Designing Novel Smart Hydrogel Formulations for the Controlled Delivery of Ocular Therapies in Contact Lens Devices

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Prepared under the supervision of Dr Peter McLoughlin, Dr Austin Coffey and Dr Laurence Fitzhenry

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DECLARATION

I hereby certify that this material, which I now submit for assessment is entirely my own work and has not been taken from the work of others, save to the extent that such work has been cited and acknowledged within the text of my work.

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ACKNOWLEDGMENTS

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ABSTRACT

The major challenge to ocular drug delivery is poor bio-availability of the delivered drug, due to the anatomy of the eye. This work presents an approach to address this problem, using novel contact lens drug delivery vehicles.

Antihistamines were used as a model drug due to their physical properties and molecular weight. 15% of the world's population suffer from allergic reactions confirming antihistamines as a relevant ocular pharmaceutical.

A novel pilot scale wet cast moulding process and methods to measure critical lens parameters were developed. This facilitated comparison of the manufactured lenses to commercial lenses. The refractive index of the lenses fabricated and commercial lenses was 1.33. Equilibrium water content was 70 % for both commercial and fabricated lenses. % of light transmitted varied from 96 - 97.5 % for fabricated lenses, which compared to 98 % for commercial lenses ACUVUE©. This is significant to the field of study as the novel manufacturing system developed allowed for an accurate assessment of drug delivery from contact lenses. Other research groups have performed drug release studies on films and discs which do not have the same thickness and shape as contact lenses. Another advantage of the manufacturing system developed was that it allowed control over lens composition and drug loading via direct casting.

Drug-laden polymer particles were investigated as a means of attenuating drug release. Zero order drug release from these drug-laden polymer particles was achieved. Drug loaded polymer particles were loaded onto contact lenses to create novel drug delivery vehicles which delivered 5.84 µg of cetirizine over 24 hours.

The activation energy for the polymerisation of HEMA with AIBN initiator was calculated to be 70.8 $kJ.mol^{-1}$. When drug-laden polymer particles were added to the HEMA monomer, the activation energy dropped to 60.7 $kJ.mol^{-1}$. This result proved that the activation energy for the polymerisation of contact lens monomers could be decreased by the presence of polymer particles. This reduction in activation energy could result in lower cost hydrogel formation, as less energy would be required. Furthermore, it was observed that formulations with two monomers exhibited an increase in activation energy of up to 17.82 $KJ.mol^{-1}$. Results obtained in this study determined that polymer particles can impact the polymerisation reaction of contact lens monomers and affect the polymers physical properties and this area warrants further investigation.

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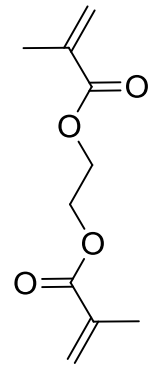

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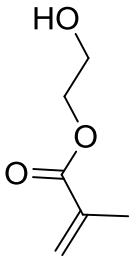

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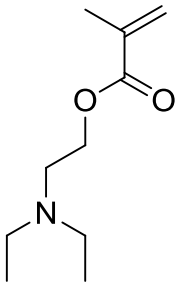

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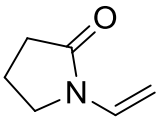

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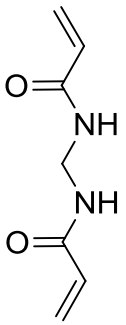

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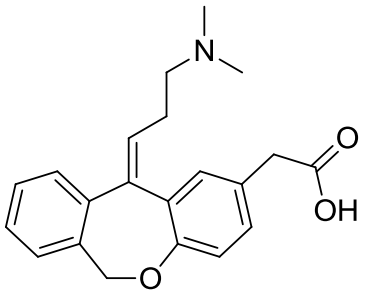

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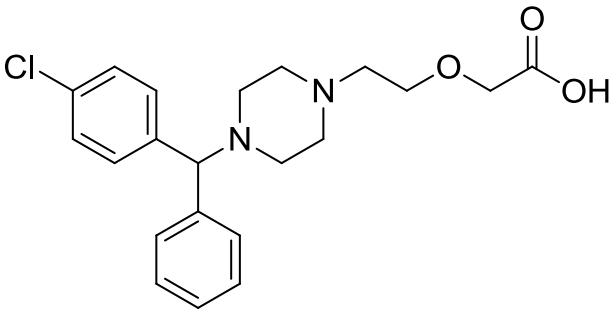

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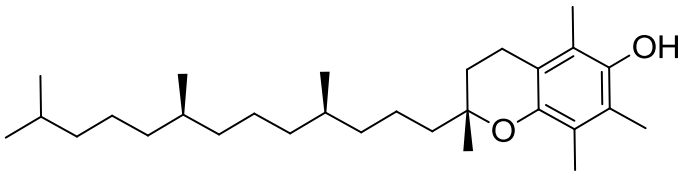

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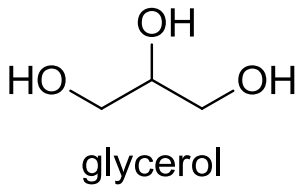

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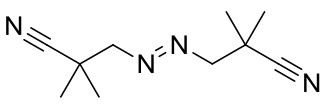

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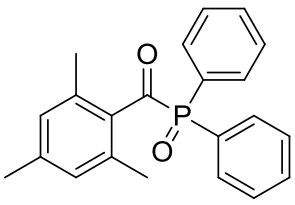


TPO

LIST OF ABBREVIATIONS

ACN	Acetonitrile
AIBN	Azobis-iso-butryonitrile
CLIDE	Contact Lens Induced Dry Eye
DEAMA	Diethyl-amino-methacrylate
NDMA	N, N-di-methyl-acrylamide
DMA	Dynamic Mechanical Analysis
DMS	Dexamethasone
DMSA	Dexamethasone Acetate
DSC	Differential Scanning Calorimetry
EGDMA	Ethylene glycol dimethacrylate
FDA	Food and Drug Administration
HEMA	2-Hydroxyethylmethacrylate
HPMC	Hydroxy-propyl-methyl-cellulose
IOP	Intra Ocular Pressure
LOD	Limit of Detection
LOQ	Limit of quantification
MAA	Methacrylic acid
MIPs	Molecularly Imprinted Polymers
NNMBA	N, N methylene bis-acrylamide
NP	Nanoparticles
NVP	N-vinyl-pyrrolidone
PVA	Polyvinyl-alcohol
PVP	Poly (vinyl-pyrrolidone)
RGP	Rigid Gas Permeable
RPM	Revolutions per minute
SCF	Super Critical Fluid
SCL	Soft Contact Lens
SDS	Sodium Dodecyl Sulphate
SEBS	Styrene-(ethylene-butene)-styrene
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis

Chapter 1

Ocular Drug Delivery

1. OCULAR DRUG DELIVERY

To deliver an active ocular drug to the eye in an effective and safe concentration is the goal of ocular drug delivery. This market is extremely large, approaching 18.7 billion dollars in 2012 [1], and is composed of a large number of drug delivery devices, such as, eye drops, ointments and creams. Ocular drug delivery is hindered by poor bioavailability of drugs. Poor bioavailability of drug in the eye is caused by the natural factors of tear turnover, drainage of the eye and subsequent loss of drug [2]. Providing controlled ocular drug delivery over time would aid the treatment of conditions such as Glaucoma, hay fever, post-operative swelling and dry eye syndrome [3]. Using contact lenses (Figure 1.1) as a medical device to facilitate drug delivery has been studied by a number of researchers and clinicians [4-6].

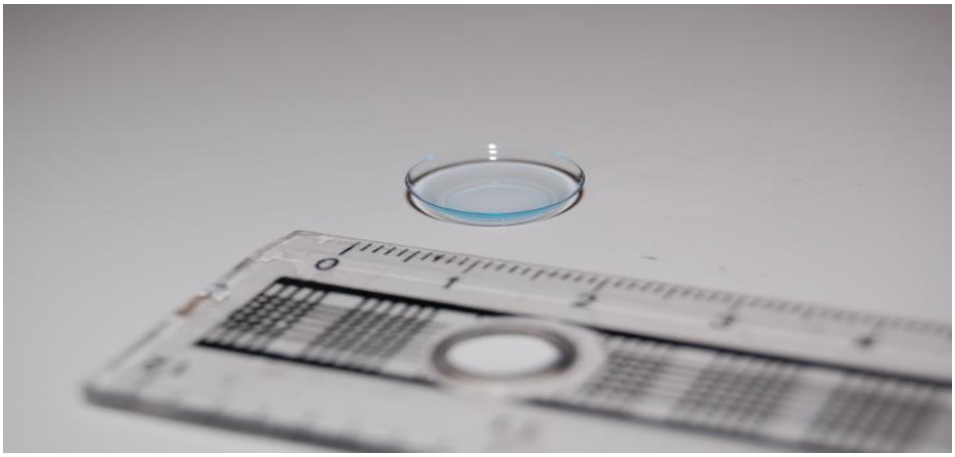


Figure 1.1: Hilafilcon daily disposable lens manufactured by Bausch + Lomb.

The aim of this research was to develop the ability to load soft contact lenses with antihistamine drugs and ensure their controlled release. Contact lenses used as medical devices would be suitable for the treatment of a variety of ocular conditions [7-11] . Novel contact lens drug delivery vehicles could provide prolonged drug delivery and increased patient comfort would ensure strong patient compliance and enhance effective disease management. Drug loading capacity of soft contact lenses has been shown to be quite low and as such has hindered their use as medical devices. Novel methods of increasing this drug loading capacity are being

investigated such as piggy back lenses, supercritical fluid drug loading and molecularly imprinted polymers [12-17].

1.1. The eye

1.1.1. Anatomy of the eye

A brief description of the eye and its surrounding structures are detailed in this section for the purpose of understanding the environment into which ocular drug delivery will occur. An eye lid provides a protective barrier for the eye. Eyelids have the ability to open and close as they are made from cells which are modified muscle tissue. Eyelids contain blood vessels and glands, which produce tears that lubricate the eye and the eyelids. Blinking distributes this lubricating fluid over the surface of the eye and removes any particles that are present in the eye. Any excess tears are removed during blinking and spill out into the surface of the eyelid or are forced into the lachrymal canal. The tear film allows oxygen to permeate it and reach the cornea, but prevents airborne particles from contacting the delicate tissues of the eye. Underneath the tear film is the conjunctiva, a transparent membrane which covers the inner eyelid and the front of the eye except for the cornea. As the conjunctiva contains blood vessels it can provide nutrients for the eye and white blood cells to protect against infections [18].

