circle inside the hydrogel matrix without affecting the upper and the lower surfaces of the hydrogel films.

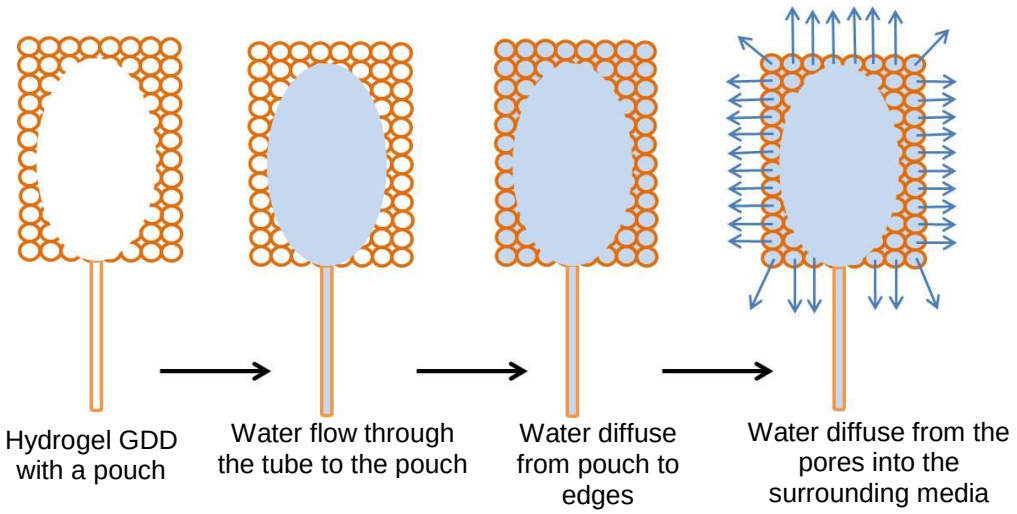


Figure 3:5 A suggested mechanism to control flow in GDD through a pouch followed by diffusion through the porous hydrogel matrix. The aqueous will accumulate in the pouch creating a pressure on the hydrogel matrix. The pressure will cause the aqueous to diffuse at a slow rate through the hydrogel matrix to the surrounding subconjunctival space.

Three different designs were created for the pouch using HEMA-MPC hydrogel films. 15% MPC hydrogel film with 1.3 mm thickness was used to create a maximum pouch using different small circles to create larger inner space. Another 15% MPC sample with the same thickness was used to create one small pouch (Figure 3:6). The femtosecond laser was able to create a pouch in the hydrogel matrix with different sizes without instant damage to the hydrogel surfaces. Before laser treatment hydrogels with 15% MPC could be bended several times without a crack. However, after laser treatment when the hydrogel film was bent around the large pouch area the surface starts to crack. The cracking indicates a loss of mechanical strength. It was thought that the larger the pouch in size, the thinner the surfaces will be which could cause breakage and cracking in the film.

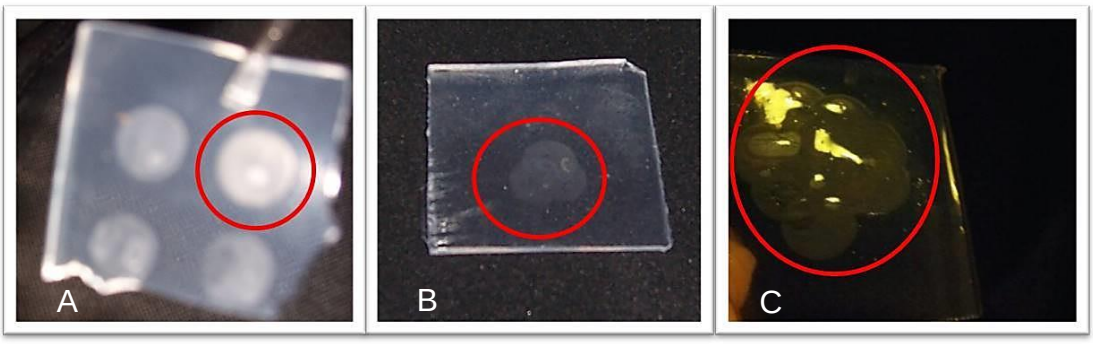


Figure 3:6 The pouch created in HEMA-MPC hydrogel films using femtosecond laser. Pouches created as single (A), triplet (B) and multi pouches (C).

When only one circle or three circles were created no immediate cracking was observed during bending. However, after preserving the films in water for more than one week the films became more fragile and cracking was noticed.

Mechanical strength is an important criterion for an implant. It was concluded that the use of a femtosecond laser is not a good method for creating the pouch.

Another approach to creating a pouch was investigated. It was thought that if the hydrogel solution was polymerised around a water soluble film or water soluble particles (such as PBS), the water soluble particles would dissolve and diffuse out of the hydrogel in water during the washing step creating a pouch. In an attempt to create a pouch in 10% MPC hydrogel film, the monomer mixture was used to fill half of the mould. PBS tablets were crushed into large particles, passed through a 40 mesh size sieve (which is equivalent to 500 µm) and placed as a solid sheet in the liquid monomer mixture. The mould was then filled with monomer mixture. Care must be taken to make sure that all the PBS particles were covered in the monomer mixture. After polymerisation the xerogel was hydrated in water, but the resulting hydrogel did not form a complete film. (Figure 3:7). To study whether the incomplete hydrogel film was a result of the presence of foreign particles and not the nature of PBS particles, the PBS particles were replaced with a thin film of polycaprolactone or filter paper and the hydrogel matrix was polymerised around the films. No complete film was also observed.

It was thought that the presence of any foreign material (particles or films) in the monomer mixture, although they did not dissolve in the monomer mixture, affected the polymerisation process around the edges of the particles or films creating an irregular film surface.

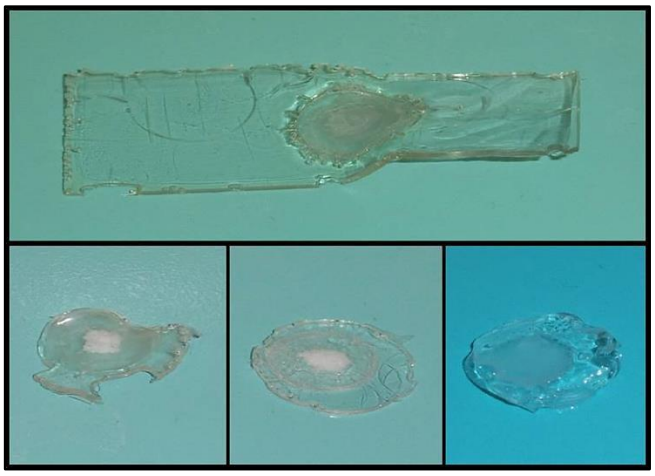


Figure 3:7 The polymerisation of 10% MPC around PBS particles. The hydrogel films formed were rough and irregular in shape.

Another approach to create a pouch was investigated which is polymerising around a small metal spatula (0.5 mm thickness). The spatula was sandwiched between the polypropylene and silicone part of the mould and the end of the spatula was immersed in the monomer matrix inside the mould. It was thought that

after hydration of the polymerised xerogel the spatula could be easily removed leaving an empty space in the middle of the hydrogel film. However, when the gel was hydrated and the spatula removed, the hydrogel thickness was different around the spatula. The thickness of the surface below the spatula was thinner with a crack compared to the one above the spatula which was thick and intact. A possible explanation is that the spatula was unable to remain suspended in the middle of the monomer matrix and leaned towards the bottom of the monomer mixture due to gravitational forces. It was concluded that if a pouch was created in the hydrogel film the properties of the film (i.e mechanical strength and smoothness of the surface) will be affected.

A new pressure control mechanism for a hydrogel GDD was suggested possible if hydrogel permeability to water flow could be controlled. However, when the permeability was measured it was very low compared to the required permeability at a flow rate of 2 µL/min. Although the pressure used for measuring the permeability was high (~30 mmHg) compared to the normal pressure of the eye (10-15 mmHg) the permeability was very low. The high pressure used and low permeability calculated suggested that the hydrogel matrix alone cannot be used to control flow in GDD.

Although the HEMA-MPC hydrogel could not be used as a control mechanism it can still be used to fabricate a combination device. The hydrogel will be used as a material for a spacer synthesis that is attached to a tube and drug will be loaded in the spacer (Figure 3:8). The hydrogel could also be used as a spacer after GFS.

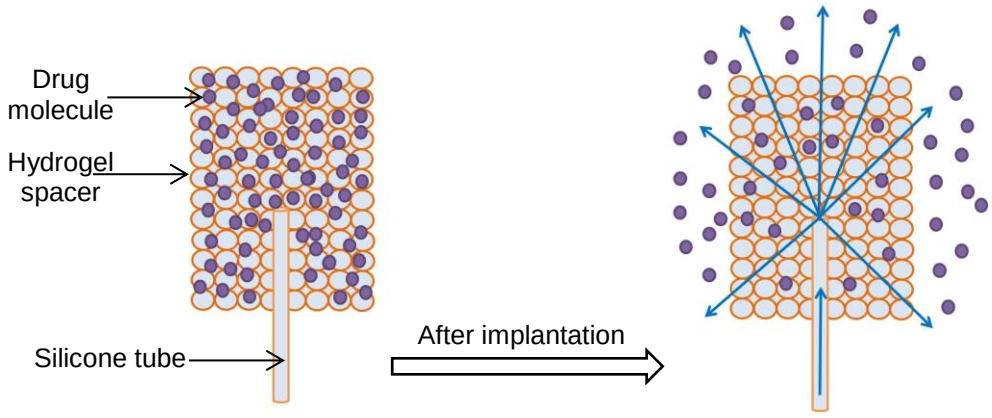


Figure 3:8 The use of HEMA-MPC hydrogel as a material for fabrication of spacer in GDD. The spacer will be attached to a silicone tube and loaded with anti-inflammatory or anti-scarring agents. Aqueous will flow through the tube only (blue arrow) and drug will be released by diffusion.

Equilibrium water content percent (EWC%) and swelling ratio (SR)

As mentioned previously, although HEMA-MPC hydrogels could not be used as pressure control mechanism they can still be utilized for drug delivery. Understanding water and swelling of HEMA-MPC hydrogels will help understanding drug loading and release from the hydrogel films. For a hydrogel to be used as drug delivery It was thought that the water content of the hydrogel may affect the rate of diffusion through the hydrogel; the more water; the better diffusion and the faster the release. The effect of MPC percentage on the EWC% of the formulation was measured. The EWC% of hydrogel formulations from S1 to S7 was calculated according to (Equation 1). The results are shown in (Figure 3:9A). The SR was also calculated for the same formulations using (Equation 2) (Figure 3:9B).

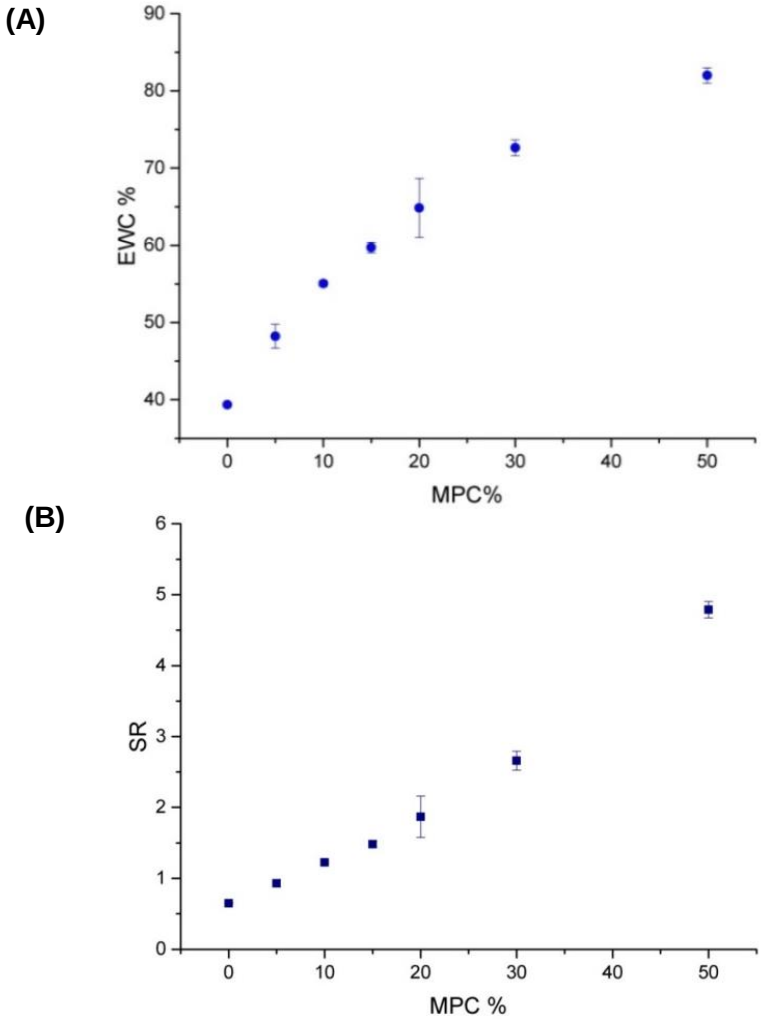


Figure 3:9 A) The effect of MPC% on EWC% as measured using Equation 1; B) The effect of MPC% on SR as measured using Equation 2. As the relative proportion of MPC was increased, there was a significant increase ($p<0.05$) in both EWC% and SR.

As the relative proportion of MPC was increased, there was a significant increase ($p<0.05$) in both EWC% and SR. MPC is a hydrophilic molecule with a zwitterionic head group that attracts water. The water present is mostly in the free (bulk) form (Morisaku et al. 2008). Kazuhiko *et al* also noticed a dramatic increase in water content when MPC was copolymerised with n-butyl methacrylate to form a hydrogel (Kazuhiko Ishihara et al. 1990).

When the MPC percentage was equal or exceeded the HEMA percentage, as in the S7 formulation (50% MPC), the water content in the formulations increased to the point where there was little mechanical strength in the films. The films were easily torn and broken. Several factors are responsible such as the arrangement of water molecules and polymer chains around each other, the solubility of the polymer chains in water and the total amount of water absorbed by the hydrogel. Although HEMA based polymer have the ability to absorb water, HEMA polymer cross-linked chains are insoluble in water, while MPC chains are, that is why HEMA hydrogels maintain a three dimensional shape in contrast to MPC hydrogels. Also MPC, compared to HEMA, has strong hydrophilic groups that attracts large amount of water. Water molecules tend to arrange themselves around the hydrophilic groups in the polymer chain and when the total amount of water increased and the relative polymer units remain the same they stretch the polymer chains and reduce the strength of the formed gels (Monti & Simonib 1992).

Effect of MPC content on the distribution of water inside the hydrogel films

Water in hydrogels is divided into three types; highly bound water, freely bound water and bulk water. It was thought that the higher water content, and larger pore size resulting from hydrogel formulations with higher percentages of MPC would have higher flow rates through the polymer matrix. It was also thought that the distribution of water inside the hydrogel may have an effect on the behaviour of the hydrogels regarding drug loading and release. To screen the different HEMA-MPC formulations the ratio between free and bound water was measured and calculated and the effect of increasing MPC percentage on the ratio was studied (Figure 3:10 and Table 3:3).

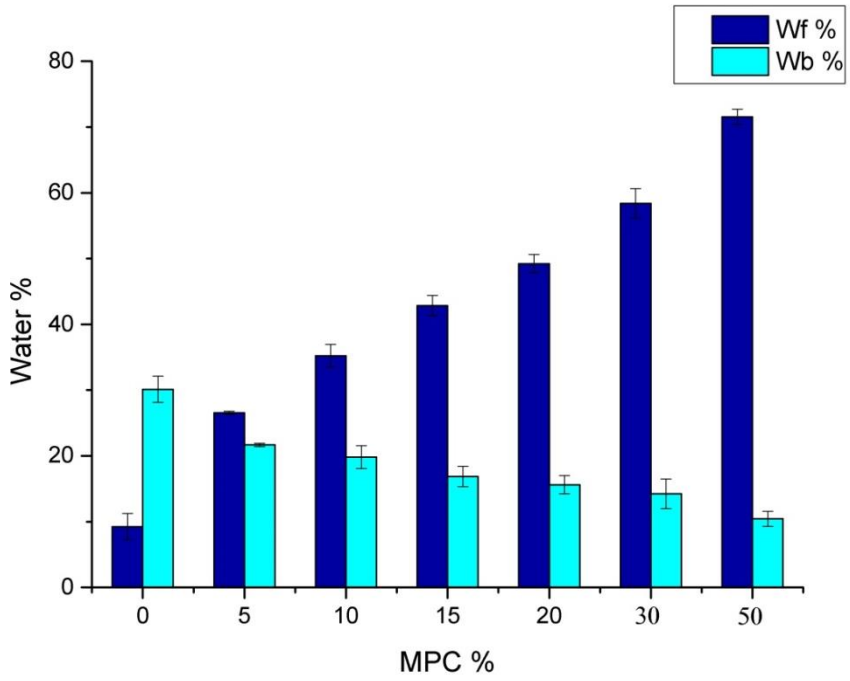


Figure 3:10 Effect of MPC% on Wf and Wb. The ratio of free water compared to the bound water was increased when the percentage of MPC increased. The Wf is significantly different between the samples ($p<0.05$).

Table 3:3 The ratio of bound water (Wb) to free water (Wf) in a hydrogel films based on MPC percentage. The ratio of free water increased with increasing MPC percentage.

MPC%	0%	5%	10%	15%	20%	30%	50%
Wb : Wf	1:0.3	1:1.2	1:1.8	1:2.5	1:3.2	1:4.1	1:6.8

Increasing the relative percentage of MPC results in an increase in free water ratio compared to bound water. The MPC repeat units attract water molecules to form a layer of water associated around the pendant zwitterionic head groups. MPC units will form weak hydrogen bonds with water molecules so the layer of water surrounding them will remain mostly free and undisturbed (Shi et al., 2012; Chen et al., 2010). On the other hand HEMA has the ability to form strong hydrogen bonds with water. As the percentage of MPC increased and HEMA is reduced more water molecules will be free or lightly bonded and can be frozen and detected by DSC.

Effect of MPC on the mechanical strength of HEMA-MPC hydrogel films

Hydrogels when completely swelled are semisolid materials and could be subjected to deformation when force applied into them. During GFS or implantation of GDD the surgeon will push the implant into the subconjunctival space using surgical instruments such as forceps and clamps. Implantable films need to have mechanical strength to withstand this stress during implantation. The films should

be able to stay intact during the surgery and be folded without break. The effect of MPC percentage on the mechanical strength of the prepared films was studied. The term mechanical strength reflects the ability of the films to stretch and to withstand force. Mechanical strength is required during implantation as force may rupture the hydrogel films.

The term that is used for quantitative determination of mechanical strength (stiffness) is called young's modulus or elastic modulus (E). A stress-strain curve is used for the determination of E value. Typical stress-strain curve has elastic and plastic regions (Figure 3:11). In the elastic region the material obeys Hooks law. Hooks law states that the force applied (stress) is proportional to the change in surface area (strain) of the material. The law states also that when no longer force is applied to the material, it returns to its original shape and size without deformation. In the stress strain curve the straight line represents Hooks behaviour of the material in the elastic region and the slope of the line is E. Materials which behave according to Hook's Law are called elastic materials. If the material deformation is permanent under force it is called plastic material (Faridmehr et al. 2014; Tranoudis & Efron 2004). An elastic behaviour is required for an implant or a device to withstand pressure during implantation without deforming.

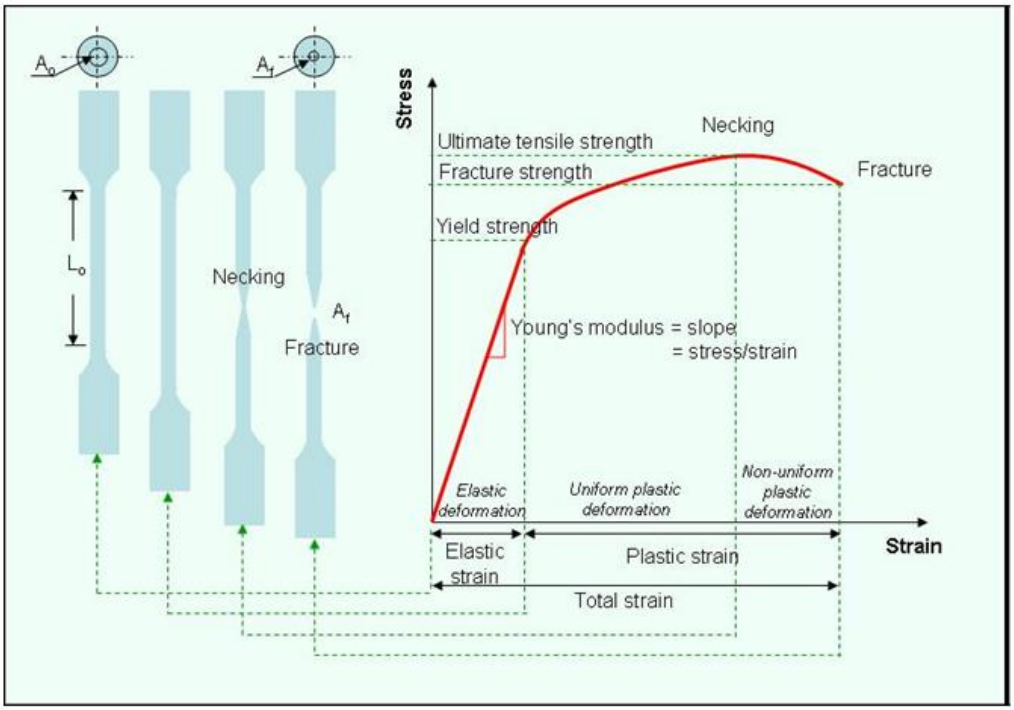


Figure 3:11 Stress strain curve demonstration the elastic and plastic regions. When the stress applied increase the strain increased in linear way (elastic region). The slop of the linear region used for the calculations of young's modulus (Faridmehr et al. 2014).

The Young's modulus (E) reflects the strength (stiffness) of films in general. The higher value for Young's modulus indicates stronger films. The Young's modulus of HEMA contact lenses was previously reported to be around 0.8 MPa and for proclear® contact lenses (which have 15% MPC) is 0.5 MPa (Young, Garofalo, Harmer, & Peters, 2010; Tranoudis & Efron, 2004). From (Figure 3:12) a decrease in Young's modulus was observed with increasing MPC% which is consistent with the previously reported data by Monti. Monti has previously reported that when the bulk water of a hydrogel (in the form of contact lenses) increased the Young's modulus reduced and becomes closer in value to Young's modulus of human cornea (0.29 MPa) (Hamilton & Pye 2008; Monti & Simonib 1992). When the percentage of MPC increased the free water percentage also increased. Free water will impart softness to the films and reduce the mechanical strength. There was a significant difference between 0% MPC and all the other formulations regarding Young's modulus ($p<0.05$).

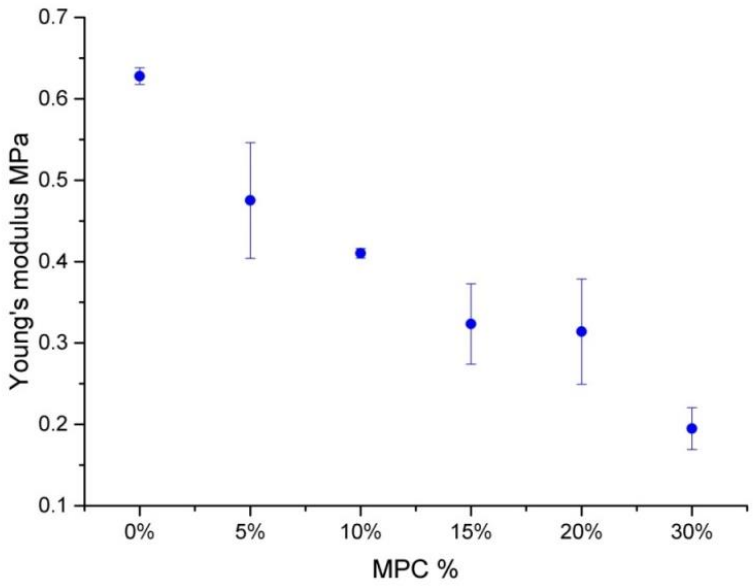


Figure 3:12 The effect of MPC% on Young's modulus (E) values. When the MPC% increased the E value reduced which indicate a reduction in the mechanical strength of the prepared films. There was a significant difference between 0% MPC and all the other formulations regarding Young's modulus ($p<o.o5$).

Another approach to investigate the mechanical properties of the prepared hydrogels was used. An attempt to measure the softness of the hydrogel surface using surface contact angle was investigated. Softness of the surface is important due to the direct contact of the surface of the hydrogels with the tissues after implantation. However, there were difficulties and limitations in conducting the experiment. The HEMA-MPC hydrogels start drying quickly when removed from

water. Hydrogels need to be mounted on the device which requires time. By the time the experiment is ready the surface of the hydrogel starts to dry and loose its softness which will give the wrong estimation comparing the softness of the surfaces of different hydrogels. To compare the mechanical strength of the prepared hydrogels only young's modulus was measured.

Effect of MPC on hydrogel swelling ratio in different solvents

Hydrogels have been investigated widely as drug delivery systems. Drug loading into a hydrogel is generally through two methods; soaking (imbibing) and *in situ* (mixing). In situ would not be a suitable method in this case, as the films require significant washing to remove unreacted monomers in the system and it is expected that a high percentage of the loaded drug will be released with the washed monomers. For the hydrogel formulation (HEMA-MPC), soaking is thought to be a better method for loading.

Loading of a drug into a hydrogel by soaking or imbibing depends on several factors such as the physicochemical properties of the drug (solubility) and the SR of the hydrogel in the loading solvent (Kim et al. 1992). If the drug is hydrophilic the loading medium will be water. However, if a hydrophobic drug needs to be loaded organic solvent should be used. Understanding loading from organic solvents requires an understanding of the behaviour of the prepared hydrogels in different solvents and the influence of MPC on that behaviour. Formulations with different percentages of MPC were studied to understand how MPC affects SR in solvents other than water. The SR was measured in methanol, ethanol, water: methanol (1:1) and water: ethanol (1:1) (Figure 3:13A). Pictures of the difference in SR between different solvents for 10% MPC hydrogels could be observed (Figure 3:13B).

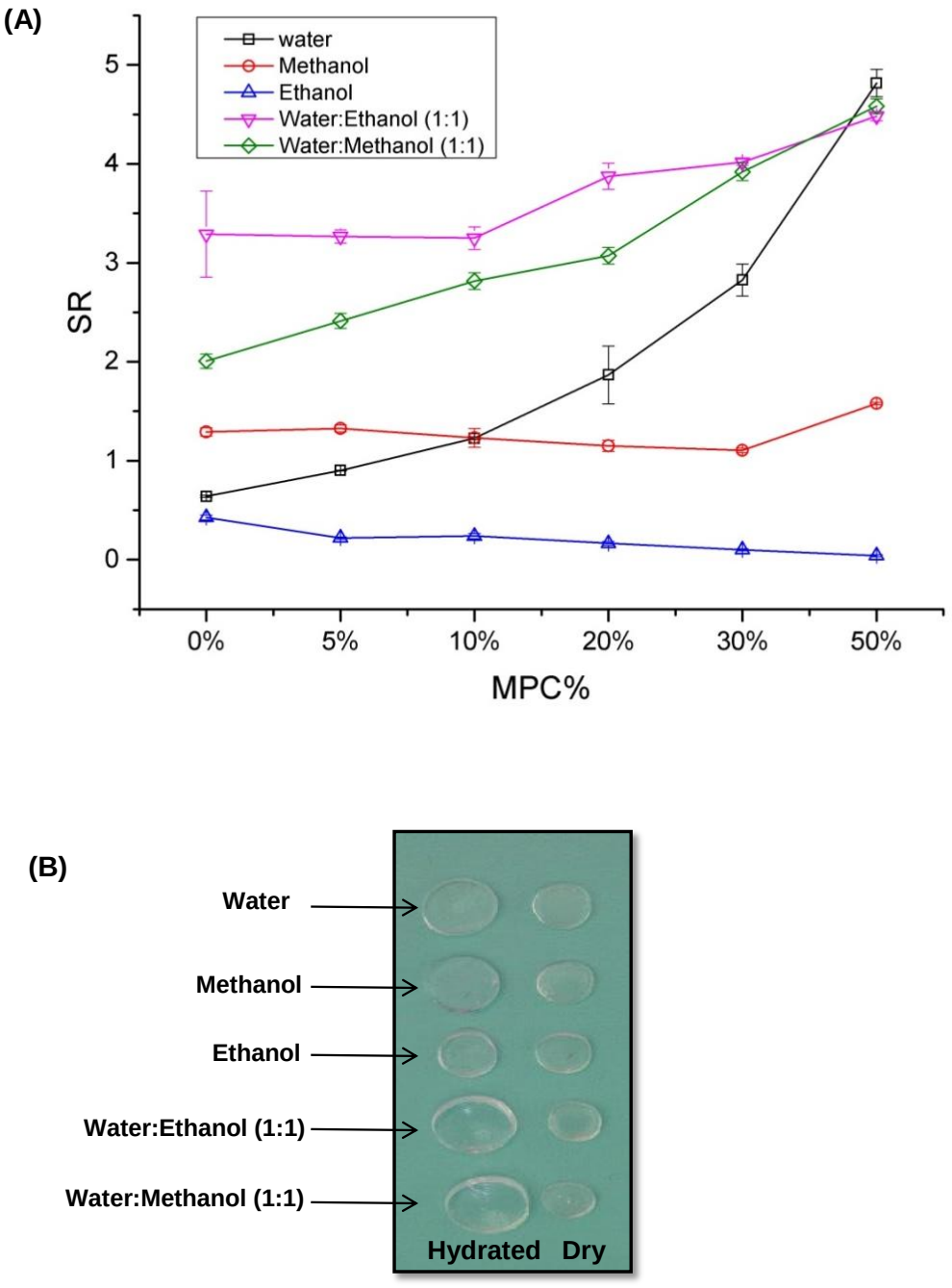


Figure 3:13 A) The effect of solvent used on SR of films with different percentages of MPC. When water alcohol mixture was used the SR increased with increasing MPC percentage; B) the SR of 10% MPC in different solvents.

The swelling behaviour of films with different percentages of MPC in methanol was also studied. When the percentage of MPC increased from 0-30% in HEMA-MPC hydrogel films, no significant difference was observed in SR ($p>0.05$). However, at 50% the SR was significantly increased ($p<0.05$). When ethanol used as solvent, there was little change in SR even with MPC above 50%. Kiritosha *et al*

reported that poly MPC hydrogels (without HEMA) swell to a less extent and has low affinity to interact with methanol and ethanol, compared to water, which may explain why in spite of the increase in the percentage of MPC no change in SR in methanol and ethanol was observed (Kiritoshi & Ishihara, 2003). The results indicates that when alcohol was used as solvent the interactions between the solvent and HEMA polymer chains predominate while when water was used the MPC polymer interactions predominate and an increase in SR was observed with increasing MPC%.

When a 1:1 mixture of methanol:water was used the SR increased with increasing MPC percentage. However, when a 1:1 mixture of water:ethanol was used this increase was only observed when MPC level increased above 20%. These findings can be explained by the difference in affinity of MPC to ethanol and methanol in which poly-MPC swells to a lesser extent in ethanol compared to methanol (Kiritoshi & Ishihara, 2002).

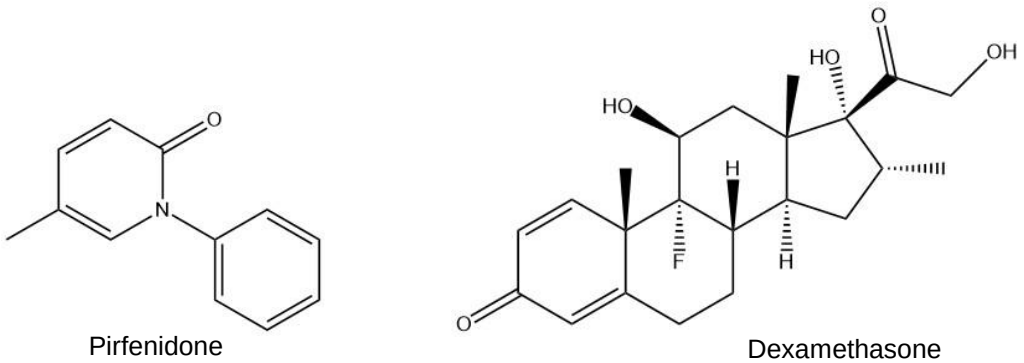
Drug loading of HEMA-MPC hydrogel films

After any surgery, a sequence of events takes place to heal the wound of the operation. The healing process is a series of complex events that starts with Inflammation and haemostasis and ends with tissue remodelling (scar formation) (Skuta & Parrish 1987). In the eye the scar formation is a problem as it may close the newly formed channel during GFS. Different methods and drugs, including the use anti-scarring agents in different dosage forms such as injections and implantable films, were investigated to modulate the wound healing process and reduce scar formation (Lockwood et al. 2013; Seibold et al. 2012). One of the methods explored was the use of a spacer at the incision site of the surgery (Hafez 2015). Different materials could be used for the synthesis of the spacer including hydrogels.

One of the advantages of fabricating a spacer from hydrogel, in addition to its biocompatibility, is the ability of hydrogels to load and release drugs. Since HEMA-MPC hydrogel films have low water permeability the loading and release of drugs will depend on drug solubility, hydrogel water content (especially the free water) and drug diffusion. To study the effect of free water on release of hydrophilic and hydrophobic drugs from HEMA-MPC films two drugs were used; pirfenidone as hydrophilic drug and dexamethasone (DEX) as hydrophobic drug.

Pirfenidone was used because of its high solubility in water (~10 mg/mL), small MW (185.2 g/mole) and a potential anti-scarring agent in the eye. Pirfenidone is available in the clinic in the form of tablets for the treatment of idiopathic

pulmonary fibrosis which is characterised by scarring of the lung tissues (Jackson & Gomez-Marin 2011). Research has shown that it also has an effect in the reduction of scarring tissues after GFS when used (Figure 3:14) (Sun et al. 2011; Mora et al. 2015; Gan et al. 2011). Topical corticosteroids such as DEX and prednisolone have been reported to effectively improve the outcomes of GFS by supressing the inflammation process. In the clinic topical eye drops of corticosteroids are prescribed after surgery.



Pirfenidone Dexamethasone

Figure 3:14 The chemical structure of dexamethasone and pirfenidone.

Drug loading into a hydrogel could be carried by two methods; *in situ* loading and soaking. For the HEMA-MPC formulations soaking was chosen as a method for loading. If the drug was loaded during polymerisation there is a possibility of losing a significant percentage of the loaded drug during washing of the hydrogel after polymerisation. Another problem is the effect of high temperature ($70^{o}C$) used during polymerisation which may cause drug degradation. In addition to that the drug may interact with the cross-linker and the monomers and cross-linked to the polymer matrix which may affect its release from the hydrogel. The soaking method was considered as the more convenient method for drug loading in this case. In the soaking method, the amount loaded and the loading efficiency of drugs in a hydrogel are affected by several factors such as EWC% of the hydrogel, swelling ratio, solubility of the drug, molecular weight of the drug and the concentration of the drug in the loading solution (Maulvi et al. 2014).

Loading and release of a hydrophobic drug (dexamethasone)

In drug releasing hydrogels there are several mechanisms involved in the release of loaded drug. These mechanisms (such as diffusion and erosion) are based on the type of hydrogel used, method of loading and the drug molecule itself (Colombo et al. 1996; Li & Mooney 2016). In conventional hydrogels diffusion of the drug from the loaded hydrogel is the driving force for the release (Satapathy et al. 2015).

Diffusion is related to the bulk water percentage in the hydrogel; the higher the bulk water content, the faster the release (Wu et al. 2016).

To test that theory and screen the formulations DEX was loaded in films containing 0%, 10% and 30% MPC and the release from the three hydrogels was compared. For the loading of DEX the hydrogel dry disks were incubated in the loading solution for 24 hours, each 10% MPC disk is 1 mm in thickness and 1 cm in diameter. The dimensions of the discs used for release represents the actual dimensions intended for a GDD or a spacer. Once removed from the incubation solution the discs were washed with ethanol for 1 minute to remove the drug molecules attached to the surface of the discs. For a hydrophobic drug molecule the solubility plays an important role in loading and release. DEX has low solubility in water (0.1 mg/mL). If HEMA-MPC hydrogel discs were loaded from aqueous solution the amount loaded and loading efficiency will be low so a 1:1 ratio water:ethanol solvent mixture was used as the loading solution. By using organic solvent at this mixture the solubility of DEX in the loading solution will increase to (1 mg/mL). DEX was loaded into a hydrogel disc (1 mm thickness, 1 cm diameter) from 1 mg/mL (1 mL) water:ethanol (1:1) loading solution. The loading of DEX was 0.40 ± 0.03, 0.3 ± 0.1 and 0.19 ± 0.03 mg/disc for 0%, 10% and 30% respectively. The dry weight of each disc was 49.2 ± 1.3, 46.5 ± 1.2 and 22.7 ± 0.2 mg and the loading efficiency was 40%, 30% and 19% for 0%, 10% and 30% respectively. The difference in UV reading between the starting solution and the solution left after incubation was used to estimate the amount of drug loaded in each disc.

To validate the method used for measuring loading samples DEX was extracted from hydrogel samples and the amount measured by extraction was compared to the amount measured by UV. 10% MPC hydrogels were used for the comparison. Methanol was used as extraction solvent because the discs swell to a higher extent in methanol compared to water or PBS. The results can be seen in (Table 3:4). There was no significant difference ($p>0.05$) between the two methods which indicated that UV method was a suitable method for loading estimation.

Table 3:4 The amount loaded and loading efficiency of DEX in 10% MPC discs calculated using two different methods (UV and extraction). No significant difference ($p>0.05$) was observed in the amount loaded and loading efficiency between the two methods.

Calculation method	Amount loaded in mg/disc	Loading efficiency%
Based on UV readings	0.3 ± 0.1	30 %
Based on extraction with methanol	0.39 ± 0.03	39 %

It was observed that the amount of drug loaded was decreased with increasing MPC percentage. Two factors affect the drug loading process; the affinity of the drug molecule to the polymer matrix and the amount of the drug soluble in the volume absorbed by the dry hydrogel matrix (the swelling of the hydrogel) (Kim et al. 1992). While the swelling of 30% MPC is higher than 0% MPC the affinity of DEX is thought to be less for 30% MPC polymer matrix. The low affinity could be due to the higher affinity of the PC head group to water molecule in favour of drug molecule. DEX has higher affinity to adsorb on hydrophobic surfaces (HEMA). When the level of HEMA reduced compared to MPC the DEX loading will be reduced. Guidi *et al* reported that the driving force of DEX loading into the hydrogel contact lenses is adsorption into the polymer matrix and DeX loading increased with increasing hydrophobicity of the lenses (Guidi et al. 2014). When HEMA was replaced with equal molar ratio of N,N-dimethylacrylamide (DMA) in the different formulations the amount of DEX loaded was higher in all DMA formulations.

The release of DEX from the loaded hydrogels was studied using an eye flow chamber developed in our lab that resembles the flow in the subconjunctival space (2 µL/min). The discs were placed in the flow chambers and the samples were collected at different time intervals and analysed using HPLC. Faster release was observed from 30% MPC compared to 0% and 10% MPC (Figure 3:15). The half-life of DEX was 3.0 ± 12.6, 1.8 ± 8.9 and 1.0 ± 3.5 day for 0%, 10% and 30% MPC respectively. Mechanism of the drug release from HEMA-MPC hydrogels is diffusion which is affected by free water inside the hydrogel matrix. Since 30% MPC has higher free water compared to 0% and 10% MPC, the release of DEX was faster.

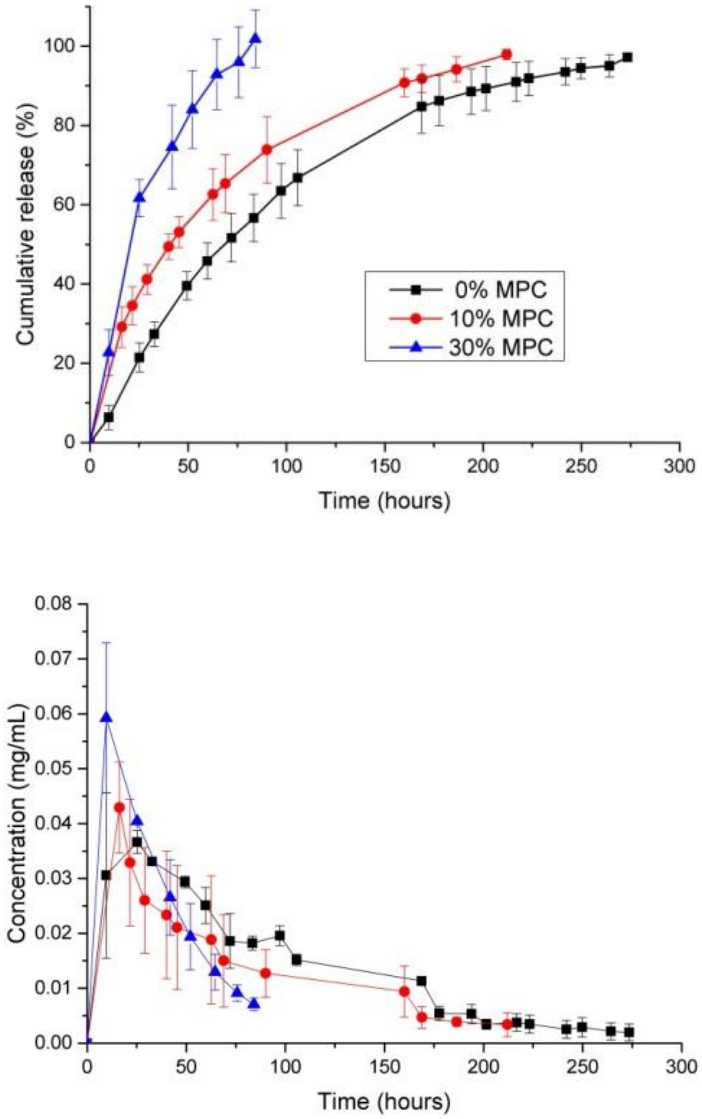


Figure 3:15 The cumulative release percent of DEX loaded in 0%, 10% and 30% MPC hydrogel films and concentration with time. The top panel is the percent of drug released and the bottom panel is the concentration of the drug released with time. The release from 30% MPC film was faster than 0% and 10% MPC.