An eye's outer surface is made up of three separate layers known as coats. An outer layer which contains the sclera and cornea is known as the fibrous coat because it is a dense, strong wall which forms a protective barrier for the eye [19]. Visually observed as a white layer underneath the tear film. Uveal tissue, or middle layer, contains the iris, ciliary body and choroid and is known as the vascular coat. Its primary role is in providing nourishment and exchanging gases. Finally the retina or inner layer, known as the nervous coat as it is responsible for vision. A further division of the eyeball can be performed which splits it into two segments, anterior and posterior, which are two humor filled chambers. The anterior segment contains the lens, iris cornea and aqueous humor. The posterior segment contains structures posterior to the lens, the vitreous humor choroid retina and optic disc [20] as presented in (Figure 1.2).

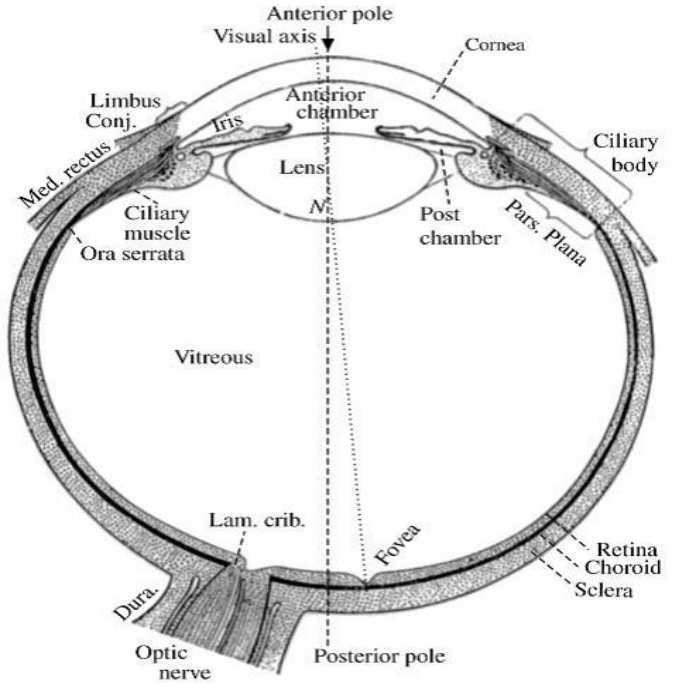


Figure 1.2: **Diagram of the eye illustrating its components [20].**

1.1.2. Tear film

A tear film has three layers, an outer lipid layer coating, an aqueous layer, which itself coats a thin layer of mucous [21]. Tear film components are secreted by a number of glands. In general the tear film is comprised of electrolytes, nutrients and a complex mixture of proteins, mucin and lipids. Tear film structure is maintained by blinking and the lipid layer is redistributed over the surface by the blinking action. Another function of the tear film is to act as a protective barrier for the eye, and provides nutrients to the cornea as well as maintaining the pH of the eye. It also provides the medium for cellular migration, cell differentiation and wound healing. These functions require a complex mixture of chemicals, including growth factors, cytokines, biologically active peptides, tumour necrosis factors, tear proteins and interleukins [22].

A cornea has to be transparent in order for clear vision, therefore it has no direct blood supply, its sole source of nutrients and waste removal is the tear film [23]. The tear film acts like an umbilical cord feeding the cornea nutrients and removing

wastes [22]. A cornea would die or be damaged without oxygen and if carbon dioxide is not removed, acidosis will occur at the surface of the cornea i.e. carbonic acid will form in the tear film due to the high concentration of carbon dioxide present, which causes pain and discomfort. Phases of the tear film are graphically illustrated in Figure1.3.

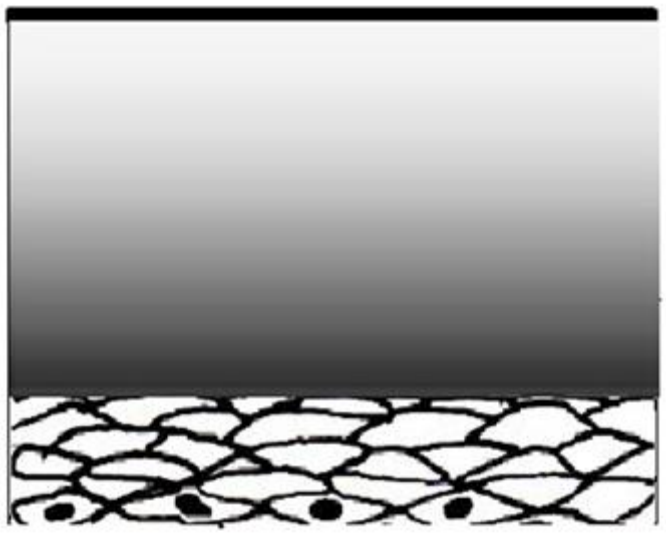


Figure 1.3: **Representation of tear film composition and approximate dimensions (adapted from [22]).**

1.1.2.1. Lipid phase

A lipid layer is the outermost layer of the tear film and is comprised of lipids such as omega hydroxyl yacyl fatty acids [24]. It is approximately 1 µm thick [22]. The lipid layer is mostly secreted by the meibomian glands, which are located in the upper and lower eyelids. These sebaceous glands consist of a single lobule or collection of lobules that form into a system of ducts. There are approximately 60-70 of these glands in the eye dispersed throughout the upper and lower eyelids. The release of lipids from the gland is controlled by blinking [25]. Meibomian glands secrete material via holocrine secretion. As the glands product is released with the remnants of the dead cells, the product is released into the cytoplasm of the cell and is released when the cell membrane ruptures. The lipid layer is responsible for the structural refractive integrity of the surface of the eye. Meibomian gland dysfunction is also one of the major causes of evaporative dry eye [26]. This outer layer is also responsible for preventing tear spill over by containing tears within the opening

between the eyelids. It also increases the stability of the tear film by interactions with the soluble components [19].

1.1.2.2. Aqueous phase

Located within the orbit of the eye, the main lachrymal gland produces the majority of the fluid in the aqueous phase of the tear film. These exocrine glands secretes their products into ducts [19]. Lacrimal glands, specifically the acinar cells within the lacrimal gland secrete the majority of the proteins present in tears. The main lacrimal gland secretes proteins which form part of the immune response. This protects the eye from infections and toxic chemicals. These proteins prevent bacteria adhering to the surface of the eye and obstruct viral attachment and block damage caused by toxins. Electrolyte composition and concentration is important to the health of the surface of the eye [21]. As these ocular tissues need to be kept hydrated as they are exposed i.e. not covered by skin.

1.1.2.3. Mucin phase

Goblet cells, as well as corneal and conjunctival squamous cells produce and secrete the mucous phase of the tear film. Goblet cells are scattered throughout the conjunctiva squamous cells [27]. Mucins are the chemical components of the mucus layer (large glycoproteins) and inorganic salts, which acts as a barrier to infection, stabilises the tear film and protects the epithelia of the eye from damage [21]. Damage can come in the form of microbial infection, physical trauma or desiccation [19]. Secretory mucins are released into the tear film and transmembrane mucins are attached to epithelial cells. The mucous phase of the tear film is represented in Figure 1.4.

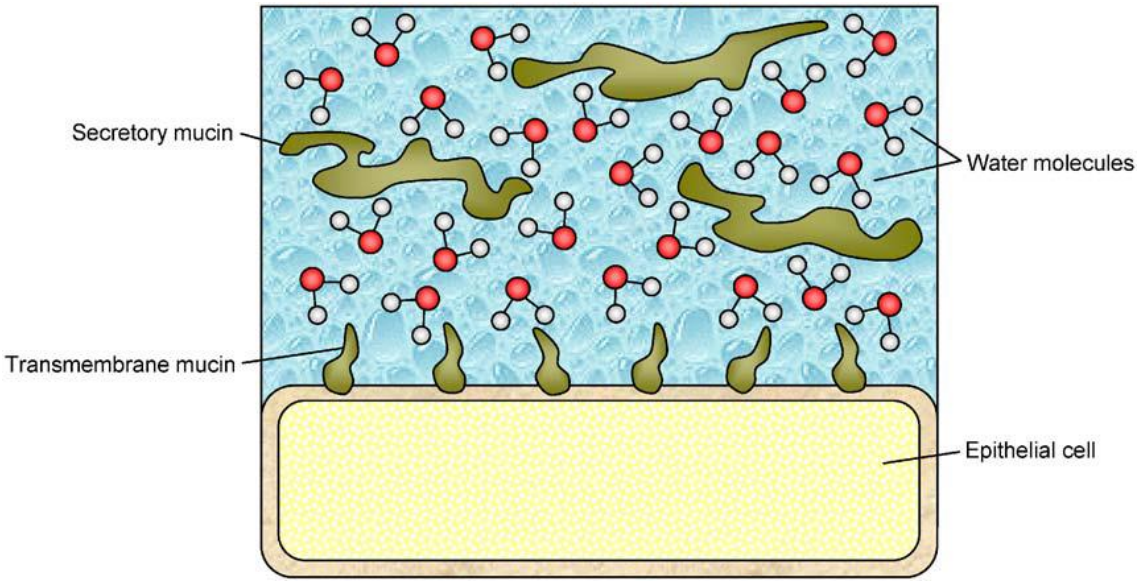


Figure 1.4: **Mucin phase diagram [21].**

1.1.3. Cornea

In order for light to enter the back of the eye it has to pass through the cornea, which is an optically transparent tissue. This is an avascular tissue (i.e. has no blood supply) as it has to be optically transparent. It receives nutrients and oxygen from blood vessels in the sclera closest to the cornea as well as from the surrounding lachrymal fluid tear film and aqueous humour. Waste products such as CO_2 are removed by the tear film [21, 23].

The cornea is approximately 520 microns thick and 12 mm wide. and is made up of five separate layers of specialised cells: epithelium, bowman's layer, stroma, descement's membrane and endothelium [22].

The epithelium is 5-6 layers of squamous stratified cells and its approximate thickness is 50-100 µm. Bowman's membrane is a homogenous sheet of cells 8-14 µm thick. This collagen layer is positioned between epithelium cells and the stroma. Approximately 90% of the stroma is water, it is 200-250 µm in depth and is an open weaved structure of collagen fibres [28]. This layer provides the structural strength for the cornea without compromising its optical clarity normally allows the diffusion of hydrophilic solutes [29]. Descement's membrane is secreted by the endothelium and is positioned between the stroma and endothelium. The endothelium is a single

layer of hexagonal cells 5 µm in depth. This layer is responsible for hydration of the cornea. It is in direct contact with the anterior chamber and water can passively transfer from the aqueous humour to the stroma [30]. An image of a cross section of a cornea is presented in Figure 1.5.

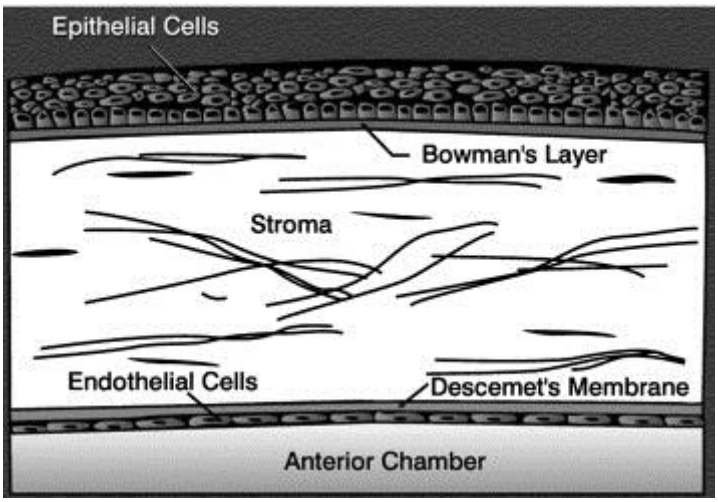


Figure 1.5: Cross section of a cornea [28].

In conclusion the ocular environment is complex, the tear film is constantly moving across the surface of the eye removing any material eluted into it. The tear film itself is a mixture of chemicals which have the ability to react with any foreign body introduced to the eye. Finally the cornea can be negatively impacted as its supply of nutrients or ability to exchange gases is impaired. In order for a drug to pass through the cornea it must be of low molecular weight and have both lipophilic and hydrophilic properties [22]. Any ocular drug delivery device must be able to deliver drugs in a controlled manner under these conditions without damaging the eye or any of its structures. To achieve this the drug delivery vehicle used must be biocompatible and able to tolerate the ocular environment. Using contact lenses for this purpose is ideal and these ocular inserts are used by millions of people daily. Furthermore a contact lens is placed directly over the cornea and this is the most direct route of drug delivery to the eye.

1.1.4. Eye permeability

Pre-corneal factors such as the tear film blinking etc. negatively impact the permeation of all drugs across the biological structures of the eye. The three types of

tissues which form the main barriers to drug permeation of the eye are the conjunctiva, sclera and cornea. The conjunctiva contains capillaries and vein like lymphatic tubes which cause substantial drug loss into the systemic system considerably reducing bio-availability. It is for this reason that the conjunctiva is considered a non-productive site for drug delivery [31]. The sclera is comprised of collagen fibres and proteoglycan embedded in an extracellular matrix. Its permeability is comparable to the corneal stroma as outlined below and is inversely proportional to the molecular radius of molecule attempting to permeate the tissue [32]. Finally the corneas two barriers to drug permeation are its epithelium cells which are lipoidal in nature, these cells present a significant barrier to hydrophilic drugs. The stroma which makes 90% of the corneal thickness is a highly hydrated structure which provides a barrier to lipid molecules. So any drug trying to permeate the cornea must have an amphipathic nature to permeate both structures.

1.1.5. Drug delivery mechanisms

In general the mechanisms which control drug release from Polymer matrices are solute diffusion, polymeric matrix swelling, and material degradation. In particular Fick's law provides a description of solute (in this case drug) transport from polymeric matrices. Fickian diffusion refers to the solute or drug transport process where the polymer relaxation time (*tr*) is much greater than the characteristic solvent diffusion time (*td*). When *tr* approaches *td*, the macroscopic drug release rate is non-Fickian or controlled release [33].

Mechanisms of drug release from polymer matrices can be further subdivided into non degradable, biodegradable and polymer dissolution.

Non degradable describes drug release from polymer matrices where the drug is entrapped within the polymer matrix. Non-degradable polymer release can be broken into two types, matrix or reservoir. In a matrix polymer drug delivery system the drug is uniformly dispersed through the polymer [34]. This differs from the reservoir system where there is an inert coating material, which functions as a rate-controlling membrane. Release rate for matrix systems remains relatively constant and are not affected by concentration gradient, but most likely is related to the thickness and permeability of polymeric membrane. *Kim et al.* observed improved drug release

characteristics of testosterone from transdermal drug delivery vehicles through an ethylene vinyl acetate membrane [35]. Whereas matrix devices are much more likely to have fickian diffusion driven delivery. As the rate of drug delivery is governed by concentration gradient and degree of polymer swelling.

Biodegradable polymers have been widely used in drug delivery devices [36-38]. In this type of drug delivery system control of drug release is obtained via the degradation of the polymer. These polymers contain labile bonds such as ester-, amide and anhydride bonds which can be broken by hydrolysis or enzymatic activity. Hydrolysis a critical factor for both degradation of the polymer and drug release.

Polymer dissolution is comprised of two transport processes solvent diffusion and chain disentanglement. Although no polymer chains are broken dissolution results in the loss of material from the polymer. *Djekic et al*. used lecithin gels for the transdermal drug delivery of Ibuprofen [39] for the treatment of arthritic pain. Drug transport in these systems can be driven by diffusion and or dissolution.

1.1.6. Ocular drug delivery methods

The ideal drug release profile would be a steady release of a therapeutic amount of drug to the eye. In practice burst release is normally obtained. Here a high % of the drug loaded (normally over 50%) is released very quickly in the first hour or less [40]. This type of rapid drug delivery will reduce the effective lifetime of any drug delivery vehicle. The following sections describe drug delivery methods which either limit the loss of drug or attempt to attenuate its release.

1.1.6.1. Contact lenses

The combination of tear fluid and cell differentiation is extremely effective at stopping the penetration of material to the internal structures of the eye. The contact lens position on the eye affords protection from the actions of blinking, tear turnover and tear evaporation [41]. However, barriers to drug delivery are still in place such as binding from proteins present in the tear fluid, adsorption or absorption of drug at other sites than the target, poor corneal permeability and metabolism of drugs [22]. The different types of cells encountered on the route through the eye need to be considered. Once drugs are released from the polymer into the tear film they have to

diffuse through the cornea. The cornea is made up of layers from, epithelium to the endothelium, which has different permeability's for hydrophilic and lipophilic drugs and provide a barrier to drug permeation [42]. An effective drug delivery vehicle will have to overcome these obstacles in order to be effective.

If a drug substance is being targeted at delivery to the aqueous humour at the back of the cornea it will need to have both hydrophilic and lipophilic properties and have a small molecular weight in order to successfully permeate the ocular tissues. The environment of the eye and the tissues which need to be permeated set the boundaries for drug delivery. The drug delivery vehicle can help overcome some of the barriers to drug delivery such as tear turnover, but the drug will have to be compatible with the ocular environment or tissues it will encounter. This creates a situation where particular materials have to be delivered by certain routes, for example, large molecular weight molecules may need to be injected as they would not pass through the cornea. The possible options for ocular drug delivery and their advantages and disadvantages are discussed in the following sections and listed in Table 1.1.

1.1.6.2. Systemic drug delivery

The blood retinal barrier prevents direct systemic drug delivery to the eye. The tight junctions of these cells provide a barrier to the passage of substance into the retinal blood supply and from there to the vitreous humour. 1-2% of plasma drug levels are attained in the vitreous of the eye [43]. This low level of penetration calls for frequent high dose administration, which causes issues with systemic side effects [44]. This method of drug delivery is wasteful and not ideally suited for ocular drug delivery. The most common systemic drug delivery systems are tablets and capsules. This type of drug delivery vehicle subjects the drug to "first pass metabolism inactivation" due to stomach acids and other digestive processes [45].

1.1.6.3. Intravitreal injection

Intravitreal injections deliver pharmaceuticals directly to the vitreous cavity of the eye. This method of drug delivery has advantages over the systemic delivery of drug

as it achieves higher levels of drug in the posterior segment, without the risk of systemic side effects and is a very successful treatment for delivering drug to the back of the eye [46, 47]. Therapeutic doses can be preserved with lower doses of drug to the patient, as the drug is directly administered to the site of use. The disadvantage of this drug delivery method is the need for it to be performed in a hospital environment and the fact that the patient suffers pain and discomfort. There are a number of medical issues with repeated ocular injections such as increased risks of cataracts and retinal detachment [48].

1.1.6.4. Intravitreal implant

Devices like Vitrasert® are surgically implanted into the eye. 4.5 mg of ganciclovir drug is coated by a laminated system of biocompatible polymers and is then compressed into a 2.5 mm disc [49]. This disc is then surgically implanted into the vitreous humor of the eye and attached to the sclera by a suture. The implant overcomes the need for repeated intravitreal injections and the implant can deliver drug to the eye for 5-8 months at a rate of 1 µg per hour. There are still risks with implantation as it is a surgical procedure and adverse reactions do occur, for example, loss of visual acuity, vitreous haemorrhage and retinal detachment. The main disadvantages of this drug delivery method are its expense and that the device needs to be removed and a new device implanted when it has been depleted.

1.1.6.5. Scleral drug delivery

In this method of drug delivery devices are surgically implanted into the sclera of the eye. The sclera is not a significant barrier to diffusion of compounds to the vitreous chamber; however, the molecular size of the drug will impact on its ability to permeate through the sclera. Scleral plugs, implants and subconjunctival injections still have the same cost and patient discomfort issues, as other intravitreal methods and are only suitable for certain types of molecules [50]. Using this area for implanting or injection is advantageous as there is a reduced risk of retinal detachment.

1.1.6.6. Carrier mediated delivery

Prodrugs are created to counteract undesirable characteristics of a drug. They are formed to assist a molecule cross a membrane or tissue barrier, by utilising biological transport and receptor systems in the eye. This drug delivery method allows molecules to pass through barriers, for example; a hydrophilic molecule can pass through a lipophilic membrane. This can be achieved by the chemical modification of the drug to change its physiochemical properties by adding a moiety to the drug which chemically and physically matches the barrier. Once the barrier has been breached drug delivery occurs by enzymatic hydrolysis releasing the drug from the carrier. This type of drug delivery has the ability to be used with a number of drugs and transport systems in the eye. The cornea alone has four transport systems which aid the delivery of peptides, nucleosides, glucose, and amino acids across it [51]. A number of di-ester ganciclovir prodrugs were synthesised by Patel *et al*. [52] The prodrugs created had high solubility and demonstrated enhanced viral potency when compared to the parent drug. Here a 16 fold increase in the aqueous solubility of the drug was achieved compared to the parent drug.