Loading and release of a hydrophilic drug (pirfenidone)

To compare loading and release of hydrophobic and hydrophilic drugs from different HEMA-MPC hydrogels, pirfenidone was loaded in hydrogel discs containing 0%, 10% and 30% MPC films and the release from the three hydrogels was compared. Using an aqueous loading solution concentration of 1 mg/mL (1 mL), the amount loaded was 0.35 ± 0.02, 0.30 ± 0.01 and 0.25 ± 0.02 mg and the loading efficiency was 35%, 30% and 25% for 0%, 10% and 30% MPC respectively. The same pattern observed with DEX in loading (decreased loading with increasing MPC percentage) was observed with pirfenidone. Although pirfenidone is water

soluble the loading was reduced with higher water content hydrogels. The low loading could be due to the higher affinity of the PC head group to water molecule in favour of drug molecule. PC will attract the free water that will occupy the pores and displace the drug molecules.

The release of pirfenidone was studied and the half-life of pirfenidone released was 1.2 ± 2.4, 0.7 ± 3.4 and 0.4 ± 2.1 day for 0%, 10% and 30% MPC respectively. More than 50% of the drug released within the first 24 h from 0% MPC and even higher percentage was released from 10% and 30% MPC (Figure 3:16). When 0.5 mg of the drug injected into the flow chamber without the hydrogel the drug was completely cleared within the first 24 hours and the half-life was 0.25 ± 1.20 days. There was no difference in half-life when 30% MPC was used compared to free drug and a slight difference was observed when compared to 10% MPC hydrogels. The higher percentage of free water and the larger pore size observed with 30% MPC will help the drug to diffuse faster outside the hydrogel into the surroundings and shorter half-life will be observed with increasing MPC percentage (Wu et al. 2016).

Although there was a small difference in the amount loaded into the hydrogel between DEX and pirfenidone, the release profile was different. The half-life of DEX was 3.0 ± 12.6, 1.8 ± 8.9 and 1.0 ± 3.5 day for 0%, 10% and 30% respectively which was higher compared to pirfenidone. Because of the low solubility DEX in PBS (100 µg/mL) the drug will be deposited in the hydrogel and released slowly. The difference in release pattern indicates that the release is controlled by the free wtaer content of the hydrogel, hydrogel polymer matrix composition, the solubility of the drug in the loading solution and the affinity of the drug to the polymer matrix.

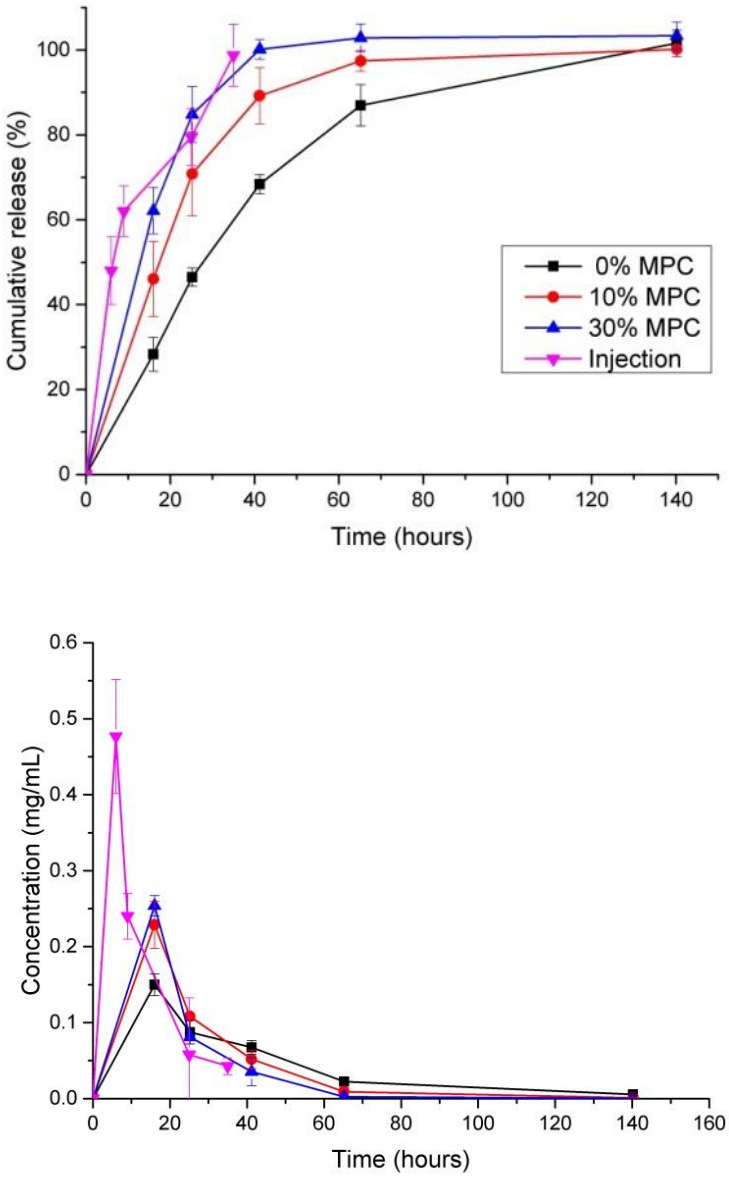


Figure 3:16 The cumulative release percent of pirfenidone loaded in 0%, 10% and 30% MPC hydrogel films and concentration with time. The top panel is the percent of drug released and the bottom panel is the concentration of the drug released with time. The release from 30% MPC film was faster than 0% and 10% MPC.

Loading of a drug molecule into a hydrogel by imbibing depends on many factors, one of them being the swelling of the hydrogel used. It is thought that the higher the SR of the hydrogel in the loading solution, the higher the amount of the drug loaded. HEMA-MPC hydrogels swell to a large extent when a water/alcohol solvent mixture was used. In order to increase the loading efficiency of pirfenidone water:ethanol (1:1) mixture was used as loading solvent. The SR of HEMA-MPC hydrogels is higher in water:ethanol mixture compared to water. It was assumed that the higher SR will allow more of the drug to be entrapped in the polymer matrix

of the hydrogel to achieve higher loading efficiency. However, the amount loaded was slightly lower compared to water. The amount loaded was 0.29 ± 0.02, 0.25 ± 0.01 and 0.15 ± 0.02 for 0%, 10% and 30% respectively which was unexpected. The same pattern was also observed in which a reduction in loading was observed with increasing MPC percentage.

For a hydrogel spacer, the balance between water content and mechanical strength is important. The presence of MPC will improve biocompatibility by increasing the free water percentage but at the same time will reduce the strength of the films. For 20 and 30% MPC films although the water content was high the mechanical strength was low. Low mechanical strength may cause problems during implantation such as rupture. Also any loaded drug will be released fast. Films made with 5% MPC have low amount of free water and low percentage of MPC which may not improve the biocompatibility of HEMA films (0% films). Although there was no significant difference in the EWC% and SR of 10 and 15 % MPC films, 15% MPC films have significant amount of free water compared to bound water. The amount of free water affects the mechanical strength of 15% MPC as they have lower Young's modulus compared to 10% MPC films. Based on these results 10% MPC film was chosen as a material for development as drug releasing spacer for ophthalmic implantation.

Effects of initiator and cross-linker on the water behaviour of 10% MPC films

One parameter that is important to be considered in the synthesis of a drug releasing hydrogel implant is the ability to load and release drugs. That ability is directly affected by water distribution inside the hydrogel; the higher the free water, the faster the release. Hydrogels with 10% MPC were chosen as the best formulation for ophthalmic implantation compared to other MPC percentages. Hydrogels with 10% MPC have higher free water content compared to bound water (1.8:1). It was assumed that modifying the water content, distribution and free to bound water ratio could be achieved by modifying the percentage of cross-linker and initiator while the HEMA and MPC percentage remain constant in the formulation. Each initiator molecule will form a polymer chain that can be cross-linked via the cross-linker molecule (eg PEGDA) to form the final polymer hydrogel structure. The higher the initiator amount, the smaller the number of chains formed and smaller the pores formed. A higher percentage of cross-linker can form hydrogels with smaller pore size. Modification of both the initiator and cross-linker was previously reported to affect the water content and distribution inside the hydrogels (Goda, Watanabe, Takai, & Ishihara, 2006; Kiritoshi & Ishihara, 2002).

For a 10% MPC formulation, the level of PEGDA was reduced from 0.56 % in the original formulation to 0.28 % and 0.14 % (which represents half and one quarter of the original amount of PEGDA respectively). The EWC% and SR were measured (Figure 3:17). No significant difference (P>0.05) was observed when the level of cross-linker was reduced to 0.28% and 0.14%. It is probably related to the fact that the level of MPC remains constant so the total amount of water molecules attracted to the zwitterionic head groups are the same. The same results were observed when the level of initiator (AIBN) was reduced from 0.74 % in the original formulation to 0.37 % and 0.185 % (which represents half and quarter the original amount of PEGDA respectively).

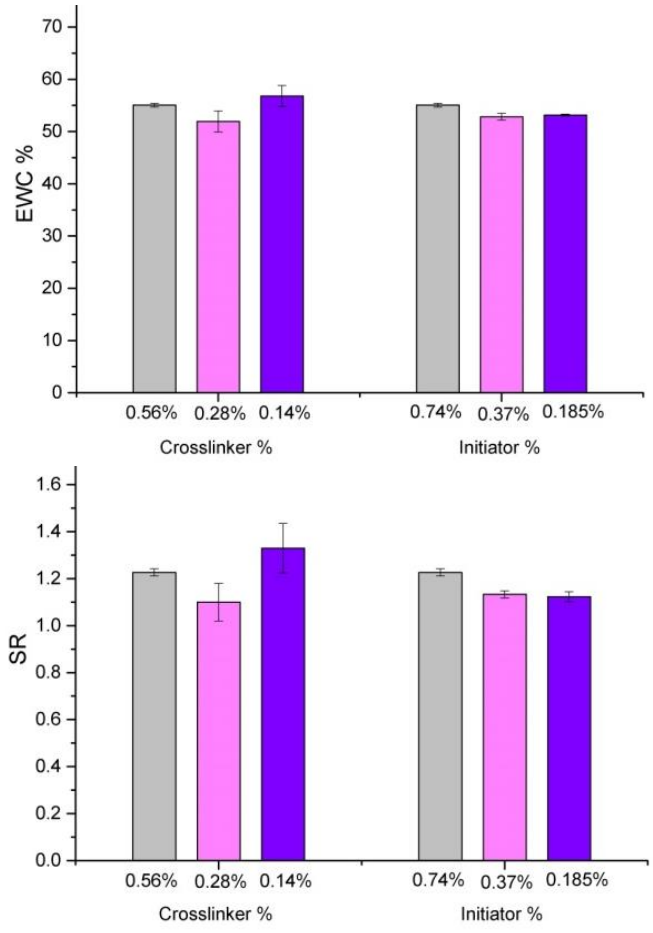


Figure 3:17 The effect of cross-linker and initiator percentages on EWC% and SR of 10% MPC gels. No significant difference (P>0.05) was observed when the level of cross-linker and initiator was reduced.

Although the EWC% was the same we investigated the possibility of changing the distribution of water inside the gels. The ratio of free to bound water was measured when the level of cross-linker and initiator changed. No significant difference (p>0.05) was observed with Wf% and Wb% when the level of cross-

linker and initiator were reduced (Figure 3:18). Based on these results the original 10 % MPC formulation with 0.56 % PEGDA and 0.74 % AIBN was found to offer the best balance between water content, mechanical strength and drug loading and release required for implantation.

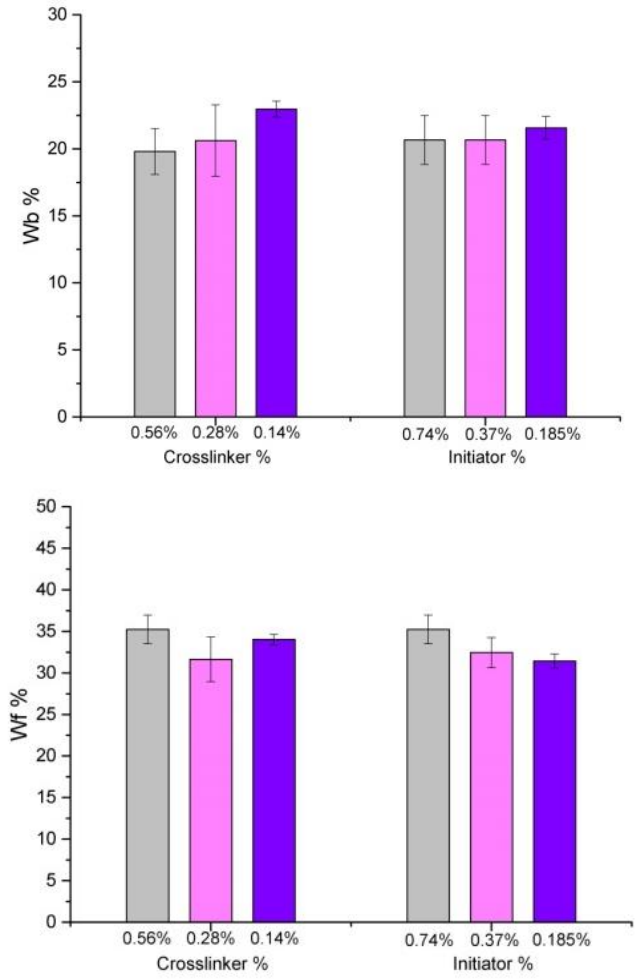


Figure 3:18 The effect of cross-linker and initiator percentage on Wf % and Wb %. There was no significant difference (P>0.05) was observed in the free and bound water ratio with decreasing cross-linker and initiator percentages.

Extended release of DEX from 10% MPC

Previous characterisation of the different HEMA-MPC formulations showed that 10% MPC has the potential to be used as drug releasing spacer. One of the aims of this chapter is to sustain anti-inflammatory drugs in the HEMA-MPC hydrogels. For 10% MPC hydrogel discs, the amount of DEX loaded in each disc was (~0.3 mg/disc). The amount loaded was low and around 50% of the drug released within the first 48 hours accompanied with a burst release. DEX low solubility in water and water organic solvent combinations restricts the concentration of the starting solution which affects the amount loaded into the discs. To overcome the problem of low solubility in water methanol was used as solvent because of the high

solubility of DEX in methanol (30 mg/mL). Although DEX has high solubility in ethanol as well but the discs does not swell in ethanol. A solution of DEX in methanol 15 mg/mL (1 mL) was used for loading DEX into the gels. Although the loading efficiency was low when using methanol as solvent 13.7% compared to 30% when water:ethanol was used, the amount loaded was 7 times higher when methanol was used. The amount loaded was 2.1 ± 0.5 mg/disc when methanol used compared to 0.30 ± 0.03 mg/disc when water:ethanol was used. When the release of the samples was studied the half-life of DEX in the discs increased from 1.8 ± 8.9 days with water:ethanol to 4.5 ± 0.9 days when methanol was used (Figure 3:19).

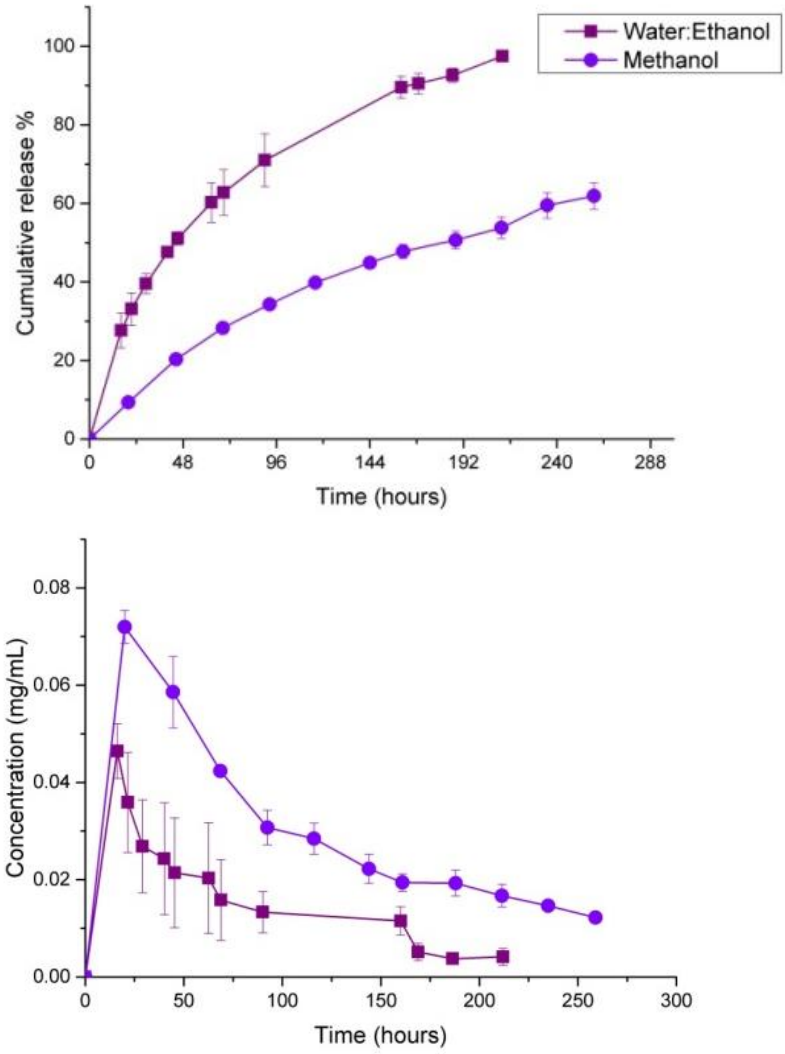


Figure 3:19 The release profile of DEX loaded into 10% MPC films from water:ethanol and methanol. The top panel is the percent of drug released and the bottom panel is the concentration of the drug released with time. The half-life of DEX was increased from 1.8 ± 8.9 days to 4.5 ± 0.9 days when methanol was used. A burst release was observed in both samples.

When methanol was used as loading solvent a 'burst release' of DEX was also observed and the concentration was high compared to the therapeutic doses of DEX used in the eye tissues (Sharma et al. 2011). In order to reduce this burst release and help to extract methanol from the discs, water was used as a washing solvent. DEX has low solubility in water and once the methanol loaded discs and placed in water the DEX starts to precipitate inside the disc creating a depot for the drug (Figure 3:20). The precipitation of DEX indicates that there is no methanol to solubilize DEX in the hydrogel matrix and the methanol used for loading is slowly removed from the hydrogel matrix by diffusion into the surrounding water during washing due to the miscibility of water and methanol.

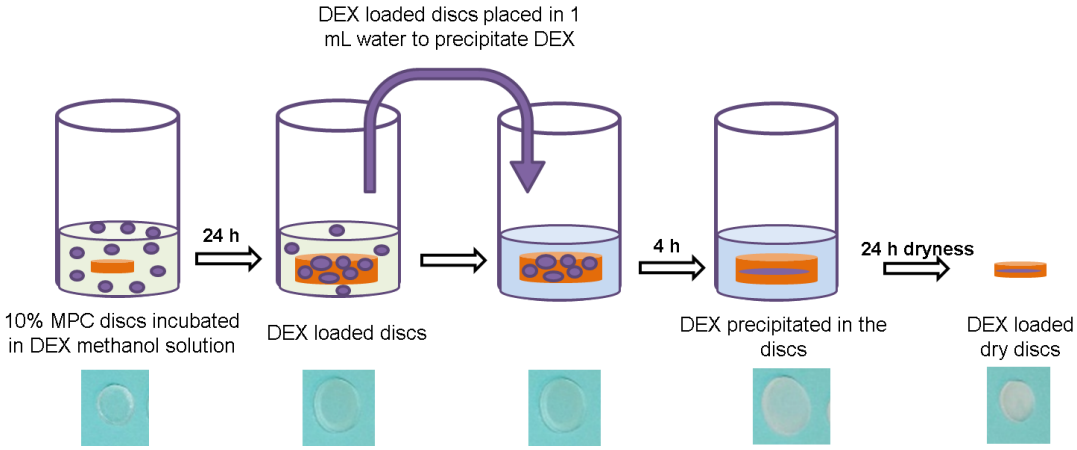


Figure 3:20 The loading of DEX from methanol. When the DEX loaded discs transferred from methanol into water DEX starts to precipitate and form a depot. We can notice the discs appearance changed from transparent to opaque once placed in water.

The depot formed will help to sustain the release of DEX from the discs. When the discs removed from incubation in methanol each disc was washed with 1 mL of water for 4 hours. The amount of DEX lost during washing was 0.2 mg which is equivalent of 10% of the amount loaded. When the release was studied a reduction in the burst release and the concentration was observed and the half-life increased to 9.7 ± 1.9 days (Figure 3:21). Washing with water also helps to eliminate the extra free DEX present inside the hydrogel matrix which causes the reduction in burst release. Also the depot created with DEX helps to sustain the release for a longer period and in a more controlled manner. The release DEX from unwashed hydrogels was studied for only 10 days because it was preliminary study to examine whether using methanol has an effect on loading and release. However, the release of DEX from washed hydrogel was studied for a longer period of time to resemble the time DEX drops used in the clinic by patients.

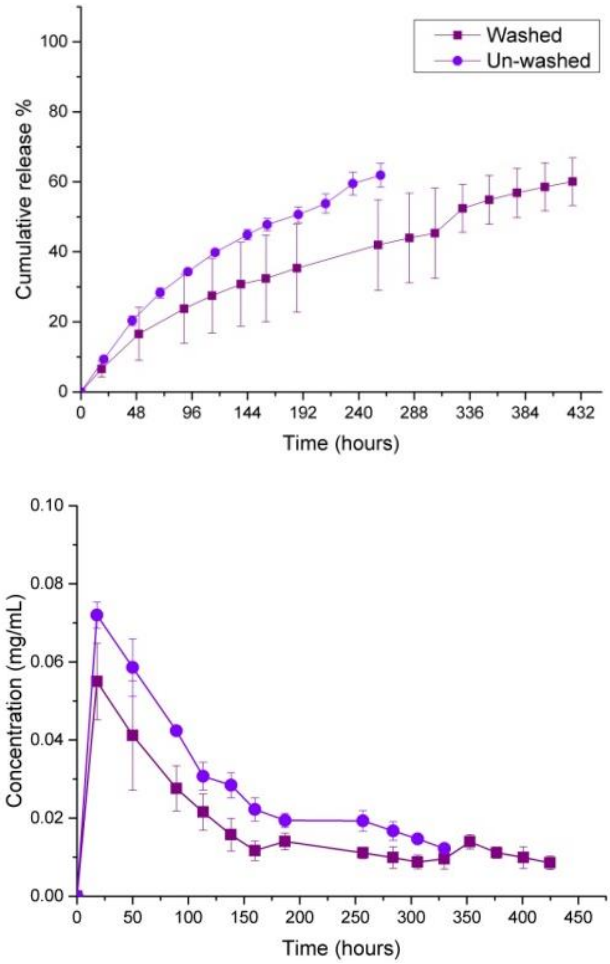


Figure 3:21 The release profile of DEX loaded into 10% MPC films using methanol before and after washing with water. The top panel is the percent of drug released and the bottom panel is the concentration of the drug released with time. The burst release and concentration was reduced after washing with water.

Loading a hydrogel with hydrophobic drug in sufficient amount is a challenging process due to the nature of hydrogels (filled with water). A hydrophobic drug (DEX) was successfully loaded into 10% MPC hydrogel discs in sufficient amount to sustain the release of DEX for 3 weeks.

Storage and delivery of hydrogel films

The intended use of the 10% MPC hydrogel film is a drug releasing spacer after glaucoma and trachoma surgery. One of the problems associated with hydrogels is the delivery in the clinic and how to maintain the hydrogel and the loaded drug stable during storage until used by the surgeon. Two possible ways of delivery, either stored in a storage solution (similar to contact lenses) to be used immediately during surgery or as dried hydrogels to be hydrated prior to use. Understanding the rate of swelling and deswelling will help to choose the best method for delivery of the hydrogel. The fully dried 10% MPC hydrogel requires at least 6 hours to reach

maximum hydration and 30 minutes to reach around 50% of hydration when placed in PBS at 25^{o}C (Figure 3:22).

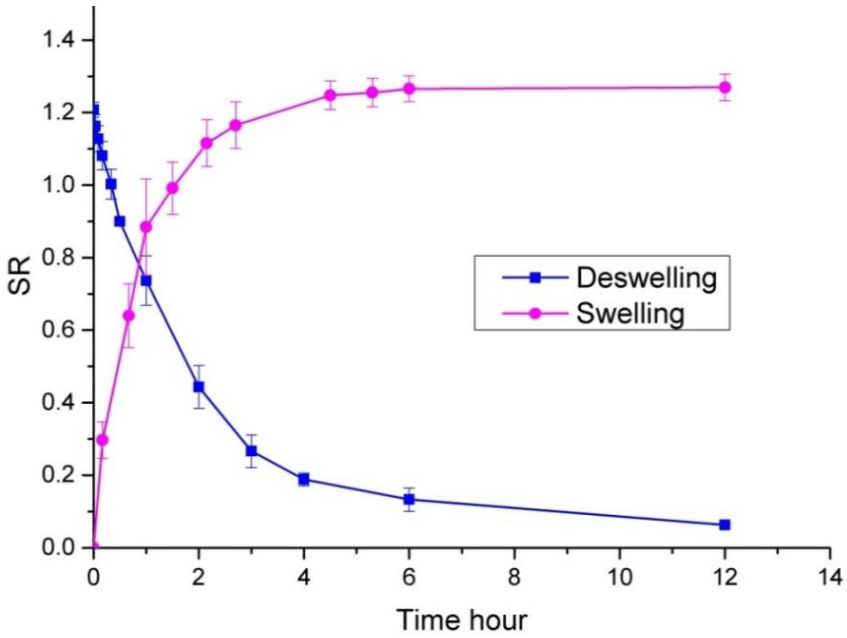


Figure 3:22 The swelling and deswelling behaviour of 10% MPC hydrogel. Rapid swelling and deswelling was observed in the first hour compared followed by slow progression in both.

Although storing a hydrogel in a storage solution will be more convenient to the surgeon, the stability of loaded drugs inside the hydrogel will be a problem. Some of the drug loaded will diffuse from the hydrogel matrix to the storage solution during storage and reduce the amount loaded. The loss of loaded drug during storage will be more significant with hydrophilic drugs. Also the stability of the drug, especially drugs subjected to hydrolysis, will be a problem. The best method is to deliver the hydrogel spacer in a dry xerogel-like form, with instructions to rehydrate them in 0.5 mL sterilised water for 1 hr prior to use. The hard glassy nature of dry hydrogels may cause irritation and discomfort if placed in dry form and rehydrating them for 1 hr will ensure at least 50% of swelling is achieved without drug loss. The remaining 50% of swelling will be completed after implantation by the flow of the aqueous humour. Complete swelling after implantation will have no effect on the dimensions of the device or spacer because once complete hydration is achieved the hydrogel return to its original intended dimensions of a GDD or a spacer.

Summary and conclusions

The aim of this chapter was to formulate and characterise a HEMA-MPC hydrogel film that could be used for the development of GDD and a subconjunctival drug releasing implant after GFS. Different formulations with increasing stoichiometries of MPC from 0% to 100% were prepared. It was noticed that the SR and water content increased with increasing MPC percentage. When the percentage of MPC increased above 30% a film was not formed after hydration. The mechanical strength films were reduced with increasing MPC percentage.

The hydraulic conductivity and permeability of the hydrogel films were too low to restrict aqueous outflow as a means to control the IOP after GDD implantation. The films were also impermeable to water passing through them. The flow rate of (1.12×10^{-18} m^2 s^{-1} pas^{-1}) for 10% MPC hydrogel was very low compared to the required flow rate in an ideal device (6×10^{-14} m^2 s^{-1} pas^{-1}). Creating a pouch by the use of a femtosecond laser or by polymerising around water soluble particles or films was also not successful for creating a hydrogel film capable of allowing aqueous outflow. While the HEMA-MPC hydrogels may not have the potential to control aqueous outflow; they were examine for potential use as a subconjunctival drug releasing implant.

To choose an appropriate formulation for drug loading and release, hydrogel films were characterised regarding the swelling in different solvents and the ability to load and release hydrophobic and hydrophilic drugs. An increase in SR was observed with increasing percentage of MPC in (1:1) water:alcohol mixture. When pure alcohol (methanol or ethanol) were used as solvents the SR was not affected by an increase percentage of MPC for ethanol. An increase in SR was only observed when the percentage of MPC was above 30% for methanol.

Formulations with 0%, 10% and 30% MPC were used for drug loading studies. The hydrogels were loaded with either a hydrophilic drug (pirfenidone) or hydrophobic drug (DEX) drug to examine the effect of MPC content (i.e. free water content) on loading and release. The drug loading was low and the release of the loaded drug was faster for hydrogels with 30% MPC for both drugs compared to hydrogels with 10% and 0% MPC.

Based on these findings hydrogels with 10% MPC offer the balance between water content, mechanical strength and drug loading and release. Since drug loading and release are related to free water, the level of cross-linker and initiator were varied to modify the ratio of free and bound water. However, there was no significant difference in free to bound water ratio when the cross-linker and

initiator percentages were reduced. Based on these results 10 % MPC formulation with 0.56 % PEGDA and 0.74 % AIBN was chosen as the best formulation for the synthesis of a hydrogel drug releasing spacer.

To increase the loading efficiency and sustain DEX release from 10% MPC hydrogels, methanol was used as solvent for DEX loading. DEX was successfully loaded into a 10% MPC hydrogel film. The amount loaded increased from 0.3 mg into 1.9 mg/disc and the release was extended for more than 3 weeks.

Although HEMA-MPC hydrogels were unable to show sufficient permeability and hydraulic conductivity to be of any use to control aqueous outflow to regulate pressure in a GDD, the hydrogel films offer a platform for development of drug releasing combination GDD and drug releasing spacer after GFS.

Chapter 4. Hydrogel implants for water soluble drugs

Fibrosis that occurs due to treatment (e.g. GFS) or due to disease shares a similar mechanism and is the cause for much blinding disease (Hsu et al. 2000; Tovell 2011; Bowman 1999). In the previous chapter HEMA-MPC hydrogel films were fabricated and characterised as a subconjunctival drug releasing spacer for GDD and after GFS to modulate wound healing. The HEMA-MPC hydrogels were characterised with the 10% MPC (which is 10% MPC, 88.7% HEMA, 0.56 % PEGDA and 0.74 % (w/w) AIBN) being selected as the optimal hydrogel formulation because it offers the best balance between water content, mechanical strength and drug loading and release. In addition to the problem of scarring caused by surgeries to lower the IOP in glaucoma, there is a condition known as trachoma where subconjunctival scarring is also the cause of blindness both due to the disease and also its surgical treatment which is widely used for recurring forms of the infection.

Trachoma affects large populations of patients in developing countries and is a major ophthalmic health problem. It is considered an endemic disease that affects poor communities in Africa and Asia. It also affects a large population in Australia. It is estimated that 229 million people live in theses endemic areas and 21 million people live with active trachoma (Taylor et al. 2014; Mariotti et al. 2009). Trachoma is a bacterial infection of the eyelid, conjunctiva and cornea that is caused by chlamydia trachomatis. The disease is spread directly through contact with the discharge from infected eyes or indirectly through poor sanitation and by flies (Kasi et al. 2004). Trachoma has different stages. In the early stages the infected eye suffers from symptoms of conjunctivitis symptoms including watery eye discharge, itching, redness, swelling and thickness of the upper eyelid (Darougar & Jones 1983). If diagnosed early, trachoma can be treated with antibiotics. In the early stages of trachoma infection antibiotics can be used. Oral azithromycin and topical tetracycline ointments are the drugs of choice (Solomon et al. 2004; Bowman et al. 2000). Other antibiotics have been studied to treat trachoma including doxycycline (Li et al. 2013).Recurrent infections will cause chronic inflammation of the eyelids which results in thickening and scarring of the upper eyelid ending with trichiasis (Gambhir & Basáñez 2007).

Recurrent trachoma inflammation causes trichiasis. Trichiasis is a term used to describe the abnormal position or growth of the eyelashes towards the eye ball due to contraction of the eyelid as a result of scarring (Figure 4:1). The abnormal eyelashes movement will cause corneal opacification, due to scratching and scarring of the cornea, and eventually blindness if untreated (Mecaskey et al.

2003). There are several treatments available depending on the severity of the condition. Epilation is sometimes used for the removal of eyelashes either using forceps or scissors. Another procedure is the destruction of lash follicles. Epilation is effective for a short period and the eyelashes grow again (Rajak et al. 2012).

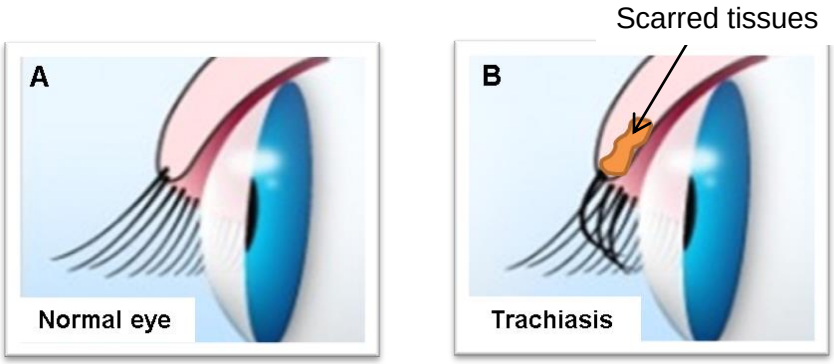


Figure 4:1 The positioning of the eyelashes in normal and trachiasis eyes (HealthFixit 2016)

A widely used treatment is the corrective surgery of the eyelid which is often referred to as the tarsal rotation procedure (Figure 4:2). The aim of the surgical procedure is to cut through the scarred tissues that cause contraction of the lid. The surgery involves rotating the marginal part of the eyelid outwards to prevent scratching of the cornea by the eyelids. A horizontal incision through the dense connective tissue located at the borders of lid margin is performed to separate it from the underlying submuscular tissues (Rajak et al. 2012). The incision will cut through the scared tissues followed by suturing of the tissue with the margin of the eyelids (Figure 4:2). The suture will be removed after two weeks and antibiotics are needed throughout the two weeks period to prevent infection. The problem with trachoma surgery is the recurrence of infection. The use of antibiotics after the surgery may reduce the risk of recurrence (Rajak et al. 2010; Burton et al. 2005). The problem again is limited patient adherence to the use of the antibiotics after surgery. Currently no treatment is available after trachoma surgery except the use of antibiotic ointments, such as tetracycline ointments (Bowman et al. 2002). Another problem to the surgery is the scarring that results from the surgery itself, which could be minimised if treated with an antifibrotic agent. MMC which is used for GFS and to implant GDDs cannot be used with trachoma surgery due to MMC toxicity.

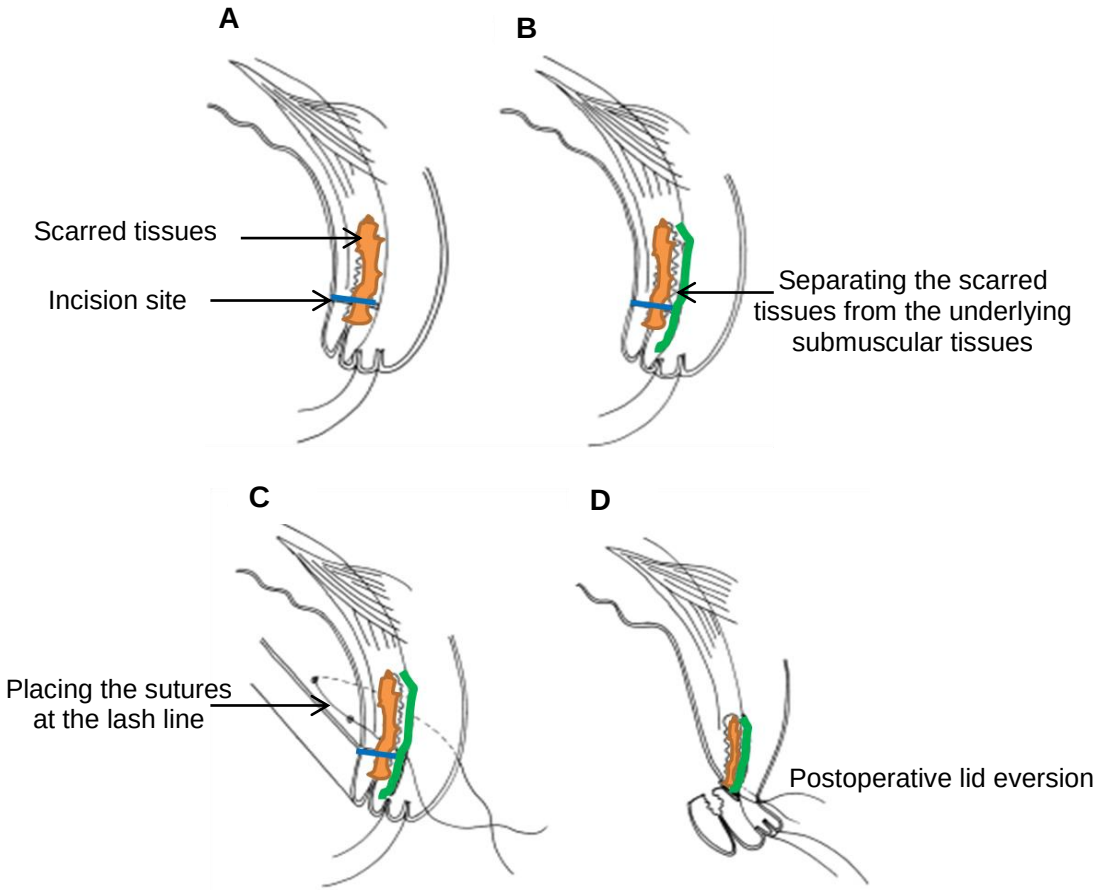


Figure 4:2 Posterior lamellar tarsal rotation. A) Horizontal incision through the tarsal plate and scarred tissues (orange). The blue line indicates the incision site; B) Separating the scarred tissues from the underlying submuscular tissues (green); C) Placing the sutures; D) Postoperative lid eversion. Adapted from (Rajak et al. 2012).

The 10% MPC hydrogel implants could be used as drug releasing lid spacer after trachoma surgery. The films could be loaded with less toxic anti-scarring agent than MMC and be implanted at the incision site during trachoma surgery. However, trachoma is different from glaucoma in terms of the type of treatment needed after surgery. For GFS anti-inflammatory or anti-scarring agent is required; for trachoma an antibiotic is also required as the site of surgery is susceptible to infection and the origin of trachoma is bacterial and one of the causes of scarring is repeated bacterial infection (Khandekar et al. 2001; Li et al. 2013). The choice of drug that is both antibacterial and anti-scarring at the same time is important.

Our lab is interested in developing anti-scarring therapeutics, dosage forms for drug delivery and exploring the possibility of using over the counter, currently used medications as new anti-scarring agents. One of the drugs that have shown promising results as anti-scarring agent is the known antibiotic doxycycline (DOXy) (Figure 4:3) (Sapadin & Fleischmajer 2006). It is a broad spectrum antibiotic of tetracycline family that has been used for the treatment of conditions caused by bacterial infections such as acne, urinary tract infections, intestinal infections, eye

infections, gonorrhea, chlamydia and periodontitis (gum infection) (Sloan & Scheinfeld 2008). DOXy is used in the clinic in several dosage forms including injection, suspension, capsules and tablets. It is also present in the form of hydrogel (Atridox®) for the treatment of periodontal disease (Do et al. 2014).

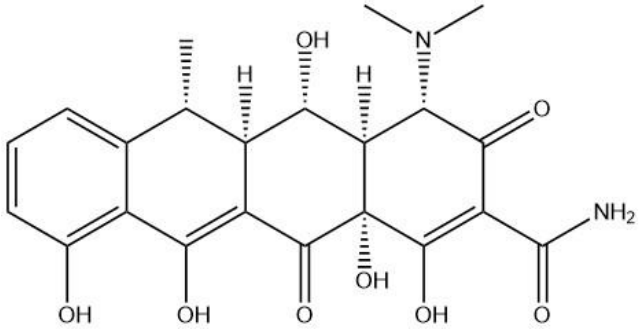


Figure 4:3 The chemical structure of doxycycline free base.

DOXy is present in three different forms; water soluble DOXy hydrochloride, water soluble DOXy hyclate and poorly water soluble DOXy monohydrate (Malmborg 1984; Joseph B Bogardus & Blackwood 1979). In addition to its antibacterial activity DOXy has also several biological activities, one of which is as an inhibitor of matrix metalloproteinases (MMPs) (Horwitz et al. 2014; Sapadin & Fleischmajer 2006; Stechmiller et al. 2010) MMPs are a group of enzymes that are capable of degrading the ECM components that are often present when there is fibrosis. MMPs depend on zinc for activation that is why they are called zinc dependant enzymes.

During the wound healing process MMPs are overexpressed by the cells migrating to the wound. Overexpression of MMPs will result in dysregulation of the ECM and formation of scared tissues (Birkedal-Hansen et al. 1993). MMP inhibitors (MMPI) could be used to remodel the ECM and prevent excessive scarring. DOXy in the hyclate form was investigated as anti-scarring agent to modulate wound healing after GFS (Georgoulas et al. 2008). DOXy was also investigated as treatment before and after trachoma surgery to improve the success rate of the surgery (Li et al. 2013; Dawson & Schachter 1985). If 10% MPC hydrogel films were able to sustain DOXy hyclate for 2 weeks, such a dosage form would be a benefit for both glaucoma and trachoma.

DOXy hyclate is water soluble (30 mg/mL). As described in Chapter 3, it is a challenge to prolong the release of a water soluble drug (pirfenidone) from a hydrogel. In an effort to slow the release of DOXy hyclate and to achieve higher loading different methods employing cyclodextrin complexation will be evaluated using the 10% MPC hydrogel films to extend the release of DOXy hyclate.