1.1.6.7. Eye drops, ointments and creams

This method of delivery is easy to use but suffers from high losses due to tear turnover and spillage, as the blink response removes the instilled material from the eye. 95% of the formulation instilled into the eye is lost, mostly ending up in the lachrymal canal [53]. To adequately treat the eye large doses need to be frequently applied. This leads to possible systemic side effects. Poor patient compliance coupled with short therapeutic dose times, are the reason for poor disease management using this type of drug delivery. The main advantages of this drug delivery method are that it is inexpensive and easy to use by the patient [48].

1.1.6.8. Achievement of effective treatment

To achieve effective treatment and drug delivery the following parameters must be considered; the drug type, disease severity, side effects, therapeutic dose needed and patient compliance. The advantages and disadvantages of these are presented in

Table 1.1. Intravitreal injections and implants deliver pharmaceuticals directly to the vitreous humour. The issue with this type of drug delivery are its expense, and patient discomfort as a surgical procedure is required. Ointments and creams are the most widely used drug delivery system because of their cost and ease of use. The disadvantage of drug delivery from ointments or creams is that the dose delivered is at a therapeutic level for a very short time. Couple this with poor compliance, i.e. the patient missing an instillation and the patient's medical condition could be untreated for long periods of time. For diseases such as glaucoma this means that the patient's disease is poorly managed and their sight could be impaired while taking the medication. Real clinical benefit could be obtained from delivering smaller doses at therapeutic levels to the tear film over long periods of time. If the device used can be positioned by the patient a significant improvement in disease management can be realised. Using a contact lens as a drug delivery vehicle can overcome many of the issues with ointments and creams without adding expense or patient discomfort from undergoing surgery [8, 10, 11, 27, 54-57]. The purpose of this study will be to develop commercially viable contact lenses and load them with drug and attenuate their release thereby creating novel drug delivery vehicles. The ability to load the lens and elute substances from it in the ocular medium will be measured by *in-vitro* studies. The ability to load comfort agents as well as active pharmaceutical ingredient is meeting a patient need as Dry Eye Syndrome (DES) affects 10-20% of the adult population and up to 70% of contact lens wearers [58]. Table 1.1 presents a comparison of ocular drug delivery methods.

Table 1.1: Ocular drug delivery method comparison [59].

Delivery method	Drug delivery Area	Advantages	Disadvantages
Contact lens	Tear film	Improved Bioavailability Increased residence time	Low load capacity Poor drug release profile
Systemic	Blood supply	Good compliance	Losses due to first pass metabolism
Intravitreal injection	Vitreal cavity	Dose controlled	Costly surgical procedure with risk of retinal detachment
Intravitreal implant	Sclera	Controlled release No patient interaction	Costly surgical procedure, Medical risk increased
Scleral	Sclera	Improved release profile	Expensive surgical procedure to implant
Carrier mediated delivery	Tear film	Improved Bioavailability	Area requires further study
Eye drops, ointments and creams	Tear Film	Ease of use	Poor drug release profile Side effects

1.2. Contact lens development

1.2.1. History of contact lenses

The first plastic material used to manufacture contact lenses was polymethylmethacrylate (PMMA). This material was used to cast lenses as it was seen to be biocompatible, as splinters of PMMA from airplane canopies removed from a number of pilot's eyes in World War II showed no signs of rejection [60]. This material was biocompatible and well tolerated by the body, but it was extremely

hard and uncomfortable for the patient. Although PMMA was not ideal it did prove the concept of using plastics [61]. Softer polymers replaced PMMA in the manufacture of contact lenses.

The hydrogel or soft contact lenses that are in common use today were invented by Otto Wichterle and his colleagues at the institute of macromolecular chemistry of the Czechoslovakian Academy of Sciences Prague in 1961 [62]. He was working on the synthesis of a new compound that could be used for implantation into the human body, poly-hydroxyethylmethacrylate or (pHEMA) [63]. He realised the potential of this material for use in the manufacture of contact lenses. However, the early models were too thick and the optical power was not reliable. In 1966 Bausch + Lomb bought the rights to the Wichterle process for 3 million dollars. By the time The FDA approved the use of these lenses in 1971 the process had been refined and Bausch + Lomb became the world's largest manufacturer of contact lenses [62]. These lenses provided comfort and ease of use for patients. The types of chemical reactions which create polymers include addition and step or condensation reactions [64]. These are discussed in the following sections.

1.2.2. Polymerisation

1.2.2.1. Addition polymerisation

A polymer formed by an addition reaction must have multiple bonds, which will react to allow the addition of other monomers. In reaction these double bonds "open up" to allow addition of the monomer units, (Figure 1.6). This reaction can continue, building large molecular weight molecules; and this growing molecule is illustrated as shown in Figure 1.6 where the monomer unit is in closed brackets and the number of repeated monomer units in the molecule is represented by the number n

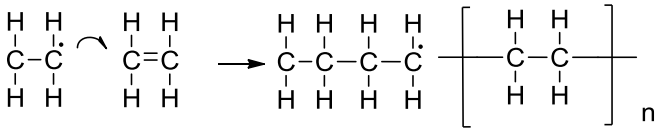


Figure 1.6: **Schematic of the addition polymerisation of ethylene.**

The polymerisation requires a source of free radicals, which is generally supplied by an initiator, a substance which decomposes under heat or UV radiation. The free radicals then break the double bonds of the monomer an example of which is shown for HEMA in Figure 1.7.

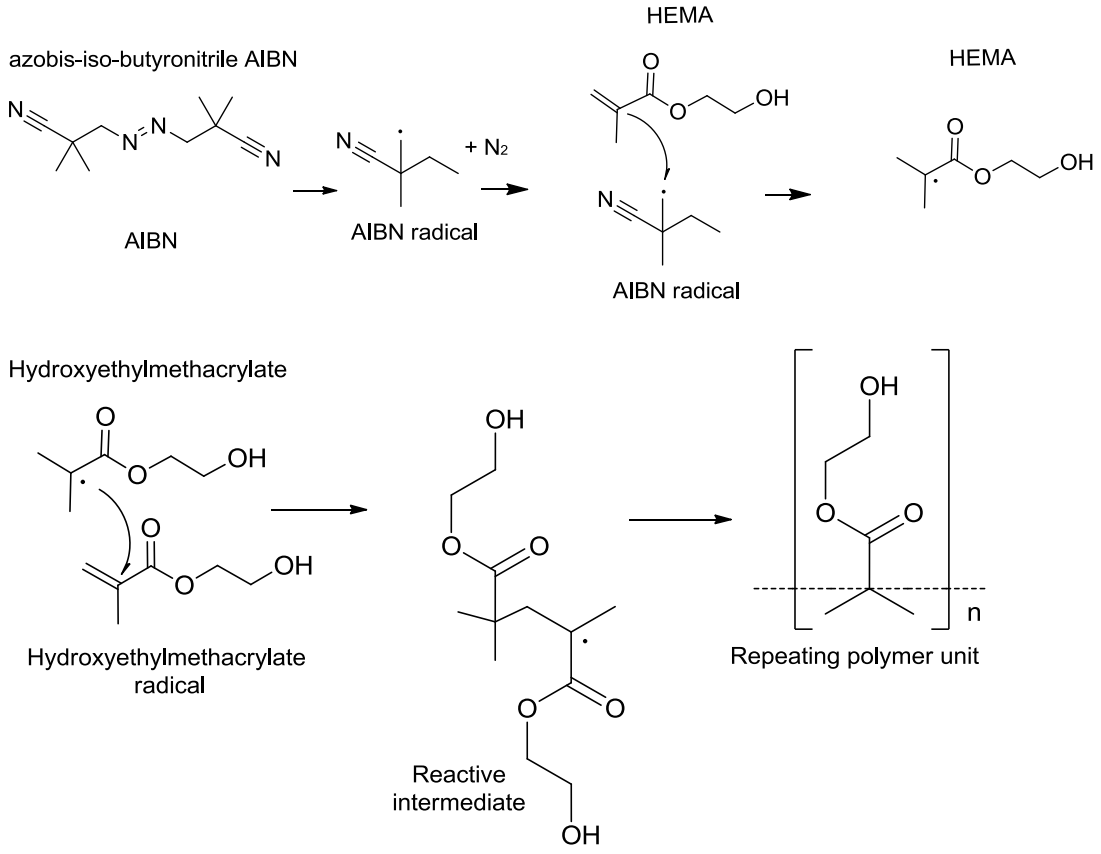


Figure 1.7: Schematic of the free radical polymerisation of HEMA.

This chain reaction occurs as the double bonds break, allowing the addition of another monomer unit. This reaction has three characteristic steps, initiation, propagation and termination. In initiation a source of free radicals is introduced to the monomer and the free radical reacts with the double bond creating an intermediate reactive species which can then continue to react. It adds on to the molecule or propagates i.e. it creates a new reactive species, one monomer unit longer. The chain length increases in this manner and longer and longer polymer chains are created. In termination the molecular reaction is completed. Termination can occur when the terminal end of the molecule reacts with a free radical or a

smaller polymer chain known as coupling termination. Disproportionation can also occur when the reactive species created captures hydrogen from another polymer chain: both molecules are stabilised in the process. The molecule which has the hydrogen atom abstracted forms a double bond and the other molecule lone pair bonds with the abstracted hydrogen to form two polymers [65].

1.2.2.2. Step reactions

Step or condensation reactions can also be used to create polymers. In these reactions monomers, with two or more functional groups react and then a smaller molecule such as water is ejected. In this reaction two monomers react to form a dimer and then again with another monomer to form a trimer. This reaction, then continues, although slowly and produces polymers of intermediate to high molecular weight. The reaction of terephthalic acid with ethylene glycol, which creates the trademarked polymer Dacron is described in Figure 1.8 [66].

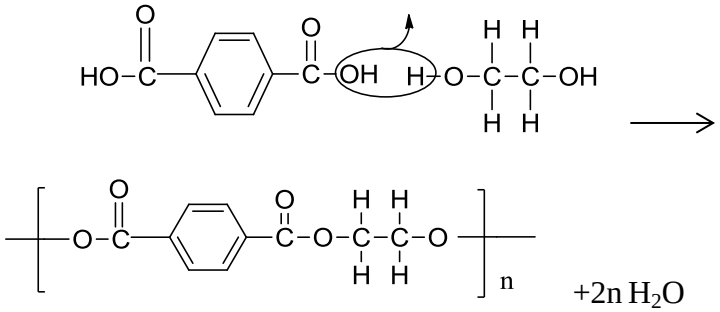


Figure 1.8: **Step reaction which forms Dacron [66].**

1.3. Hydrogels

Hydrogels are a class of hydrophilic cross-linked polymers which absorb substantial amounts of water [5]. Hydrogels can be either physically cross-linked where polymer chains are linked via ionic interactions such as alginates and polysaccharides or chemically cross-linked where the polymer chains are chemically bonded to each other an example of which would be methacrylates [67]. In this work the focus will be on chemically cross-linked hydrogels as they are used to manufacture contact lenses. These insoluble materials maintain structure and shape due to their chemical cross-linking, which provides mechanical strength. HEMA, or in its polymerised form poly HEMA is used in a wide variety of pharmaceutical applications [14, 68-

70] . These materials are widely used in the manufacture of contact lenses [3, 14, 60, 71-74] as well as implantable medical devices used for drug delivery [53, 69, 75-79] Maintaining physical shape is vital for contact lens drug delivery devices as they must fit the eye and be transparent. Polymers can be prepared by a number of techniques: suspension [80, 81] bulk [82, 83], solution [84-86] and precipitation [38, 87, 88] polymerisation. In regard to ocular drug delivery from contact lenses, photo or heat initiated bulk polymerisation reactions are used to manufacture these polymers, especially for the high volume daily disposable contact lens market [60]. The Use of multiple monomers to produce lenses can have a large impact on the physical properties of the resultant lens. The ability to alter the swelling, physical strength or hardness and permeability of contact lenses are vital to ensure patient comfort [89]. EGDMA is a commonly used cross-linker used in the manufacture of contact lenses and HEMA hydrogels and can be used to increase the physical strength of the polymers produced [90, 91]. These materials are commonly used in the manufacture of both contact lenses and controlled release drug-laden polymer particles [4, 92-94].

1.3.1. Hydrogel contact lens properties

A hydrogel can be defined as a "cross-linked network of hydrophilic polymers". They have the ability to swell and keep their three dimensional structure [95]. Hydrogel physical properties lend themselves to their application in contact lens manufacture [89]. In order for a lens to function, it must be optically transparent (optical materials that are used for contact lenses must transmit 90% of visible light) and have sufficient mechanical strength. Its surface must interact with the tear film and be biocompatible. As the cornea receives oxygen from the air, the polymer must be oxygen permeable to prevent acidosis and itching and discomfort for the wearer. [19]. If the material does not have sufficient oxygen permeability acidosis and hypoxia will occur under the lens and the CO_2 produced will acidify the tear film causing discomfort to the wearer [96]. To ensure the contact lens can remain in place on the cornea some fluid or ion permeability of the lens is needed. Its refractive index must match that of the cornea so that vision is not impaired. Dimensional

stability is also very important so that the material will swell in a uniform manner and it will be able to maintain its dimensions under ocular conditions.

Mechanical strength is also an important factor in contact lens manufacture as its effect on patient comfort and durability is profound. Contact lenses must be soft and flexible for comfort, but have the necessary strength so they can withstand handling. When the lens is in place in the eye it will be deformed by the action of blinking. When hydrated the material is soft and flexible because water acts as a plasticizer. Hydrogels are viscoelastic so they deform in a time dependent manner when a stress is applied and they recover in the same manner when the stress is removed as long as the yield point has not passed beyond its elastic limit. The tensile modulus of the material determines how the eyelid will deform the lens. Strength will suggest how the material will perform when handled. These parameters can be altered by changing the composition of the polymer or how the polymer is processed [60].

Surface characteristics of the polymer determine the wettability of the lens manufactured. The term wettability refers to the ability of a liquid to spread onto a solid surface [97]. This parameter impacts the comfort and the stability of the tear film under the lens. Assessment of the wettability of contact lens materials is made using contact angle measurement. The contact angle is dependent on a number of factors, most importantly, surface tension of the test liquid and the surface used. This method provides information on the interaction of the lens with the tear film. How the lens will impact both tear formation and stability can be determined by this method.

The water content of chemical hydrogels is also an important factor for comfort and strength. The more water absorbed by the hydrogel the more flexible it will become. The high water content of hydrogels ensures they are comfortable on the eye, but they have consequently lower oxygen permeability due to the high water content of the swollen lenses, which leads to discomfort when worn for more than eight hours [89].

Ion Permeability is required to prevent the contact lens from adhering to the cornea and allow movement of the lens on the tear film. The lens can be deformed during

blinking; ion transport through the lens will allow the tear film to reform and prevent it adhering to the cornea. Movement of salt through the matrix is more difficult as sodium ions have to be in a shell of H_2O. As sodium is a large constituent of the tear film this ion is very influential in preventing the lens from adhering to the eye. The movement of salt across the hydrogel matrix *in vivo* is governed by the permeability of the polymer and is an artefact of its partition coefficient and diffusivity. Silicone hydrogels are two phase hydrogels as they are produced from a mixture of hydrophilic and hydrophobic monomers. Sodium cannot pass through the silicone phase of the hydrogel. This means that the phase separation and distribution of the silicone phase throughout these hydrogels is crucial to its ion permeability [98].

The refractive index (RI) of the lens must be similar to the tear film, which is 1.337 [29]. RI varies with water content. This relationship is linear for non-silicone or conventional hydrogels up to approximately 70% water content [99]. Therefore, any impact on the water content of the lens will impact its refractive index.

The dimensional stability of a hydrogel is measured by its ability to return to its original shape when stress is applied to it. The dimensional stability of the hydrogel is affected by any factor that will change its water content, as water acts as a plasticiser. This data is crucial if the lens is to be manufactured by lathing where the dry hydrogel is lathed and then swelled. This swelling factor must be accurately known to ensure the correct power of a lens is achieved.

The Swelling factor (SF) can be obtained using Equation 1.1 [99].

$$\boldsymbol{SF = \frac{Wet\ dimension}{Dry\ dimension}} \qquad \textbf{Equation 1.1}$$

The lens geometry and size vary with manufacturer and on the magnifying power or dioptre required for visual correction. Table 1.2 details, examples of 3 commercial contact lens dimensions [100].

Table 1.2: **Contact lens geometry, water content and dimensions [100].**

Material	Etafilcon	Balafilcon	Lotrafilcon
Water content	58%	36%	24%
Base curve (mm)	9.0	8.6	8.6
Diameter (mm)	14.2	14	13.8
Centre thickness (mm) -3.00 power	0.084	0.09	0.08

Etafilcon= (HEMA) copolymerised with sodium methacrylate and 2-ethyl-2-hydroxymetyl,1-3 propane diol tri methacrylate
Balafilcon= Trimethoxy silane (TRIS) copolymerised with N-vinyl-pyrrolidone (NVP).
Lotrafilcon= Fluoroether macromer copolymerized with a TRIS monomer and N, N-di-methyl-acrylamide (NDMA)

1.3.2. Contact lens manufacture

Contact lenses are manufactured by a number of methods, three of the most common are lathe cutting, spin casting and cast moulding. Critical attributes for contact lens manufacture are optical clarity, visual correction and cost. A lens must be affordable and the manufacturer has to be able to make a profit. The manufacturing process and hydrogel properties have to be simplified and controlled in order to manufacture a high volume product, which can meet these critical quality attributes [63].

1.3.2.1. Lathe cutting lens manufacture

This manufacturing process begins with a button of dry hydrogel material, once it is secured to a back surface of a lathe it is spun about a central axis. Two diamond tipped cutters are used to cut the button, one cuts the posterior surface and shape of the lens and the other reduces the thickness of the lens from the button, this occurs simultaneously. The hydrogel buttons must be kept dry and the humidity of the environment controlled so the hydrogel disc can be cut accurately. When cutting is completed the surface of the lens are smoothed by polishing using abrasives which are suspended in oil and the lens is then hydrated in saline and inspected, autoclaved and packaged. This method of manufacture is very labour intensive and more expensive than spin casting or cast moulding [101]. It does, however, have applications for the manufacture of niche lenses with unusual or very high prescriptions.

1.3.2.2. Spin casting contact lens manufacture

A lens form is created by pouring liquid monomer into a concave mould which is spun at a high rate about a central axis in an oxygen deprived atmosphere, as the posterior surface of the lens is being exposed to atmosphere while it is being cured [102]. If oxygen is present it will scavenge initiator and inhibit polymerisation. This will affect the surface of the lens. The speed of rotation, amount of monomer and tool shape, establishes final lens parameters. Ultraviolet light and or heat are used to initiate polymerisation and form the final lens. The edges of the lens can be polished once the polymerisation is complete and then inspection, hydration, re-inspection, packaging and autoclaving can occur.

1.3.2.3. Cast moulding contact lens manufacture

A set of matching male and female polypropylene moulds are created using highly polished steel tools or non-ferrous materials [103] by an injection moulding process. Use of non-ferrous materials can produce moulds with high levels of accuracy and surface finish as presented in Figure 1.9.

Figure 1.9: **Polypropylene contact lens moulds.**

State of the art moulding machines can manufacture high quality, reproducible moulds in high volumes, cheaply. Maintaining quality at a low cost is essential for the manufacture of daily disposable lenses. Final lens quality is chiefly dependent on the quality of the mould. A continuous highly automated manufacturing process is needed to meet these requirements. Monomers are filled into the female mould (contact lens shape is defined by the mould) a male mould is then placed on top. A

process known as “liquid edge moulding” as the edge is formed by the pressure of the mould lid and the amount of monomer added to the mould. This action creates the lens edge and squeezes out any excess monomer to leave the edge intact. Considerable expertise and engineering are involved in mould manufacture to ensure that this happens consistently and forms an edge which is comfortable for the patient. Moulds can then be placed in an oven for curing or cured by ultraviolet radiation as the polypropylene mould is transparent to UV light. When the lens is removed from the mould there is no need to finish or polish the lens and the moulds can be discarded. Finally the lens is hydrated in saline visually inspected and packed in blister packs and autoclaved [63].

1.3.3. Contact lens classification.

There are a number of classification systems for contact lenses. The American Food and Drug Administration (FDA) have two classification systems for contact lens materials. These classifications are presented in Tables 1.3 and 1.4. The first FDA classification system categorises contact lenses based on water content and whether the polymer is ionic or non-ionic for soft contact lenses, and the second classification system assigns groups based on the chemical composition of the lenses.

Table 1.3: FDA soft contact lens material classifications based on water content [104].

Group	Material
I	Low water content (<50%), non-ionic polymers
II	High water content (>50%), non-ionic polymers
III	Low water content (<50%), ionic polymers
IV	High water content (>50%), ionic polymers

Table 1.4: FDA classification of soft contact lenses by material composition [104].