Hypothesis and aims

Hydrogel films with 10% MPC were fabricated as a potential material for GDDs or drug loaded subconjunctival spacer after GFS. It was hypothesised that a hydrogel implant could be used in other parts of the subconjunctiva such as the eyelid as a drug releasing implant after trachoma surgery. It was also hypothesised that a hydrophilic drug, doxycycline (DOXy), could be formulated in the hydrogel to display a sustained release profile. It was though possible that a diffusion barrier could be created through the hydrogel polymer matrix to delay doxycycline release.

Hydrogels with 10% MPC were loaded with DOXy from different solvents and different concentrations to determine the best loading conditions. The films were also modified in an attempt to slow the diffusion of DOXy by introducing cyclodextrin as a DOXy complexation agent and potentially as a diffusion barrier within the hydrogel. The CD will be introduced using different approaches such as the formation of an IPN, semi-IPN network and embedding already cross-linked CD particles into the hydrogel matrix. The change in SR of the hydrogels after the addition of CD and the release of DOXy from CD modified films will be studied.

Results and discussion

Loading and release of doxycycline in 10% MPC hydrogel films

In the previous chapter hydrogels with the formulation (by weight percent) 10% MPC, 88.7% HEMA, 0.56 % PEGDA and 0.74 % (w/w) AIBN were found to be the most suitable for potential GDD and spacer development. The same formulation is intended as a starting point to develop a hydrogel formulation that could be considered for use after trachoma surgery. Delivering DOXy through 10% MPC hydrogels may improve the success rate after trachoma surgery.

Water comprises a large part of the hydrogel matrix after hydration due to the ability of hydrogels to absorb and hold water in the hydrogel structure. Hydrogels can absorb up to one hundred times the dry weight during hydration (Vervoort et al. 1998; Kono & Fujita 2012; Appel et al. 2012). For a hydrogel it is thought that the loading efficiency of hydrophilic drugs will be high and accordingly the release of these drugs will be fast (Paradiso et al. 2014). Fast release was observed previously when pirfenidone was loaded into 0%, 10% and 30% MPC containing hydrogel films. Pirfenidone is a hydrophilic drug, with high solubility in water (~10 mg/mL) and low MW (185.226 g/mol) (Richeldi et al. 2011). These findings are in agreement with the results reported by Hiraishi *et al* (Hiraishi et al. 2008). The group reported that the release of another hydrophilic drug, chlorhexidine, *in vitro* from a methacrylated based resin with low water content was slower than the release from high water content resin.

For a hydrophilic drug the amount of drug loaded and the loading efficiency depends on the concentration of the loading solution and the volume absorbed by the dry hydrogel. The amount loaded of timolol maleate was increased from 24.5 µg to 280 µg/lense when the loading solution concentration increased from 100 µg to 1 mg/mL (Maulvi et al. 2014). Drug load can often depend on the degree of hydrogel swelling. Drug loading is usually estimated using Equation 8 where V_s is the absorbed volume by the dry gel, W_p is the weight of the dry gel and C_o is the concentration of the loading solution (Kim et al. 1992)

$$\text{Lower amount loaded} = (V_s / W_p) \times C_o \quad \textbf{(8)}$$

To study the effect of swelling and concentration of loading solution on the amount of DOXy loaded into 10% MPC hydrogels, a soaking method was used. Dry xerogel (10% MPC) discs of 1cm diameter and 1mm thickness, and with approximate weights for each dry disc of 46.5 mg were used to analyse the amount of drug loading. One of the factors that affects loading is the concentration of the

loading solution. To estimate maximum loading and loading efficiency, each dried disc of 10% MPC hydrogel was placed in 1 mL of DOXy aqueous solutions with varying concentrations (6 mg/mL, 15 mg/mL and 30 mg/mL) for 24 hours. The amount loaded was estimated as the difference in UV absorbance between the loading solution before and after incubation. Each disc was removed from the loading solution and dried under vacuum for 24 hours.

When the concentration of the loading solution was increased the amount of drug loaded was significantly increased (Table 4:1). Although the maximum amount loaded was 7.5 mg when loading from 30 mg/mL solution, 15 mg/mL was chosen as the optimum loading solution for further studies. It was reported that DOXy has the ability to supress cell contraction when used in a range of (100-400 µg) (H. Li et al., 2013). From the UV readings it was calculated that a loading solution of 15 mg/mL will load 3.6 mg into each disc (weight of the dry disc is 48 mg) which is estimated to be enough for two weeks if the drug is released at a rate of 200 µg /day.

Table 4:1 The amount loaded and loading efficiency of DOXy in 10% MPC discs. The solutions used for loading were (6 mg/ml, 15 mg/ml (3ml), 30 mg/ml; 1 ml).

Loading solution in PBS	Amount loaded mg/disc	Loading efficiency %
6 mg/mL (1 mL)	1.20 ± 0.08	24.10 ± 2.05
15 mg/mL (1 mL)	3.60 ± 0.09	25.8 ± 0.8
30 mg/mL (1 mL)	7.50 ± 0.09	25.0 ± 0.3

Another factor that can affect loading is the volume absorbed by a dry hydrogel. According to equation 7 the higher volume absorbed will result in higher loading. The swelling ratio (SR) for HEMA hydrogels containing 10% MPC changes with different solvents. The SR of 10% MPC hydrogels in water and methanol was found to be 1.20 ± 0.02 and 1.20 ± 0.09 respectively. When a (1:1) water:alcohol mixture is used, the SR was increased to 2.80 ± 0.08 for water:methanol (1:1) and 3.2 ± 0.1 for water:ethanol (1:1) mixture. A higher SR indicates a higher volume of the solvent is absorbed by the dry hydrogel matrix.

It was assumed that by maximising the swelling of the films during the loading process the amount loaded would be increased. It was also assumed that using an alcohol:water (1:1) mixture for loading will result in a better entrapment of the drug. By swelling first in the alcohol:water mixture the drug will be loaded in higher amount compared to using water alone, and the volume absorbed by the hydrogel will be higher. After drying to remove the loading solvent the hydrogel will

re-swell again in PBS; however, it will swell to a lesser extent and less volume will be entrapped in the hydrogel matrix (Figure 4:4). This decrease in swelling may entrap the drug molecule deeper into the hydrogel matrix and will slow the release of the drug.

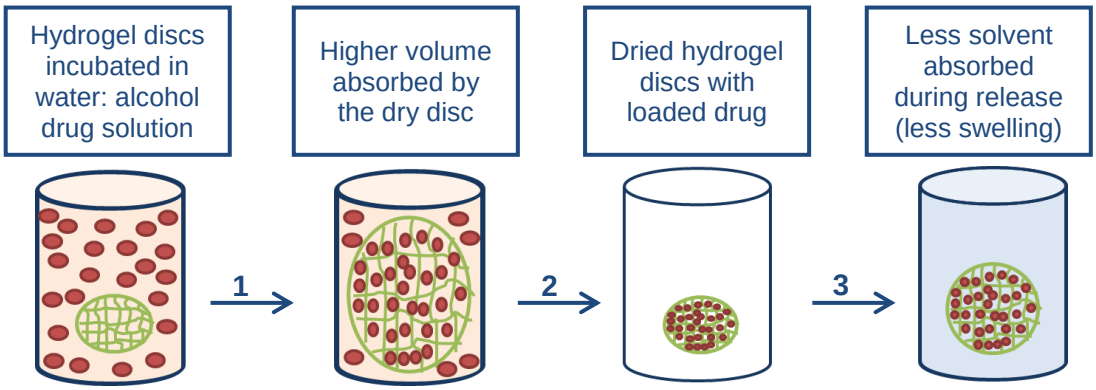


Figure 4:4 The proposed mechanism for Improved loading and release from different solvent systems.1) The dried hydrogel disc placed in water: alcohol (1:1) mixture to achieve maximum swelling. The volume absorbed by the hydrogel is high which potentially load high amount of the drug; 2) After removal from incubation solution and drying, the drug is precipitated in the hydrogel matrix; 3) Less swelling will be observed when the loaded discs placed in the releasing buffer which may allow slow release of the loaded drug.

Hydrogel discs with 10% MPC were loaded from different solvents and solvent combinations using a concentration of (15 mg/mL, 1 mL) and the release of DOXy from the loaded discs was studied. It was assumed that the amount loaded from water:alcohol mixture (1:1) will be high compared to water or methanol; however, there was no significant difference ($p>0.05$) in the amount loaded, and therefore the loading efficiency, when using different solvents and solvent combinations except where water:ethanol (1:1) was used ($p<0.05$). When water:ethanol (1:1) was used the amount loaded and loading efficiency was lower (Table 4:2). This may be due to the affinity of the drug for the hydrogel matrix. It is possible that when the loading solution is rich with alcohol, the alcohol will have a stronger interaction with the polymer matrix, displacing the drug and filling the internal volume of the hydrogel matrix, and any extra swelling and increase in size will be filled by alcohol not the drug.

Table 4:2 The amount loaded, loading efficiency and half-life of DOXy in 10% MPC discs from 15 mg/mL 1 mL loading solution using different solvents.

Loading solution	Amount loaded mg	SR	Loading efficiency %	Half-life days
Water	3.7 ± 0.4	1.20 ± 0.02	24.6 ± 4.1	1.20 ± 0.01
PBS	3.60 ± 0.09	1.20 ± 0.01	24.0 ± 0.8	-
Water: Ethanol (1:1)	2.4 ± 1.8	3.2 ± 0.1	16.0 ± 2.8	0.9 ± 0.1
Water: Methanol(1:1)	3.8 ± 1.8	2.80 ± 0.08	25.3 ± 9.4	0.9 ± 0.1
Methanol	3.5 ± 5.5	1.20 ± 0.09	23.3 ± 8.4	1.1 ± 0.3

The release of DOXy from the loaded discs was studied and the release profile was compared using the *in vitro* eye flow chamber. The release of DOXY from water or methanol loading was studied first for a week. The release was stopped when no more DOXy was detected from the collected samples using HPLC (Figure 4:5). However, only ~ 70% of the estimated loaded drug was released during that period. A possible explanation is that the amount of the drug loaded into the discs was overestimated and the real amount was lower. Another explanation is that the drug degraded through that period and the degradation products was entangled in the hydrogel matrix.

Following the study of DOXy release from water and methanol, the release was studied from water:ethanol or water:methanol (1:1) loading. The release was faster compared to water and methanol. More than 80% of the loaded drug was released after 4 days compared to 60% using water or methanol (Figure 4:5). The experiment was ended after 4 days because the fast release of DOXy render the loading using water:ethanol or water:methanol (1:1) solution inappropriate for extended release. From the release experiment we concluded that the type of solvent used for loading has no effect on loading and release of DOXy from 10% MPC hydrogels.

Based on the above observation DOXy was loaded and released from 10% MPC hydrogel discs. However, it was noticed from the release profile of DOXy (Figure 4:5) that the half-life of DOXy is 48 hours and there was burst release of the drug in the first 24 hours. In an effort to extend the half-life of DOXy in the films several modifications to the method were evaluated using the 10% MPC films.

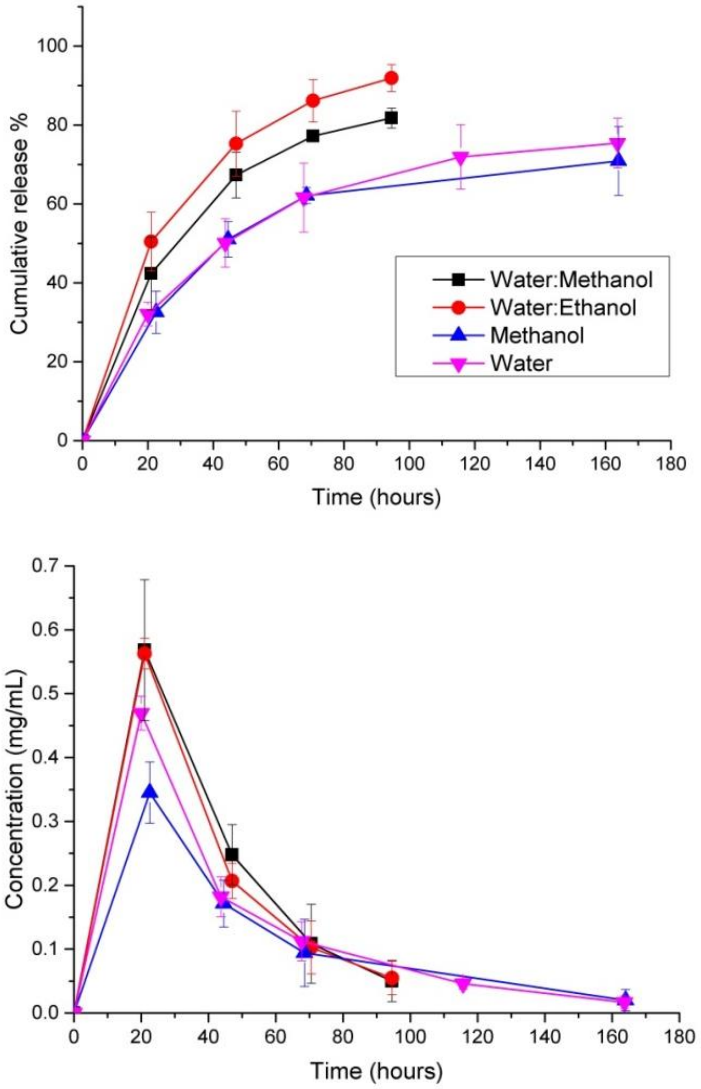


Figure 4:5 The release profile of DOXy from 10% MPC discs loaded from 15 mg/mL 1.0 mL loading solution using different solvents. The top panel is the percent of drug released and the bottom panel is the concentration of the drug released with time. The solvents used are water, water:ethanol 1:1, water:methanol 1:1, ethanol and methanol.

The use of beta-cyclodextrin (β-CD) in HEMA-MPC hydrogels

The transport of molecules from loaded hydrogels to the surrounding medium is through the solubilisation of the drug in the water filled regions of the hydrogel matrix followed by drug transport through the polymer chains into the polymer surfaces (Amsden 1998). When the release of a molecule from a hydrogel is controlled by diffusion, the release rate is affected by the following factors; path length (*l*) and molecular diffusivity (D). The duration of release can be estimated by l^2/D (Peng et al. 2010). The release profile can be increased by either increasing the path length (*l*) or decreasing diffusivity. Creating a diffusion barrier can prolong the path for the molecule to diffuse out of the polymer matrix (Hsu et al. 2014). Rad *et al* reported that the release of betamethasone from silicone based contact lenses

was reduced when vitamin E was used as hydrophobic diffusion barrier (Rad et al. 2016). The release time of 90% of the loaded betamethasone (3.5 hours) from three commercial contact lenses was increased to (264-360) hours after using Vitamin E as diffusion barrier in the contact lens matrix.

Molecular diffusivity is affected by the solubility of the material in the volume fraction of the hydrogel, the size of the molecule, the mobility of the polymer chains, the pore size of the hydrogel and drug polymer affinity (Amsden 1998; Korsmeyer et al. 1983; Nakamura et al. 2004). DOXy hyclate rapidly diffused from 10% MPC hydrogel discs. To slow the diffusion of DOXy from the polymer matrix, beta-cyclodextrin (β-CD) was introduced. By incorporating β-CD into the polymer matrix, the affinity of DOXy to the hydrogel matrix could be increased and the release rate could be decreased.

The concept of affinity based drug delivery has been investigated in recent years. In affinity based systems the drug will have higher affinity to the hydrogel matrix compared to the surrounding medium. The high affinity between the drug and the hydrogel matrix will help in modulating drug loading and release from the hydrogel matrix to increase loading and slow diffusion (Thatiparti et al. 2010; Vulic & Shoichet 2014; Wang & von Recum 2011). Jeon *et al* described the formation of affinity based heparin-alginate hydrogels for growth factor delivery (Jeon et al. 2011). Due to the high affinity of growth factors to heparin, the heparin-alginate hydrogel was able to sustain the release of the growth factors in PBS for 3 weeks compared to 5 days using alginate hydrogel alone.

CDs are cyclic oligosaccharides that are widely used in medicine as a complexing agent and there are several marketed products where CDs are used as excipients (Sharma & Baldi 2014; Rao et al. 2001; Szejtli 2005; Loftsson & Brewster 2010). Through the formation of inclusion complexes the physicochemical properties of the drug molecules will be changed such as solubility, stability, permeability and toxic side effects (Davis & Brewster 2004; Concheiro & Alvarez-Lorenzo 2013; Vilanova & Solans 2015).

Jóhannsdóttir *et al* reported an increase in the solubility of cyclosporine A from 0.008 mg/mL to 0.5 mg/mL when 5% (w/v) of α-CD was used for the formulation of eye drop (Jóhannsdóttir et al. 2015). Moriwaki *et al* reported an increase in the solubility of albendazole from 13.62 µmol/L to 276 µmol/L when β-CD was used in a concentration of 16.3 mmol/L (Moriwaki et al. 2008). Abdur Rouf *et al* reported that the solubility and dissolution rate of rapamycin was improved with complexting with CD (Abdur Rouf et al. 2011). In the dissolution test only 25% of the pure rapamycin was dissolved after one hour compared to 80% of the complex

when an equal amount of the drug was added. Kanjickal *et al* reported that alteration in the release of cyclosporine A from PEG hydrogels after sterilization could be avoided by using cyclosporine A-CD complex instead of the free drug in the hydrogel (Kanjickal et al. 2005).

In the area of ophthalmic drug delivery CDs are widely used in formulation as an excepient, particularly in eye drops and several marketed products utilise CDs to increase drug solubility (Sharma & Baldi 2014; Cal & Centkowska 2008). Several commerical eye drops such as Voltaren Ophthalmic® and Clorocil® are available in the clinic in which CDs were used to improve the solubility of the poorly soluble drugs (Loftsson & Stefánsson 2002) .

CDs can form inclusion complexes because they have an interior hydrophobic cavity that can accommodate hydrophobic drugs or hydrophobic parts of drug molecules that can fit in the cavity to form the complex (Del Valle 2004). Such a drug-CD inclusion complex can be more water soluble than drug alone due to the solubilising OH groups on the outer surface of CDs (Figure 4:6) (Pinho et al. 2014). Moriwaki *et al* reported an increase in the solubility of albendazole from 13.62 µmol/L to 276 µmol/L when β-CD was used in a concentration of 16.3 mmol/L (Moriwaki et al. 2008).

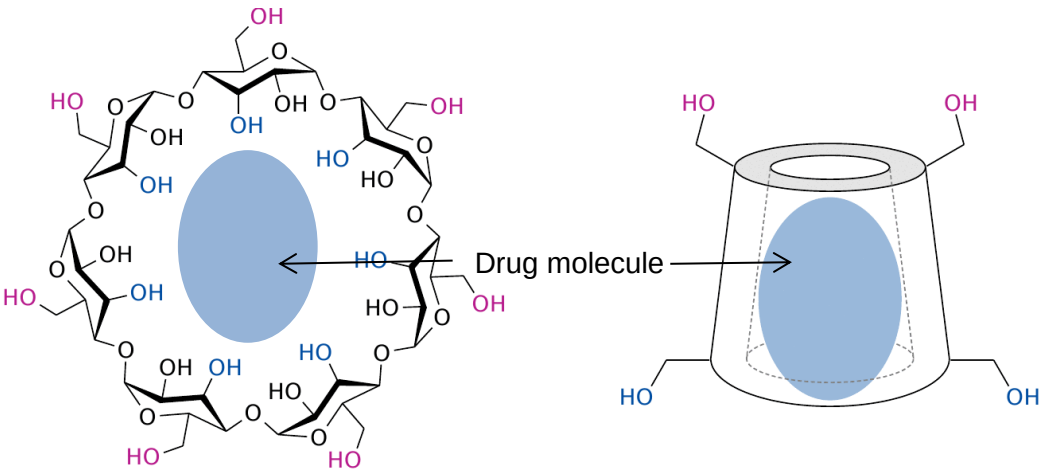


Figure 4:6 Structure and arrangement of β–CD molecule that have 7 glucose units. The drug molecule is complexed with the inner cavity of the CD molecule. (http://watcut.uwaterloo.ca/webnotes/Pharmacology/deliveryCarriers.html)

The drug-CD complex is formed by non-covalent interactions between the drug and CD, therefore the reaction is reversible. Formation and dissociation of the CD-drug complex depends on the stability of the complex formed (Bibby et al. 2000). When a strong complex is formed the dissociation rate of the drug from the complex will be low (Stella et al. 1999). Corre *et al* reported that the decrease in intestinal absorption rate of diphenhydramine in rat model from two CD molecules

was related to the strength of the inclusion complex formed (Corre et al. 1998). The absorption rate was reduced by 36% when dimethyl-β-CD was used compared to only 8% when hydroxypropyl-β-CD was used because of the high stability of the complex formed with dimethyl-β-CD compared to hydroxypropyl-β-CD. The stability constant was 895 M^{-1} for dimethyl-β-CD compared to 494 M^{-1} for hydroxypropyl-β-CD.

CDs are available in three main forms α-, β- and γ-cyclodextrin which contain 6, 7 and 8 molecules of glucose respectively and derivatives of the three types are also available such as hydroxypropy-β-CD, hydroxypropyl-γ-CD, dimethyl-β-CD and sulphated-β-CD (Vyas et al. 2008; Crini 2014). The molecules differ in the size of the inner hydrophobic cavity and solubility in water. The difference in the properties allowed the formation of complexes with different properties and complexing wide range of drugs (Otero-espinar 2010; Lezcano et al. 2002). Rodriguez-Aller *et al* reported different stability of latanoprost when complexed with different CD molecules as eye drops (Rodriguez-Aller et al. 2015). Latanoprost stability was not improved compared to the commercial eye drops when α-and γ-CD were used as complexing agents; however the stability was improved when β-CD derivatives were used. Only 33.5% of the initial latanoprost remained after 12 months in direct sunlight from the commercial eye drop compared to 81.9% and 79.6% for dimethyl-β-CD and propylamino-β-CD respectively. Bayomi *et al* reported that the improvement of photostability of nifedipine by complexation with CD was affected by the type of CD used (Bayomi et al. 2002). The degradation rate constant of nifedipine was smaller when dimethyl-β-CD and hydroxypropy-β-CD compared to β-CD which was 0.12, 0.29 and 0.47 for dimethyl-β-CD, hydroxypropy-β-CD and β-CD respectively.

CD hydrogels have been investigated as delivery systems to slow the release of loaded drugs (Concheiro & Alvarez-Lorenzo 2013; MacHín et al. 2012). Li *et al* reported that a supramolecular self-assembled hydrogel made of α-cyclodextrin and a biodegradable poly(ethylene oxide)–poly[(R)-3-hydroxybutyrate]–poly(ethylene oxide) (PEO–PHB–PEO) triblock copolymer was able to sustain the release of a model drug dextran-FITC (Li et al. 2006). The release of dextran *in vitro* was sustained for a month when the supramolecular hydrogel was used compared to using α-CD-PEO alone in which 100% of the loaded dextran was released in less than 5 days.

CD Hydrogels could be made entirely from CD cross-linked molecules or the CD molecules introduced into the polymer matrix of the hydrogel (Concheiro & Alvarez-Lorenzo 2013). Drug-CD complex formation is governed by the rates of

association (Ka) and dissociation (Kd) (Bibby et al. 2000). When a drug is loaded into CD hydrogels, the rate of drug release will depend on two mechanisms; affinity of the drug to CD and diffusion from the hydrogel matrix. The loaded drug has to associate and dissociate through several CD molecules before diffusing outside the hydrogel (Figure 4:7). There are several approaches used to introduce CD into a hydrogel polymer matrix such as co polymerisation with CD, the use of CD grafted polymers and the formation of IPN with CD (Li et al. 2015; Rosa dos Santos et al. 2009; Xu et al. 2010; Ribeiro et al. 2012).

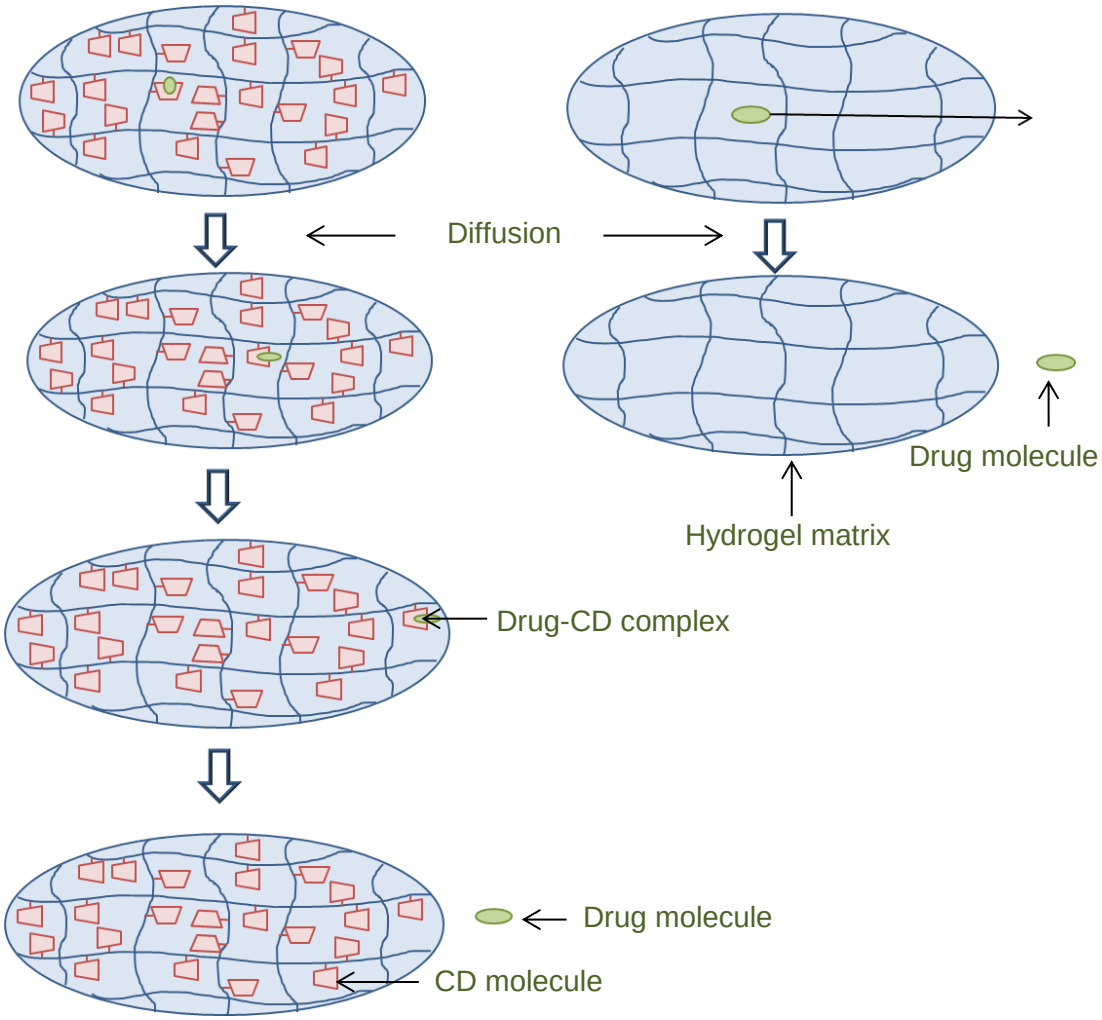


Figure 4:7 Schematic representation of drug release from hydrogels with CD molecule and hydrogels without CD. The drug molecule will have to complex and de-complex several CD molecules before release from the hydrogel while it diffuse without obstacles in CD free hydrogels.

Moya-Ortega *et al* reported the formation of semi-IPN of γ-CD and polyacrylic acid for the sustained delivery of dexamethasone (Moya-Ortega et al. 2010). Dexamethasone release was sustained for 1 week with the semi-IPN hydrogel compared to 1 day when using γ-CD hydrogel only. Mennini *et al* reported

that both the solubility and the release profile of hydrophobic drug dehydroepiandrosterone were improved when loaded in CD hydrogel for vaginal delivery (Mennini et al. 2016). The release of dehydroepiandrosterone from a combination hydrogel made with HP-β-CD and γ-CD was sustained for more than 8 hours *in vitro* using dissolution apparatus. Kono and Teshirogi reported the formation of β -cyclodextrin-grafted carboxymethyl chitosan hydrogels (CD-g-CMCs) as a controlled delivery system (Kono & Teshirogi 2015). The amount of acetyl salicylic acid absorbed into the (CD-g-CMCs) was 23.9 µmol/g compared to 2.4 µmol/g for CMC hydrogels without CD. When *in vitro* release was studied the amount released from CMC hydrogels was 86% in the first 2 hours compared to 41% using (CD-g-CMCs).

Although CD has been used as complexing agent for hydrophobic drugs it has also been investigated as complexing agent for hydrophilic drugs to modulate the physicochemical properties of the complexed drugs (Ikeda et al. 2002; Mariangela de Burgos M de Azevedo et al. 2011). β–CD was used as complexing agent for DOXy monohydrate and hyclate to reduce photosensitivity and increase stability of DOXy (H. Zhang et al. 2013; Wang et al. 2013; He et al. 2011). Kogawa *et al* reported that DOXy hyclate has the ability to form inclusion complex with β–CD if they were mixed in equimolar ratios (Kogawa et al. 2014). Introducing β-CD into 10% MPC hydrogel films may slow the diffusion and sustain the release of loaded DOXy.

Formation of a complex of DOXy hyclate with β-CD was examined by dissolving the two molecules in equimolar amounts in 5 mL DW and sonicated for one hour then freeze dried to achieve the dry powder of the complex. There are several methods that could be used to confirm the formation of inclusion complex such as DSC, thermogravimetric analysis (TGA) and FTIR (Poorghorban et al. 2015; Figueiras et al. 2015). A complex was confirmed when DSC was measured for the individual molecules, the physical mixture and the complex. The physical mixture was prepared by mixing CD and DOXy powders in equimolar ratios. The melting peak of DOXy at ~170^{o}C disappeared when the complex was formed. The DOXy melting peak was present when physical mixture was formed (Figure 4:8). These results suggest that β-CD has the ability to form inclusion complex with DOXy. Similar DSC profile was reported by Kogawa *et al* when complexed DOXy with β-CD (Kogawa et al. 2014).

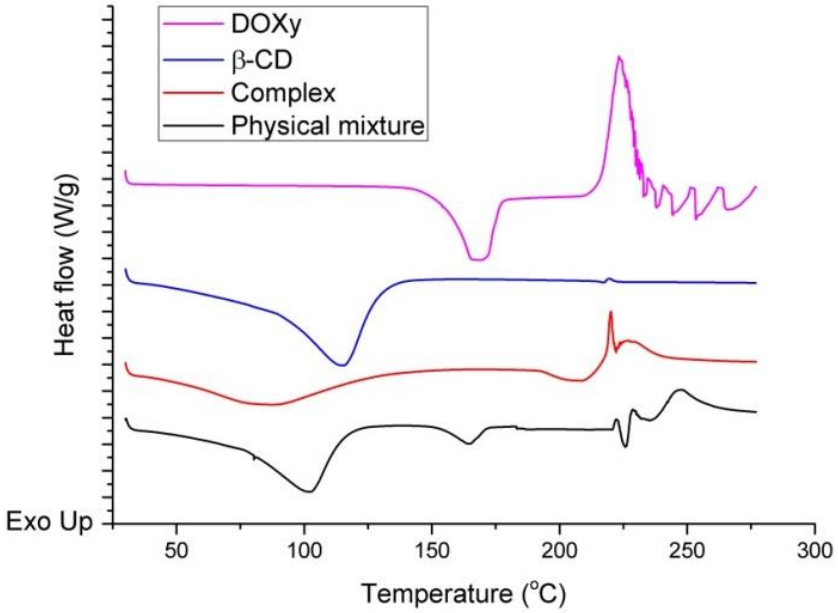


Figure 4:8 The DSC analyses of β-CD, DOXy and the physical mixture and complex of both β-CD and DOXy. In the DSC graph the melting peak of DOXy was not observed in the complex and was observed in the physical mixture which indicates the formation of inclusion complex.

Formation of 10% MPC- 89.5% HEMA film with pendant CD

Hydrogel films with 10% MPC-89.5% HEMA and pendant β-CD were prepared. Glycidyl methacrylate (GMA) was used as a monomer to conjugate CD into the hydrogels polymer chains. GMA was previously used as a monomer for the formulation of hydrogel soft contact lenses with pendant CD molecules as controlled drug delivery systems (Rosa dos Santos et al. 2009). GMA should be polymerised first with the hydrogel matrix as a co monomer followed by activation of the hydrogel for the conjugation of CD into GMA. GMA has an acrylate group at one end and epoxide at the other end. The acrylate group will help GMA to attach to the hydrogel network on one end and reacts with CD through the epoxy group on the other (Figure 4:9) (Ribeiro et al. 2012).

Nava-Ortíz *et al* reported the functionalization of polyethylene and polypropylene films with β-CD using GMA (Nava-Ortíz et al. 2009). GMA was first grafted into the films. After grafting, the films were incubated for 24 hr with continuous stirring at 70^{o}C in a mixture of DMF (2 mL) and 0.5 M NaCl (8 mL) with β-CD.

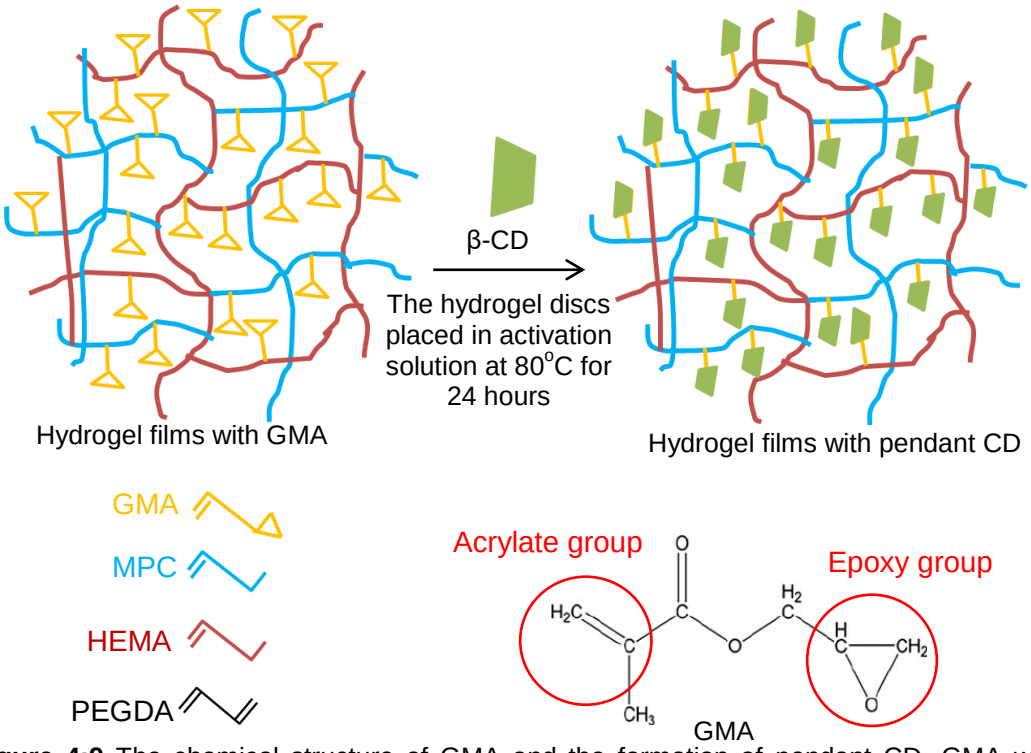


Figure 4:9 The chemical structure of GMA and the formation of pendant CD. GMA was attached through the acrylate group into the HEMA-MPC hydrogel film and conjugated to the CD through epoxide group through activation solution.

Hydrogel films with 10% MPC and pendant CD were fabricated. The GMA was used as a co-monomer during the formulation of 10% MPC hydrogel. The CD was then conjugated by reaction of the epoxy group on the GMA. For the preparation of the hydrogels, to 1 mL of 10% MPC monomer solution mixture (that contains 88.7% HEMA, 10% MPC, 0.74% AIBN and 0.56% (w/w) PEGDA) different amounts of GMA (1%, 2.5%, 5% and 9% (w/v)) were added. The mixture was polymerised to synthesise hydrogels with GMA cross-linked in the polymer matrix. After polymerisation, the xerogels were left to hydrate in water and washed to remove the unreacted monomers. Hydrogels with higher percentages of GMA (5% and 9% (w/w)) did not hydrate when placed in water and remained in a hard and glassy xerogel form, due to the large percentage of GMA in the polymer mixture. Hydrogels with (1% and 2.5% (w/w)) was hydrated when placed in water for washing. To activate the epoxy group and conjugate β-CD to the polymer matrix, the GMA containing hydrogels need to be incubated in activation solution. The activation solution is composed of dimethyl formamide (DMF), sodium hydroxide (NaOH) and β-CD. Discs of 1 cm in diameter were cut from the hydrated films and placed in the activation solution at 80°C for 24 hr. In the presence of alkaline conditions ($NaOH_{(aq)}$) and high temperatures the epoxy ring will open and form a covalent bond with the hydroxyl group of the β-CD (Figure 4:10).

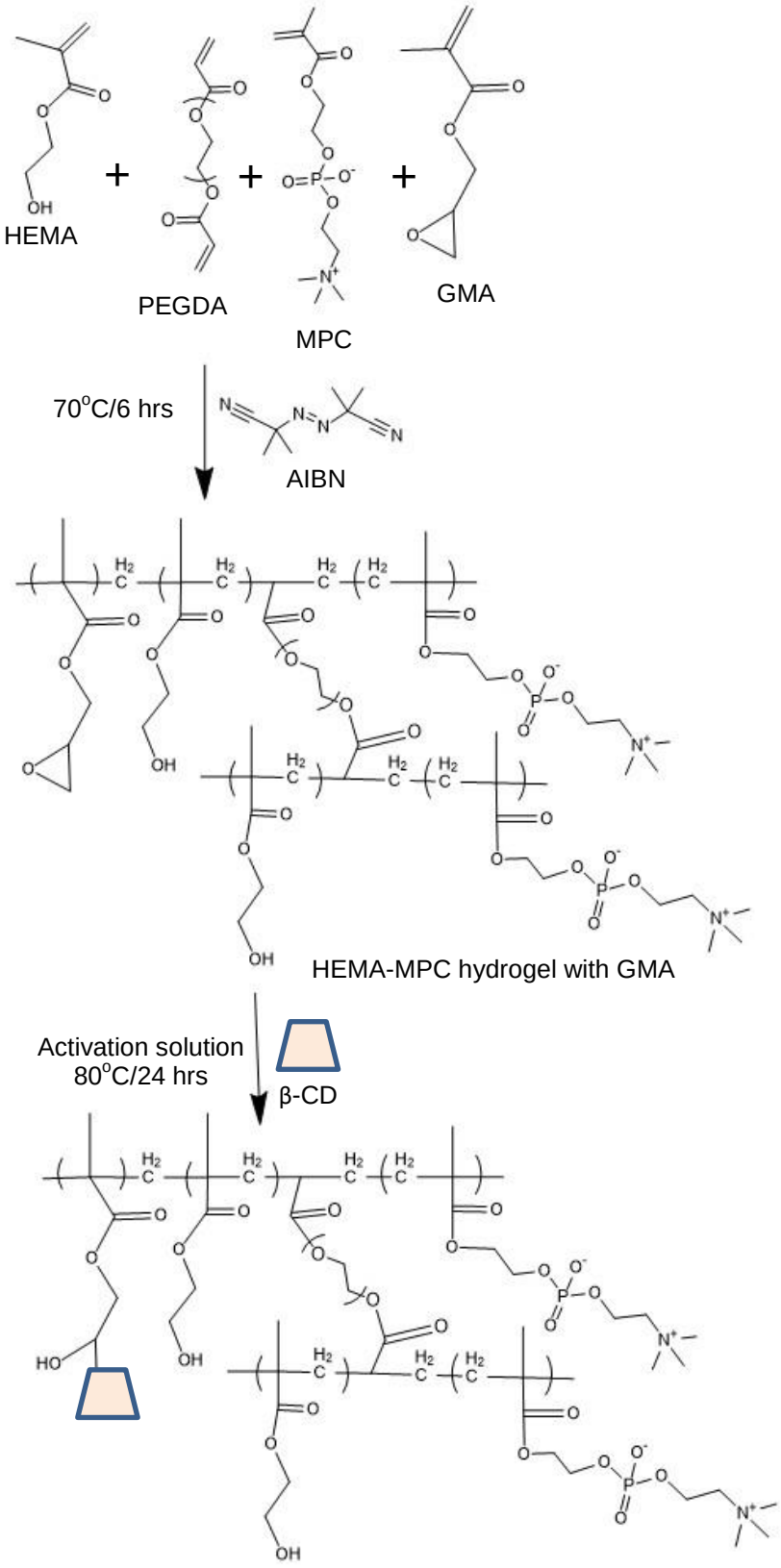


Figure 4:10 The fabrication of 10% MPC hydrogel with GMA. Monomer mixture of 10% MPC was first polymerised with GMA. Followed the polymerisation and washing the hydrated hydrogel placed in activation solution at 80°C for 24 hours for the conjugation of β-CD into the hydrogel.

β-CD molecules have several hydroxide groups (OH) and it is possible that more than one OH group may participate in the reaction and form a covalent bond with GMA, thereby two or more GMA molecules could be attached to the same β-CD molecule. The multiple cross-linking of the same β-CD molecule may increase the cross-linking density of the hydrogel formed and potentially affect the properties of the hydrogel formed.

When the discs were removed from the activation solution and washed with water for 3 days they were fragile and easy to break. The loss of mechanical strength is probably due to the presence of DMF which is a strong organic solvent. To examine the effects of DMF on the hydrogel the 10% MPC hydrated disc without GMA was placed in DMF for 24 h. When removed the disc was fragile and easily fragmented. Mechanical strength is an important parameter for a spacer or a device as it is intended for implantation for a long period of time. Reduction in the mechanical strength of the films after soaking in the activation solution, render them unusable for implantation. No further action was taken regarding the pendant β-CD using this activation method.

In an effort to incorporate β-CD into the hydrogel without using DMF, the β-CD solution was mixed with 10% MPC monomer solution and GMA, to create an *in situ* polymerisation method. Monomer solution of 10% MPC (1 mL) was mixed with 1 ml β-CD solution and different amounts of GMA (0.5, 0.2 and 0.1 g). These are labelled formulations number 1, 2 and 3 respectively. The β-CD monomer solution is 20% (w/v) in 0.2 M NaOH solution (Figure 4:11). It was hoped GMA will be polymerised into the HEMA-MPC hydrogel matrix through the acrylate group and at the same time conjugated to β-CD through the epoxy group.