Group	Material
1a	Poly 2-HEMA, but ≤0.2% by weight of any ionisable chemical (e.g., MAA)
1b	Poly 2-HEMA, but >0.2% by weight of any ionisable chemical (e.g., MAA)
2a	A copolymer of 2-HEMA and /or other hydroxyalkylmethacrylates, di-hydroxyalkylmethacrylates or alkylmethacrylates but ≤0.2% by weight of any ionisable chemical.
2b	As in group 2a but >0.2% ionisable chemicals.
3a	A copolymer of 2-HEMA with N-vinyl lactam and/or alkyl acrylamide but ≤0.2% by weight of ionisable chemicals.
3b	As in group 3a but >0.2% ionisable chemicals.
4a	A copolymer of alkyl methacrylate with N-vinyl lactam and/or alkyl acrylamide but ≤0.2% by weight of ionisable chemicals.
4b	As in group 4a but >0.2% ionisable chemicals.
5	Soft lens material formed from polysiloxanes.

1.4. Drug-loading of contact lenses

In order for the contact lens to elute a compound into the eye it must first be loaded into the contact lens. Hydrogel matrices are capable of swelling and absorbing water. Diffusion will allow the drug to diffuse into or out of a hydrogel matrix depending on the drug concentration of the solution the hydrogel is placed in [105]. A number of major factors are involved in drug loading and the choice of drug loading technique used. The size of the drug molecule to be loaded is critical as large molecules (e.g. polymers) will not be able to diffuse into the hydrogel matrix. The amount of material required to be loaded into the hydrogel and the compatibility of the material to be loaded with the hydrogel matrix are also of critical importance. Compatibility of drug and hydrogel also needs to be considered as hydrophilic polymers will absorb hydrophilic materials, but will have difficulty accommodating drugs with hydrophobic moieties and vice versa [2]. Techniques currently in use for drug loading hydrogels are described as follows.

1.4.1. Soaking of contact lenses

Soaking is the simplest way of loading drug into hydrogel contact lenses. The contact lens is soaked in a solution of the drug to be loaded. The drug enters the hydrogel matrix over a number of hours and when equilibrium is reached the lens can be removed and inserted into the patient's eye [106] as illustrated in Figure 1.10.

Figure 1.10 Image of contact lens being drug loaded via soaking.

Alternatively the drug can be instilled directly into the eye using, for example an eye dropper while the patient is wearing the contact lens. The benefits of this approach include: its simplicity, ease of loading water soluble drugs and the fact that standard commercial lenses can be used in drug loading studies. Loading is based on a simple diffusion profile, as is the drug elution; no control over release is possible, so it must be combined with other techniques in order to provide controlled drug delivery. This lack of control of drug release is the methods major disadvantage. Schultz and Poling loaded brimonidine tartrate and timolol maleate (two common glaucoma drugs) into contact lenses [107]. The contact lenses were loaded via soaking in a drug solution. The level of drug uptake into the contact lens and its release was then measured. It was observed that the drug both diffused into and out of the contact lens in approximately one hour. The feasibility of using contact lenses as a drug delivery vehicle was proved by the study, but it highlighted the issue of retaining the drug in

the lens for prolonged release. Also, only relatively small amounts of material could be loaded (lens dry weights are approximately 20 mg) and small molecule water soluble drugs are practical for this method of loading [105]. Soluri *et al.* demonstrated ACUVUE® commercial contact lenses had a capacity of 461.78 µg per lens of Ketotifen fumarate, however, the release profile was rapid with 61 % of the loaded drug being released in 120 minutes [6]. Extended release has however been demonstrated by Chauhan *et al.* where they observed release of 0.3 µg a day over 90 days from silicon contact lenses loaded with dexamethasone. The lenses were loaded via soaking the lenses in an ethanol solution of the drug [106]. Soaking has also been used to load drug-polymer particles into contact lenses. Here the lenses were soaked in an ethanol drug particle solution. 2.5 µg a day of timolol was released over 60 days from the lenses loaded with the drug loaded polymer particles.

1.4.2. Super critical fluid drug loading of hydrogels

A super critical fluid (SCF) is defined as a substance above its critical temperature and pressure [108]. An SCF will completely fill any container it is placed in, but it cannot be liquefied once it is heated above its critical temperature. A meniscus will not be evident for an SCF as no phase separation will occur [108]. Once a single phase solvent has been created in this manner it can be used in a number of processes where phase separation is undesirable, for example drying, where the meniscus or capillary forces could inhibit the process of mass transport [109].

Super critical fluids have unique properties which can be used to enhance many chemical processes. The loading of materials into hydrogel matrices is of most interest to this study. The most commonly used super critical fluid is carbon dioxide (CO_2). The material is cheap, non-toxic, non-flammable and as CO_2 is a gas at ambient conditions, it can be easily removed from the polymer after processing [108].

Supercritical solvents such as carbon dioxide (CO_2) can be used to drug load polymer matrices instead of organic solvents. This approach has benefits for the patient as they are not at risk from residual solvents present in the polymers as the process facilitates the removal of the supercritical fluid [109]. A significant beneficial

attribute of supercritical fluids is their ability to swell and plasticise the hydrogel matrix. This increases the interstitial space in the matrix and facilitates diffusion into the polymer matrix. The physical properties of SCF's make them ideal for drug loading hydrogels. Firstly, they swell the hydrogel matrix. Secondly, they have the ability to be tuned so that the solubility of the SCF for specific materials or drugs can be changed, with the addition of solvents. Finally the SCF can be easily removed from the hydrogel matrix when the equilibrium of drug loading is achieved. Depressurisation at the end of the process returns the CO_2 to its gaseous state and as long as this is performed in a controlled manner no damage is done to the hydrogel matrix and the drug remains in the hydrogel [109].

Yanez *et al*. demonstrated loading of 55 $mg.g^{-1}$ of flurbiprofen into Hilafilcon contact lenses using a number of processing cycles to increase the drug loading [4]. Increasing the number of processing cycles, improved the amount of drug which could be loaded and the mechanism proposed was that each cycle created more drug specific cavities which caused both an increase in drug loading and extended drug delivery. This compares to drug loading using lenses in an aqueous solution of < 1 $mg.g^{-1}$. Drug release occurred over 3-4 hours for lenses loaded using super critical solvent compared to 1-2 hours for lenses loaded by soaking in an aqueous solution of flurbiprofen. Intra Ocular Pressure (IOP) medications such as timolol maleate and acetazolamide have been loaded into Balafilcon lenses, using super critical fluids by Costa *et al*. [14]. The solubility of the drug can be improved by the addition of a co-solvent. Ethanol at 5, 10 and 15 % mole concentration was added to the SCF to increase the polarity of the fluid and increase the solubility of the drugs in the SCF. The critical operational conditions of the SCF process are temperature and pressure. These parameters can be manipulated to increase the swelling of the hydrogel matrix. The ability to tune these parameters allowed improved impregnation of commercial Balafilcon contact lenses with both timolol maleate and acetazolamide.

1.4.3. Piggy back drug loading of contact lenses

Piggy back contact lenses were introduced in the 1960's. The system originally consisted of a rigid gas permeable (RGP) lens worn on top of a soft contact lens

[110]. It was used when the patient required an RGP lens, but could not tolerate wearing this hard lens (Figure 1.11).

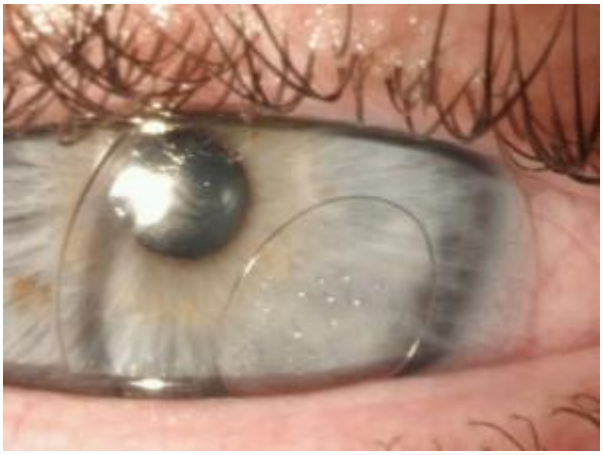


Figure 1.11: Piggy back contact lens drug delivery device [111].

This idea was then expanded by adding a drug plate between two SCL lenses. This provides a pocket or reservoir of drug. Drug loaded into the reservoir diffuses through the soft contact lens (SCL) into the tear film. Sano, *et al [111]*. created a drug plate by coating and freeze drying a PVA (poly vinyl alcohol) disc containing levofloxacin with a block styrene-(ethylene/butene)-styrene (SEBS) polymer solution. A drug plate was placed between a hydrophilic SCL and a hydrophobic contact lens. A hydrophilic lens was placed on the eye followed by the drug plate and then the hydrophobic lens placed on top. Levofloxacin was released from this device over an 8 hour period. Use of the non-hydrophilic SCL helped to inhibit the burst release profile of levofloxacin from the device. There are issues with this approach as the reduction in oxygen permeability which is caused by the combination of the two SCL's and the drug plate. Weissman *et al* [96] observed tear oxygen tension or pO_2 values of 100 mm Hg from piggy back lenses (200 µm) but this reduced to 60 mm Hg when scleral lenses of a greater thickness were used (400-500 µm). Also, there was no attenuation or control of the drug release. The material was released in a burst release profile and the majority of drug was released in less than two hours. Even if the burst release profile could be corrected and controlled drug release was possible, the effect of poor oxygen permeability would allow a patient to tolerate wearing these lenses for short periods of time only. Therefore, until extremely high oxygen permeable materials are available this type of device is not feasible. This is borne out

by the relatively small amount of literature available on these types of drug delivery lenses.

1.4.4. Drug loading via molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are synthetically manufactured polymers which have specific recognition capabilities [112]. Based on natural systems, they have the ability to recognise and react with specific molecules. Selectivity is fashioned by a process which involved the binding of a molecule to a receptor site. This binding can occur through the creation of non-covalent bonds/interactions. Biomolecules such as enzymes control biological activities in this manner and achieve high selectivity. Selectivity obtained from molecular imprinting was observed, by Polyakov in 1931 while working with gelatinous silica for use in chromatography [113]. In molecular imprinted polymers this selectivity is created when pre-polymerisation template monomer interactions are form template specific arrangements around the template drug these arrangements are then permanently fixed when the monomers are polymerised.

The benefits of this imprinting technique are increased loading of analytes into polymers and increased residence time of these loaded analytes in the polymers when placed in biological fluids [114]. The polarity of the template is a critical factor for controlled drug delivery, and the template and polymer polarities need to be similar for controlled drug delivery [55]. Polymer and analytes of interest are mixed under controlled conditions and regions of selectivity for these analytes are created in the polymer matrix. The analytes can then be removed by washing, leaving the receptor sites available to load drug and then release it slowly when required. Figure 1.12 displays a schematic representation of the process of molecularly imprinting.