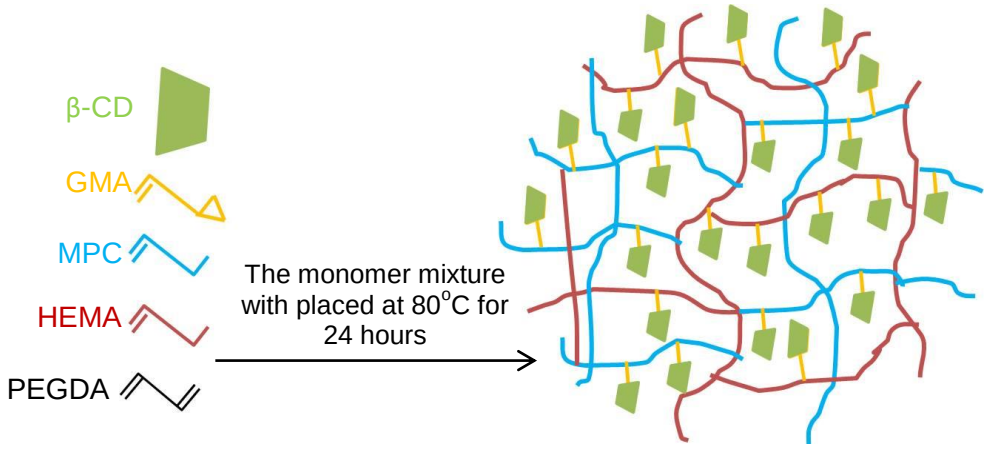


Figure 4:11 The preparation of HEMA-MPC hydrogel films with pendant CD using GMA as conjugating agent *in situ*. The monomer mixture with CD placed at 80oC for 24 hours in the incubator.

Incomplete and brittle films were formed with formulations numbered 1, 2 and 3 (Figure 4:12). A possible reason is the presence of large amount of β-CD in the mixture. β-CD only hydrogels are brittle and it is possible that the presence of high amount of β-CD in the formulations reduced the mechanical strength of the films. However, even when the volume of β-CD monomer solution used in the formulation was reduced to 0.5 mL in formulations numbered 4, 5 and 6 no proper film was formed (Figure 4:12). The hydrogel films formed were incomplete (filled with bubbles) and brittle around the edges. Since no proper film could be formed using *in situ* approach, a different approach was needed to incorporate β-CD into the hydrogel.

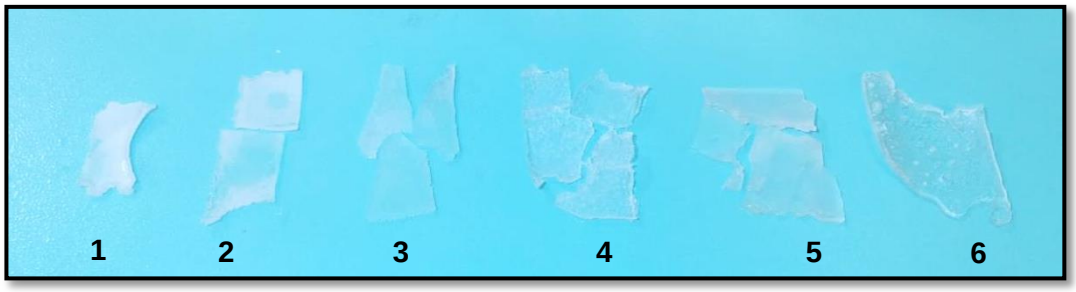


Figure 4:12 Different formulations were prepared by varying the ratio of 10% MPC monomer solution with 20% β-CD monomer solution and GMA. Formulations numbered 1, 2, 3 contain equal volumes of both monomer solutions and different amounts of GMA (0.5, 0.2 and 0.1 g) respectively. Formulations numbered 4, 5, 6 contain (2:1) ratios of 10% MPC monomer solution: β-CD monomer solution and varying amount of GMA (0.5, 0.2 and 0.1 g) respectively. Complete films could not be formed by any of the formulations.

Embedding CD cross-linked particles in 10% MPC-HEMA films

Preparation of films with pendant β-CD using GMA appeared to be unachievable in these MPC hydrogels. β-CD could be introduced as embedded particles in the hydrogel matrix. If β-CD were used as powder in the hydrogel mixture it will be washed with the unreacted monomers due to its solubility in water. Converting β-CD into insoluble form would help to immobilise the particles within the hydrogel film. Chemically cross-linked CD particles (micro and nano) were previously reported and prepared to be used as sorbents and drug carriers for drugs (Trotta et al. 2012; Trotta 2011; Yamasaki et al. 2006; Torne et al. 2013; Trotta & Cavalli 2009). Swaminathan *et al* reported the synthesis of β-CD nanosponges for the delivery of antitumor camptothecin (Swaminathan et al. 2010). During the release study *in vitro* only ~25% of the loaded camptothecin was released from the nanosponges after 24 hours.

β–CD cross-linked hydrogel was used for the preparation of β–CD cross-linked particles. The β–CD monomers were chemically cross-linked using

poly(ethylene glycol) diglycidyl ether (PEGDE) as cross-linker for the formation of β–CD hydrogel (Figure 4:13). After polymerisation, the cross-linked hydrogel was crushed and passed through a sieve to create the microparticles. Understanding the properties and water behaviour of the β–CD hydrogels will help to choose the best formulation for the synthesis of the β–CD cross-linked particles.

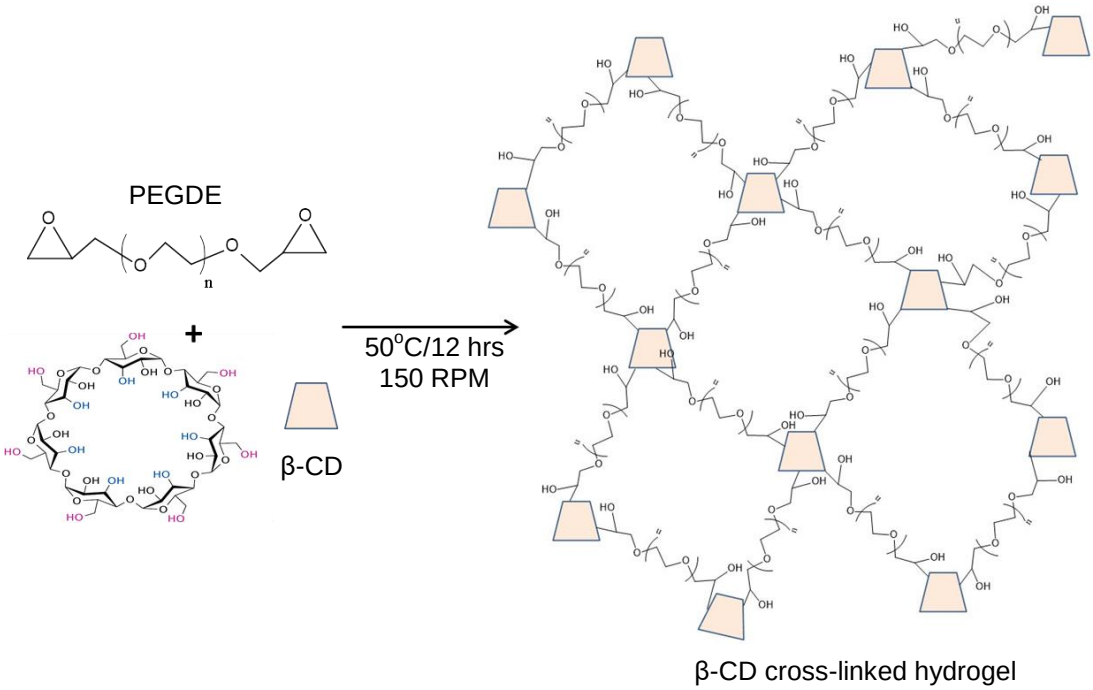
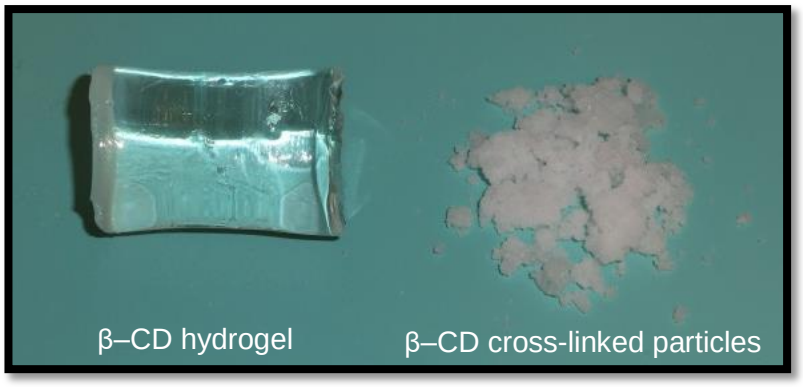


Figure 4:13 Formation of cross-linked β-CD hydrogels. The CD monomers mixed with polyethylene glycol diglycidyl ether (PEGDE) as cross-linker and polymerised at 50°C for 12 hours at 150 RPM in 0.2 M NaOH. The epoxy group on the cross-linker interacts with the hydroxyl groups on the CD molecule through covalent bonding.

To study the effect of the ratio of the cross-linker PEGDE to β–CD solution on the properties of the prepared hydrogels the level of cross-linker was varied (1:1, 1:2, 2:1) with β–CD solution. β–CD was dissolved in 0.2 M NaOH at a concentration of 15% (w/v). The EWC% and SR of the formulations were measured (Table 4:3). There was only a slight difference in SR and EWC% observed between the hydrogels. However, when the level of PEGDE to β–CD solution was 2:1, the hydrogel formed was too hard to pass through the 40 mesh sieve. The softness could be increased by reducing the percentage of PEGDE compared to β–CD solution. The ratio of cross-linker chosen for the synthesis of the CD particles was (1:1) (Figure 4:14). This ratio will offer the advantage of a higher cross-linker density and the ability to pass through the 40 mesh sieve to produce the microparticles. The microparticles were freeze dried to be used for embedding in 10% MPC hydrogels.

A



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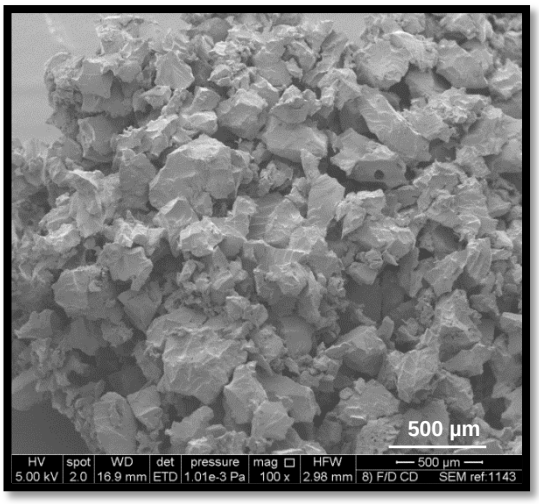


Figure 4:14 The preparation of β–CD microparticles; A) The CD hydrogel was first formed the crushed and passed through 40 mesh sieve to create the particles; B) SEM image of the cross-linked CD particles with scale bar of 500 µm.

Table 4:3 The EWC% and SR of CD hydrogels prepared with different ratios of cross-linker to monomer. The water content and SR was higher when the level of cross-linker was low.

15% CD:PEGDE	EWC%	SR
1:1	86.1% ± 0.7	5.9 ± 0.2
2:1	90.6% ± 0.5	5.6 ± 0.3
1:2	85.4% ± 0.5	9.7 ± 0.2

Hydrogels with 10% MPC were polymerised around the cross-linked β-CD particles. Cross-linked β-CD particles are hydrogels so polymerising HEMA-MPC around the cross-linked β-CD particles could create a hydrogel inside a hydrogel and add an additional barrier to slow the release of loaded DOXy. The β-CD particles were added to 10% MPC monomer solution in two different concentrations 1% and 2% (w/v). When the β-CD particles added to 10% MPC monomer solution, suspension was formed. A suspension made with 1% (w/v) β-CD particles was injectable through a 21G needle. A suspension made with 2% (w/v) was non injectable, even when the needle size was increased to 19G.

A hydrogel suspension of 10% MPC and 1% (w/v) was injected into the mould and placed in the oven for polymerisation. As a xerogel the film was opaque (compared to the clear 10% MPC) and after hydration the films were still opaque, confirming that the β-CD particles were embedded in the film and not washed out during the removal of unreacted monomers (Figure 4:15). Films with cross-linked β-CD particles had similar strength to the films without β-CD particles which indicated that the presence of β-CD particles did not affect the mechanical properties of the original 10% MPC film. Qualitative determination was used to compare the strength of the hydrogels with and without crosslinked β-CD particles. Hydrogels made with 10% MPC could be bended more than 20 times in one minute without breaking so as the 10% MPC hydrogels with crosslinked β-CD particles.

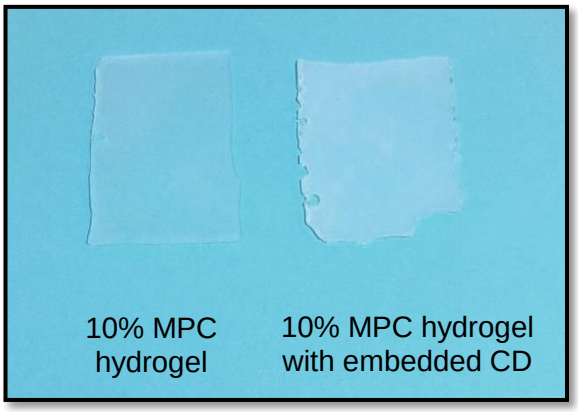


Figure 4:15 10% MPC hydrogel made with embedded β-CD particles compared to standard 10% MPC film. The film with β-CD is opaque compared to standard 10% MPC films.

The EWC% and SR in different solvent systems were measured for 10% MPC hydrogels embedded with 1% (w/v) β-CD particles to study the effect of the presence of β-CD particles on 10% MPC behaviour. The EWC% of hydrogels with embedded β-CD particles was higher (65.4 ± 0.1) compared to 10% MPC hydrogels (55.1 ± 0.3). The increase in water content could be attributed to the hydroxyl groups present in the β-CD. When the ratio of free to bound water was measured for 10% MPC with embedded β-CD particles, the majority of water was free. The ratio of free to bound water was (2:1) for the 10% MPC with embedded β-CD particles compared to (1.8:1) in 10% MPC hydrogels. β-CD hydrogels have higher free water compared to bound water when examined. The ratio of free to bound water was (3.1:1). The SR in water was also increased in the presence of CD particles; however, there was no difference in SR in solvents other than water (Table 4:4).

Table 4:4 The effect of embedding CD particles on the SR of 10% MPC hydrogels in different solvents.

Solvent used	SR	
	10% MPC	10% MPC with CD particles
Water	1.20 ± 0.02	1.80 ± 0.09
Methanol	1.20 ± 0.09	1.2 ± 0.2
Water:Methanol (1:1)	2.80 ± 0.08	2.6 ± 0.3
Water:Ethanol (1:1)	3.2 ± 0.1	3.7 ± 0.3
Ethanol	0.20 ± 0.03	0.20 ± 0.01

For the loading process a hydrogel disc of 1 cm diameter and 1 mm thickness was used. The loading of DOXy in each disc was increased from 3.7 ± 0.4 mg in the 10% MPC films to 6.6 ± 0.4 mg when the hydrogel films were embedded with β-CD particles. Unfortunately, there was no significant difference in the release profile of DOXy from the β-CD embedded hydrogels compared to 10% MPC hydrogels (Figure 4:16). A possible explanation is that the affinity of DOXy for β-CD is low. The low affinity may have caused faster rate of dissociation compared to association between β-CD and DOXy. It is also possible that because of the high water solubility of DOXy the drug would prefer to be in solution form rather than the inner cavity of the β-CD molecule.

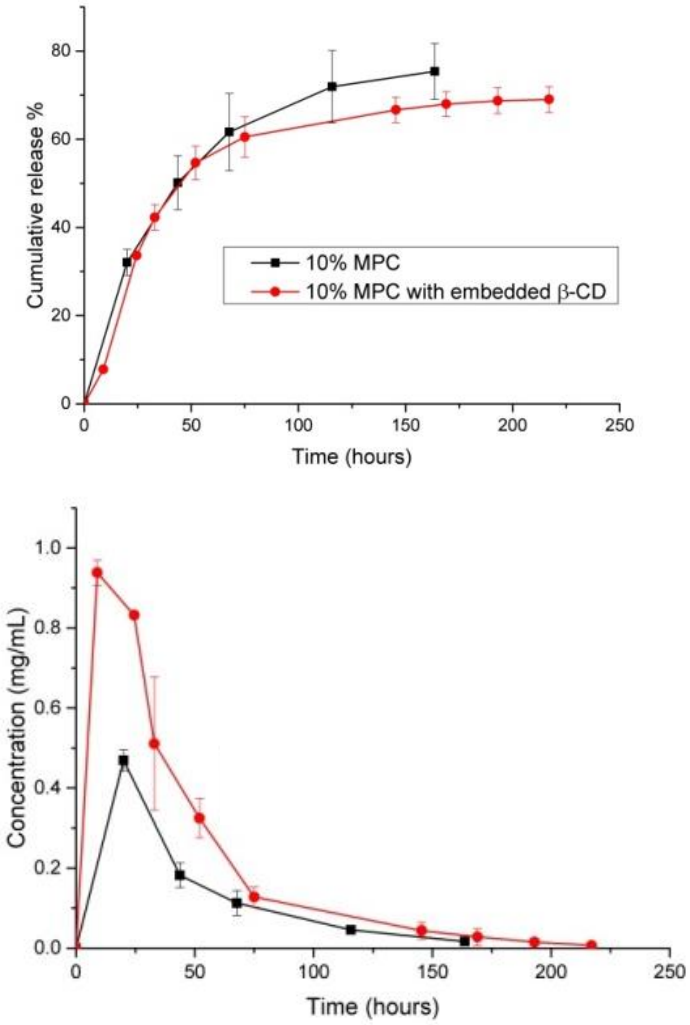


Figure 4:16 The release of DOXy from 10% MPC and 10% MPC hydrogels embedded with β-CD cross-linked particles. The top panel is the percent of drug released and the bottom panel is the concentration of the drug released with time. No difference in the release profile was observed.

Formulation of an IPN derived from 10% MPC-HEMA and β-CD

As described previously IPN and semi-IPN are terms used to describe hydrogels made by the polymerisation of two orthogonal monomer systems at the same time (IPN) or polymerisation of a monomer mixture around a polymer (semi-IPN). Each monomer system undergoes separate polymerisation with polymer chain addition (or growth) occurring simultaneously for each polymer. In principle, an IPN can result in an entangled mixture of two or more polymers that would be difficult to achieve by mixing alone. IPN and semi-IPN technique are usually used to impart strength to hydrogels or manipulate the physical and chemical properties such as swelling and mechanical strength (Lohani et al. 2014; Aminabhavi et al. 2015; Samanta & Ray 2014).

Full-IPN of cross-linked β-CD and 10% MPC was prepared using *in situ* approach. The two monomer mixture will be mixed in equal volume ratios. (Figure 4:17). To ensure that each monomer system undergoes separate polymerisation, HEMA-MPC monomers were polymerised in the presence of PEGDE as a replacement of PEGDA and β-CD monomer mixture was polymerised in the presence of PEGDA as a replacement for PEGDE separately. Both mixtures remained liquid and did not polymerise to a hydrogel which indicates that the two systems can undergo separate polymerisation at the same time.

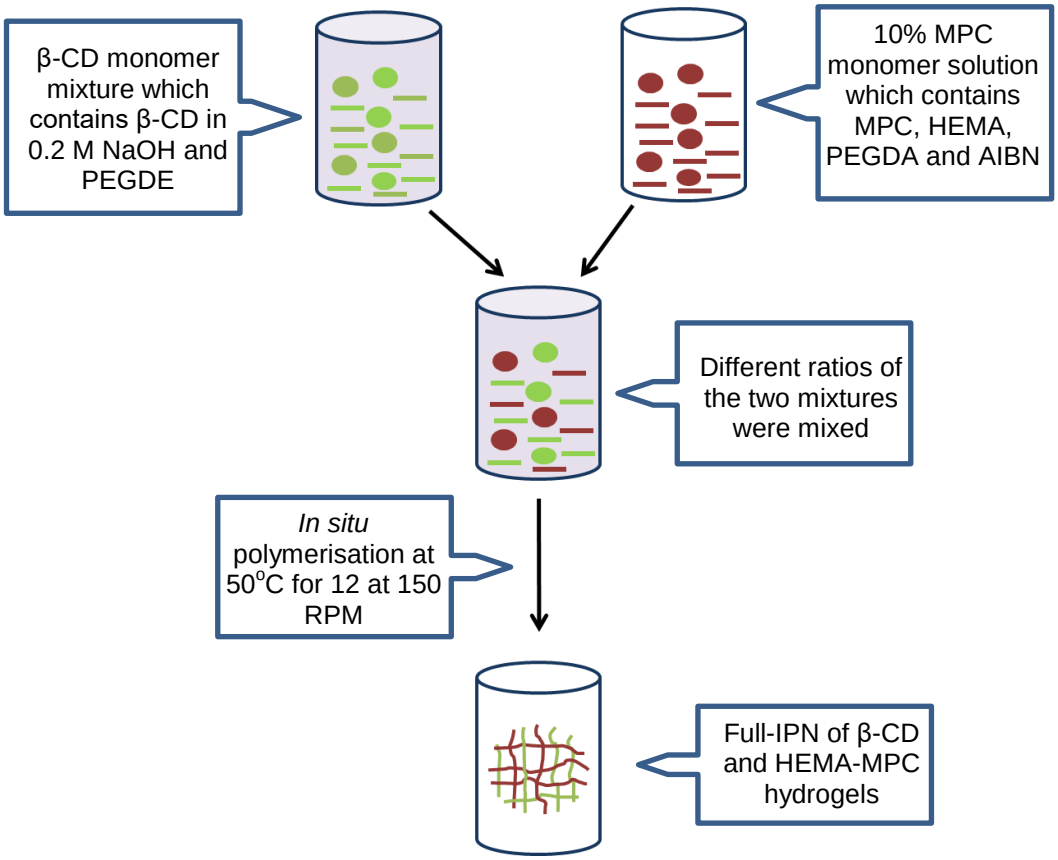


Figure 4:17 The *in situ* polymerisation of IPN of β-CD and 10% MPC monomer solution. The two monomer mixtures are mixed together and polymerised at the same time.

β-CD hydrogels are polymerised under different conditions than 10% MPC hydrogels (50°C/12 hours at 150 RPM). To ensure that 10% MPC could be polymerised in conditions similar to β-CD the monomer solution of 10% MPC was placed for polymerisation at (50°C/12 hours at 150 RPM). A xerogel was formed and there was no difference in appearance between hydrogels prepared at 70°C for 12 hours and hydrogels prepared at (50°C/12 hours at 150 RPM).

Three different formulations were prepared in which the β-CD monomer solution and 10% MPC monomer solution were mixed in equal volume ratios (1:1). The difference between the three formulations is the composition of β-CD monomer

solution used. Three different β-CD monomer solutions were prepared with varying percentages of β-CD (15%, 30%, 50% (w/v)) in 0.2 M NaOH. The amount of PEGDE as cross-linker in each β-CD solution was 50% (v/v). The percentage of β-CD in the mixture was varied to identify the maximum amount of β-CD that could be applied into the monomer mixture and still maintain a film. The mixture then placed in the incubator at 50^{o}C for 12 h at 150 RPM. Before incubation all the mixtures were white in colour. No complete film was formed and all the three films were broken and difficult to handle (Figure 4:18).

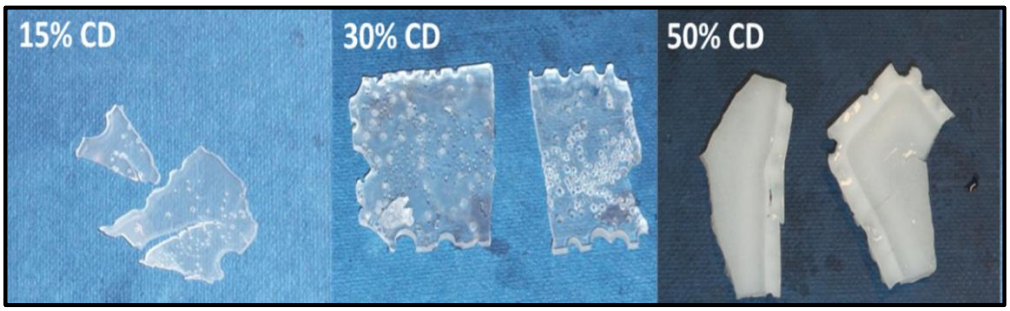


Figure 4:18 IPN hydrogel films made by *in situ* polymerisation of two monomer solutions. β-CD monomer solution and 10% MPC monomer solution were mixed in equal ratios (1:1). Three different β-CD monomer solutions were prepared with varying percentages of β-CD (15%, 30%, 50% (w/v)) in 0.2 M NaOH. The amount of PEGDE as cross-linker in each β-CD solution was 50% (v/v). For all the formulations prepared no proper film was formed.

The closest film to the 10% MPC films superficially were the films made with 30% β-CD monomer solution. To explore whether changing the ratio of β-CD monomer solution to 10% MPC monomer solution will have an impact of the formulation, different formulations with different ratios of 30% β-CD to 10% MPC monomers were prepared. The ratios in which the two monomer solutions were mixed were (1:1, 1:2, 2:1 30% β-CD:10% MPC). Varying the ratio between the two monomer solutions did not affect the properties of the films regarding the texture, the films prepared were brittle (Figure 4:19).

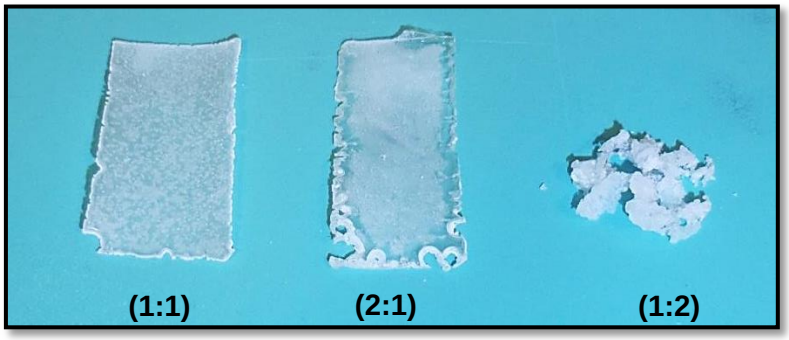


Figure 4:19 Hydrogel films with different ratios between 10% MPC-89.5% HEMA monomer solution : 30% CD monomer solution (1:1, 2:1, 1:2) were prepared. Formulation with 1:2 ratio was not in the form of film and very brittle. The other two films were weak and the CD was not distributed uniformly through the film

Since the formation of an IPN was not possible using an *in situ* approach, a sequential approach was investigated for the preparation of IPN of 10% MPC and β-CD. Sequential polymerisation is basically two steps polymerisation. The first step is to polymerise the first monomer mixture in the presence of the other mixture followed by polymerisation of the second monomer mixture (Figure 4:20).

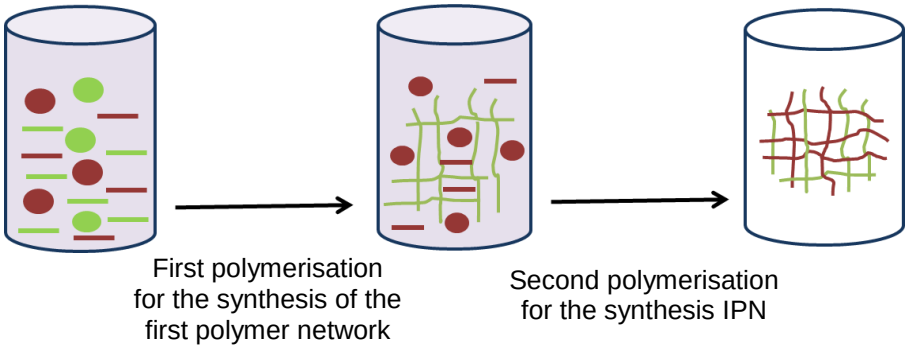


Figure 4:20 Sequential approach used for IPN synthesis. Polymerisation of the first monomer mixture in the presence of the second monomer mixture followed by polymerisation of the second monomer mixture to have an IPN hydrogel.

The polymerisation of the 10% MPC monomer mixture first took place followed by the polymerisation of 30% β-CD monomer mixture. To avoid using the higher temperature required by AIBN (70°C), the initiator was replaced with TEMED/APS system. The TEMED/APS system is capable of starting the polymerisation process at room temperature eliminating the need of higher temperature. After 15 minutes of the addition of TEMED to start the polymerisation process, the mould was transferred to an incubator to start the polymerisation of β-CD monomer solution. The films prepared were smooth similar to 10% MPC; however they were opaque which is a possible indication of the formation of an IPN (Figure 4:21).

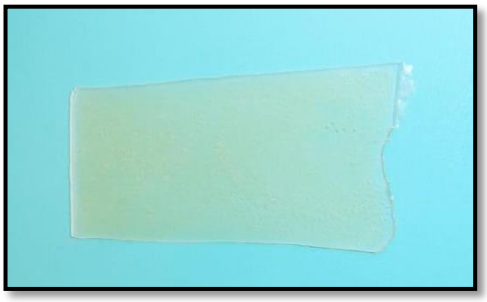


Figure 4:21 IPN hydrogel film fabricated from sequential polymerisation of 10% MPC monomer solution and β-CD monomer solution. First TEMED/APS system was used for the polymerisation of 10% MPC followed by polymerisation of β-CD by placing the hydrogel in the incubator at 50^{o}C for 12 hours at 150 RPM. A hydrogel film was formed.

No difference was observed in SR when water, ethanol and methanol were used. There was an increase in SR when alcohol mixture was used in IPN formulations compared to standard 10% MPC films (Table 4:5). The loading of the IPN hydrogel was 5.9 ± 0.9 which was also significantly higher than the loading of the hydrogel without β-CD. Unfortunately, similar to using embedded β-CD particles no difference in DOXy release profile was observed between 10% MPC hydrogels and the IPN hydrogel discs (Figure 4:22).

Table 4:5 The SR of IPN of β-CD and 10% MPC compared to 10% MPC hydrogels, no difference was observed in SR when water, ethanol and methanol were used. There was an increase in SR when alcohol mixture was used in IPN formulations compared to standard 10% MPC.

SR	Water	Ethanol	Methanol	Water:Ethanol (1:1)	Water:Mthanol (1:1)
IPN	1.60 ± 0.04	0.10 ± 0.03	1.100 ± 0.004	4.0 ± 0.1	3.10 ± 0.09
10% MPC	1.20 ± 0.02	0.20 ± 0.03	1.20 ± 0.09	3.2 ± 0.1	2.80 ± 0.08

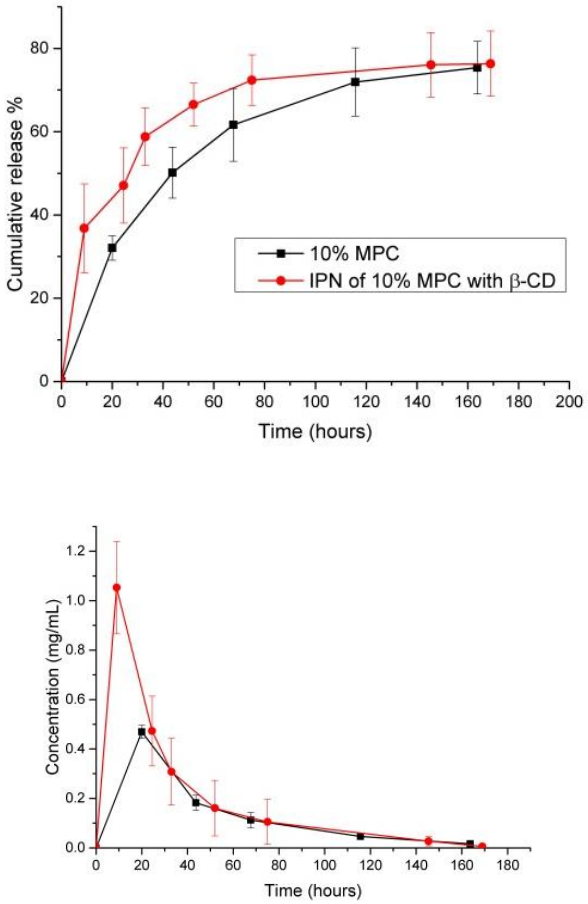


Figure 4:22 DOXy percent of drug release and concnetration from 10% MPC and IPN of 10% MPC with CD. The drug follows similar profile in release from both hydrogels.

Different approaches were used for the incorporation of β-CD into 10% MPC polymer matrix. The β-CD was successfully incorporated into the hydrogel by using β-CD cross-linked particles or sequential IPN. However, there was no difference in release profile of DOXy in the presence of β-CD compared to standard 10% MPC.

Another approach was considered for investigation which is the introduction of vitamin E (VE) as a diffusion barrier to slow the diffusion of DOXy from the hydrogel. VE is a large antioxidant hydrophobic molecule that is soluble in organic solvents and precipitate in the presence of aqueous environment. VE as diffusion barrier was studied to slow the release of water soluble drugs from hydrogel contact lenses (Peng et al. 2010). To create diffusion barrier inside a contact lens VE was loaded in the soluble form using organic solvent (typically ethanol). After loading the organic solvent was extracted with water which causes the VE molecules to aggregate and precipitate inside the lens creating the barrier. Longer time will be required for the drug molecules to diffuse from the lens and the release will be slowed (Figure 4:23).

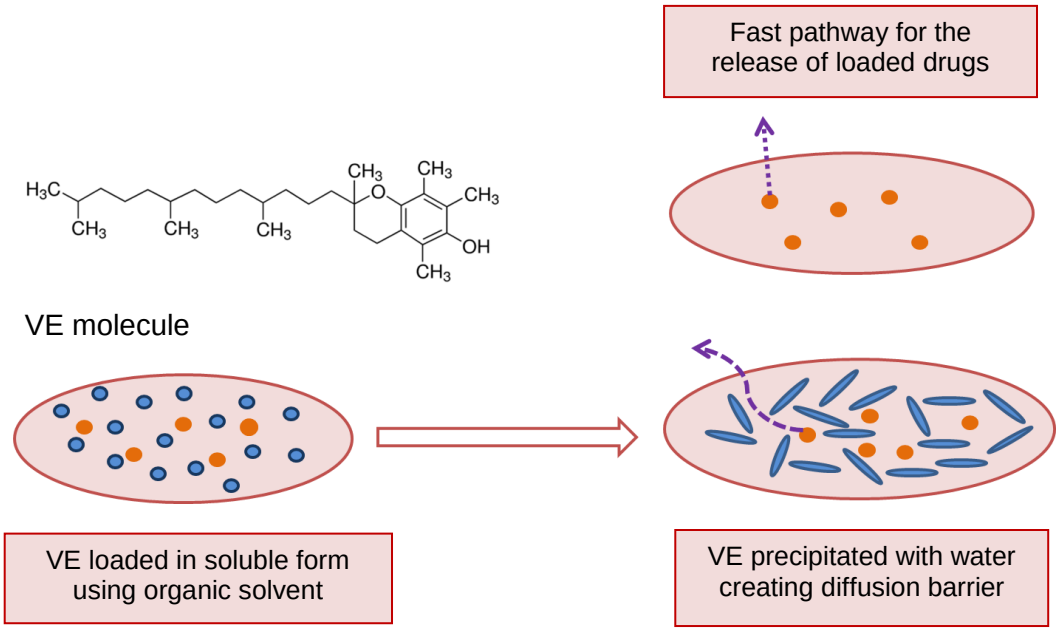


Figure 4:23 VE as diffusion barrier with its chemical structure. VE was loaded using organic solvent then precipitated using water to create a barrier that prolong the diffusion pathway of the loaded drugs.

Methanol was used to load 10% MPC hydrogel discs with VE. VE could possibly be loaded by three methods; first by loading the discs first with VE followed by DOXy, second by loading both VE and DOXy from the same solution and third by loading DOXy first followed by VE. Unfortunately each approach has its own limitation. For the first approach VE was successfully loaded into 10% MPC hydrogel discs. The dried discs were placed in methanol solution of VE (0.1 g/mL, 1 mL) for 24 hours. After loading the discs were removed from the loading solution and placed in 2 mL DW for another 24 hours. The discs were clear after methanol loading and were opaque after soaking in water. This indicates that VE was precipitated in the hydrogel. However, soaking of VE loaded discs in DOXy solution for 24 hours (similar to 10% MPC hydrogel) for loading of DOXy was insufficient to load therapeutic dose of DOXy. Longer soaking times (e.g. weeks) will be required. DOXy is unstable molecule in aqueous solution and hydrolysed quickly which made the approach unsuitable for DOXy loading.

Loading from the same solution was also considered by soaking dried discs of 10% MPC in DOXy and VE methanol solution. The limitation of this approach is the inability to calculate the loading of DOXy accurately into the discs. Both UV and HPLC cannot be used to estimate loading because the HPLC method for DOXy required aqueous buffer which will precipitate the VE inside the HPLC column and damage it. UV cannot be used because it cannot separate the readings of both

molecules. There is also the possibility that VE will occupy the whole space in the hydrogel reducing the amount loaded below the required therapeutic level.

Theoretically loading of DOXy could be performed first followed by loading of VE. However, the limitation is that significant amount of the loaded DOXy could be lost during loading of VE from methanol solution by diffusing through the hydrogel or by the washing step to remove methanol. Due to the limitation of VE and the experimental conditions VE was not used as diffusion barrier.

The aim of DOXy loaded hydrogel was to be used after trachoma surgery. The aim was to sustain the release of DOXy for 2 weeks and based on our current results the release was sustained for one week using the flow rate of ~2 µL/min, which is the aqueous flow in the subconjunctival space. However, the flow in the eyelid area is much slower than the subconjunctival space. When implanted in the eye lid the low flow will possibly cause slower diffusion of DOXy and slower release. It was difficult to measure the release at very low flow rates because of the flow chamber design. The space of the flow chamber is 400 µL designed to mimic the bleb after GFS and going to flow rate slower than 2 µL /min is going to slowly fill the inner space of the flow chamber. The slow filling of the chamber will allow most of the loaded drug to be released and gives the wrong estimation on burst effect and release profile of the drug. Up to our knowledge there is currently no treatment after trachoma surgery in the clinic or in development and the DOXy loaded film could be the proper solution to improve success rate after trachoma surgery.

Summary and conclusions

The aim of the work described in this chapter was to explore the possibility of using 10% MPC-HEMA hydrogel films as drug releasing implant in the eyelid for potential use after trachoma surgery. DOXy hyclate has been identified as an excellent drug candidate to treat patients after trachoma surgery. It was anticipated that DOXy loading into the hydrogel would be challenging, but there is a great need to try to formulate DOXy for sustained local delivery to the eyelid. Different loading solutions and different loading solvents were used to load DOXy into a 1 cm hydrogel disc. Loading from DOXy (15 mg/mL) in 1 mL from PBS was found to be a satisfactory loading method compared to other concentrations and solvents. The amount loaded was 3.6 mg for each disc. Although the loading was high, the release was only maintained for less than a week at flow rate of 2 µL/min, which is better than would be expected by an injection.

β-CD was introduced as affinity barrier to slow the diffusion of DOXy from the hydrogel matrix. No compliant film could be formed using an *in situ* IPN of β-CD

and 10% MPC-89.5% HEMA was prepared, incomplete films were produced. Although a semi-IPN network could be fabricated using β-CD polymer, the apparent high average molecular weight of the polymer could cause batch-to-batch variability during synthesis. A hydrogel film with cross-linked β-CD particles embedded in the hydrogel matrix was also fabricated. The presence of β-CD particles did not affect the SR of the standard 10% MPC films. However, there was no difference in the release profile of DOXy with and without β-CD particles. This result implied the β-CD particles were too densely cross-linked and were insoluble to allow for DOXy solubilisation.

A new approach was investigated for the synthesis of IPN of β-CD and 10% MPC by sequential polymerisation. A hydrogel film was formed and the cross-linked β-CD network did not affect the SR of the standard 10% MPC films. However, it was also observed that the presence of an IPN did not affect the release profile of DOXy from the hydrogel.

Currently there is no available treatment after trachoma surgery in the clinic. Although the hydrogel film sustained the release of DOXy for one week, the films could still be beneficial after trachoma surgery. Since the presence of β-CD did not affect the release of DOXy, hydrogel films with 10% MPC are a protential candidate for future development as a drug releasing spacer after trachoma surgery. The use of both DOXy hyclate for early release in combination with DOXy hydrate within the 10% MPC-HEMA film may be warranted because the poorly soluble DOXy would be released over a sustained period after the acute phase which would be treated by the more fast release of the DOXy hyclate.

Chapter 5. Hydrogel formulation for the vitreous body

The possibility of using a hydrogel as a drug implant to help control subconjunctival scarring on the eye after surgery was investigated in the work that is described in previous chapters. Most blinding eye conditions occur intraocularly at the back of the eye (posterior segment) (Bunce & Wormald, 2006; Jonas et al., 2014). Drug delivery to the posterior segment is challenging due to the physiological and anatomical barriers of the eye (Kang-Mieler et al. 2014). In an aging population, wet AMD is one of the main causes of visual impairment for the elderly population. The most common treatments available for AMD are anti-vascular endothelial growth factor (anti-VEGF) antibody based therapeutics. IVT injections of anti-VEGF medicines are often required every 4-8 weeks to slow the progression of wet AMD, and these injections may be required for many years or even decades (Ambati & Fowler 2012; Abedi et al. 2014; Peden et al. 2015). Multiple IVT injections are associated with complications such as inflammation, vitreous haemorrhage and retinal detachment. There is significant and real patient discomfort with undergoing IVT injections; including a financial burden placed on patients and healthcare systems (Falavarjani & Nguyen 2013). New formulations that can prolong the duration of action of an injected medicine that results in reduced dosing frequency are sought by patients, healthcare professionals and providers.

Several approaches have been investigated to improve the duration of action of IVT injected proteins to better achieve sustained ophthalmic therapy that would decrease the number of injections required by patients. Strategies include protein PEGylation, and the use of particulates (micro and nanoparticles), gels and liposomes were used to increase the duration of action and reduce the frequency of dosing (Yu et al. 2014; Fletcher et al. 2016; Xie et al. 2015; Li et al. 2012; Pan et al. 2011; Pisal et al. 2011).

Rauck *et al* were able to sustain the delivery of bevacizumab after IVT injection into New Zealand white rabbit eyes using poly(ethylene glycol)-poly-(serinol hexamethylene urethane) thermoresponsive gels over 9 weeks compared to bevacizumab bolus injection. Clinical examination of the rabbit eyes injected with the gel for inflammatory response showed no signs of inflammation and the intraocular pressure was maintained at baseline during the period of the study. Histological examination of the retinal tissues of the rabbit eyes after 10 weeks shows no distinguishable morphological differences between the gel injected eyes and the control (Rauck et al. 2013).

Abrishami *et al* reported the formation of liposome encapsulated bevacizumab for IVT injection. According to their results the concentration of bevacizumab on the rabbit eyes after liposome injection was three times higher than the therapeutic amount after 42 days and the clearance of the drug from the vitreous was slower compared to bevacizumab injection (Abrishami et al. 2009). Liposomes have long been studied in a range of applications, but there remain no protein based formulations that have been approved.

Varshochian *et al* reported the synthesis of albumin-PLGA nanoparticles in an effort to extend the residence time of bevacizumab in the vitreous cavity. When the nanoparticles were injected into the vitreous of New Zealand albino rabbits the calculated half-life of bevacizumab from the nanoparticle was 8.4 days compared to 5.2 days in the control rabbits that had received injected bevacizumab (Varshochian et al. 2015).

Lovett *et al* reported that bevacizumab release was sustained for 3 months when injected in the vitreous of Dutch belted rabbits in the form of silk fibroin hydrogels. During the period of the study no obvious inflammatory or biocompatibility problem was observed in the animals. The level of bevacizumab in the vitreous after 3 months of gel injection was equivalent to the level detected after 1 month of bevacizumab only injection which indicates sustained behaviour of the gel (Lovett et al. 2015).

Although the above discussed examples show promise as sustained formulations for protein delivery, there is a limitation regarding the methods used for release studies. The release was either studied using animal model (preferably rabbits) or *in vitro* release using test tubes. Animal eyes are anatomically and physiologically different from the human eye which may possibly give wrong estimation of the release of the protein. Using in vitro test tubes does not represent the actual volume of the vitreous or the actual fluid flow in the back of the eye.

Extensive research has been described to develop methods for the sustained delivery of proteins; however no dosage form has been approved in the clinic for ocular delivery. One key limitation is the nature of the protein molecule (Fu et al. 2000). Proteins are challenging molecules to be formulated into extended release dosage forms because proteins must maintain their tertiary folded structure to be biologically active. Protein based medicines can also be immunogenic and display other toxicities, for example, increased susceptibility to infection as seen with anti-TNFα antibodies (Ali 2013).

Protein instability, during and after formulation, is a defining challenge. Protein therapeutics display relative long half-life when directly injected into the eye.

For example, the half-life of IVT ranibizumab (~40 kDa, 0.5 mg) and bevacizumab (~150 kDa, 1.25 mg) is approximately 6.7-10.0 days and 7-11 days in humans respectively (Krohne et al. 2008; Meyer et al. 2011; Zhu et al. 2008; Beer et al. 2006; Krohne et al. 2012).

Unfortunately IVT injection is still required every 4-6 weeks. Aflibercept (molecular weight 110 kDa) is a Fc fusion protein that is also targeted to VEGF. This medicine is given in a 2 mg dose which is approximately a 1.8 molar equivalent compared to the ranibizumab dose. The increased amount of aflibercept in a 50 μL IVT injection allows this medicine to be given every 6-8 weeks whereas ranibizumab must be given monthly. Aflibercept is by far the most preferred IVT anti-VEGF because it can be dosed less frequently than ranibizumab. If a formulation for an anti-VEGF medicine could be developed that would allow dose once every 3 or 4 months, there would be much interest.

Manufacturing processes are also important to preserve the activity of the proteins. Proteins are liable to unfolding and deactivation when subjected to organic solvents or harsh preparation conditions, which is often necessary when considering the formulation of proteins with some clinically used polymers, such as poly(lactic co glycolic) acid (PLGA) (Mohammadi-Samani & Taghipour 2014). Even successful loading of PLGA particles with proteins could be followed by phase separation due to the difference in solubility of PLGA (water insoluble) and the protein (water soluble). It is preferred to prepare protein dosage forms in aqueous environment to preserve the activity of the protein.

Hydrogels have developed as an interesting field for the possible sustained delivery of proteins. Hydrogels are capable of imbibing a amount of high water content and are reported to be biocompatible. Many hydrogel systems that have been described do not require organic solvents during preparation (Shi et al. 2013; Stile et al. 1999; Vermonden et al. 2012). Proteins are also subjected to degradation by proteases and the entrapment of proteins inside a hydrogel network would be expected to protect the therapeutic protein from proteolytic degradation and while prolong its duration of action (van de Weert et al. 2005). It is also possible to consider hydrogels with a low cross-link density to allow formulation in the form of injectable solutions. If the composition of the hydrogel includes a stimuli responsive polymer that can collapse, it is possible to have an injectable solution that upon a stimulus would solidify to become an implant. Thermoresponsive hydrogels potentially offer the advantage of injectable implant to extend the release of a loaded protein (Klouda 2015).

Thermoresponsive hydrogels change their conformation with temperature, often collapsing from an open solubilised, water associated structure to a more dense, collapsed and insoluble form. The so called sol-to-gel transition changes the swelling and pore size of the hydrogel. In the soluble form, the hydrogel is soft, fully swollen with water and can pass freely through a small needle. At a temperature known as the volume phase transition temperature (VPTT), the polymer can deswell and collapse forming a viscoelastic implant (Huynh et al. 2011). The thermal responsiveness in these hydrogels is regulated by the balance between hydrophilic and hydrophobic interactions between the polymer chains and their surroundings (Stewart et al. 2011).

One of the most widely investigated thermoresponsive polymers is poly(N-isopropylacylamide) (NIPAAm) which can be made in the presence of a cross-linker to give a hydrogel. NIPAAm hydrogels are swollen in water below a VPTT of ~33^{o}C. As the temperature rises about ~33^{o}C many NIPAAm hydrogels then collapse and deswell pushing the water out of the hydrogel network (Stile et al. 1999; Lue et al. 2011; Schild 1992). The preparation of NIPAAm hydrogels can be conducted in aqueous solution avoiding organic solvents, which is advantageous for protein delivery. The swollen hydrogel has a relatively large pore sizes which allow the drug to be released quickly at room temperature. Once injected and collapsed inside the human body the drug molecules will be entrapped inside the hydrogel structure potentially creating a depot and the pore size between polymer chains will be reduced which will slow protein diffusion from the hydrogel (Drapala et al. 2011). As previously discussed in Chapter 1 the characteristics and physical properties of NIPAAm hydrogels (e.g. VPTT) can be influenced by co-monomer, cross-linker type and the synthetic process (e.g. initiator and cross-linker stoichiometry) (Obeso-Vera et al. 2013; Yildiz et al. 2006). In the case of proteins, the use of a hydrophilic cross-linker could create a hydrophilic environment around the loaded protein that helps to preserve its stability and activity during formulation and use.

Developing and comparing new formulations for IVT protein injections require a proper *in vitro* model that mimics the eye in terms of aqueous flow. One of the problems facing the preclinical development of ophthalmic IVT protein formulation is the lack of proper *in vitro* ocular flow model. The main route for elimination of a protein from the vitreous after injection is through aqueous outflow into the anterior chamber (Krohne et al. 2008; El Sanharawi et al. 2010). Proteins are large, charged molecules, so do not readily permeate through the retina as do low molecular weigh molecules such as steroids. *In vitro* models to study elimination of proteins through the anterior route are very limited and do not allow

proper examination of the clearance of proteins in a similar way to the human eye. Most research in preclinical development is conducted either through animal studies or other *in vitro* dissolution models. Animal studies are expensive. Animal eyes are anatomically different from human eyes (in terms of volume of the vitreous and aqueous flow rates) and carry the risk of developing antidrug antibodies (ADAs). ADAs accelerate the clearance of the protein resulting in inaccurate estimations of clearance and half-life values in the vitreous (Shankar et al. 2006; Chirmule et al. 2012).

Different *in vitro* techniques were described to evaluate IVT ophthalmic preparations including the use of computational modelling of the eye, permeation models and USP dissolution apparatus (Westebbe et al. 2013; Missel 2002; Durairaj et al. 2009). These models are rough and do not reflect the human eyes in terms of volume, scale and rate and direction of the aqueous flow. For example the USP dissolution apparatus used had a flow rate of 1.5 mL/min in a chamber of 8-19 mL which is different from the flow rate of 2.0 µL/min and the vitreous volume which is 3.5-9 mL (Fotaki 2011). Another example is the use of single compartment non-flow model to evaluate distribution in the vitreous by mimicking saccades eye movement (Repetto et al. 2005).

To address these issues and to accelerate preclinical studies, our group has recently developed a 2-compartment, aqueous flow model called the PK-Eye to be used for ocular drug development. The model has been shown to estimate the human clearance times of proteins from the vitreous body (Awwad et al. 2015). The PK-Eye was used to study the release profile and half-life of injectable bevacizumab and injectable NIPAAm hydrogels loaded with bevacizumab and infliximab for comparison.

Hypothesis and aims

An ideal sustained delivery system for proteins in the vitreous should be (i) injectable, (ii) biocompatible, (iii) maintain both physical and chemical stability of the encapsulated protein during formulation and its stability over the period of the release, and (iv) maintain the level of the protein within the therapeutic concentration. Thermoresponsive injectable hydrogels could be used as a potential delivery system for proteins due to the properties related to biocompatibility, injectability and mild preparation conditions.

It is hypothesised that the properties of a thermoresponsive NIPAAm hydrogel can be mediated by varying cross-linker stoichiometry and structure. The aim of this chapter was to examine formulations that could be the basis to develop

an injectable extended release thermoresponsive NIPAAm hydrogel for bevacizumab delivery. Three different hydrophilic, macromolecular cross-linkers of varying molecular weight (PEGDA, PC 3059 and acrylated HA) were examined. The use of macromolecular cross-linker may allow the formation of aqueous environment around the protein that may help in the mixing process and help with maintaining protein stability. Another possible advantage of using macromolecular cross-linker is creating an extra diffusion barrier to the protein encapsulated inside the hydrogel.

The hydrogels were synthesised and then characterised in respect to VPTT, swelling ratio (SR), percentage water retention (WR%) and structure. The characterisation of the hydrogels formed the basis of a screening process to identify a possible formulation that will potentially extend the release of the protein in the vitreous. The release of bevacizumab was then studied using a specialised PK-Eye model that mimics protein clearance from the posterior segment.

Results and discussion

Preparation of NIPAAm hydrogels using PEGDA-700

NIPAAm hydrogels were prepared with different amounts of polyethylene glycol diacrylate PEGDA (Mn 700) by free radical polymerisation. Since these hydrogels are intended for protein delivery, it was important to create an aqueous environment surrounding the protein during synthesis and delivery. PEGDA was chosen as cross-linker because of its hydrophilic nature that attracts large amount of water molecules around the polymer chains creating an aqueous environment and human biocompatibility when cross-linked into hydrogels (Li et al. 2014). NIPAAm hydrogels made with PEGDA (Mn 575) was reported to have minimal effect on retinal function when administered as IVT injection in rats (Turturro et al. 2011).

The high amount of water in the hydrogel matrix improves biocompatibility but also associated with burst release effect. Burst release can be of an advantage as initial loading dose; however the duration of release could be compromised and the possibility of dose related side effects could be increased. Optimisation of the formulation to have sufficient amount of water for biocompatibility and at the same time controlling release is important and could be achieved by controlling formulations parameters; in particular cross-linking density.

Four different NIPAAM hydrogels were prepared with increasing PEGDA stoichiometries i.e. 2, 4, 8, 12 and 15 µL. The prepared hydrogels were screened to observe the effect of cross-linker on the physicochemical properties of the prepared hydrogels in order to identify the best formulation for protein delivery. Different mixtures of monomers were prepared by dissolving equal amounts of NIPAAm (40 mg) monomers and ammonium persulfate (APS) (4 mg) with different amounts of PEGDA in 1 mL DW prior to polymerisation. Before the addition of TEMED (20 µL) the reaction mixture was in liquid form. Polymerisation was initiated by the addition of TEMED. Hydrogel formation was observed approximately after one minute. The mixture became viscous and no free movement was observed indicating the formation of the hydrogel (Figure 5:1). All or large percentage of the liquid monomer mixture was thought to be converted into a hydrogel since there was no free flowing liquid remained in the glass vial. The reaction mixture was allowed to polymerise at 4^{o}C for 24 hours. PEGDA amount below 4 µL displayed a free-flowing liquid even after the addition of TEMED. Therefore, 4 µL was the minimum amount of cross-linker required for the formation of a hydrogel.

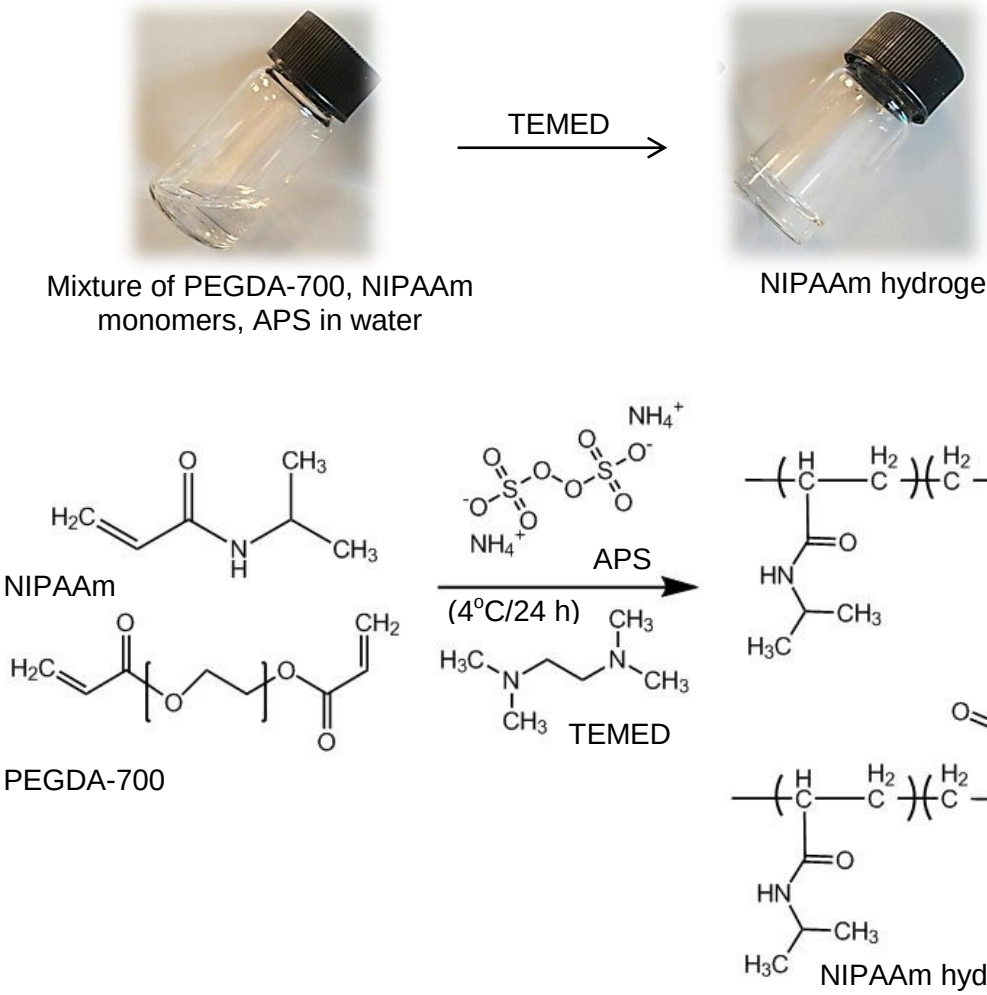


Figure 5:1 The chemical structure of the starting materials for NIPAAm hydrogel formulation and the hydrogels formed. NIPAAm hydrogels were prepared with different percentages of PEGDA as cross-linker by free radical polymerisation at 4°C for 24 hours. In the above picture the free flowing monomer mixture of PEGDA-700, NIPAAm and APS was converted into a viscous hydrogel by the addition of TEMED at room temperature.

Hydrogels that were prepared with 4 and 8 µL of PEGDA were transparent in colour, whereas hydrogels with 12 and 15 µL were opaque. Increased cross-linking density due to the addition of increasing PEGDA resulted in less soluble hydrogels, which was indicated by the reduction in transparency of the formed hydrogels (Figure 5:2A). SEM results displayed a more compact hydrogel structure with increased cross-linker percentage, which is consistent with the formation of denser hydrogels (Figure 5:2B). The cross-linker acts as junction point (cross-linking point) that brings the polymer chains together and reduces the mobility of the polymer chains.

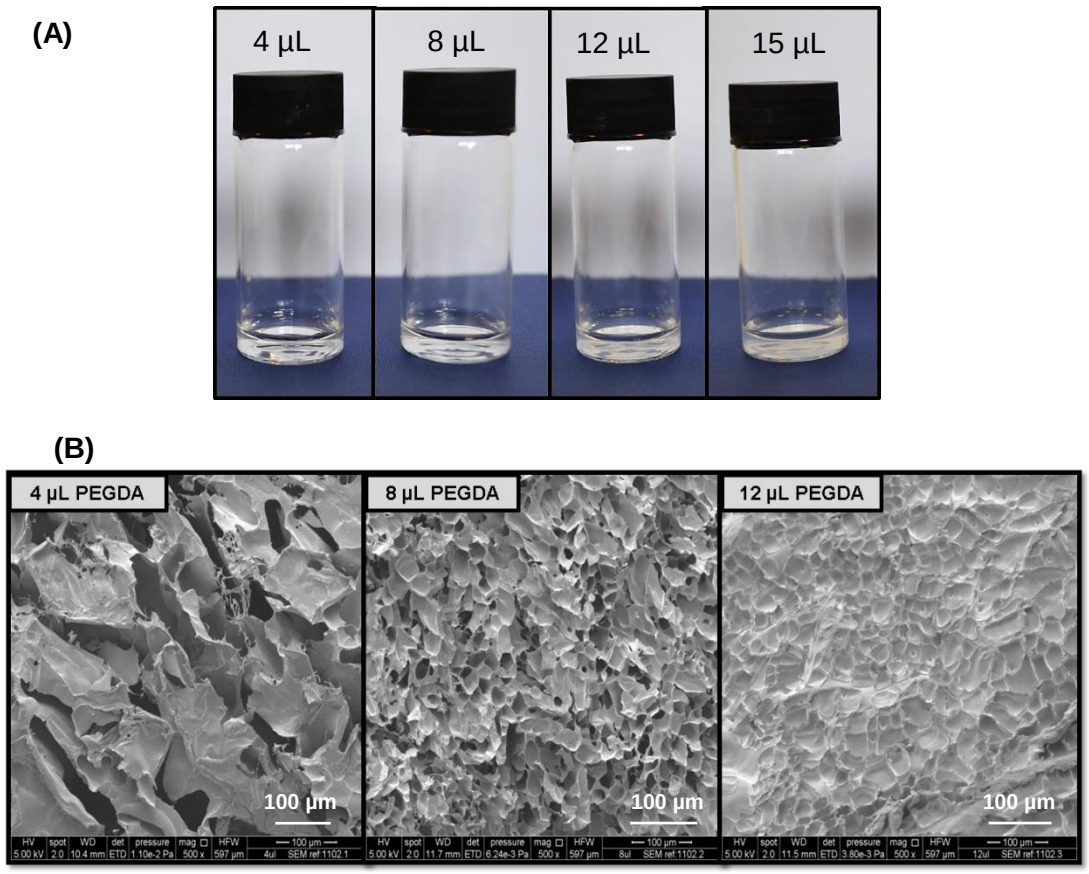


Figure 5:2 A) Picture of the prepared NIPAAm hydrogels showing a decrease in transparency with increasing cross-linker amounts. Hydrogels made with 12 and 15 µL PEGDA were opaque compared to transparent hydrogels made with 4 and 8 µL; B) SEM images of NIPAAm hydrogels prepared with different amounts of PEGDA cross-linker. More compact structure was observed with increasing cross-linker amount. The scale bar is 100 µm.

An important criterion to consider for IVT injections is the ability of a hydrogel to pass easily through a small needle without undue back pressure. The injectability of each hydrogel was qualitatively evaluated by passing the contents through a 23G needle (Table 5:1). The hydrogel was loaded into a 2.5 mL syringe by first removing the plunger of the syringe and placing the hydrogel inside the syringe with a spatula. After loading of the hydrogel into the syringe body the plunger returned into the syringe. Hydrogels made with 4 µL and 8 µL of the PEGDA cross-linker were easily injectable. Hydrogels with 12 µL of the cross-linker could be injected only with extra pressure while 15 µL hydrogels were not injectable. Passing the hydrogel through a small needle did not break the hydrogel and the hydrogel remain intact after injection. Hydrogels with 4 and 8 µL PEGDA allowed higher mobility of the polymer chains, which are similar to fluids and allowed them to be freely injected through small needle.

Table 5:1 The qualitative determination of injectability of NIPAAm hydrogels formulated with different amounts of PEGDA using 23G needle.

PEGDA amount	Injectability
4 µL	Easy
8 µL	Easy
12 µL	Difficult
15 µL	Non-injectable

Measuring the viscosity of the prepared hydrogels was considered to relate the viscosity and injectability of the hydrogels. However, there were difficulties to do the experiment in the facilities available at UCL School of Pharmacy. In order for the hydrogel to be examined by the rheometer available, the hydrogel needs to be completely soluble in water. NIPAAm hydrogels cannot be dissolved in water so the hydrogels cannot be tested using the available rheometer.

Hydrogel characterisation

There was a need to determine the effect of cross-link density on the thermoresponsiveness of the hydrogels. Drapala *et al* reported an increase in VPTT and SR at 25^{o}C of NIPAAm hydrogels with increased amounts of PEGDA (Mn 575) (Drapala et al. 2011). The VPTT, SR and WR% were determined for the 4 prepared hydrogels to better understand the effects of the different cross-link densities. Three batches (n=3) for each type of cross-linker were prepared for the characterisation of the hydrogels.

Effects of different percent incorporation of cross-linker on VPTT

An important factor for biomedical thermoresponsive hydrogels is that the VPTT is often desired to be lower than the physiological body temperature (~37^{o}C). The VPTT was determined by DSC to correlate changes with cross-link density. Hydrophilic interactions predominate at temperatures below the VPTT due to the formation of more strong non-covalent interactions such as hydrogen bonds between water and the hydrogel polymer matrix. The collective interactions of polymer and water are greater than polymer-polymer interactions, so the polymer remains in solution. When the temperature is increased above the VPTT, non-covalent hydrophobic interactions dominate between polymer chains. Water-polymer interactions, in particular hydrogen bonds, become less dominant so the polymer becomes less soluble. At the VPTT, the hydrophobic interactions of the isopropyl pendant groups of NIPAAm cross-linked chains become stronger than the hydrogen bonds between the amide pendant groups and the surrounding water

molecules (Zhang et al. 2003). The internal volume of the polymer decreases as the polymer begins to collapse onto itself. Polymer-polymer chain interactions will continue to increase until the polymer precipitates and the hydrogel is transitioned from a swollen to deswollen (collapsed) form resulting in a reduced internal volume (Yıldız et al. 2002; Sato Matsuo & Tanaka 1988).

It was previously reported that the VPTT of NIPAAm hydrogels is ~33^{o}C (Schild 1992). The onset temperature of the DSC endothermic peak was considered as the VPTT. The endothermic peak increased to a slightly higher temperature with an increasing amount of cross-linker (Figure 5:3). The VPTTs were 34.3 ± 0.1, 35.5 ± 0.1, 35.6 ± 0.4, and 36.2 ± 0.2^{o}C as the PEGDA cross-linker was increased from 4 to 15 µL respectively. The sharpness of the endothermic peak was reduced with increasing amounts of cross-linker indicating a reduction in the thermal responsiveness of the hydrogel.

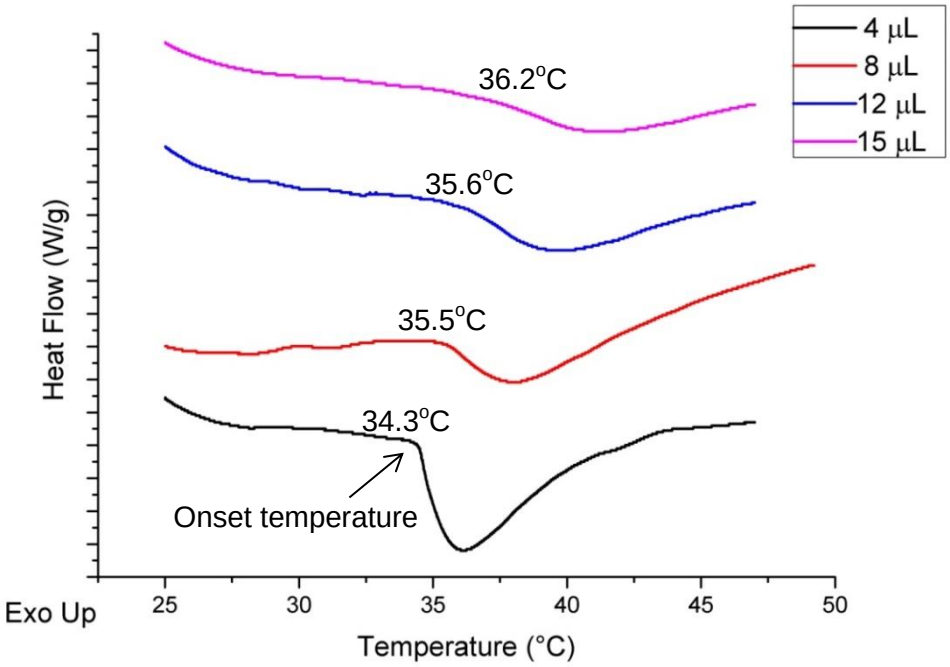


Figure 5:3 DSC graph representing volume phase transition temperature (VPTT) of NIPAAm hydrogels with varying PEGDA concentrations. The onset temperature of the DSC endothermic peak was considered as the VPTT. It was observed that there was a shift in endothermic peak to a higher value with increasing amount of cross-linker. The VPTT were 34.3 ± 0.1, 35.5 ± 0.1, 35.6 ± 0.4, and 36.2 ± 0.2^{o}C for 4, 8, 12 and 15 µL respectively.

The mechanism of VPTT can also be explained thermodynamically by Gibbs energy of the system in which ΔG represents the change in free energy, ΔH represents the change in enthalpy, ΔS represents the change in entropy at T temperature.

$$\Delta G=\Delta H-T\Delta S$$

At temperatures below VPTT there is large number of hydrogen bonds that helps in keeping the polymer matrix in the dissolved (swelled) form favouring the enthalpy of dissolution. When the temperature increases the enthalpy of the system will be reduced and the entropy will be increased. The hydrogen bonds becomes weak and phase separation occur which is characterised by the collapsing of the hydrogel (Alarcón et al. 2005). Alteration of the number of hydrogen bonds in the polymer matrix can shift the VPTT (Lutz, J. F. 2008). PEGDA is a moderately large molecular weight cross-linker that is hydrophilic. PEGDA has an oxygen molecule in each repeat unit, so PEGDA can form hydrogen bonds with water molecules. Higher energy will be required to thermodynamically disturb the system when higher level of PEGDA present in the formulation which explains the increase in VPTT when the PEGDA level increased.

Cross-linker effects on hydrogel swelling ratio (SR) and water content

The SR is used to describe the swelling capacity of a hydrogel when at equilibrium within the surrounding media. An increase in weight of the hydrogel is observed due to water imbibing into the dry hydrogel matrix. It can also be used to estimate the water content in a fully swollen (room temperature) and deswollen thermoresponsive hydrogel (body temperature) (Park & Hoffman 1994). The SRs of the NIPAAm-PEGDA cross-linked hydrogels prepared were measured at 25, 37 and 48^{o}C (Table 5:2). There was a significant decrease ($p<0.05$) in the SR for all the hydrogels when the temperature was increased from 25 to 37^{o}C.

Table 5:2 The SR of NIPAAm hydrogels prepared with different percentages of PEGDA as cross-linker at three different temperatures (25, 37 and 48^{o}C).

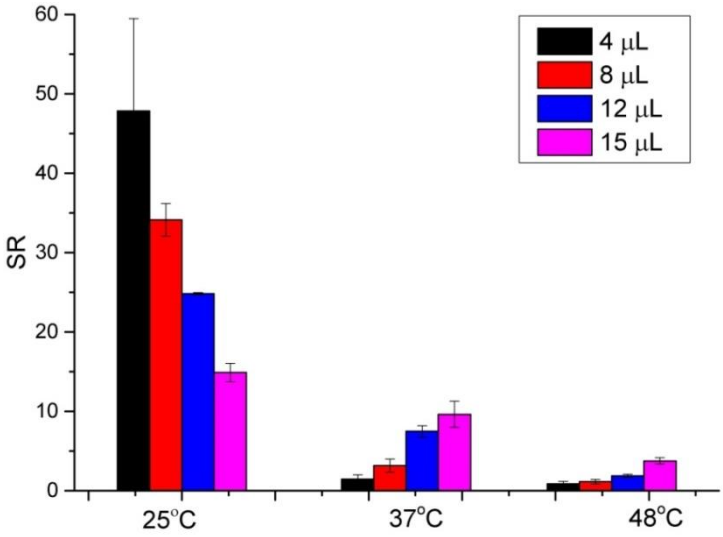
Temperature	SR			
	4 µL	8 µL	12 µL	15 µL
25^{o}C	47.8 ± 11.6	34 ± 2	24.8 ± 0.1	14.9 ± 1.1
37^{o}C	1.4 ± 0.5	3.2 ± 0.8	7.5 ± 0.7	9.6 ± 1.6
48^{o}C	0.8 ± 0.3	1.1 ± 0.3	1.9 ± 0.2	3.7 ± 0.4

When the SR is measured at a temperature below the VPTT (25°C), the hydrogel is more soluble with more internal water due to the hydrogen bonds

between the hydrophilic groups on the polymer chains and water. A hydration shell is formed around the hydrophobic groups further leading to more water uptake. All of the NIPAAM hydrogels retain a larger amount of water at 25^{o}C compared to 37^{o}C. At 25^{o}C, the SR was the highest with 4 µL cross-linker and dropped with increased amounts of PEGDA. With higher cross-link density, the mobility of the polymer chains forming the hydrogel matrix will be reduced and hydrogels with small pore size and compact structure will be formed. Increased cross-link density causes a reduction in hydrogel swelling with increased cross-linker percentage. PEGDA amount of 4 µL was not significantly different from 8 µL ($p>0.05$); however it was significantly different ($p<0.05$) from 12 and 15 µL hydrogels (Figure 5:4A). When the temperature increased above the VPTT (37^{o}C), all the collapsed polymer hydrogels displayed a much lower SR (Table 5:2). When a hydrogel collapsed, large amount of the water inside the hydrogel was pushed out and small amount of water remained entrapped inside the hydrogel.

Drapala *et al* reported that the SR of different NIPAAm hydrogels with different amounts of PEGDA (Mn 575) at 37^{o}C was independant on the cross-linker amount (Drapala et al. 2011). Based on that it was thought that the SR at 37 and 48^{o}C would not be affected by the increase in the amount of cross-linker used and all the hydrogels will behave in the same manner above the VPTT of the hydrogel; however when the temperature was increased above VPTT, the SR increased with increasing cross-linker amount (Figure 5:4A). Although all the hydrogels prepared were able to collapse and deswell when the temperature increased from 25^{o}C to 37^{o}C the degree of collapse was different and dependant on the cross-linker amount. At 37^{o}C and 48^{o}C there was no significant difference ($p>0.05$) in SR between 4 and 8 µL, but there was a significant difference between 4, 12 and 15 µL ($p<0.05$). In contrast to what Drapala *et al* reported it was found that in our system PEGDA when present in a higher amount (12 and 15 µL) would reduce the thermoresponsiveness and the ability of the hydrogel to collapse. The incomplete collapse of the hydrogels will allow more water to be entrapped compared to hydrogels made with 4 and 8 µL, which may influence the release of the entrapped protein. The visual difference in swelling of the hydrogels at 25 and 37^{o}C is presented in (Figure 5:4B).where the hydrogels appeared transparent at 25^{o}C and opaque at 37^{o}C.

(A)



(B)

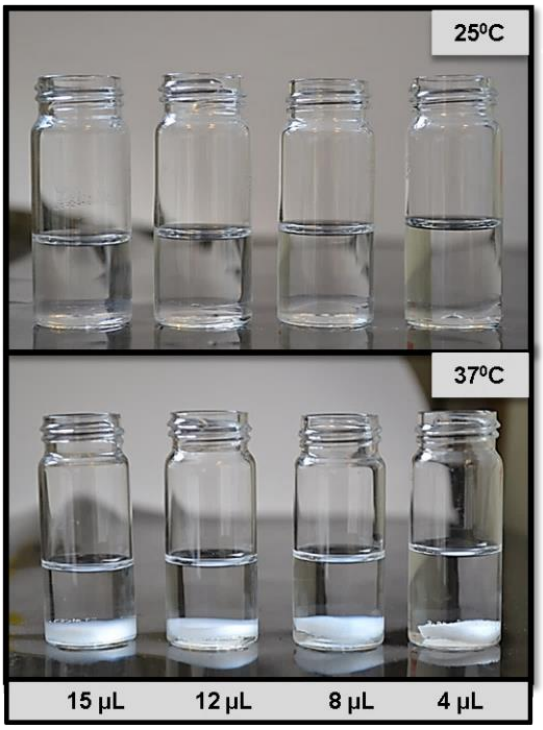


Figure 5:4 A) A graph representing the SR of NIPAAm hydrogels prepared with different percentages of PEGDA as cross-linker at three different temperatures (25, 37 and 48°C). At 25°C the SR of 4 µL hydrogel was not significantly different from 8 µL ($p>0.05$); however it was significantly different ($p<0.05$) from 12 and 15 µL. At 37°C and 48°C there was no significant difference ($p>0.05$) in SR between 4 and 8 µL, but there was a significant difference between 4, 12 and 15 µL ($p<0.05$); B) Pictures of the hydrogels showing the visual difference in swelling of the hydrogels at 25 and 37°C. Hydrogels at 25°C were transparent but turned opaque when the temperature increased to 37°C.

Effect of percentage cross-linker on water retention percent (WR%)

WR is the weight of water retained inside a hydrogel above the VPTT versus the weight of the initially absorbed water before hydrogel collapse. The WR% gives an indication of how much water will be entrapped inside the hydrogel after collapse. Water retention characterises the change in volume and the time required to achieve that change when the temperature increases above the VPTT of the hydrogel. Hydrogels that take a longer time to collapse will allow some of the encapsulated drug to diffused into the surroundings and reduce the amount of drug entrapped during collapse. Also the degree of collapse of a hydrogel is important. Hydrogels that completely collapse and retain small amount of water in the collapsed inner structure risk losing large amount of the entrapped drug. The drug will be expelled with the expelled water resulting in a low entrapment and high initial burst release, which will affect the release profile of the encapsulated drug.

The effect of increasing the amount of cross-linker on WR% was measured (Figure 5:5). The thermal responsiveness of the lowest cross-linked NIPAAM hydrogels (4 µL PEGDA) was faster compared to more highly cross-linked hydrogels (8-15 µL PEGDA). With increased amounts of PEGDA, the NIPAAM hydrogels became less responsive to temperature changes while encapsulating a higher amount of water in after collapse. A significant difference in WR% was seen between the lowest cross-linked hydrogel (4 µL PEGDA) compared to the higher two cross-linked hydrogels (12 and 15 µL PEGDA). There was no significant difference in WR% ($p>0.05$) between the two lower cross-linked dense hydrogels (4 and 8 µL PEGDA). The difference in WR% between the hydrogels suggests that hydrogels with lower percentage of cross-linker may control the diffusion of the drug from the collapsed hydrogel in a more controlled manner compared to hydrogels with higher percentages of cross-linker.

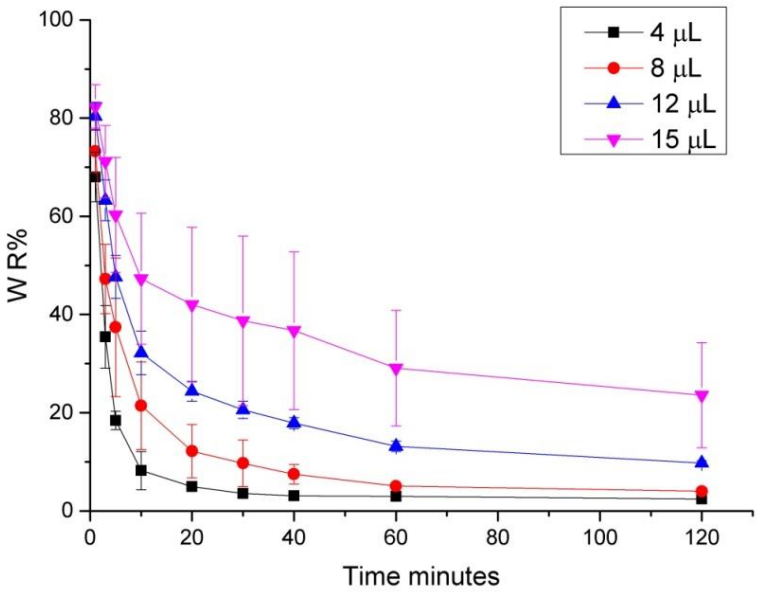


Figure 5:5 A graph representing the water retention percent (WR%) of NIPAAm hydrogels prepared with various percentages of PEGDA as cross-linker. There was no significant difference in WR% ($p>0.05$) between 4 and 8 µL hydrogels; however here was a significant difference ($p<0.05$) when 4 µL was compared to 12 and 15 µL hydrogels.

In thermoresponsive hydrogels drug diffusion is a two stage process; initial burst release followed by a slow extended release of encapsulated drugs. During a collapse of a thermoresponsive hydrogel, large amounts of water will be expelled causing the initial burst release and reducing the inner volume of the hydrogel. The initial burst release results in a proportion of entrapped drug into the collapsed hydrogel matrix creating a depot surrounded by a skin like layer at the interface between the hydrogel surfaces. The surrounding media that acts as a controlling mechanism for the release of the remained drug (Schild 1992). The entrapped drug will diffuse slowly through the interface. Several factors will control the diffusion of encapsulated drug in a thermoresponsive hydrogels; volume of water entrapped, the solubility of the drug in water and the MW of the drug (Varshosaz & Hajian 2004). Proteins are soluble in water so it was suggested that when high amount of water entrapped in the hydrogel matrix after collapse, the fraction of protein soluble will increase and faster release will be observed.

In vitro release of antibody from NIPAAm hydrogels using the PK-Eye

Protein loading into a hydrogel could be achieved through several methods such as soaking (imbibing), cross-linking, two phase partitioning and *in situ* polymerisation, (Bromberg & Ron 1998; Kim et al. 1992). Proteins, such as bevacizumab with a molecular weight of 150 KDa, are large molecules and simple imbibing of the dry hydrogel into the protein solution for loading may result in low loading effeciency (He et al. 2016). When the loading of ovalbumin into dextran hydrogel by imbibng was studied, only 9% (w/w) of the protein from the loading solution was loaded into the hydrogel (Gehrke et al. 1998). Theortically, the problem of low loading effeciency could be avoided by repeated cycles of swelling and drying of the hydrogel in the loading solution; however the approach is difficult with proteins because of the possibility of protein denaturation and aggregation by repeated drying (Frokjaer & Otzen 2005).

Cross-linking of a polymer in the presence of the protein could also be used for protein loading. The protein need to be thouroughly mixed with the polymer before cross-linking; however it is difficult to achieve homogenous mixing with macromolecules and there is a possibility that the cross-linker used could interact with the protein itself instead of the polymer (Bromberg & Ron 1998).

Two phase partitioning involves the partitioning of the protein from polymer solution into the hydrogel. The protein should have low solubility in the polymer solution compared to the hydrogel matrix (Bromberg & Ron 1998). High loading of dextran gels with BSA was achieved by partitioning of BSA from PEG polymer solution (low affinity with BSA) into the dextran gel phase (High affinity with BSA) (Gehrke et al. 1991).

In situ polymerisation is preferred for efficient protein loading compared to other methods. Polymerising the protein in the presence of monomers and cross-linker allows the entrapment of the protein within the hydrogel matrix and could result in higher protein encapsulation. The polymerisation proces can result in better entanglement of the protein within the polymer matrix due to proper mixing of the protein with the small monomers (Leach & Schmidt 2005). When using *in situ* polymerisation it is important to consider polymerisation processes that would not be detrimental to the stability of protein. For our work, it is hypothesised that the combination of NIPAAm polymerisation in aqueous enviroment and at $4^{o}C$ will maintain the stability of protein.

Both methods of imbibing and polymerising in the presence of bevacizumab were used and compared for NIPAAm hydrogel loading. In the imbibing method, 8 µL PEGDA hydrogel was formulated. After the polymerisation, the hydrogel was freeze-dried for 3 days. The freeze-dried NIPAAm hydrogel was soaked in 1 mL of bevacizumab solution (12.5 mg/mL in PBS). The hydrogel was incubated for 48 hr at 4^{o}C (Figure 5:6). After incubation, the hydrogels were removed from the incubation solution and then rinsed gently 5 times, 4 mL each time in PBS; each wash was 15 minutes long. Each wash fraction was analysed by HPLC to calculate how much of the protein was lost during the wash; it was calculated to be ~28% (~3.5 mg). The drug loading was the difference between the starting solution and the amount lost during washing. Bevacizumab final loading was 72.3 ± 6.6% (~9 mg). In the *in situ* method, the polymerisation reaction of the hydrogel was performed in 1 mL protein solution (25 mg/mL) instead of 1 mL DW. The encapsulation was 100%. A hydrogel was formed similar to the one made without the protein with no signs of heterogeneity or precipitation.