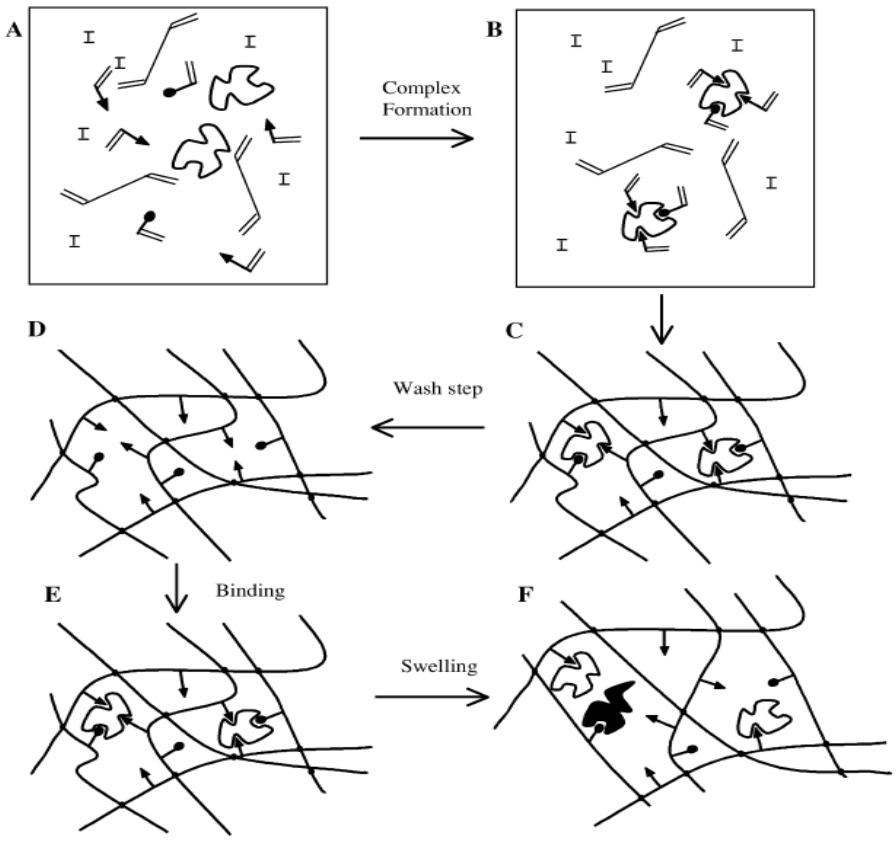


Figure 1.12: **Schematic of the selectivity being created via molecular imprinting in synthetically manufactured polymers [112]. A = initial mixing of drug template and monomer, B = formation of a pre-polymerisation complex, C = polymer formation, D = removal of template by washing, E = rebinding, F= Polymer swelling creates areas of differing affinity [112].**

1.4.5. Molecular imprinting and drug delivery

Molecular imprinting as a technique was developed separately by Wulff and Mosbach in the 1970s [112]. They used the technique to create selective stationary phases for use in HPLC analysis. In 2002 Hiratani used molecularly imprinted polymers to increase the amount of material that could be loaded into a contact lens [16]. This increased the available reservoir in the contact lenses, the drug loaded has been estimated at only one tenth of the dose available in a topical instillation [115]. With such a small reservoir available the ability to increase drug loading amount for the contact lens was critical.

The general preparation of molecularly imprinted polymers uses a high concentration of cross-linker and organic solvents as porogens. This leads to very hard polymers with solvent residues which are not suitable for use as contact lenses.

To overcome these issues an alternative strategy using 0.32 - 8.34 mol % cross-linker and no solvents was pursued. These weakly cross-linked imprinted hydrogels have the physical characteristics required for biocompatibility, drug delivery and increased drug loading [16]. In this study methacrylic acid MAA and ethylene glycol dimethacrylate EGDMA, timolol maleate and photo-initiator were mixed to form pre-polymerisation complexes. As long as the EGDMA was present in concentrations of greater than 60 mM, the imprinted hydrogels absorbed significantly more timolol maleate than non-imprinted hydrogels. Up to three times the amount of drug was absorbed by imprinted hydrogels, when compared to the drug absorbed by non-imprinted hydrogels. Also release from the hydrogels was sustained for 48 hours with an EGDMA concentration of 80 mM. This demonstrates that the concentration of the cross-linker is critical to the formation of sufficient binding cavities for the template molecule.

1.4.5.1. Configurational biomimetic imprinted polymers

Natural systems have the ability to specifically bind, to target molecules in a three dimensional configuration. These three dimensional configurations have the ability to identify and bind molecules due to their functional groups and structural characteristics i.e. enzyme substrate interactions [112]. Configurational biomimetic imprinting CBIP attempts to mimic these natural interactions for specific molecules by examining natural systems such as signalling and genetic mechanisms and finding monomers with similar structural components to proteins and nucleic acids. It then attempts to match natural recognition interactions that occur in the body with monomers that have structural moieties which are similar to proteins, with the aim of creating recognition within a polymerised hydrogel. Figure 1.13 illustrates the CBIP process. Venkatesh *et al.* [74] has demonstrated that it is possible to load and control release Ketotifen fumarate, an antihistamine from hydrogel networks. The hydrogels were manufactured using CBIP.

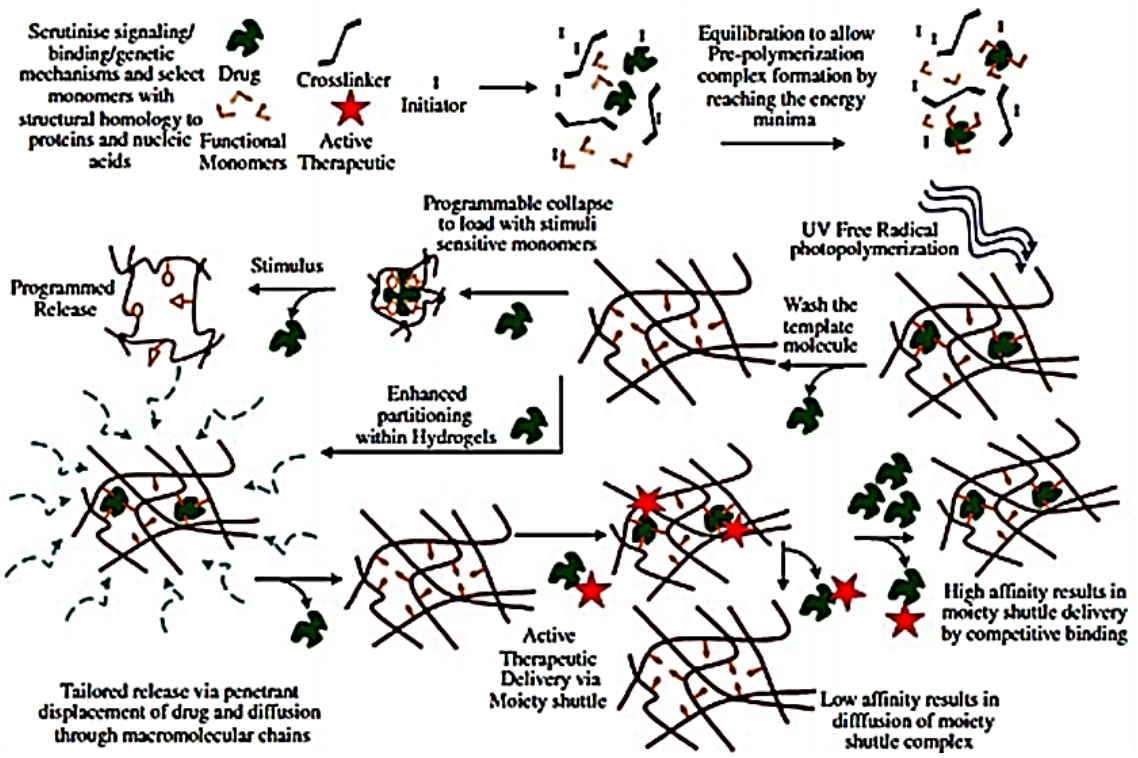


Figure 1.13: **Schematic of configurational biomimetic imprinting (CBIP) [74].**

It was observed that the rate of polymerisation decreased as the concentration of template drug in the pre-polymerisation monomer/template mix increased. This may be due to the template drug causing restrictions in the movement of the monomers and the free radical polymerisation. This decrease in reaction rate provides some evidence for the assembly of monomer template configurations before polymerisation. Pre-polymerisation complexes form template specific cavities in the final polymer which remain after the template is removed. These binding pockets have an increased affinity for the template molecule and afford slower release of template from the hydrogel matrix. In this study Venkatesh *et al.* [74] observed six times higher loading of ketotifen (0.05 mmol/g) in imprinted polymers, when compared to control polymers. The release of ketotifen at therapeutic concentrations was observed for an extended time. The drug was released over a period of six days. 80% of the ketotifen was released in 5 days, this compares to 2 days for complete release from the control polymers.

1.4.6. Loading polymers with comfort agents

The most commonly used treatments for contact lens induced dry eye (CLIDE) are instilled wetting agents. Most of these instilled treatments are listed as artificial tears which treat the symptoms of dry eye but not the cause. These wetting agents add moisture to the eye as well as absorbing tear fluid and releasing this back during blinking. They can also increase the stability of the tear film by increasing its viscosity [21]. The inclusion of comfort agents into a hydrogel matrix has distinct advantages for the delivery of these agents to the eye. Approximately 50% of contact lens users suffer from contact lens induced dry eye (CLIDE) [116]. The ability to maintain or prolong patient comfort is of key interest to manufacturers of commercial lenses and patients. The ability to elute a comfort agent or nutraceutical from a contact lens would have the ability to alleviate discomfort or treat CLIDE.

Ciba vision manufactures Nelfilcon A which is currently the only comfort eluting contact lenses on the market. “Fresh look dailies”, one of the marketed brands of this product releases, Poly Vinyl Alcohol (PVA) at a rate 0.062 %. h^{-1}. This lens is a polymer of PVA partially acetalised with N-formylmethyl acrylamide. These lenses release, PVA into the tear fluid as unbound material from the lens is eluted over time [57]. The lenses were found to elute PVA when the surface tension of the lens storage solution was observed to reduce over time. From this discovery non functionalised PVA was added to the contact lens matrix to increase the level of unbound PVA for elution. The release of PVA increases tear film stability and patient comfort [57].