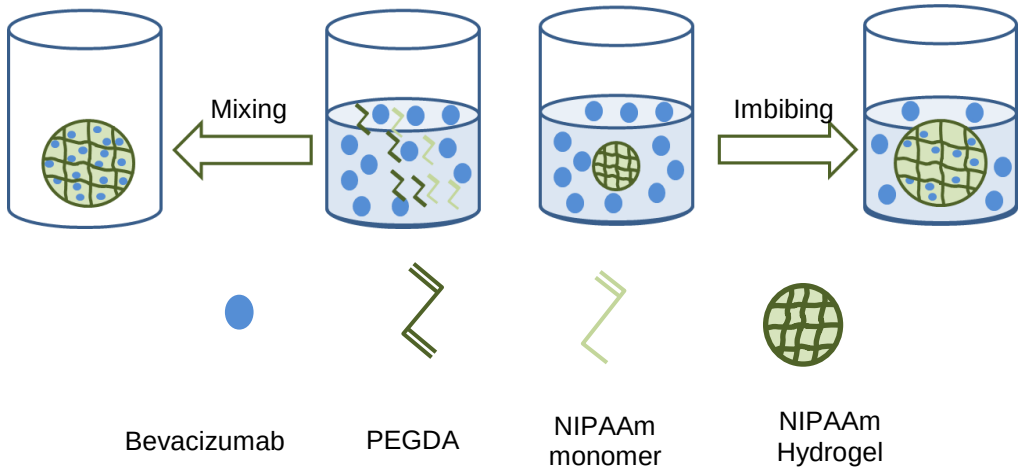


Figure 5:6 Schematic representation of the preparation of bevacizumab loaded NIPAAm hydrogels through imbibing and mixing methods. Through imbibing a portion of the protein is loaded into the hydrogel matrix while using mixing all the protein in the mixture entrapped in the hydrogel.

The release of bevacizumab from the hydrogels was compared to its injection form: (i) 100 µL of NIPAAM hydrogel prepared by mixing, (ii) 250 µL of NIPAAm hydrogels prepared by imbibing and (iii) 100 µL injection (all equivalent to 2.5 mg bevacizumab). All the formulations were injected into the posterior part of the PK-Eye model at 37^{o}C (Figure 5:7). The release profile of bevacizumab from the NIPAAm hydrogel prepared by imbibing was similar to its injection form; there was no significant difference ($p>0.05$) in the half-life which was 1.9 ± 0.3 days and 2.3 ± 0.8 for the hydrogel and the injection respectively. However, the hydrogel

prepared by *in situ* polymerising showed an extended release profile. The half-life of bevacizumab was 3.7 ± 1.2 days. The large size of the protein allowed better entanglement during polymerisation compared to the imbibing method.

The *in situ* method was used to prepare hydrogels with different PEGDA stochiometries i.e. 4, 8 and 12 µL. The clinical dose of bevacizumab is (1.25 mg, 50 µL). In previous work with the PK-Eye model, the *in vitro* half-life of bevacizumab (1.25 mg, 50 µL) was reported to be 1.2 ± 0.1 days and the half-life of 2.5 mg of bevacizumab in PBS was 2.3 ± 0.8 days (Awwad et al. 2015). The PK-Eye was used to study the release of the four prepared samples of bevacizumab loaded hydrogels. The injection of bevacizumab solution (2.5 mg, 100 µL) and bevacizumab loaded hydrogels (4 µL. 8 µL and 12 µL) (2.5 mg, 100 µL) were investigated after injection in the posterior cavity of the model. After polymerisation, no effort was made to remove the unreacted monomer by washing as it was important to first determine if there was an extended release profile compared to bevacizumab alone.

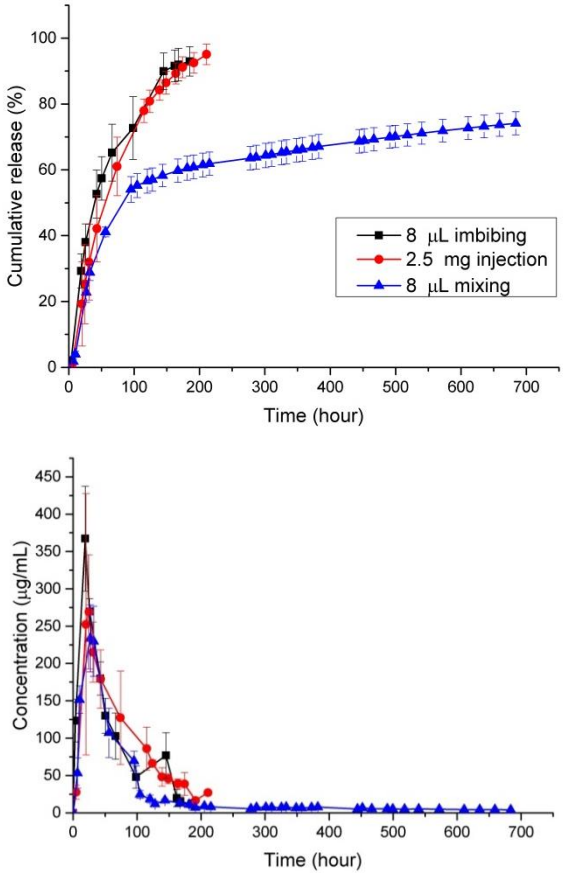


Figure 5:7 A graph representing the release of bevacizumab after injecting 100 µL of NIPAAM hydrogel prepared by mixing, 250 µL of NIPAAm hydrogels prepared by imbibing and 100 µL injection (all equivalent to 2.5 mg bevacizumab) into the PK-Eye. There was no significant difference ($p>0.05$) in the half-life of bevacizumab from the injection and imbibing method, the half-life was 1.9 ± 0.3 days.

A half-life of 2.0 ± 0.01, 3.7 ± 1.2 and 2.60 ± 0.03 days was observed with 4, 8 and 12 µL PEGDA respectively with a protein release of ~74%, ~87% and 95% respectively after a month (Table 5:3). The release of bevacizumab was studied for one month to compare it with the monthly injection administered in the clinic. After 10 days ~61.9 ± 3.6%, 63.2 ± 8.3% and 75.0 ± 1.9% of bevacizumab was released from 4, 8 and 12 µL PEGDA respectively and this is in contrast with the injection of the free antibody which was nearly cleared within 10 days with about 95.1% ± 3.1 of the injected dose having been released. There was no significant difference in release ($p>0.05$) between 4 and 8 µL PEGDA but there was a significant difference when compared to 12 µL PEGDA ($p<0.05$). Bevacizumab was released faster from 12 µL PEGDA hydrogels.

Table 5:3 Summary of *in vitro* bevacizumab release from the PK-Eye. Bevacizumab (2.5 mg, 100 µL) injection and NIPAAm hydrogels of bevacizumab (2.5 mg, 100 µL) made with PEGDA (4 µL. 8 µL and 12 µL) were injected via the injection port of PK-Eye model.

Bevacizumab	Rate constant (d^{1})	Half-life (days)	Drug released (%) after one month
(2.5 mg, 100 µL) injection	0.32 ± 0.09	2.3 ± 0.8	95.1 ± 3.1
(2.5 mg, 100 µL) 4 µL	0.350 ± 0.002	2.0 ± 0.01	74.2 ± 0.3
(2.5 mg, 100 µL) 8 µL	0.20 ± 0.06	3.7 ± 1.2	87.6 ± 6.4
(2.5 mg, 100 µL) 12 µL	0.270 ± 0.003	2.60 ± 0.03	95.8 ± 2.2

Abbreviations: *k*: rate constant.

The release of bevacizumab followed a bimodal display profile from the NIPAAM hydrogels (Figure 5:8A), whereas the free antibody followed a first order kinetic profile. All three hydrogels showed a burst release after a week followed by a decrease in protein concentration with time. The burst release is thought to be a result of the immediate collapse of the hydrogel at 37^{o}C. After a week, the profile was close to zero-order kinetics, which is characterised by a constant release with time (Figure 5:8B).

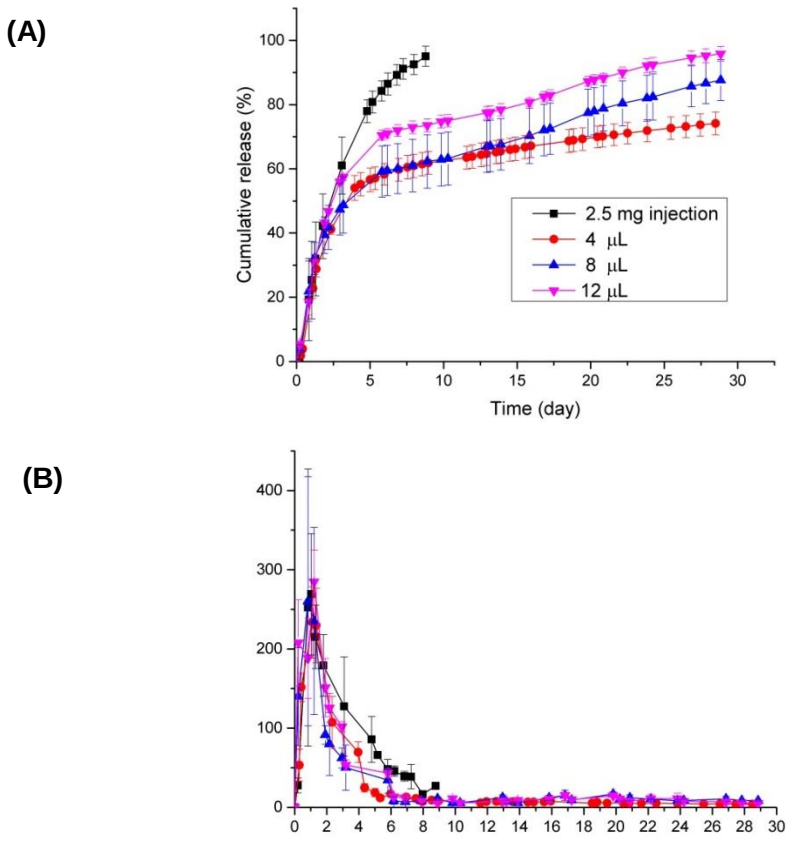


Figure 5:8 The release profile of Bevacizumab (2.5 mg) injection and NIPAAm hydrogels made with different amounts of PEGDA (4, 8 and 12 µL) in PBS, pH 7.4 with half-life values of 2.01 ± 0.01 days, 3.66 ± 1.21 days and 2.55 ± 0.03 days respectively for (4, 8 and 12 µL) respectively.

Protein release rate is controlled by (i) the initial burst release during collapsing of the hydrogel above VPTT, (ii) the pore size of the hydrogel formed after collapse and (iii) the amount of water retained in the hydrogel structure after collapse. Antibody release which occurs upon hydrogel collapse will cause a burst release. Once the hydrogel collapses, the pore size within the hydrogel would be expected to be less than when the hydrogel is swollen with water. The remaining protein would then be expected to be entrapped within the collapsed hydrogel resulting in a more sustained release profile. There will also be less entrapped water with a higher relative amount of bound water which could further slow the diffusion of the protein through the hydrogel. Increased cross-link density appeared to give a higher burst with little difference in the rate of release for the entrapped fraction (Figure 5.8). Burst release is expected with thermoresponsive hydrogels due to the expulsion of water during collapsing at VPTT. Derwent and Mieler reported an immediate burst release of BSA and IgG from NIPAAm hydrogels made with different amounts of PEGDA-575 as cross-linker. The different amounts of cross-linker used had no effect on the burst release (Kang Derwent & Mieler 2008).

NIPAAm hydrogels made with 4 and 8 µL PEGDA showed similar characteristics, behaviour and sustained the release of protein to the same extent *in vitro*. At 25^{o}C the SR of 4 µL is higher than 8 µL, which may cause the loss of higher percentage of the protein during washing. Washing is required to remove the unreacted monomers from the hydrogel before injection. Also hydrogels made with 4 µL is very soft and difficult to handle. Based on these observations 8 µL PEGDA was chosen as the best formulation to sustain the release of bevacizumab in the posterior segment.

Effect of protein on the physical properties of the loaded hydrogels

Encapsulating or entangling a large molecule during the polymerisation process when formulating a hydrogel may affect the physical properties of the prepared hydrogels. A more macroporous and hetrogenous network structure of cross-linked NIPAAm hydrogel is expected when the hydrogel is prepared in the presence of a large molecule to be entrapped. Zhang *et al* reported that a macroporous NIPAAm hydrogel was prepared when polymerised in the presence of PEG. SEM images demonstrated larger pores in the hydrogel matrix when the MW of PEG increased from 300 to 2000 and the SR at 25^{o}C was also increased (Zhang et al. 2001).

The physical properties of the 8 µL PEGDA cross-linked NIPAAm hydrogels polymerised in the presence of bevacizumab were evaluated. The protein loaded hydrogels were characterised regarding VPTT, SR and WR%. VPTT shifted from 35.5 ± 0.1^{o}C for the empty hydrogel to a lower value of 34.2 ± 0.5^{o}C for the bevacizumab loaded hydrogels, which was still well below the physiological temperature (Figure 5:9). Proteins will compete with water molecules to occupy the internal volume of a hydrogel and less water will be entrapped resulting in reduced formation of hydrophilic bonds. Less energy (which indicates lower VPTT) will be required for the hydrophobic interactions to predominate over the hydrophilic ones.

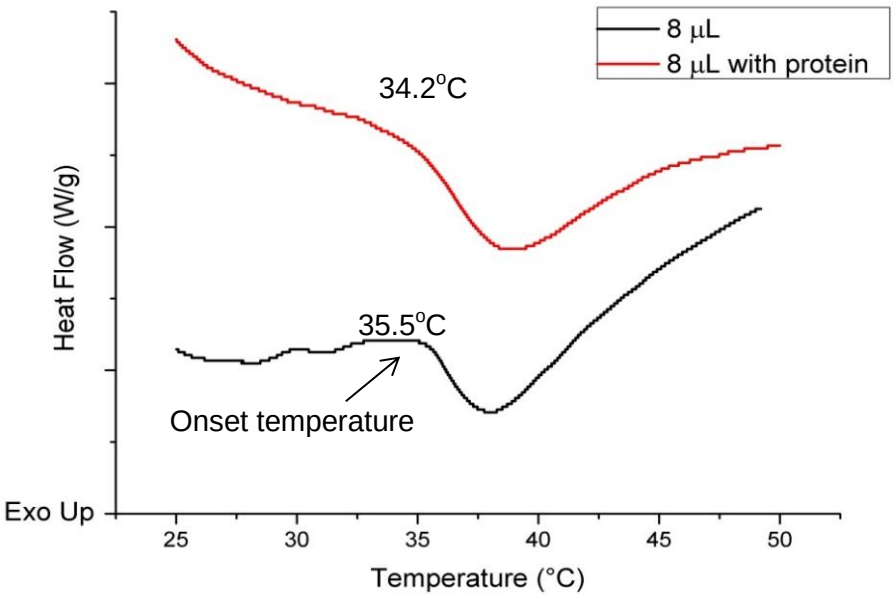


Figure 5:9 DSC graph on the effect of the protein on VPTT of 8 µL NIPAAm hydrogel. The VPTT shifted from 35.5 ± 0.1°C for the unloaded hydrogel to 34.2 ± 0.5°C when bevacizumab was loaded.

The SR of bevacizumab loaded hydrogels were 34.13 ± 2.05, 3.2 ± 0.8 and 1.1 ± 0.3 at 25, 37 and 48°C respectively. There was a significant difference in SR at 25°C ($p<0.05$) between the bevacizumab loaded and unloaded hydrogel. However, there was no significant difference in the SR at 37 and 48°C ($p>0.05$) (Figure 5:10A). Hydrogels polymerised in the presence of bevacizumab at 25°C were denser and opaque compared to empty hydrogels; however, when they collapsed at 37°C they were both opaque and white in colour.

The difference in swelling at 25°C could be related to the MW of bevacizumab. Bevacizumab is a large molecule and when incorporated inside the hydrogel during the polymerisation process will be entangled into the polymer matrix. The entangled protein will restrict the movement of the polymer chains and occupy the space that is usually occupied by the free water. This will restrict the swelling of the hydrogel at 25°C. At 37°C even the presence of a large molecule did not have an impact on SR and did not alter the behaviour of the hydrogel.

Since proteins are hydrophilic in nature they will not have an impact on hydrophobic bonds formation. Therefore, the deswelling of NIPAAM hydrogels will not be affected by the presence of protein. This was also confirmed by the results of WR%, which showed no significant difference for both loaded and unloaded hydrogels because ($p>0.05$) (Figure 5:10B). The SEM images showed a relatively denser and more compact structure with the protein loaded hydrogels as compared to unloaded hydrogels at 25°C (Figure 5:10C).

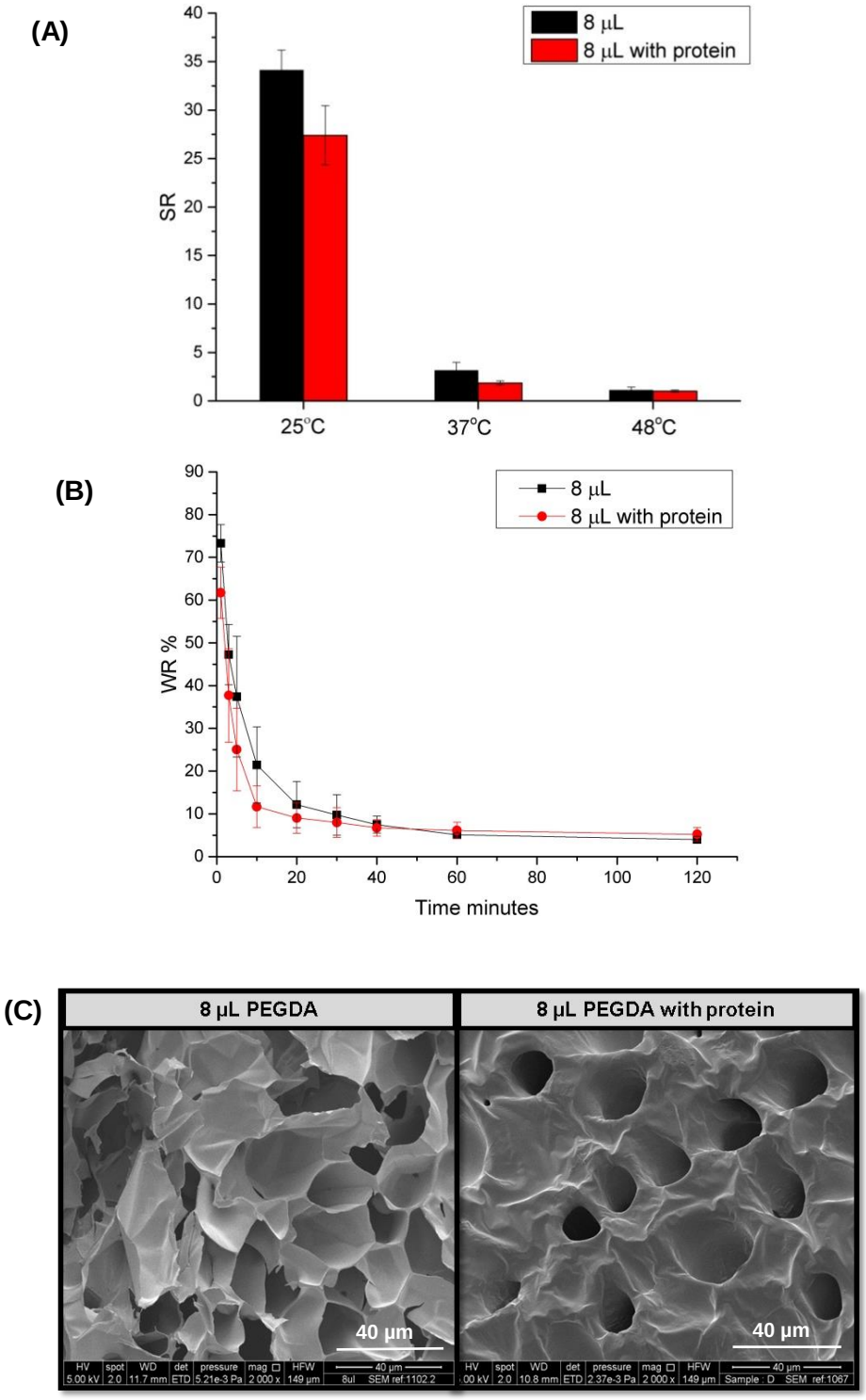


Figure 5:10 A) A graph demonstrating the effect of loaded bevacizumab on SR of 8 µL NIPAAm hydrogel. A significant difference in SR at 25°C ($p<0.05$) was observed between bevacizumab loaded and unloaded hydrogel, no significant difference was observed at 37 and 48°C ($p>0.05$); B) A graph demonstrating the WR% of loaded and unloaded hydrogels. There was no significant difference observed in WR% ($p>0.05$) in hydrogels with and without the protein; C) Scanning electron microscopy (SEM) images of NIPAAm hydrogels with and without protein made with 8 µL PEGDA. The structure of the loaded hydrogels was relatively denser and more compact compared to unloaded hydrogels at 25oC. The scale bar is 40 µm.

Washing the hydrogel by some process would be an important step to remove unreacted monomers, which can be toxic if tested *in vivo*. Contact lenses are washed after fabrication to remove monomeric and oligomeric material. In the case of an antibody-loaded hydrogel, it was expected that some of the antibody would be lost during the wash step.

To study the effects of washing the hydrogels on the release profile and percentage of protein lost during washing, bevacizumab NIPAAm hydrogels were prepared using 8 µL PEGDA. After polymerisation, the hydrogels were rinsed gently 5 times, 4.0 mL each time in PBS. Each wash was 15 minutes long. Each wash fraction was analysed by HPLC to calculate how much of the antibody was lost during the wash. It was calculated to be ~28% (~18.4 mg remaining). In a 100 µL volume, the final concentration of bevacizumab after washing was ~1.84 mg/100 µL. To compare washed and unwashed hydrogels, approximately 125 µL of the washed gel (equivalent to ~2.3 mg of bevacizumab) was injected into the posterior cavity of the model with PBS. A half-life of 3.1 ± 1.3 days in PBS was observed with the washed hydrogel, which was not significantly different ($p>0.05$) from the unwashed hydrogel i.e. 3.7 ± 1.2 days (Figure 5:11).

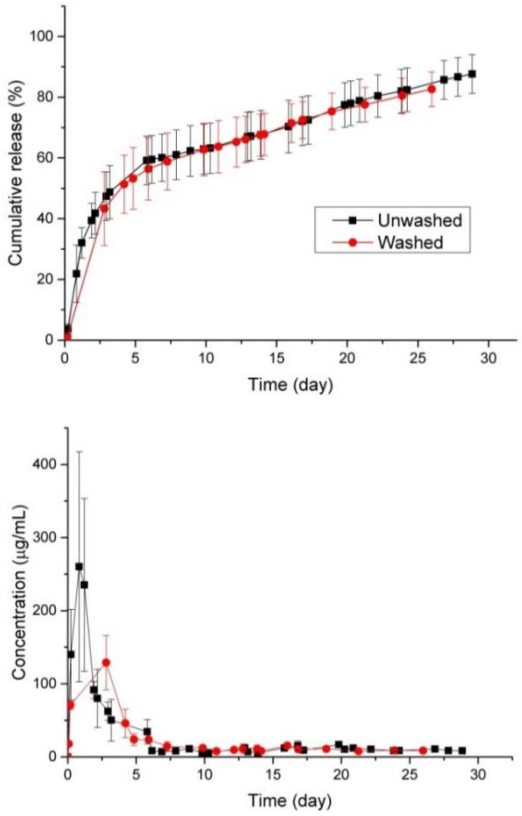


Figure 5:11 The release profile of unwashed and washed bevacizumab 8 µL NIPAAm hydrogels in PBS, pH 7.4. The half-life of unwashed and washed hydrogels was 3.7 ± 1.2 and 3.13 ± 1.3 days respectively. No significant difference ($p>0.05$) was observed in the half-life between washed and unwashed hydrogels.

The release profiles of bevacizumab from washed and unwashed hydrogels were similar. However, the concentration of bevacizumab measured during the first 3 days was lower in washed hydrogels compared to the unwashed ones. During polymerisation some of the protein will be entrapped close to the surface and they will be washed when the monomers were washed. Removal of the protein close to the surface will reduce the initial burst release associated with the hydrogel (Figure 5:11).

Incorporation of phosphorylcholine PC into NIPAAm hydrogels

One of the advantages of using PEGDA as the cross-linker to make the NIPAAm hydrogels is the biocompatibility of the PEG structure. The biocompatibility of the NIPAAM hydrogel as currently made is important because these hydrogels would be expected to be nondegradable in the body remaining in contact with tissues for a long period. As discussed in chapter 1, phosphorylcholine (PC) containing hydrogels have the advantage of increased biocompatibility due to the presence of zwitterionic pendant chain that resembles cell membranes.

Chang *et al* reported that NIPAAm hydrogels made by copolymerisation of NIPAAm and zwitterionic sulfobetaine methacrylate (SBMA) shows improved biocompatibility over standard NIPAAm hydrogels. When the absorption of three major plasma proteins (γ-globulin, fibrinogen, and human serum albumin) was compared between NIPAAm hydrogels and NIPAAm-co-SBMA hydrogels, the relative protein adsorption on the surface was reduced below 12% for the copolymer hydrogel. Also blood platelet activated and adhered on the NIPAAm hydrogel surface was considerably reduced in the presence of zwitterionic groups compared to NIPAAm hydrogels with no zwitterionic groups (Chang et al. 2010).

MPC (similar to sulfobetaine) belongs to the family of zwitterionic monomers. To explore the possibility of copolymerisation of MPC with NIPAAm for the synthesis of MPC-co-NIPAAm thermoresponsive hydrogel, NIPAAm formulation was prepared with 8 µL PEGDA and 5 mg of MPC was added to the formulation mixture before polymerisation. After polymerisation a transparent hydrogel was formed; however when the VPTT was measured no thermoresponsiveness was observed. When the amount of MPC was reduced to 1 mg there was a slight response to temperature changes (Figure 5:12). Although there was a thermal responsiveness when 1 mg MPC was used, it was very weak and the VPTT was 36.3 ± 0.3 ^{o}C, which was very close to body temperature. It was concluded that this formulation was not thermally responsive. A possible explanation is that a combination of PEGDA and MPC form strong hydrogen bonds with water. The

increased number of hydrophilic interactions on the polymer chains will require higher temperatures to disturb the hydrophilic lipophilic balance causing the hydrogel to collapse.

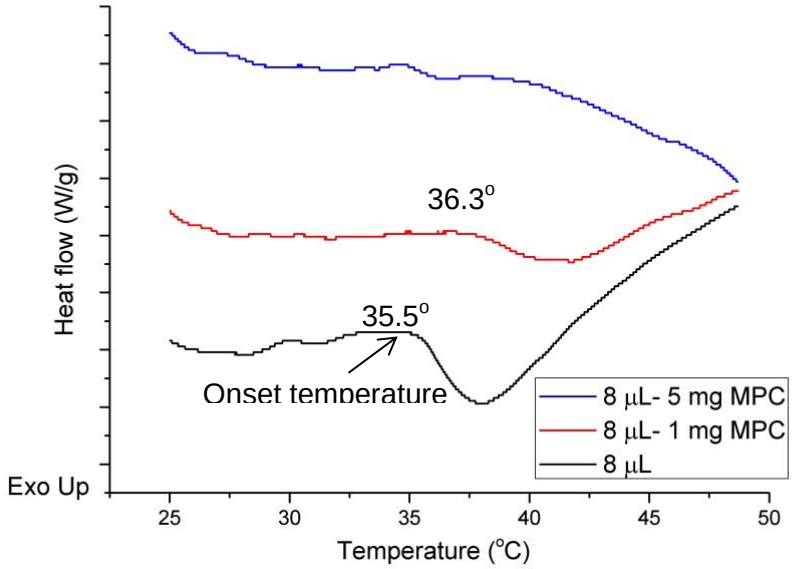


Figure 5:12 DSC graph representing the VPTT of 8 µL hydrogels prepared with 5 mg or 1 mg MPC compared to NIPAAm without MPC. There was no thermal responsiveness when 5 mg MPC was added and the VPTT was 36.3 ± 0.3 when 1 mg MPC was added compared to 35.5 ± 0.09; however the VPTT was close to body temperature and the onset of temperature changes was not sharp.

In order to add PC zwitterionic group into the NIPAAm hydrogels and maintain the thermal responsiveness, PEGDA was replaced with PC bearing cross-linker. PC cross-linker is described in the literature and used to improve the properties of hydrogels (Kiritoshi & Ishihara 2004). US patent application number by Michael Driver also describes the synthesis of different acrylated PC molecules for various applications (Driver 2013).

A proprietary PC cross-linker (PC 3059) was supplied by Vertellus biomaterial (UK) for use in this work. 8 µL PEGDA as cross-linker in NIPAAm hydrogels was replaced with equivalent weight of PC as cross-linker. When PEGDA was replaced with equivalent weight of PC (9 mg which is equivalent to 13% (w/w)) no hydrogel was formed. The mixture solution remained as free flowing liquid after the addition of TEMED. The reaction mixture remained liquid even when the amount of PC increased to 15 mg (equivalent to 20% (w/w)). A hydrogel was only formed when the amount of PC added was 20 mg (equivalent to 25% (w/w)).

Effect of cross-linker change on the behaviour of NIPAAm hydrogel

It is expected that many important properties of NIPAAm hydrogel would be affected by the dramatic change of cross-linker from PEGDA to a PC derived macromolecular cross-linker. To study whether replacing PEGDA with a PC derived cross-linker would have an impact; hydrogels made with 8 µL PEGDA and 20 mg PC (equivalent to 25% (w/w)) were prepared and compared in respect to VPTT, SR, WR% and *in vitro* release. To eliminate the effect of any other factors, except the cross-linker type, on the behaviour of the hydrogels prepared the two hydrogels were prepared in the exact same conditions. A 1 mL bevacizumab solution (25 mg/mL) was used to prepare bevacizumab loaded PC hydrogels, which is equivalent to the loading for bevacizumab loaded PEGDA hydrogels.

Both monomer mixtures for the hydrogels polymerised after 1 minute of TEMED addition. In both cases, the hydrogels were transparent and easily injectable through a 23G needle. With PC, the VPTT shifted to a slightly lower value (33.8 ± 0.1^{o}C) compared to PEGDA-NIPAAm hydrogels (35.5 ± 0.1^{o}C) (Table 5:4). The PC cross-linker was expected to attract more water than PEGDA. The presence of the zwitterionic pendant chain in the PC repeat unit in its structure can either be free or lightly bound water, which requires less energy to break and lower the VPTT. This could be a contributing factor to the sharp decrease in WR% after 5 minutes when PC was used as compared to PEGDA (Figure 5:13). However, the overall behaviour is the same and they both respond by deswelling with an increase in temperature. There was no significant difference ($p>0.05$) in SR between PEGDA and PC hydrogels in all the temperatures tested (25, 37, 48^{o}C).

Table 5:4 Volume phase transition temperature (VPTT) and swelling ratio (SR) at 25, 37 and 48^{o}C of NIPAAm hydrogels made either with 8 µL PEGDA or 20 mg PC 3059. With PC, the VPTT shifted to a slightly lower value (33.8 ± 0.1^{o}C) compared to PEGDA-NIPAAm hydrogels (35.5 ± 0.09^{o}C) There was no significant difference ($p>0.05$) in SR between PEGDA and PC hydrogels in all the temperatures tested.

Cross-linker type	SR			VPTT	Injectability
	25^{o}C	37^{o}C	48^{o}C		
8 µL PEGDA	34 ± 2	3.2 ± 0.8	1.1 ± 0.3	35.5 ± 0.1	Easy
20 mg PC 3059	37.6 ± 3.2	3.8 ± 1.9	1.4 ± 0.3	33.8 ± 0.1	Easy

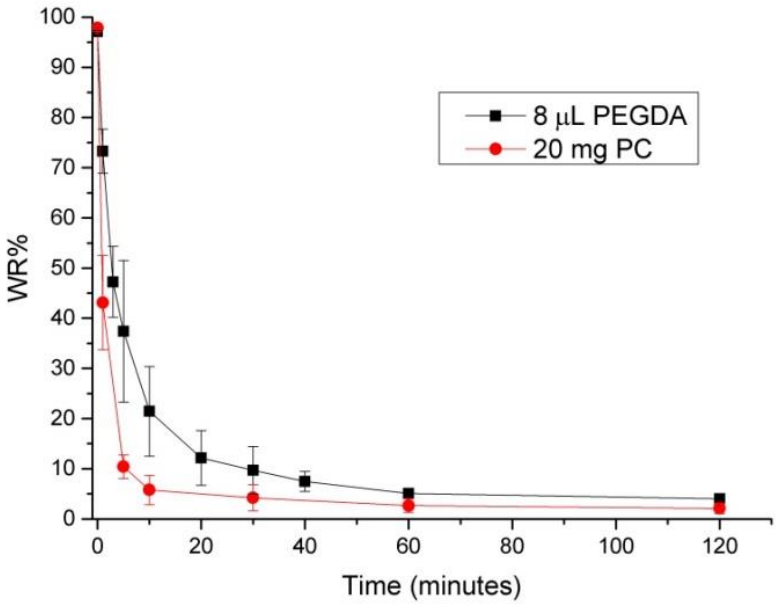


Figure 5:13 The water retention (WR%) of hydrogels synthesised with 12 μL PEGDA and 20 mg PC. A sharp decrease in WR% after 5 minutes when PC was used as compared to PEGDA; however, the overall behaviour is the same (no difference was observed in WR%).

No significant difference ($p>0.05$) was observed in the release of bevacizumab from both hydrogels (Figure 5:14). The release of hydrogel loaded protein is thought to be affected by the amount of water entrapped in the hydrogel structure after collapsing. Both hydrogels swell to the same extent and entrap similar amounts of water at 37^{o}C, which provides the basis for a similar release profile of bevacizumab to occur.

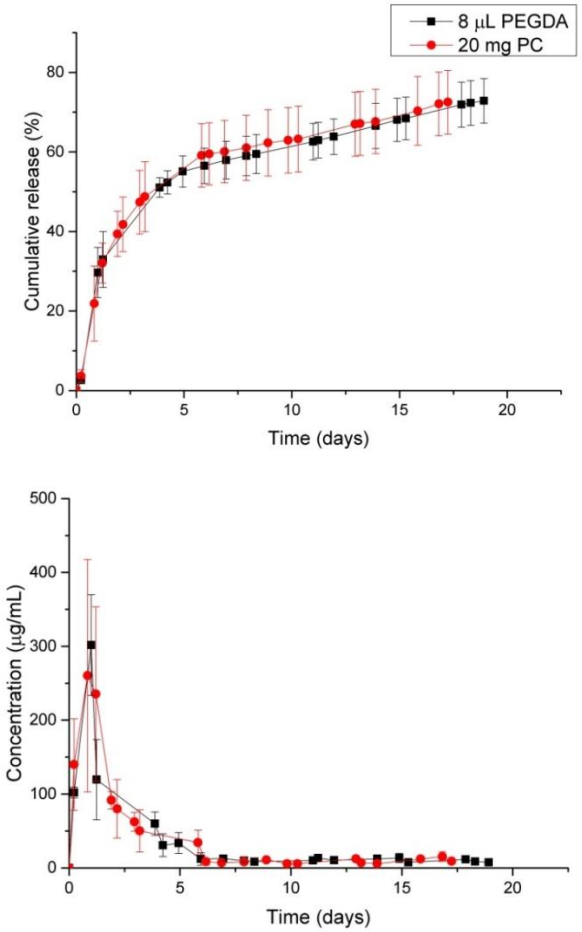


Figure 5:14 The release of bevacizumab from NIPAAm hydrogels made with 20 mg PC cross-linker compared with 8 µL PEGDA cross-linker. No significant difference ($p>0.05$) was observed in percent of drug released and concentration between the two hydrogels.

Effect of bevacizumab on the physical properties of the PC cross-linked hydrogel

From previous experiments it was noted that the presence of the antibody during the polymerisation process had no effect on the physical characteristics of the PEGDA cross-linked NIPAAm hydrogels. The same was observed with PC cross-linked hydrogels. A VPTT of 33.8 ± 0.1°C and 33.3 ± 0.3°C was observed for the empty and bevacizumab loaded hydrogels respectively, which were not significantly difference ($p>0.05$) (Figure 5:15A). The SR of bevacizumab loaded hydrogels was 25.4 ± 1.4, 3.1 ± 1.3 and 1.0 ± 0.2 at 25, 37 and 48°C respectively. There was a significant difference is SR at 25°C ($p<0.05$) between bevacizumab loaded and unloaded PC cross-linked hydrogel but no significant difference ($p>0.05$) was observed at 37 and 48°C (Figure 5:15B). A more compact structure was observed in SEM when the PC cross-linked hydrogel was loaded with bevacizumab (Figure 5:15C).

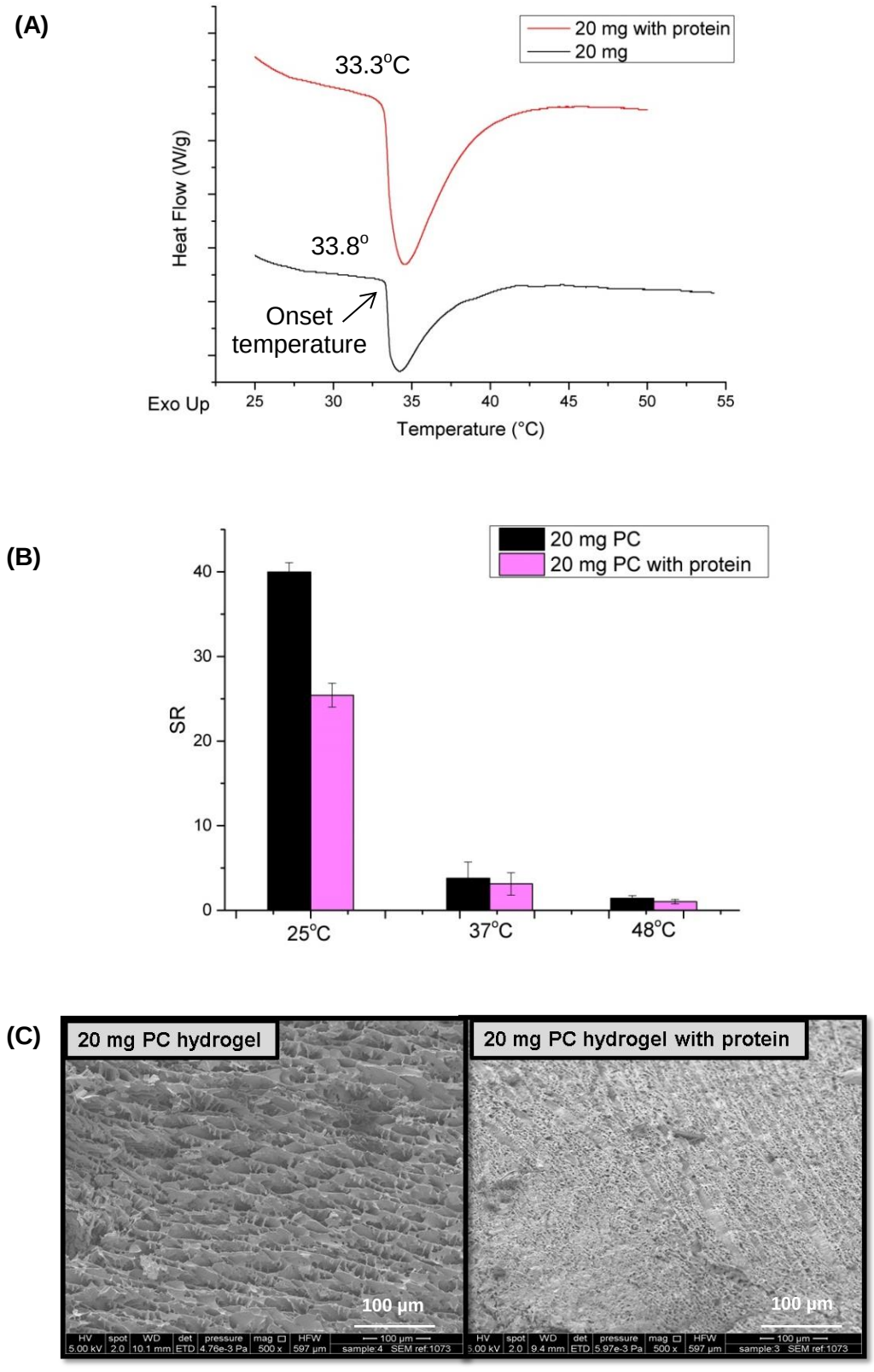
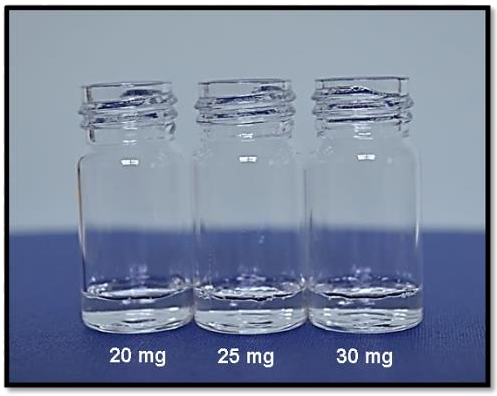


Figure 5:15 The effect of the protein on the physical characterisation of 20 mg PC NIPAAm hydrogel; A) VPTT shifted from 33.8 ± 0.1°C for the unloaded hydrogel to 33.3 ± 0.3°C for the bevacizumab loaded hydrogels; B) A significant difference in SR at 25°C ($p<0.05$) was observed between loaded and unloaded hydrogel, no significant difference was observed at 37 and 48°C ($p>0.05$); C) A more compact structure was observed with loaded hydrogels compared to empty ones. The scale bar is 100 µm.

Effect of increased PC cross-linker on NIPAAm hydrogels

NIPAAm hydrogels formulated with 8 µL PEGDA and 20 mg PC displayed similar behaviour and release characteristics of bevacizumab. When increasing the stoichiometry of PEGDA from 4 to 12 µL the resulting NIPAAm hydrogels became non-injectable. In analogous fashion, the amount of PC cross-linker was increased from 20 mg to 30 mg (equivalent to 33.5% (w/w)). For the preparation of the hydrogels, NIPAAm monomers and APS were dissolved in 1 mL of DW with increasing stoichiometries of PC i.e 20, 25 and 30 mg (equivalent to 25%, 30%, 33.5% (w/w) respectively). The mixture was free flowing liquid in the vial. All the hydrogels transparent and converted to a gel after the addition of TEMED (Figure 5:16A). SEM showed a more compact structure with increased amounts of PC (Figure 5:16B).

(A)



(B)

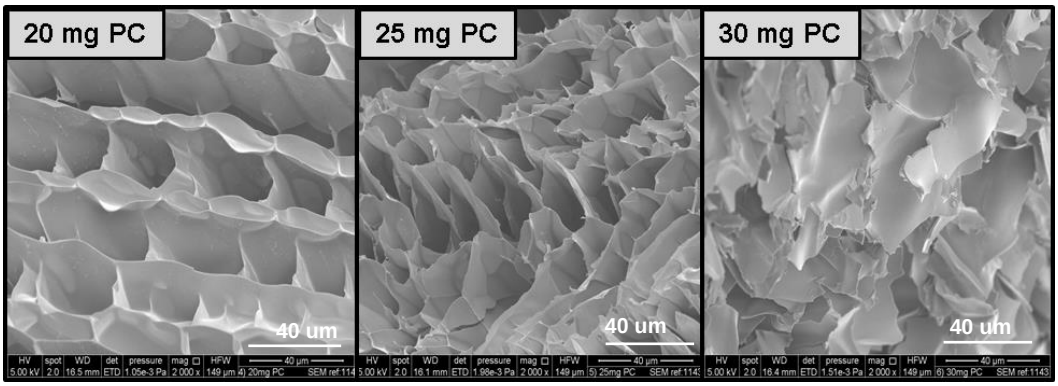


Figure 5:16 A) Picture of the prepared NIPAAm hydrogels using PC 3059 as cross-linker. Gels made with 20, 25 and 30 mg cross-linker are all transparent; B) SEM images of NIPAAm hydrogels shows no difference in structure when the cross-linker percentage increased. The scale bar is 40 µm.

The injectability of each PC hydrogel was also determined qualitatively with a 23G needle (Table 5:5). The hydrogel was injectable with a PC amount up to 30% (w/w), however the gel was difficult to inject above 33.5% (w/w).

Table 5:5 The qualitative determination of injectability of NIPAAm hydrogels synthesised different amounts of PC 3059 using 23G needle.

PC 3059	Injectability
20 mg	Easy
25 mg	Easy
30 mg	Difficult

A successful thermoresponsive hydrogel should have a VPTT below the physiological pH. The VPTT for the PC cross-linked hydrogels was ~34˚C and there was no significant difference ($p>0.05$) in the VPTT between the hydrogels made with the different amounts of PC (Figure 5:17). The endothermic peak remained sharp when the percentage of PC increased which indicates that a higher percentage of PC as cross-linker didn't reduce the thermal responsiveness of the prepared gels.

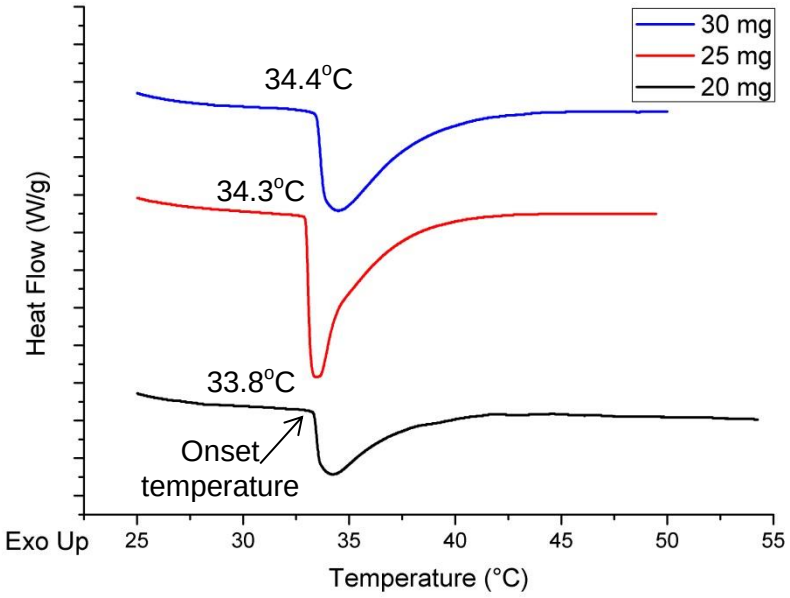


Figure 5:17 Volume phase transition temperature (VPTT) of NIPAAM hydrogels with varying PC 3059 concentrations determined by DSC. The VPTT was 33.8 ± 0.1, 34.3 ± 0.05 and 34.4 ± 0.1 for 20, 25 and 30 mg PC respectively.

The swelling ratio of NIPAAm hydrogels prepared was measured at 25, 37 and 48°C. Similar to PEGDA, there was a significant decrease ($p<0.05$) in SR for all hydrogels when the temperature increased from 25 to 37°C (Table 5:6).

Table 5:6 The SR of NIPAAm hydrogels prepared with different amounts of PC 3059 as cross-linker at three different temperatures (25, 37 and 48^{o}C).

Temperature	SR		
	20 mg	25 mg	30 mg
25^{o}C	37.6 ± 3.2	26.5 ± 4.7	23.0 ± 2.0
37^{o}C	3.8 ± 1.9	5.2 ± 1.8	5.1 ± 0.5
48^{o}C	1.4 ± 0.3	2.4 ± 1.3	2.8 ± 1.3

At 25^{o}C there was a significant difference ($p<0.05$) in the SR between the 20 mg, and 25 and 30 mg PC cross-linked hydrogels. No significant difference was observed ($p>0.05$) between 25 and 30 mg PC hydrogels. This is consistent with the results observed when PEGDA. Higher cross-linking density is associated with less swelling in the surrounding medium. At 37 and 48^{o}C, no significant difference was observed in any of the hydrogels ($p>0.05$) (Figure 5:18A). The behaviour of the PC cross-linked hydrogels is different compared to PEGDA cross-linked hydrogels at 37^{o}C. This is probably related to the nature of the cross-linkers and the interaction between the cross-linker and water. While it is known that PEGDA can form hydrogen bonds with water, PC groups attract free water around the PC polymer chains. The visual difference in swelling of the hydrogels at 25 and 37^{o}C is presented in (Figure 5:18B).

The effect of PC on WR% was also measured. Higher amounts of PC results in higher water entrapment inside the hydrogel (Figure 5:18C) due to the presence of PC head group. The same pattern was also noticed with PEGDA. This indicates that hydrogels made with a hydrophilic cross-linker behave the same way regarding water content and swelling.

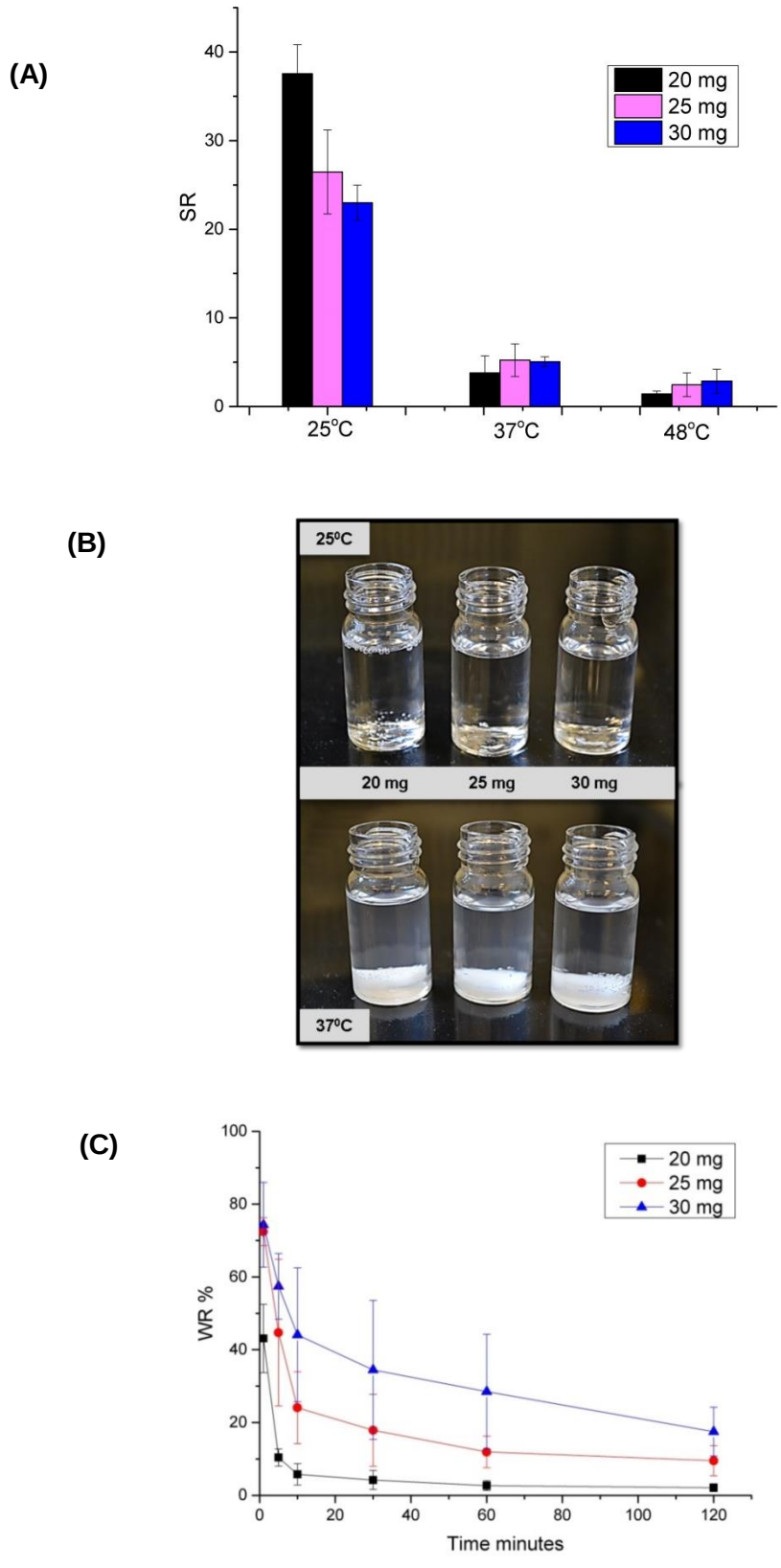


Figure 5:18 A) The swelling ratio SR of NIPAAm hydrogels prepared with various amounts of PC 0598 as cross-linker. The SR was measured at three different temperatures (25, 37 and 48°C); B) Pictures of the hydrogels at 25 and 37°C; C) The WR% of PNIPAAm hydrogels prepared with various amounts of PC 3059 as cross-linker.

NIPAAm hydrogels made with 20, 25 and 30 mg (equivalent to 25, 30 and 33.5% (w/w) respectively) PC were screened regarding physical properties of the formulated hydrogels and the effect of PC cross-linker on the general behaviour of NIPAAm hydrogels. Although the NIPAAm hydrogel made with 33.5% (w/w) PC was thermoresponsive, it was difficult to inject this hydrogel. Hydrogels made with 25% and 30% (w/w) PC showed similar characteristics and thermal behaviour when compared to PEGDA.

Acrylated hyaluronic acid (Ac-HA) cross-linker in NIPAAm hydrogel synthesis

Most of the non-biodegradable delivery systems for posterior segment require removal after the drug is fully released. There are some exceptions where there is no need to remove the delivery system when the drug is fully released, for example Iluvien®. Iluvien® is a small, non-biodegradable, injectable polymer drug matrix that release fluocinolone acetonide in the vitreous up to 3 years for the treatment of diabetic macular edema (Kane et al. 2008).

NIPAAm hydrogels made with PEGDA-700 or PC 3059 displayed a sustained release of bevacizumab for more than a month; however they are non-biodegradable. Turturro *et al* reported that only a small transient effect was observed in the retinal functions during the first few days after injection of NIPAAm hydrogel and the data returned to normal base line after one week of injection (Turturro et al. 2011). Surgery may be required to remove the empty hydrogels when several injections are used and the empty hydrogels starts to accumulate in the vitreous and affect the ocular tissues. To avoid surgery in these cases, the use of biodegradable cross-linker for NIPAAm hydrogel synthesis could be an option. Biodegradable NIPAAm hydrogels were reported to be synthesised by incorporating a biodegradable monomer unit into the polymer backbone structure or cross-linker (Fujimoto et al. 2009; Zheng et al. 2015; Huang & Lowe 2005). Lee and Cheng reported the synthesis of biodegradable NIPAAm hydrogels using biodegradable polycaprolactone diacrylate as cross-linker (Wang & Li 2011). To study the possibility of using a biodegradable cross-linker and its effect on the physical and chemical properties of the hydrogel, NIPAAm hydrogel with hyaluronic acid (HA) as cross-linker was prepared and analysed.

Hyaluronic acid (HA) is natural polysaccharide polymer with repeating units of D-glucuronic acid and N-acetyl-D-glucosamine disaccharide. It is a common component in many parts of the human body such as extracellular matrix, synovial fluid and the vitreous humour. It has the ability to absorb thousand times its dry weight of water (Kogan et al. 2007; Mero & Campisi 2014). It is a widely

investigated and used component for drug delivery due to its biodegradability and biocompatibility (Figure 5:19A). The commercially available form of HA is called sodium hyaluronate and it is used for the synthesis of eye drops, eye gels and injections (Pavelka & Uebelhart 2011; Widjaja et al. 2014; Mayol et al. 2008).

In our lab an acrylated HA (Ac-HA 50 kDa) molecule was synthesised to be used as biodegradable hydrophilic cross-linker for NIPAAm hydrogel synthesis. The same method and percentages of monomers used for the preparation of PEGDA and PC NIPAAm hydrogels was used for the synthesis of Ac-HA NIPAAm hydrogels. Different amounts of Ac-HA were used for the hydrogel synthesis (2, 4, 7 and 10 mg which represents 3.2%, 6.3%, 10.5% and 14.4% (w/w) respectively). The monomer mixtures start to gel approximately one minute after the addition of TEMED (Figure 5:19B).

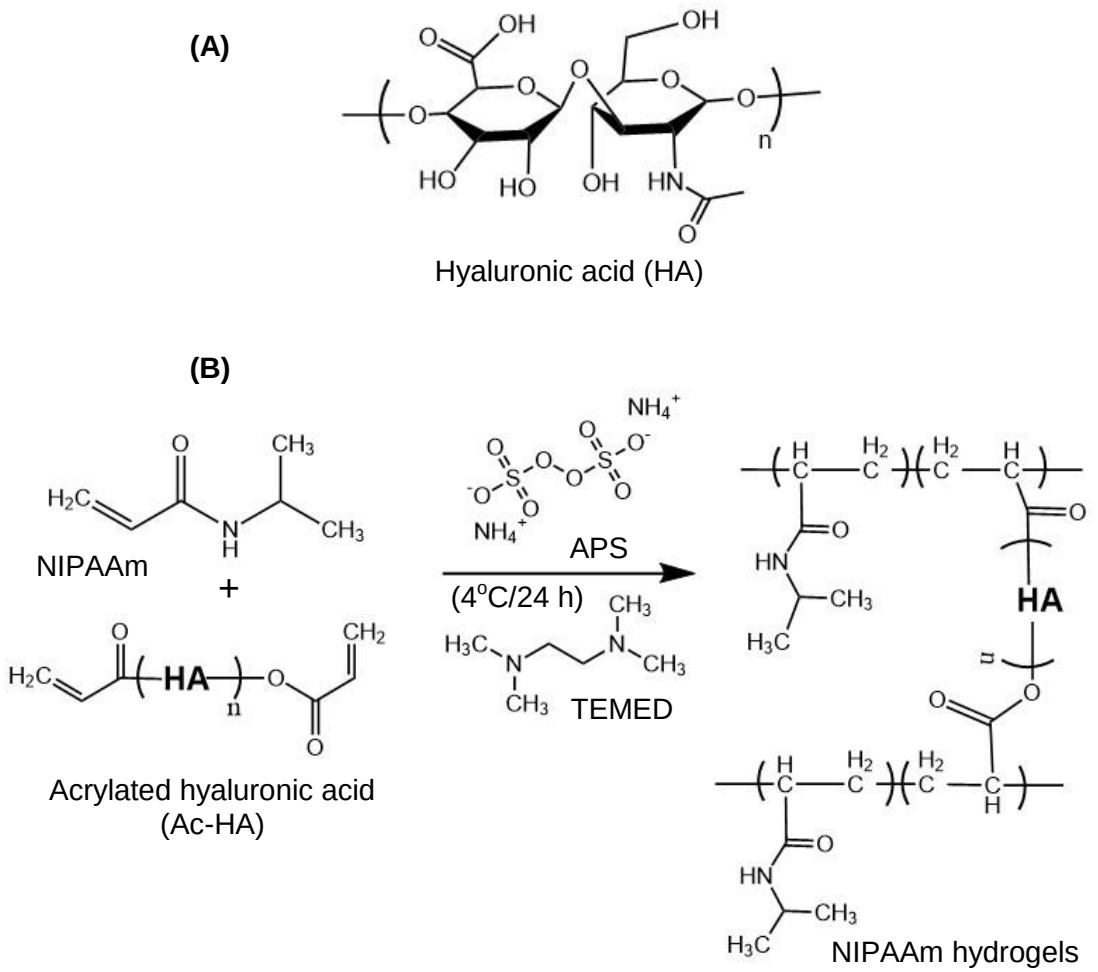
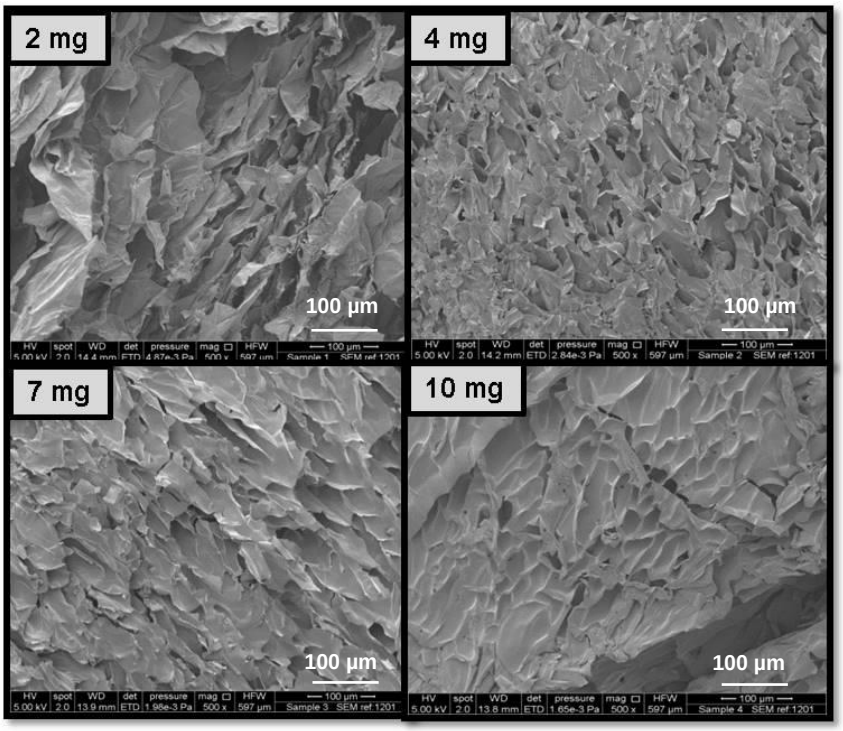


Figure 5:19 A) The chemical structure of hyaluronic acid (HA), B) the starting materials for NIPAAm hydrogel formulation and the hydrogels formed. NIPAAm hydrogels were prepared with different percentages of Ac-HA as cross-linker by free radical polymerisation.

The injectability of the hydrogels was determined using a 23G needle. Hydrogels with 7 and 10 mg Ac-HA were very hard to inject and displayed VPTT values very close to body temperature with no significant difference in SR (from 25^{o}C to 37^{o}C). Similar trend to PEGDA and PC3059 in SR at 25^{o}C was observed; when the cross-linker amount increased the SR reduced which can be observed by the SEM images of the freeze dried hydrogels (Figure 5:20A). Hydrogels with 2 and 4 mg showed thermal responsiveness and both were injectable (Figure 5:20B and Table 5:7).

(A)



(B)

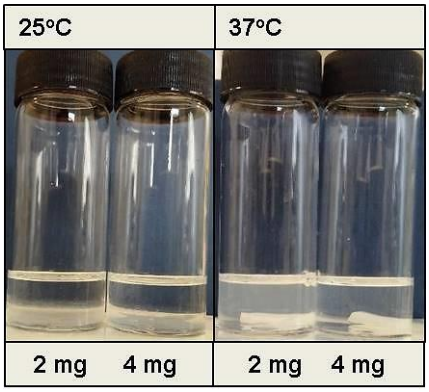


Figure 5:20 A) SEM images of NIPAAm hydrogels made with different amounts of Ac-HA. Denser hydrogels were prepared with increasing Ac-HA amount. Scale bar is 100; B) Pictures of the hydrogels at 25 and 37^{o}C. The hydrogels at 25 are transparent and became opaque at 37^{o}C.

Table 5:7 Different physical properties of NIPAAm hydrogels made with different amounts of Ac-HA. All data presented was done in triplicate (n=3) and presented as mean and standard deviation (± STD) except for VPTT (one experiment).

Ac-HA mg	VPTT ^{o}C	SR		Injectability
		25^{o}C	37^{o}C	
2	33.7	165.4 ± 2.9	102 ± 8	Easy
4	34.5	105.1 ± 8.4	94.1 ± 5.9	Easy
7	36.4	79.8 ± 2.9	78.2 ± 3.4	Difficult
10	36.9	62.5 ± 4.8	60.2 ± 0.7	Non-injectable

NIPAAm hydrogels made with 2 and 4 mg Ac-HA were loaded with infliximab to study the release of the protein. Infliximab was used instead of bevacizumab because of the lack of resources for bevacizumab (Infliximab and bevacizumab have the same MW 150 kDa). The aim was to prepare Ac-HA NIPAAm hydrogel with 10 mg infliximab by dissolving AC-HA, NIPAAm and APS in 1 mL of protein solution (9.4 mg/mL); however the AC-HA was hard to dissolve and properly mixed with the protein solution. The reduction in solubility of HA could be attributed to the addition of acrylate groups in the HA structure. OH groups were replaced with acrylate which possibly affected the solubility by reducing the groups available for hydrogen bond formation. Proper mixing between the monomers and the protein is important to achieve uniform distribution and proper entangling of the protein. To help in the mixing process Ac-HA, NIPAAm and APS were first dissolved in 200 µL of PBS. When a clear solution was formed 1 mL of protein solution was added taking the total volume into 1.2 mL. A hydrogel was formed after addition of TEMED with a concentration of protein 7.8 mg/mL. The same washing process used for PEGDA NIPAAm hydrogel was used for washing Ac-HA NIPAAm hydrogels.

During washing the amount of drug lost was quantified. After washing, hydrogels with 2 and 4 mg lost 2.5 mg ± 0.2 (~26.7%) and 1.8 mg ± 0.2 (~19.5%) of the loaded protein respectively. The final concentration of protein in the hydrogels was 5.8 mg/mL and 6.3 mg/mL for 2 mg and 4 mg AC-HA NIPAAm hydrogels respectively. The amount of protein lost during washing of 4 mg hydrogels was significantly lower than the amount lost during washing 2 mg hydrogels. This was expected because the SR of hydrogels made with 2 mg was significantly higher than the SR of 4 mg hydrogels. Based on these findings hydrogel with 4 mg cross-linker was further characterised for release studies.

The release of 4 mg AC-HA NIPAAm hydrogels from the PK-Eye model was studied and compared with bevacizumab. The amount of infliximab injected was 1.1 mg (170 µL) of the hydrogel and after 10 days only ~25% of the drug was released

compared to ~60% from 8 µL PEGDA and PC NIPAAm hydrogels (Figure 5:21). The large MW of the HA provided a proper entangling to the protein and created another barrier for the diffusion of the protein from the collapsed hydrogel.

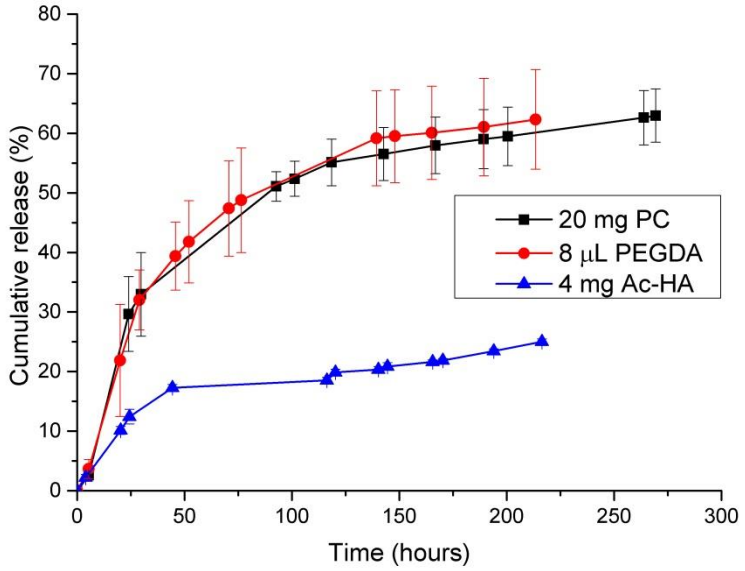


Figure 5:21 A graph comparing the release of bevacizumab and infliximab from NIPAAm hydrogels made with different cross-linkers. After 10 days only ~25% of the drug was released from NIPAAm hydrogels made with Ac-HA compared to ~60% from 8 µL PEGDA and PC NIPAAm hydrogels.

In hydrogels made with PC and PEGDA the presence of protein did not have a major effect on the overall physicochemical properties of the hydrogels except for swelling at 25°C, it was significantly lower in the presence of protein. With AC-HA as cross-linker the hydrogel was still injectable after loading with the protein and the VPTT was 35.5°C compared to 34.5°C for the empty hydrogel. However, with Ac-HA there was a significant difference is SR not only at 25°C but also at 37°C (Table 5:8). HA is a viscous material that is used to increase viscosity of solutions. When used as cross-linker the viscosity of the hydrogel will increase reducing the SR significantly at 25°C. At 37°C the higher viscosity of HA may also prevent complete collapse of the hydrogel.

Table 5:8 Different physical properties of NIPAAm hydrogels with and without infliximab.

Ac-HA NIPAAm hydrogel	VPTT °C	SR		Injectability
		25°C	37°C	
Empty hydrogel	34.5	105.1 ± 8.4	94.1 ± 5.9	Easy
Infliximab loaded hydrogel	35.5	33 ± 6	20.3 ± 3	Easy

When comparing SR of hydrogels made with PEGDA or PC 3059 vs hydrogels made with Ac-HA at 25°C and 37°C, a significant difference was observed (Table 5:9). The structure of HA molecule is dominated by the presence of OH groups that are capable of forming hydrogel bonds with water. The combined effect of large MW of Ac-HA (50 kDa) and the abundance of OH group will attract large amount of water and increase the swelling of the hydrogel compared with PEGDA and PC (PEGDA Mn is 700 Da).

Table 5:9 The SR of NIPAAm hydrogels prepared with different hydrophilic cross-linkers.

Cross-linker type	SR	
	25°C	37°C
8 µL PEGDA	34 ± 2	3.2 ± 0.8
20 mg PC 3059	37.6 ± 3.2	3.8 ± 1.9
4 mg Ac-HA	105.1 ± 8.4	94.1 ± 5.9

NIPAAm hydrogels made with 2, 4, 7 and 10 mg Ac-HA were screened regarding physical properties of the prepared hydrogels and the effect of cross-linker amount on the general behaviour of NIPAAm hydrogels. Hydrogels made with 7 and 10 mg Ac-HA were non injectable and non thermoresponsive. Although hydrogel made with 2 and 4 mg Ac-HA were injectable and thermoresponsive, only 4 mg hydrogels were used for release studies. Higher percentage of the loaded protein lost during the washing of unreacted monomers from 2 mg hydrogels compared to 4 mg. Only ~25% of the loaded protein was released after 10 days. NIPAAm hydrogels made with 4 mg Ac-HA are good candidates for developing sustained formulation for protein delivery.

Summary and conclusions

NIPAAm thermoresponsive hydrogels were prepared by free radical polymerisation using different macromolecular hydrophilic cross-linkers i.e. PEGDA, PC 3059 and Ac-HA. The *in vitro* release of an antibody was studied in the PK-Eye model. This *in vitro* model that mimics the aqueous flow of the human eye provides a good estimate of antibody clearance from the vitreous body. NIPAAm hydrogels (derived from 40 mg of NIPAAm monomer) containing 4, 8, 12 and 15 µL PEGDA were prepared and characterised regarding their physical properties. With larger amounts of PEGDA, the hydrogels were less thermoresponsive and retained larger amounts of water in at 37°C. In addition, the injectability of these hydrogels also reduced. No significant difference was observed between 4 and 8 µL cross-linker regarding SR, VPTT and WR%.

In vitro studies in the PK-Eye showed a bimodal release profile characterised by an initial burst followed by a zero-order release after a week for one month. No significant difference was observed with the different cross-linker stochiometries. The presence of protein during polymerisation did not appear to affect the physical properties of the prepared hydrogels (i.e. VPTT, injectability and SR at 37^{o}C).

To prepare hydrogels with potentially improved biocompatibility and to examine the effect of changing the cross-linker type on the properties of the hydrogel, 4 µL PEGDA was replaced with 20 mg of a preparatory molecule (PC 3059) as a cross-linker with 40 mg NIPAAm. Changing the cross-linker had no significant difference in the behaviour of the prepared hydrogels regarding their properties such as VPTT, SR, WR% and the release of loaded bevacizumab. Three different amounts of PC 3059 was investigated i.e. 20, 25 and 30 mg. No significant difference in VPTT and SR were observed with increased amounts of cross-linker. The presence of protein during the polymerisation process again, surprisingly, did not alter the physical properties of the prepared hydrogels. Hydrogels made with 20 mg PC2059 retained less water in their structure and were further investigated for their release properties. Both hydrogels are potential candidates for further study to be the basis for an extended release formulation for intravitreal protein delivery.

Another hydrophilic cross-linker was examined for the synthesis of NIPAAm hydrogels. Different NIPAAm hydrogels with increasing stoichiometries of Ac-HA (50 kDa) were prepared (2, 4, 7 and 10 mg). When the amount of Ac-HA ws increased, the thermal responsiveness was reduced and the SR was alsoreduced at 25^{o}C. This hydrogel behaves in similar way to PEGDA-NIPAAm cross-linked hydrogels. Preliminary release studies of Ac-HA loaded hydrogels demonstrated better control of the loaded protein compared to PEGDA and PC NIPAAm hydrogels with the release of 25% only of the encapsulated protein from Ac-HA hydrogels compared to 60% from PEGDA and PC hydrogels in 10 days.

NIPAAm hydrogels with different properties were prepared by using different types of hydrophilic macromolecular cross-linkers. The type and percentage of cross-linker in the formulation significantly affects the characteristics of the hydrogel to sustain the release of the encapsulated antibody.

Chapter 6. General discussion and conclusion

Blinding eye diseases are considered a major economic and social problem in developed and developing countries, generally affecting aging populations. Most blindness and severe visual impairment are caused by conditions that cause damage in the back of the eye; such as glaucoma and AMD (Bunce & Wormald 2006).

People suffering from glaucoma is estimated to be 60.5 million in 2010 and the number is estimated to increase to 111.8 million in 2040 (Tham et al. 2014; Quigley & Broman 2006). The only proven treatment for glaucoma is to lower the intraocular pressure (IOP) in the eyeball. Current treatments have a variable long-term success rate (Manickavasagam & Oyewumi 2013). Eye drops require lifelong use, are costly and are associated with local side effects. Compliance remains a major problem with eye drops with no solution to compliance emerging (Tsai 2009; Sleath et al. 2006). Laser treatment to open the drainage meshwork can lower pressure, but local fibrosis can occur (Wise 1987). Both the use of eye drops and laser treatments often fail to achieve long term reduction in IOP required to slow disease progression.

The lowest IOP can be achieved by creating a new drainage channel that allows aqueous to flow out of the eye into the subconjunctival tissues. Aqueous drainage can be achieved be trabeculectomy (or glaucoma filtering surgery-GFS) or by surgically implanting a glaucoma drainage device (GDD) (Schwartz et al. 2006; Chiselita 2001). There are many GDDs that have been developed, but limitations remain. Successful surgery is associated with a better clinical outcome and quality of life with lower healthcare costs compared to standard long term medical therapy. Unfortunately surgery has not become the primary treatment option because of its complexity and the post-surgical treatments that are often needed due to localised fibrosis (Skuta & Parrish 1987). Complications such as accurately control aqueous outflow due to blockage caused by local fibrosis results surgical failure with a return to higher IOP and disease progression.

GDDs are increasingly being used as first line treatment when surgery is called for. There are two key problems with current GDDs; (i) lack of control on aqueous outflow in the first few weeks before the fibrous capsule formation which may cause hypotony, (ii) lack of long term and consistent control of IOP among wide range of patients, (iii) formation of a thick fibrous capsule around the implant due to excessive scarring and foreign body response which may cause blockage of the tube and elevated IOP. The material used for the fabrication of implantable

devices, including GDDs plays an important role in the long-term success of the device. If the material used is highly biocompatible, less foreign body response is expected and a thin, less dense fibrous capsule may be formed around the implant. GDDs with spacer plates typically control aqueous outflow through a pressure controlled valve or use of a suture through the drainage tube with diffusion through the fibrous capsule formed around the implant. Consideration of new mechanisms for flow control based on different principles may help to developing new GDDs with higher success rate compared to the current GDDs.

An ideal GDD should; (i) have minimal foreign body response, (ii) be able to control the IOP to 10-12 mmHg over a wide range of patients for a prolonged period of time, (iii) be easily implanted and (iv) be affordable for patients around the world. It is thought that a GDD made from a hydrogel may offer a solution to the problems associated with the current devices available in the clinic.

Hydrogels are widely used in biomedical research because they are known to be biocompatible materials due to their tissue like properties (flexibility, softness and high water content). Hydrogels are polymer networks that allow the transport of gases, liquids and dissolved substrates (Hoare & Kohane 2008; Devi & Nautiyal 2014; Buwalda et al. 2014). Example applications where hydrogels are widely used and investigated include tissue engineering, 3D cell culture, wound dressing, medical adhesives and drug delivery systems (Yang et al. 2011; Huynh et al. 2011; Appel et al. 2012).

Initially it was hypothesised that a biocompatible hydrogel material could be used as a flow restrictor in a GDD. It was thought that the flow through a hydrogel material could be adjusted based on the characteristics of the hydrogel to allow aqueous flow. It was hoped that cross-link density and polymer properties could be found that allowed the flow of water at approximately 2 µL/min with a pressure resistance of 10 mmHg. It was hypothesised that hydrogel properties would allow aqueous flow to be impeded by resistance through the hydrogel network that would result in pressure control for a GDD.

It was further hoped that with an appropriate hydrogel material could be used to moderate the lower foreign body response to reduce the chance of a fibrous capsule forming around the device. Avoiding capsule formation is important to ensure unmanageable IOP increases do not occur over time. Long-term implantable devices into the subconjunctiva (including GDDs) currently require the administration of an anti-inflammatory and/or anti-fibrotic agent to reduce the foreign body response to the device after the trauma of surgery and implantation. Anti-inflammatory or anti-scarring agents could be loaded to the hydrogel to

formulate a combination device that would further reduce the foreign body response.

HEMA and MPC based co-polymers were selected as hydrogel components to control aqueous outflow as a means to regulate pressure because of these polymers are widely used in the eye (contact and intraocular lens). Much is known about the use of hydrogel materials in the manufacture of and coating of lens. It was not clear at the outset of this research what was the permeability and the capacity of water to actually flow through a hydrogel, it was necessary to evaluate gels derived comprised of different relative amounts of MPC to HEMA. A small library of hydrogel films ranging from 0 to 100 % (w/w) MPC were prepared and characterised in respect to appearance, water permeability and water content. From the outset it appeared that as the relative amount of MPC increased within the hydrogel films, the total water content and network pore size increased.

The critical properties needed for aqueous flow to control pressure were water permeability and the hydraulic conductivity of the hydrogel. These properties are not usually measured for hydrogels used in drug delivery but sometimes measured in ophthalmic lens development. In the case of contact lens, oxygen permeability is the key parameter that must be optimised. Water permeability and hydraulic conductivity of 10% MPC-HEMA hydrogels was measured under pressure by dynamic approach. Hydrogels fabricated with 10% MPC was chosen as a representation of the HEMA-MPC formulation. It was thought that if sufficient water flow through the 10% MPC hydrogel matrix, the formulation could be manipulated. Unfortunately, the fabricated hydrogel film displayed low permeability. The permeability and water flow was low at high pressure (~30 mmHg) which indicates that the films cannot be used as flow control mechanism for GDD.

Low water permeability was unexpected. One thought was that the low permeability was related to how water was distributed inside the hydrogel network where the ratio of bound water could be high compared to free water due to the presence of hydrophilic monomers such as MPC and HEMA. However, when the bound water ratio was measured the ratio of free water was high compared to bound water. The results suggest that for this particular HEMA-MPC formulation the water type has no effect on permeability.

Another rationale for the low permeability to water flow may be due to gel blocking. Water flow and water transport may be two different processes. Clearly when hydrated films were placed on a bench, the water quickly evaporated, much as what happens in a contact lens. Likewise the xerogel becomes hydrated. These observations indicate there is water transport. However this transfer of water

appears to differ from transfer due to flow. In this case it is possible that under the pressure of flow, hydrogel chain conformations change resulting in polymer chains actually blocking flow (Höhne & Tauer 2014; Wack & Ulbricht 2007; Berg et al. 1994).

To avoid aqueous flow through an entire hydrogel film, the incorporation of a chamber or pouch was considered. The pouch may exert extra pressure from inside the hydrogel to speed the flow rate through the hydrogel surfaces. The pouch will be deflated at low pressure and when the pressure increases, due to accumulation of aqueous inside the pouch, the pouch will expand (similar principle to balloons) and the aqueous will flow. Two approaches were examined to create a pouch; (i) femtosecond laser and (ii) gel film fabrication around a water soluble positive mould. A femtosecond laser was used to create a pouch in a fully hydrated hydrogel. Unfortunately, after the laser treatment the film surface became fragile and cracked. This preliminary experiment suggested that ablation of the internal volume of the hydrogel would be difficult to achieve. One reason for this may be due to the chaotic nature of the hydrogel, or because upon vaporisation by the laser there was a high localised increase in pressure/heat that caused the cracking.

Since the femtosecond laser was not useful to create an internal cavity within the hydrogel pouch, A PBS tablet was crashed and placed in the mould before conducting the polymerisation to fabricate the hydrogel. It was thought that by polymerising around a soluble material, the material could be dissolved during the washing process of the hydrogel to leave an empty pouch. No candidate hydrogel films worthy of further study were fabricated when polymerising around particles. The presence of the particles affected the polymerisation process in the surrounding area producing an irregular thickness to the film around the particles.

To further confirm that the polymerisation process is affected by the presence of foreign material during the polymerisation and not the nature of the PBS tablet itself, the polymerisation of 10% MPC monomer mixture was conducted in the presence of a metal spatula, filter paper and polycaprolactone film. No proper film was formed with each of the materials used. A pouch or a continuous internal volume could not be created. After much experimentation, it was determined that HEMA-MPC hydrogel films could not be used to restrict flow as a means to control pressure. The concept of using flow through hydrogels as a mean to control flow through GDD could still be investigated in future work by using different polymers and monomers for the GDD fabrication. A different concept for the hydrogel could also be investigated such as the synthesis of macroporous hydrogels.

Although we were unable to use a hydrogel as a flow-restricting barrier to

control outflow pressure, it was still possible to consider a hydrogel-derived spacer that could be used in a conventional GDD. The hydrogel material would be expected to have a lower propensity for causing a severe foreign body response. Additionally since GDDs do need co-administration of an anti-fibrotic agent, a hydrogel GDD spacer could be used also to release an appropriate drug within the subconjunctival space after GFS.

Hydrogel formulations were examined in terms of mechanical strength, drug loading and drug release. Young's modulus (E) of the fabricated films was measured as an indication of mechanical strength. As the strength of films increases, the modulus also tends to increase. The modulus inversely varied with the amount of MPC.

The drugs DEX (hydrophobic) and pirfenidone (hydrophilic) were loaded into MPC discs to study the effect of water content on drug release. The discs were 1 cm in diameter and 1 mm thickness in fully hydrated state. Fully dried discs were used for loading. The MPC was varied from 0-30% in the discs that were evaluated. For pirfenidone, the amount loaded was 0.35 ± 0.02, 0.30 ± 0.01 and 0.25 ± 0.02 mg/disc for 0%, 10% and 30% MPC respectively. For dexamethasone, the amount loaded was 0.40 ± 0.03, 0.3 ± 0.1 and 0.19 ± 0.03 mg/disc for 0%, 10% and 30% respectively. Initially it was thought that the loading would be increased with increasing MPC percentage because the swelling of the hydrogel increased with increasing MPC percentage. It came as a surprise that the loading decreased with the increasing MPC percentage for both drugs. The affinity between the drug molecule and the polymer matrix could explain the results. DEX has higher affinity to adsorb on hydrophobic polymer chain surfaces (HEMA). When the level of HEMA was reduced compared to MPC (highly hydrophilic), DEX loading was reduced.

The release of both drugs was studied using an eye flow chamber that mimics the aqueous flow in the subconjunctival space at a rate of 2 µL/min. Both drugs were released faster from the 30% MPC compared to 0% and 10% MPC hydrogels. The half-life of DEX was 3 ± 12, 1.8 ± 8.9 and 1 ± 3 day and the half-life of pirfenidone was 1.2 ± 2.4, 0.7 ± 3.4 and 0.4 ± 2.1 day for 0%, 10% and 30% MPC respectively. In non-stimuli responsive non-biodegradable hydrogels diffusion of the drug from the loaded hydrogel is the driving force for the release (Satapathy et al. 2015). Diffusion is also related to the percentage of free water in the hydrogel, where it is thought that the higher the bulk water content the faster the release (Wu et al. 2016). Since 30% MPC hydrogels have higher free water compared to 0% and 10% MPC hydrogel, the diffusion was faster from 30% MPC.

A GDD spacer must have sufficient mechanical strength to be implanted and sufficient water content to ensure biocompatibility. The presence of MPC will increase the water content, which potentially may improve biocompatibility but at the same time will reduce the strength of the films and cause faster release of the loaded drug. Hydrogels with 20% and 30% MPC have low mechanical strength with high free water. The half-life of both DEX and pirfenidone was short when released from 30% MPC compared to 0% and 10% MPC. Hydrogels with 5% MPC have higher relative mechanical strength and less free water but they have low percentage of MPC compared to the other formulations which could impact biocompatibility long-term. Hydrogels with 10% and 15% MPC showed similar characteristics regarding water content; however, hydrogels with 10% MPC are mechanically stronger and have larger fraction of free water compared to 15% MPC. Hydrogels made with 10% MPC were chosen as the best formulation for the fabrication of a drug releasing subconjunctival spacer after GFS. The 10% MPC hydrogels offer the best balance between water content, mechanical strength, drug loading and drug release.

Both drugs (DEX and pirfenidone) still did not have an optimal release profile from 10% MPC hydrogel discs. As discussed previously, the high ratio of free water facilitates the diffusion of loaded drugs in hydrogels. It was thought that by modifying the ratio of cross-linker and initiator in the 10% MPC formulation, the free to bound water ratio could be modified. It was previously reported that modification of both initiator and cross-linker have an effect on water content and distribution inside the hydrogels (Goda, Watanabe, Takai, & Ishihara, 2006; Kiritoshi & Ishihara, 2002). It was thought that by increasing the ratio of bound to free water in the formulation, the loaded drugs would more slowly diffuse to prolong its release.

To modify the water amount and distribution the percentage of cross-linker (PEGDA) was reduced from 0.56% to 0.28% and 0.14% while keeping the 10% MPC formulation mixture constant (88.7% HEMA, 10% MPC, 0.74% (w/w) AIBN). The initiator was also modified. The percentage of AIBN was reduced from 0.74% to 0.37% and 0.18% while keeping the 10% MPC formulation mixture constant (88.7% HEMA, 10% MPC, 0.56% (w/w) PEGDA). There was no significant difference in the free to bound water ratio when either the cross-linker or initiator was decreased. Based on the experiments that were conducted, the 10 % MPC formulation with 88.7% HEMA, 0.56 % PEGDA and 0.74 % (w/w) AIBN was chosen as the best formulation to fabricate a hydrogel derived drug releasing GDD spacer.

When a GDD implanted or a patient undergoes GFS, medication is required for a period of 4-8 weeks after surgery with non-steroidal anti-inflammatory drops often being prescribed. DEX eye drops are often used to reduce local ocular inflammation. Loading the hydrogel spacer with DEX to sustain its local could eliminate the side effects associated with using eye drops such as low efficacy, low patient adherence to treatment and increased sensitivity of an already inflamed eye tissue due to the presence of preservatives (Ghate & Edelhauser 2008; Noecker 2001).

DEX loading was 0.3 mg/disc and the half-life for release was 1.8 ± 8.9 days when water:ethanol (1:1) was used as the loading solvent for 10% MPC. Water:ethanol (1:1) was replaced with methanol as solvent for loading in an effort to maximise hydrogel loading by mediating gel swelling and increasing drug concentration in the soluble phase during loading. The maximum solubility of DEX in water alcohol mixture is limited. However, if ethanol or methanol were used alone a solution of 15 mg/mL of DEX could be prepared. Ethanol could not be used because 10% MPC hydrogels have low SR in ethanol (0.20 ± 0.03). On the other hand, the SR of the hydrogel is similar between water and methanol (1.20 ± 0.02 and 1.20 ± 0.09 for methanol and water respectively). The toxicity of methanol was a factor to be considered. To extract methanol from the loaded hydrogel and to create a depot of DEX inside the hydrogel matrix, the loaded discs were washed with 2 mL of water for 4 hours followed by vacuum drying for 24 hours. The loading increased from 0.3 mg to 1.9 mg/disc and the half-life increased from 1.9 days to 9.7 days.

For future work release could be studied using hydrogel discs with different dimensions and study the effect of thickness, surface area and shape on the release of loaded drugs. There is a wide range of drugs that could be used as anti-inflammatory or anti-scarring after GFS (such as ilomastat) and the loading and release of different drugs from HEMA-MPC hydrogel could be further investigated. There is also the potential for loading more than one drug on the same hydrogel to have synergistic effect. Two or more drugs could be loaded either from the same solution or loading one drug first followed by drying of the hydrogel and loading the other drug molecule.

Currently there are no GDDs in the clinic that possess a drug eluting spacer. In the case of GFS, there are also no clinically approved drug eluting spacers that could be used after trabeculectomy. A drug eluting spacer could offer a solution to the major problem of foreign body response that is associated with GDDs. Large-scale production, efficacy, ensuring sterility and minimising costs are important

parameters to be considered. The same techniques used for contact lens fabrication and sterilisation could be modified for the fabrication of a GDD and drug eluting spacer.

As discussed previously scarring of the subconjunctival tissues is a common complication after GDD implantation and GFS. An effort was made to load anti-scarring drug into the 10% MPC hydrogel discs fabricated. One of the drugs that show promising results as anti-scarring agent is the known antibiotic doxycycline (DOXy) (Li et al. 2013; Dawson & Schachter 1985). Another advantage of fabricating a DOXy releasing hydrogel spacer is the possibility of utilising the spacer for other subconjunctival scarring conditions (e.g. trachoma).

Trachoma is the number one cause of blindness caused by an infection, often occurring in resource limited regions of the world. The cause of blindness is due to subconjunctival scarring in the eyelid resulting in lid contracting and turning inwards so when the eye blinks, the eye lashes scratch the cornea causing blindness. Trachoma surgery often fails because the eyelid contracts due to scarring resulting in blindness. Ideally topical antibiotics (e.g. tetracyclines) should be used for two weeks after surgery; however, patient adherence in many resource limited regions to topical treatments is very low and currently there is no available treatment after trachoma surgery. The 10% MPC hydrogel films could be used as a solution to the problem of trachoma surgery. The hydrogel film could be loaded with an anti-scarring agent and implanted at the incision site during the surgery.

DOXy, in the hycale form, is highly soluble in water (~30 mg/mL). When a hydrophilic drug (pirfenidone) was previously loaded into 10% MPC hydrogel disc and the release was studied using eye flow chamber, the half-life was less than one day (0.7 days). For a hydrophilic drug the amount of drug loaded and the loading efficiency depends on the concentration of the loading solution and the volume absorbed by the dry hydrogel (Kim et al. 1992). Both parameters (drug loading concentration and volume absorbed by the hydrogel) were examined regarding the effect on amount loaded and release profile.

DOXy loading solutions with varying concentrations (6 mg/mL, 15 mg/mL and 30 mg/mL) were used. The amount loaded was 1.20 ± 0.08, 3.60 ± 0.09 and 7.50 ± 0.09 mg/disc for 6 mg/mL, 15 mg/mL and 30 mg/mL respectively. The loading increased with increasing concentration. Although the maximum amount loaded was 7.5 mg when loading from 30 mg/mL solution, 15 mg/mL was chosen as the optimum loading solution for further studies. It was reported that DOXy has the ability to supress cell contraction when used in a range of (100-400 µg) (H. Li et al., 2013). If loading solution concentration used was 15 mg/mL, the amount loaded

in each disc will be 3.6 mg which is estimated to be enough for two weeks if the drug is released at a rate of 200 µg /day.

The 10% MPC hydrogel swell to a different extent in different solvents. The amount of solvent absorbed into the dry hydrogel may affect the loading and release profile of DOXy. Different solvents and solvent combinations were evaluated to optimise DOXy release. The loading efficiency of DOXy into a 10% MPC hydrogel disc was 24.6 ± 4.1, 24 ± 0.8, 16 ± 2.8, 25.3 ± 9.4 and 24.3 ± 8.4% for water, PBS, water:ethanol (1:1), water:methanol (1:1) and methanol respectively. Although high loading efficiency was achieved, the release of the DOXy was fast with 50% of the loaded drug released within 24 hours. The half-life of DOXy was 1.2 ± 0.01, 0.9 ± 0.1, 0.9 ± 0.1 and 1.1 ± 0.3 for water, water:ethanol (1:1), water:methanol (1:1) and methanol respectively.

The release of DOXy was studied in the eye flow rig that mimic the subconjunctival flow. Usually the flow rate of 2 µL/min is only in the subconjunctival space at the GFS site while the flow rate in the subconjunctival space in the eyelid is much smaller. Keeping that in mind, the release of DOXy from 10% MPC films is expected to be slower in the eyelid and the half-life is expected to be longer. Ideally the release of DOXy should have been studied at flow rate lower than 2 µL/min; however the flow rate from the peristaltic pump can go down only to 1 µL/min. Even if the flow rate was reduced, the inside volume of the flow rigs will cause a problem in estimation of half-life. The volume of the flow chamber is 400 µL (similar to the bleb created by the GFS) which will allow longer contact time of the fluid with the hydrogel and faster release (as seen in our results). For a future work a design of new flow chamber with low internal volume (100 µL instead of 400 µL of the current version) will be required. The new flow chamber with a flow rate of 0.2 µL /min instead of 2 µL /min will result in a more accurate estimation to the flow in the trachomatous eye lid.

While DOXy loaded hydrogels have the potential to be used after trachoma surgery, slower release is required if they are to be used after GFS. In an attempt to slow the diffusion of DOXy from 10% MPC hydrogels, a diffusion barrier was created inside the hydrogel. β-cyclodextrin (β-CD) was incorporated into the hydrogel matrix to create an affinity drug releasing system. By incorporating β-CD into the polymer matrix, the affinity of DOXy to the hydrogel matrix could potentially be increased and the release rate could be decreased. DOXy has the ability to form inclusion complex with β-CD and the multiple association and dissociation between β-CD and DOXy in the hydrogel matrix may slow the release of DOXy. Different approaches were investigated to conjugate β-CD including formation of a film with

pendant β-CD, embedding of cross-linked β-CD particles into the polymer matrix and formation of IPN of 10% MPC and β-CD.

For the preparation of 10% MPC hydrogel with glycidyl methacrylate (GMA) in the polymer matrix, GMA was mixed with 10% MPC monomer solution before polymerisation. The ratio of GMA used in the formulation was (1%, 2.5%, 5% and 9% (w/w). After polymerisation and washing to remove leachables, the films were incubated for 24 hr with continuous stirring at 70^{o}C in a mixture of DMF and 0.5 M NaCl with β-CD. The activation solution will activate the epoxy group in GMA to start the conjugation reaction with β-CD. However, this approach was infeasible. Films fabricated with 5% and 9% (w/w) GMA were unable to hydrate and remained glassy even after incubation in water for more than a week. Although films fabricated with 1% and 2.5% (w/w) GMA were hydrated in water, the films started to break apart after incubation in the activation solution.

In an effort to incorporate β-CD into the hydrogel without using an activation solution, the β-CD solution was mixed with 10% MPC monomer solution and GMA to be polymerised at the same time (*in situ* polymerisation). A monomer solution of 10% MPC was mixed in equal volumes with β-CD solution and increasing amounts of GMA (0.5, 0.2 and 0.1 g/mL). It was hoped GMA could be polymerised into the HEMA-MPC hydrogel matrix through the acrylate group and at the same time conjugated to β-CD through the epoxy group. Unfortunately no compliant film was formed after polymerisation. Even when the volume of 10% MPC monomer solution used was double the volume of β-CD solution, no proper film was formed. β-CD molecules have several hydroxide groups (OH) and it is possible that more than one OH group may participate in the reaction to form a covalent bond with GMA, and two or more GMA molecules could be attached to the same β-CD molecule. The multiple cross-linking of the same β-CD molecule may increase the cross-linking density of the hydrogel formed and potentially affect the properties of the hydrogel formed.

Preparation of films with pendant β-CD using GMA appeared to be unachievable in these MPC hydrogels. β-CD could be introduced as embedded particles in the hydrogel matrix. If β-CD were used as embedded particulate in the hydrogel mixture it could be washed with the unreacted monomers due to its solubility in water. Converting β-CD into insoluble form would help to immobilise the particles within the hydrogel film.

For the preparation of 10% MPC hydrogels with embedded β-CD cross-linked particles, β-CD hydrogel was first prepared. The β-CD hydrogel was then passed through a 40 mesh sieve to create microparticles and the particles were

freeze dried to be used in the 10% MPC film. Different β-CD cross-linked microparticles were prepared with different ratios of cross-linker (PEGDE) to β–CD solution (1:1, 1:2, 2:1). When the level of PEGDE to β–CD solution was 2:1, the hydrogel formed was very hard to pass through the 40 mesh sieve. The softness increased by reducing the percentage of PEGDE compared to β–CD solution. The ratio of cross-linker to β–CD solution chosen for the synthesis of the cross-linked CD particles was 1:1. The ratio offered the advantage of higher cross-linker density and the ability to pass through the 40 mesh sieve to produce the microparticles.

A monomer solution of 10% MPC was prepared and β-CD cross-linked particles were added into the monomer solution in two different concentrations (1% and 2% (w/v)). The combination used for polymerisation was 10% MPC with 10% (w/v) β-CD particles. Formulation with 20% (w/v) of β-CD cross-linked particles in 10% MPC monomer solution was non-injectable into the mould through 21G needle. Even when the needle size changed to 19G, suspension made with 2% (w/v) was still non injectable. The ability to inject the formulation into the mould is important to avoid air bubbles and to ensure uniform distribution of the formulation mixture through the mould.

After polymerisation of the monomer mixture with 1% (w/v) β-CD cross-linked particles a film was formed. The presence of the particles slightly increased the SR, free water ratio and water content in the hydrogel films. The EWC% was (65.4 ± 0.1) and (55 ± 0.3), the free to bound water ratio was (2:1) and (1.8:1) and the SR was 1.8 ± 0.1 and 1.2 ± 0.1 for 10% MPC with and without embedded β-CD particles respectively. The increase in water content could be attributed to the hydroxyl groups present in the β-CD structure that attracts water molecules. It is worth mentioning that when the ratio of free water to bound water was measured for cross-linked CD particles, the ratio of free to bound water was high (3.1:1). The hydrogel discs were then used for drug loading and release studies using eye flow rig.

Although the amount of DOXy loaded increased from 3.7 ± 0.1 mg/disc for non β-CD embedded hydrogels to 6.6 ± 0.1 mg/disc for β-CD embedded hydrogels, the release profile was the same. A possible explanation is that the affinity of DOXy for cross-linked β-CD is low. The low affinity may have caused faster rate of dissociation compared to association between β-CD and DOXy. It is also possible that because of the high water solubility of DOXy the drug would preferentially partitioned too much into solution rather than the inner cavity of the β-CD molecule.

A new approach was investigated for the incorporation of β-CD into the polymer matrix. An IPN of 10% MPC and β-CD hydrogels was prepared by *in situ*

polymerisation. It was thought that by using an IPN the hydrogel would have different properties compared to 10% MPC hydrogel alone or cross-linked β-CD hydrogels alone. Hydrogel formulations were prepared in which the β-CD monomer solution and 10% MPC monomer solution were mixed in equal volume ratios (1:1) and polymerised into a hydrogel. Various percentages of β-CD (15%, 30%, 50% (w/v)) were used for the formulations. The percentage of β-CD in the mixture was varied to identify the maximum amount of β-CD that could be applied into the monomer mixture and still maintain a film. After polymerisation the films were brittle.

Since formation of IPN was not possible using *in situ* approach, sequential approach was investigated. In the sequential approach the 10% MPC monomer solution was polymerised first, in the presence of 30% (w/v) β-CD monomer solution, followed by polymerisation of the β-CD monomer solution. Equal volumes of both monomer solutions were used for the IPN preparation. When the polymerisation process completed, a hydrogel film was formed. Unfortunately, no difference in release profile was observed with DOXy.

Further work with DOXy is still possible since this active drug substance is widely used and is known to have anti-bacterial, inflammatory and anti-fibrotic effects. The concept of utilizing a complexing agent in the hydrogel matrix as complexation or diffusion barrier could still be investigated in future work. One possible aspect is formation of polyelectrolyte complex with DOXy. DOXy is a weak base and if a weak acid polymer or monomer (such as acrylic acid) incorporated in the hydrogel matrix a complex could be formed. The complex may slow the release of the loaded DOXy. The possibility of using the concept of microparticulates for DOXy delivery could also be investigated. For example PLGA coated microparticles could be embedded in the hydrogel matrix to slow the release of DOXy.

Another possible approach to increase the duration of action of DOXY is the use of DOXy monohydrate rather than DOXy hyclate. DOXy monohydrate is less soluble than the hyclate form and work conducted with an MSc student at the end of this PhD project has shown potential to utilise CD as an affinity agent within a hydrogel for DOXy when the monohydrate form was used. Much work remains however to better understand DOXy affinity and rebinding effects to CD. Maintaining DOXy stability also remains an issue.

As discussed previously, much blindness and visual impairment worldwide are related to glaucoma and AMD. While hydrogels could be considered to overcome some of the current treatment problems associated with current GDDs and GFS for glaucoma, it was also thought that hydrogels could be investigated to

overcome problems with current AMD treatments. There are many other conditions that require direct administration of medicines to the vitreous cavity.

IVT injections of antibody based medicines are the first line treatment for AMD. IVT injections can have complications such as inflammation, vitreous haemorrhage, retinal detachment, patient discomfort and place a financial burden on patients and healthcare systems when IVT injections are required repeatedly over a long period (Falavarjani & Nguyen 2013). Sustained release protein formulations designed for the back of the eye have the potential to reduce the frequency of IVT injections. Solid implants for sustained drug delivery to the vitreous are available in the clinic; however implants are only used for the delivery of low molecular weight, poorly soluble drugs such as steroids. Proteins are hard to formulate as solid implants because it is difficult to maintain tertiary structure of proteins. Protein stability is difficult to maintain because proteins are susceptible to unfolding and aggregation. Protein therapeutics such as antibodies are currently not formulated as implants or even as formulations that can be incorporated into a pump, such as some insulin formulations. Formulations that can reduce the frequency of administration and can maintain the stability of the protein during storage and release are desperately needed in the clinic.

Hydrogels with 10% MPC that were considered for use in the subconjunctival space could not be considered for use in the vitreous cavity because of the need for implantation. From the outset, use of a hydrogel for the back of the eye would require that the hydrogel formulation be injectable. Implantation of a hydrogel device would require a surgical procedure which long term would be expensive and limit patient access. Also it would be expected that the polymerisation conditions used for the HEMA-MPC hydrogels, e.g. relatively high temperature or UV initiation conditions would be difficult to achieve while maintaining protein stability. It was clear that an in situ approach is necessary to consider a hydrogel strategy using a protein as the active drug substance since. Mixing a large molecule such as a protein into a pre-formed hydrogel was considered not to be feasible.

Another type of hydrogel was investigated as a potential injectable formulation for therapeutic proteins destined for the vitreous cavity. Thermoresponsive injectable hydrogels were considered as a potential formulation strategy for the intraocular use of therapeutic proteins. Biocompatibility, injectability and mild preparation conditions render thermoresponsive hydrogels as possible candidates for development of depot protein formulations. Thermoresponsive hydrogels that are in principle designed to be swollen and able to flow at room

temperature could then collapse into a semisolid implant once the surrounding temperature increased above hydrogel VPTT. Clearly issues related to the potential burst release of protein upon gel collapse and the need to consider the ultimate fate of the hydrogel are important, non-trivial issues that require effort to solve. However it was felt that first it was important to determine what could be achieved to prolong the duration of action for a protein from an injectable thermoresponsive hydrogel.

NIPAAm derived thermoresponsive polymers are widely examined for biomedical applications, including as the basis of extended release formulations. NIPAAm thermoresponsive hydrogels are frequently made with small molecular weight cross-linkers to allow the complete collapse of the hydrogel at physiological temperature. It was thought that if a large molecular weight hydrophilic cross-linker was used, a thermoresponsive hydrogel could still be formed. The hydrophilic cross-linker was considered to be able to provide a more favourable to maintain the stability of the protein within the hydrogel during formulation, collapse and release. The type and relative incorporation of the cross-linker was expected to affect the properties of the hydrogel formed. PEGDA with a Mn of 700 was chosen as cross-linker because of its hydrophilic nature and its reported biocompatibility in the human body (Li et al. 2014; Turturro et al. 2011).

NIPAAm hydrogels with increasing relative amounts of PEGDA (4, 8, 12 and 15 µL) were prepared both in the presence and absence of protein (bevacizumab or infliximab). These hydrogels were prepared in water with a mild initiating system that have the ability to start the polymerisation reaction at ambient temperatures and continue the polymerisation reaction at 4^{o}C (TEMAD/APS system) in an effort to avoid loss of protein stability. The antibody loaded NIPAAm hydrogels were characterised and compared regarding physicochemical properties in particular thermal responsiveness, injectability and antibody release. Increasing the relative percentage of PEGDA in the formulation was accompanied by reduction in thermal responsiveness and injectability. The VPTTs were 34.3 ± 0.1, 35.50 ± 0.09, 35.6 ± 0.4, and 36.2 ± 0.2^{o}C as the PEGDA cross-linker was increased from 4 to 15 µL respectively. The degree of collapse was also reduced with increasing PEGDA percentage. The degree of collapse could be estimated based on the SR of the hydrogel at 37^{o}C; the higher the SR the lower is the degree of collapse. The SR was 1.4 ± 0.5, 3.2 ± 0.8, 7.5 ± 0.7 and 9.6 ± 1.6 for 4, 8, 12 and 15 µL PEGDA respectively.

Two methods for protein loading were screened to choose the method that offers maximum loading and prolonged release of the loaded protein; *in situ* polymerisation and imbibing methods. The release was studied using the PK-Eye

model that mimics the aqueous outflow in the eye and was developed to estimate human protein clearance times from the vitreous cavity. Both simulated vitreous and phosphate buffer solutions are used in the PK-Eye. Phosphate buffer solution was used to evaluate the majority of the NIPAAm hydrogel formulations because release experiments can be conducted more quickly and still compare different formulations. Although bevacizumab loading was surprisingly similar from the two methods (~72% after washing) the release profile was significantly different ($p<0.05$) which indicated there was a difference in the mixing method. The half-life of bevacizumab when mixed by imbibing was 2.3 ± 0.8 compared to 3.7 ± 1.2 when the antibody was incorporated during polymerisation. The large size of bevacizumab (150 kDa) allowed better entanglement when mixed during NIPAAm polymerisation compared to imbibing method.

NIPAAM derived hydrogels from polymerisation of 40 mg of NIPAAm with 4, 8 and 12 µL PEGDA were loaded with bevacizumab (2.5 mg) using *in situ* polymerisation. The NIPAAm monomer was dissolved in 1 mL of bevacizumab drug solution (25 mg/mL) followed by addition of PEGDA and polymerisation. Bevacizumab release was compared. A half-life of 2 ± 0.01, 3.7 ± 1.2 and 2.60 ± 0.03 days was observed with 4, 8 and 12 µL PEGDA respectively with a protein release of ~74%, ~87% and 95% respectively after a month. Although the calculated half-life was relatively similar between the prepared hydrogels and bevacizumab injection (2.3 ± 0.8 day for the injection), the release profiles were different. Nearly all of the bevacizumab was cleared after 10 days when injection was used (~95.1% ± 3.1) compared to ~61.9 ± 3.6%, 63.2 ± 8.3% and 75.0 ± 1.9% for 4, 8 and 12 µL PEGDA respectively. The release of bevacizumab followed a bimodal display profile from the NIPAAM hydrogels with the first phase following a first order profile for the first week and then a more sustained pesedo-zero order behavior for the remaining three weeks of the release study.

Entangling a large molecule during the polymerisation process when formulating a hydrogel may affects the physical properties and mixing of a protein within the hydrogel. The effect of encapsulating bevacizumab during NIPAAm polymerisation on the properties of the prepared hydrogels were studied. A slight reduction in VPTT from 35.5 ± 0.09^{o}C for unloaded hydrogel to 34.2 ± 0.5^{o}C for loaded hydrogel. Although the SR was significantly different between loaded and unloaded hydrogels ($p<0.05$) at 25^{o}C, there was no significant difference in SR at 37^{o}C. The SR of bevacizumab loaded hydrogels were 34.1 ± 2.1 and 3.1 ± 0.8 at 25 and 37^{o}C respectively. The entanglement of the protein in the hydrogel network restricts the movement of the hydrogel polymer chains resulting in reduced SR at

25^{o}C. It appeared that the presence of the antibody had little impact on hydrogel collapse at 37^{o}C. Overall, protein encapsulation during polymerisation had no effect on the general properties of the prepared hydrogels, which could imply that mixing was not as good as could be achieved. It is possible that polymer formation occurred where the majority of the forming polymer did not entangle and interact with the antibody. Hence the observed macroscopic properties are indicative of hydrogel rich region of the samples that were evaluated. Release profiles did suggest that some mixing had occurred, but the release profiles were not to the extent that might have been hoped for.

NIPAAm hydrogels fabricated with 15 µL PEGDA were non-injectable and hydrogels fabricated with 12 µL PEGDA were difficult to inject. The release of bevacizumab was also significantly faster ($p<0.05$) when compared to hydrogels made with 4 and 8 µL PEGDA. NIPAAm hydrogels made with 4 and 8 µL PEGDA showed similar characteristics, behaviour and sustained the release of protein to the same extent *in vitro*. At 25^{o}C the SR of 4 µL is higher than 8 µL, which may cause the loss of higher percentage of the protein during washing. Washing is required to remove the unreacted monomers and other leachable oligomeric substrates from the hydrogel before injection. Also hydrogels made with 4 µL were very soft and difficult to handle. Based on these observations 8 µL PEGDA was chosen as the best formulation that would considered for further work to sustain the release of bevacizumab in the posterior segment.

After the hydrogel polymerisation process, removal of unreacted monomers is essential as they are toxic in nature. Bevacizumab loaded hydrogels with 12 µL as cross-linker was washed and the release of the protein was compared between the washed and the unwashed hydrogels. Although ~28% of the loaded protein was lost during washing, there was no significant difference ($p>0.05$) in half-life and release profile between washed and unwashed hydrogels; the half-life was 3.1 ± 1.3 and 3.7 ± 1.2 days for washed and unwashed hydrogels respectively.

NIPAAm hydrogels are non-biodegradable and will be in direct contact with eye tissues for a long period of time. In an effort to improve biocompatabilty it was thought to incoprporate MPC in the formulation. MPC-co-NIPAAm thermoresponsive hydrogel with 8 µL PEGDA was prepared. Although a hydrogel was formed when MPC was incorporated in the formulation, the thermal responsiveness was reduced. Even when the percentage of MPC in the formulation was reduced to ~0.6% (w/w), weak thermal responsiveness was observed.

In order to add PC zwitterionic group into the NIPAAm hydrogels and maintain the thermal responsiveness, PEGDA was replaced with PC bearing cross-

linker. The same method used for the formulation of PEGDA cross-linked NIPAAm hydrogels (in situ polymerisation) was used for the synthesis of PC cross-linked NIPAAm hydrogels. When 8 µL PEGDA was replaced with PC 3059 as cross-linker no difference in behaviour was observed (25% (w/w)). PC 3059 is a macromolecular cross-linker provided to us from Vertellus. The cross-linker structure is complex and the molecular weight characteristics are not known. However, approximately 1% MPC is incorporated in PC3059. The VPTT of the PC3059 cross-linked NIPAAm hydrogels was significantly lower ($p<0.05$) (33.8 ± 0.1^{o}C) with PC compared to PEGDA (35.5 ± 0.09^{o}C) cross-linker. There was no significant difference ($p>0.05$) in SR between PEGDA and PC NIPAAm hydrogels at 25 and 37^{o}C. There also was no significant difference ($p>0.05$) in the release profile of bevacizumab from both hydrogels. Also similar to PEGDA, bevacizumab loading had no effect on the general properties of the prepared PC cross-linked hydrogels. PC 3059 is promising alternative of PEGDA as cross-linker with a potential of higher biocompatibility. These observations were surprising and were consistent with less than optimal mixing of the antibody within the hydrogel.

From the previous work with PEGDA it was noticed that when the percentage of cross-linker increased in the formulation, hydrogels with different properties are formed. To examine whether the characteristics of all of the hydrophilic macromolecular cross-linkers are similar, hydrogels with increasing percentage of PC cross-linker (25% to 33.5% (w/w)) were prepared and compared regarding the physical properties of the hydrogels that were formed. Similar to PEGDA, the hydrogels fabricated using higher percentages of PC were difficult to inject and the VPTT was slightly increased with higher cross-linker percentage. The difference in behaviour between PEGDA and PC cross-linked hydrogels was observed in the collapse of the hydrogel at 37^{o}C. The SR was not significantly increased ($p>0.05$) when PC percentage increased (as observed with PEGDA). The SR was 3.8 ± 1.9, 5.2 ± 1.8 and 5.1 ± 0.5 for 25%, 30% and 33.5% (w/w) respectively. A possible explanation is the hydrophilicity of the cross-linker used. While it is known that PEGDA can form hydrogen bonds with water, PC groups attract free water around their polymer chains that could be easily expelled during the collapse of the hydrogel. The results indicate that the interaction of the molecule with water is also important and should be considered.

Both PEGDA and PC are non-biodegradable molecules. It was interesting to examine whether a biodegradable macromolecular hydrophilic cross-linker will behave in a similar way to non-biodegradable ones. It is also of an advantage if the NIPAAm hydrogel could be formulated as biodegradable hydrogel. Ac-HA

synthesised in our lab was used as biodegradable hydrophilic cross-linker. HA is a common component in many parts of the human body such as extracellular matrix, synovial fluid and the vitreous humour and widely investigated component for drug delivery due to its biodegradability and biocompatibility (Widjaja et al. 2014; Mayol et al. 2008; Kogan et al. 2007).

Different amounts of Ac-HA were used for the hydrogel synthesis (2, 4, 7 and 10 mg) in the presence of NIPAAm (40 mg). The injectability was reduced and thermal responsiveness was reduced with increasing amounts of Ac-HA when used as a cross-linker. The hydrogels became non thermoresponsive when 7 and 10 mg Ac-HA were used. No significant difference in SR ($p>0.05$) at 25 and 37^{o}C was observed with 7 and 10 mg Ac-HA hydrogels. NIPAAm hydrogels made with 2 and 4 mg Ac-HA were loaded with infliximab (9.4 mg/mL). Infliximab and bevacizumab displayed similar MW (150 kDa). When both protein loaded hydrogels were washed to remove unreacted monomers, ~26.7% of infliximab was lost from hydrogels made with 2 mg Ac-HA compared to ~19.5%. The SR of hydrogels made with 2 mg was significantly higher ($p<0.05$) than the SR of 4 mg hydrogels. The higher SR indicated high water content which allows faster diffusion of the protein to the surrounding washing medium. Since protein loss was significantly evident with hydrogels made with 2 mg Ac-HA, it was decided that hydrogels fabricated with 4 mg should be used for preliminary protein release studies.

The initial studies on infliximab release from 4 mg Ac-HA cross-linked hydrogels revealed a difference in release profile when compared to 8 µL PEGDA and 25% (w/w) PC cross-linked hydrogels. Only ~25% of the loaded protein was released after 10 days from Ac-HA cross-linked hydrogels compared to ~60% from 8 µL PEGDA and 25% (w/w) PC cross-linked hydrogels. The difference could be attributed to the molecular nature of HA. HA is used as viscosity agent in pharmaceutical formulations and the slow release of infliximab from Ac-HA cross-linked hydrogels is a combined effect of thermal responsiveness of the NIPAAm hydrogel and viscosity enhancing effect of HA.

The aim of our work was to screen different macromolecular hydrophilic cross-linkers for the formulation of sustained release protein thermoresponsive hydrogel as IVT injection. The three investigated molecules PEGDA, PC 3059 and Ac-HA demonstrated promising results in the ability to form a dosage form that is injectable, thermoresponsive and sustain the loaded protein for more than one month. The formulations also allow the formation of hydrogels with different concentrations of the protein by manipulation of the concentration of the starting protein solution during the polymerisation process.

A possible future work is the study of the potential proper NIPAAm hydrogel formulations with proteins of different molecular weights and study whether the molecular weight will have an impact on the properties of the hydrogel. Optimisation of the loading of the protein after washing of the hydrogel could also be investigated. The protein lost during washing could be compensated by rehydrating of the protein loaded dry hydrogel in protein solution. Biocompatibility and animal studies should be considered following optimisation of the formulation.

The work described in this thesis set out to examine the use of hydrogels in two different parts of the eye: (i) subconjunctiva and (ii) vitreous cavity. The use of hydrogels in the form of implants was investigated in the subconjunctival space to improve current treatments available for glaucoma. Although HEMA-MPC hydrogels were unable to show sufficient permeability and hydraulic conductivity to be of any use to control aqueous outflow to regulate pressure in a GDD, the hydrogel films offer a platform for development of drug releasing combination GDD and drug releasing spacer after GFS.

Different hydrogel based formulations for the sustained delivery of protein in the vitreous using thermoresponsive hydrogels was investigated.using macromolecular hydrophilic cross-linkers. Modification in the type and percentage of cross-linker used allowed the preliminary screening and development of formulations that sustained the protein for more than one month.

Appendix

Dynamic approach for flow measurements

The equations for determining hydraulic conductivity were derived from first principles. A schematic representation (Figure 7:1) of the apparatus is shown in the below figure to develop the analysis. The analysis is based on D'Arcy's law. D'Arcy's law states that flow is proportional to the applied pressure. Thus for a membrane disc of radius a (m), flow per unit area is designated by:

$$\frac{\boldsymbol{F}}{\boldsymbol{\pi\, a^2}} = \boldsymbol{L\ P},$$

Where L is the hydraulic conductivity of the membrane (m/s/Pa) and P is the applied pressure (Pa).

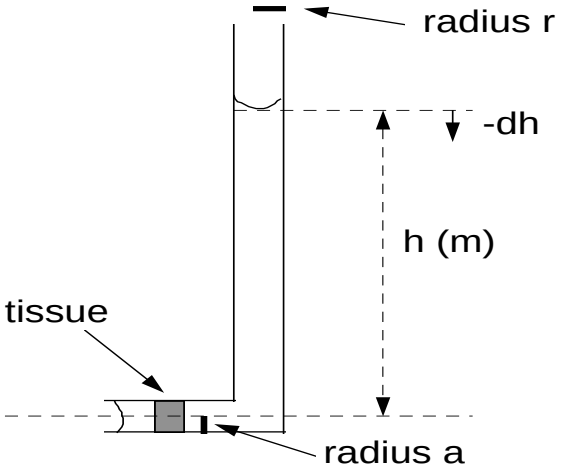


Figure 7:1 A schematic representation of the apparatus used for permeability and hydraulic conductivity measurements.

Since

$$\boldsymbol{F} = \frac{\boldsymbol{dV}}{\boldsymbol{dt}}, \tag{7.1}$$

$$\frac{\boldsymbol{dV}}{\boldsymbol{dt}} = \boldsymbol{\pi\, a^2\ L\ P}, \tag{7.2}$$

Pressure due to the column of fluid is given by P = p g h

Where p is the density of water (1000 Kg/m^3), g is the gravitational constant 9.81 m/s^2 and h is the height of column in meters. Hence:

$$\frac{\boldsymbol{dV}}{\boldsymbol{dT}} = \boldsymbol{\pi\, a^2\ L}\ \mathrm{p}\ \boldsymbol{g\ h}, \tag{7.3}$$

After a time dt, change in volume is dV = - π r^2 dh, where r is the radius of the capillary column, i.e., column height falls. Therefore:

$$-\frac{\pi r^2 dh}{dt} = \pi a^2 L \text{p} g h, \quad (7.4)$$

Rearranging:

$$\frac{dh}{h} = -\frac{a^2 L \text{p} g}{r^2} dt, \quad (7.5)$$

Integrating:

$$\int \frac{dh}{h} = -\frac{a^2 L \text{p} g}{r^2} \int dt, \quad (7.6)$$

Thus

$$\ln h = -\frac{a^2 L \text{p} g}{r^2} t + c, \quad (7.7)$$

Where c is the constant of integration.

Boundary conditions are: t= 0, h = h_o and hence c = ln h_o

Hence:

$$\ln h = -\frac{a^2 L \text{p} g t}{r^2} + \ln h_0 , \quad (7.8)$$

$$\ln h - \ln h_0 = \frac{a^2 L \text{p} g t}{r^2} , \quad (7.9)$$

$$ln\left(\frac{h}{h_0}\right) = -\frac{a^2 L \text{p} g t}{r^2}, \quad (7.10)$$

From $\log_b a = c$, $b^c = a$

$$\frac{h}{h_0} = e^{-\frac{a^2 L \text{p} g t}{r^2}}, \quad (7.11)$$

Or

$$h = h_0 e^{-\frac{a^2 L \text{p} g t}{r^2}}, \quad (7.12)$$

From equation (7.10) a plot of (ln $h/h_{0)}$ against t should yield a straight line of gradient G.

$$G = -\frac{a^2 L \text{p} g}{r^2}$$

This gradient can be obtained by linear regression of the above plot. If the gradient is designated G, then the hydraulic conductivity L is given by:

$$L = -\frac{r^2}{a^2 \text{p} g} G, \quad (7.13)$$

Then permeability is calculated using L

$$K = L \times T, \tag{7.14}$$

Where T is the thickness in (m).

Calculations of permeability

As an example of the calculations used to measure permeability, the calculation for hydrogel made with 10% MPC is shown below. The values of water height (h) were taken at different time intervals and the results are listed (Table 7:1). These measured values were plotted (Figure 7:2). From the figure we measure the gradient to calculate the hydraulic conductivity using equation (7.13). The hydraulic conductivity was then used to calculate the permeability of the sample using equation (7.14). The permeability of all the received films was calculated in the same way as 10% MPC.

Table 7:1 The values of height (h) of water column taken at different time intervals in seconds.

h cm	Time	h m	h/h^0	ln h/h^0	Time seconds
27	12:25 pm	0.27	1	0	0
26.7	2:50 pm	0.267	0.989	-11.061×10^{-3}	8700
26.5	4:06 pm	0265	0.9814	-18.775×10^{-3}	13260
26.4	4:47 pm	0.264	0.9778	-22.45×10^{-3}	15720
23.8	9:23 am	0.238	0.88148	-126.153×10^{-3}	75480
23.7	10:36 am	0.237	0.878	-130.109×10^{-3}	79860
23.6	11:38 am	0.236	0.874	-134.675×10^{-3}	83580
23.3	1:09 pm	0.233	0.8629	-147.456×10^{-3}	89040

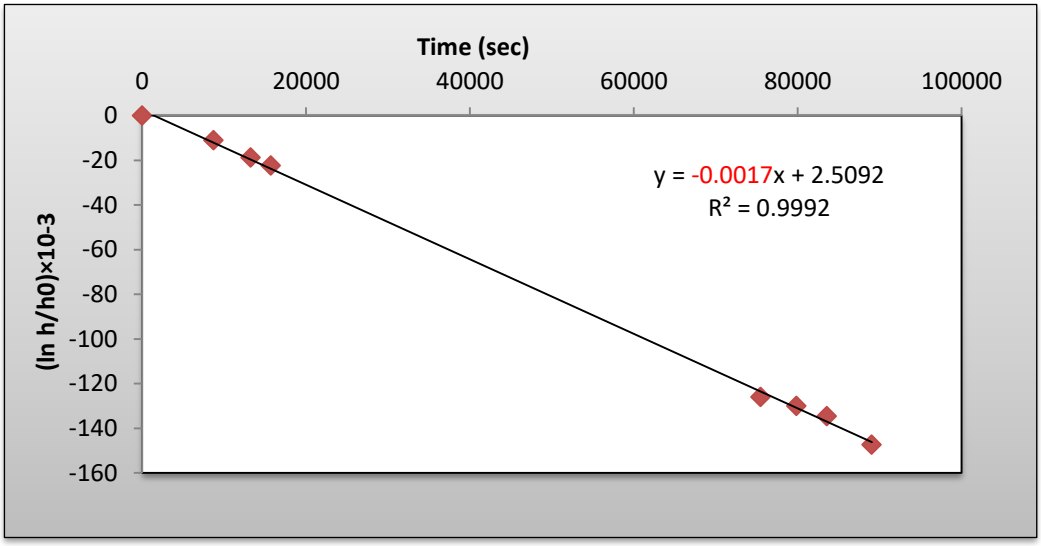


Figure 7:2 The time verses ln h/h0 will yield a gradient which is -0.0017 for sample number (7/28). This gradient used to calculate the permeability of the sample tested.

The time verses ln h/h0 will yield a gradient which is -0.0017 for 10% MPC film. The gradient used to calculate the permeability of the sample tested.

$$L = -\frac{r^2}{a^2 \ \rho \ g} \ G$$

$$L = \frac{(1.75 \times 10^{-3})^2}{(3 \times 10^{-3})^2 \times 1000 \times 9.81} \ 0.0017 \times 10^{-3}$$

$$\mathrm{L} = 5.897 \times 10^{-11} \ \mathrm{m\ s^{-1}\ pas^{-1}}$$

$$\mathrm{K} = \mathrm{L} \times \mathrm{T}$$

$$\mathrm{K} = 5.897 \times 10^{-11} \times 1 \times 10^{-3}$$

$$\mathrm{K} = 5.897 \times 10^{-14} \ \mathrm{m^2\ s^{-1}\ pas^{-1}}$$

The estimated permeability required to flow through a devise with the following dimensions (1 mm thickness, 2 cm2 surface area) and with estimated flow rate of (2 µl/m) to lead to a pressure of (10 mmHg) was calculated using equations (7:14).

$$\mathrm{Q} = 2\ \mu\mathrm{l/min} = 1.6 \times 10^{-11}\ \mathrm{m^3/s}$$

$$\mathrm{T} = 1\ \mathrm{mm} = 1 \times 10^{-3}\ \mathrm{m}$$

$$\mathrm{A} = 2\ \mathrm{cm^2} = 2 \times 10^{-4}\mathrm{m^2}$$

$$\mathrm{P} = 10\ \mathrm{mmHg} = 1333.2\ \mathrm{pascal}$$

$$\mathrm{K} == \frac{Q \ T}{\Delta P \ A}$$

$$\mathrm{K} = = \frac{1.6\times10^{-11}\ m^3/s \quad 1\times10^{-3}\ m}{1333.2\ pascal \quad 2\times10^{-4} m^2}$$

$$\mathrm{K} = 6 \times 10^{-14}\ \mathrm{m^2\ s^{-1}\ pas^{-1}}$$

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