White *et al*. [11] have demonstrated controlled release of 1000 µg of hydroxyl-propyl-methylcellulose (HPMC) over 60 days. HPMC is a wetting agent which promotes tear film stability. HPMC is widely used in artificial tear solutions which are instilled into the eye to treat dry eye syndrome. As with all instilled treatments, patient compliance, convenience, residence time and bioavailability are poor. The ability to load a contact lens with this agent would greatly improve the delivery of this material. The release of the HPMC was controlled by the addition of cross linking and functional monomers. This allowed the ability to tune the release of

material from the lens. White *et al*. also chose to use silicone hydrogels as the final product would be used for extended wear and the benefits of the comfort agent would be more beneficial [11].

1.4.7. Microemulsions used for drug delivery

A microemulsion can be defined as a stable dispersion of two immiscible liquids, stabilised by surfactants; it is normally clear as long as the dispersed droplets are less than 100 nanometers in diameter. The systems are oil, water and surfactant with droplets in the 10-100 nm range. These systems have long term stability are easy to prepare and can solubilise drug particles. Characteristics of microemulsions make them an ideal drug delivery vehicle or component. Microemulsions have been investigated as delivery mechanisms for transdermal [78], pulmonary [117], nasal [118], and ocular drug delivery [119].

Tween 80 was mixed with a 2% NaCl solution with continuous heating and stirring to form a 30% (w/w) solution. Separately a solution of canola oil and Panodan SDK at a ratio of 1:5:1 (w/w) was prepared. These solutions were then mixed with heating to form a clear microemulsion [53]. This microemulsion was then added to various mixtures of monomer (HEMA) and cross-linker (EGDMA) and cured to create polymer films. HEMA, EGDMA and micro-emulsion mixtures were cured at 60 ^{0}C for 24 hours. The polymer created was washed to remove any oligomers present. Particle size of the micro emulsion and the % drug loading must be minimised so optical clarity is not impacted.

Drug loading the polymer films was achieved by simply placing the particle laden hydrogels in a solution of the desired drug. Once the polymerised hydrogel has been washed to remove unreacted monomers from hydrogel matrix to ensure there was no interference from these oligomers during the loading. The desired drug in this case was added by dissolving it in the oil prior to forming the emulsion. This is effective for the loading of hydrophobic drugs and achieved controlled release with 95% of the loaded drug being released over 9 days.

Oil and water nano-emulsions were prepared by Ammar *et al* [120]. Tween 80, isopropyl mistrate and Cremphor EL were mixed with propylene glycol, transcutol P, Miranol C2M surfactants until transparent emulsions were produced. These nano emulsions were loaded with drug and mixed until once again a clear emulsion was produced using a vortex mixer. The nano-emulsions produced showed drug action in the eye for 4-6 hours, which compared to 3-4 hours for the marketed product used as a comparison.

A Microemulsion developed for the intra nasal drug delivery of udenafil has been reported by Jong *et al* [118]. Here Capmul MCM L8 was used as the oil and labrasol and di-ethylene glycol mono ethyl ether were the surfactant and co-surfactant. Three times more Udenafil permeated the skin in (2 hours) from the emulsion than did from controls which did not use the microemulsions.

1.4.8. Surfactant laden hydrogel drug delivery vehicles

The use of surfactants and the resultant micelles created when drug and surfactants are mixed have been studied to determine their ability to increase loading and attenuate release for desired molecules in polymer systems[121]. Lipophilic micelles in the polymer matrix facilitated lipophilic interactions with the drug in the polymer matrix, it is typically a two stage process where the nano or micro particles are created and then polymerised with the monomers or monomer mixtures to produce the drug delivery device. The polymer surfactant interactions or aggregations fall into three categories, firstly the hydrophobic interactions of the polymer backbone with the non-polar surfactant tail. Secondly hydrogen bonding depending on the functional groups present in the polymer and the ability of the surfactant to act as a Lewis base and finally the electrostatic force interactions from the polar surfactant heads and charged moieties within the hydrogel[122] .

Surfactants can produce micelles which amplify the attraction for the drug in the polymer matrix. Micelles can be manufactured in a one-step synthesis and can produce polymers with suitable physical characteristics for use as contact lenses. The release of cyclosporine from polymer moulds 100 µm and 200 µm in thickness has been demonstrated by Kapoor *et al*. [121]. Surfactant loaded gels were prepared by

mixing the monomer with a drug and then adding water containing the surfactant. The mixture was degassed, a photo initiator added and UV polymerised. Cyclosporine was released at a steady rate for up to 20 hours. Also the rate of release was independent of gel thickness. This indicates that the cyclosporine has to diffuse through the hydrogel and that the concentration of cyclosporine in the hydrogel and the micelle aggregates are in equilibrium. The micelle aggregates are in effect delaying release of cyclosporine from the hydrogel the structure of cyclosporine is presented in Figure 1.14.

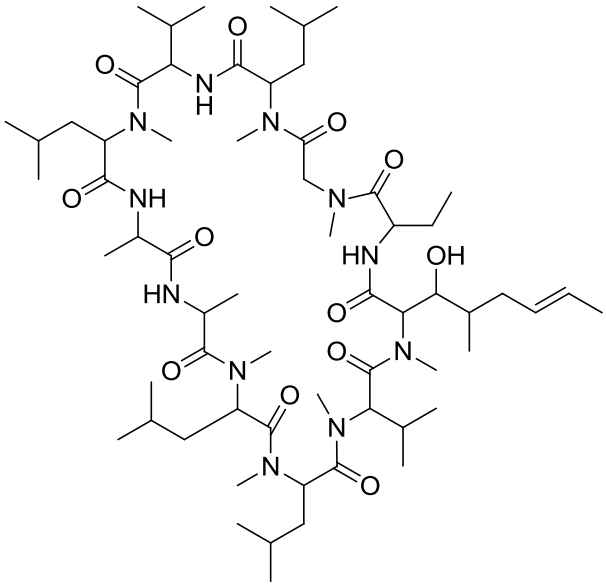


Figure 1.14: Structure of cyclosporine

The most effective surfactant in terms of extending release of cyclosporine was brij-78. This was most likely due to its high partition coefficient. However, brij-700 which has a higher partition coefficient, but hydrogels using this surfactant did not have extended release profiles when compared to brij-78 hydrogels. This details the importance of other factors such as critical aggregation concentration and the relationship between hydrophilic chain length and the fraction of hydrophobic segments. Also brij-78 was not effective for extending the release of other drugs from polymerised HEMA hydrogels such as dexamethasone and dexamethasone acetate. The choice of surfactant, drug and polymer matrix will be critical for the success of extending release. The physical characteristics of the hydrogels were compatible with use as contact lenses.

1.4.9. Diffusion barriers used to attenuate drug delivery

Diffusion barriers are materials implanted in the polymer matrix to convolute and retard the diffusion of the analyte of interest through the polymer matrix. Vitamin E is a natural antioxidant, it reacts with free radicals in the body and by doing this, protects cells from damage [123]. Vitamin E has been used to slow the release of loaded drugs from polymer matrices [7]. This relatively large molecule (molecular weight 430.7) sits in the polymer matrix and blocks channels of release for the drug which has diffused into the polymer matrix. Peng *et al*. demonstrated that it was possible to load commercial contact lenses with vitamin E and used it to change the diffusion rate of drug from polymers [124] . Loading silicon polymers (ACUVUE®) with 10 and 40% w/w vitamin E increased the duration of timolol to 6.5 hours compared to 1.5 hours for other silicon contact lens polymers. This study also presented similar results for increasing drug elution time for other hydrophilic drugs, namely flucanozole and dexamethasone sodium phosphate. Previous research had changed the composition of the polymers to alter the diffusion rate of drugs. This study illustrated that it was possible to load materials into a viable contact lens polymer composition and change its drug diffusion rate without altering the polymers ability to be used as a contact lens. This was advantageous as polymers which have a history functioning well when used to manufacture contact lenses can be used rather than developing new materials.

Dexamethasone release was significantly increased by Kim *et al.* [125]. Release of Dexamethasone from commercial silicon contact lenses was increased from a couple of hours to 7-9 days when loaded with 30% vitamin E. It was observed that the water content of the lens impacted the drug release rate. Larger amounts of water in the lens lead to reduced drug elution time, most likely due to the hydrophobicity of the drug. Figure 1.15 contrasts commercial silicon lenses to vitamin E loaded lenses, a slight yellow hue can be seen in the lenses after loading with vitamin E.

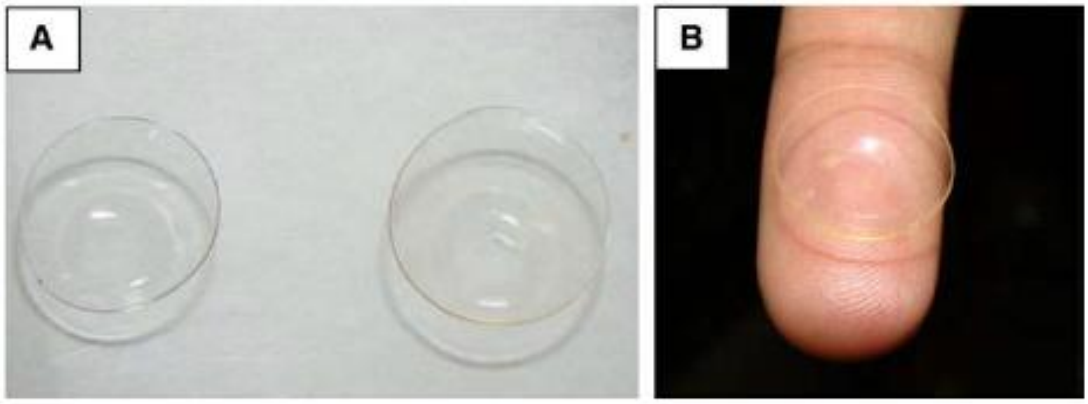


Figure 1.15: **Visual comparison of commercial silicon lenses (A) to vitamin E loaded lenses (B) [125].**

1.4.10. Drug-laden polymer drug delivery

Hydrogels are a versatile class of polymer and can be prepared and formed into diverse physical forms, monolithic slabs, films, gels and particles. These particles, films and gels etc. can be drug loaded and release of drug from the polymer matrices is dependent on the diffusion of the drug through the polymer. The ability of the polymer matrix to release drug can be regulated by controlling chemical cross-linking of the hydrogel, which attenuates release of the drug enmeshed within the polymer [58, 76, 82, 126, 127]. Drug loaded polymer particles can have the drug dispersed through the polymer particles in a number of different manners. Firstly, the drug particles are in the core of the sphere or particle where the drug can be present as a liquid or solid known as a microcapsule. Secondly the drug can be present in discrete nano domains throughout the particles which are still termed as microcapsules in literature. Finally, the drug can be dispersed throughout the polymer matrix where there are no discrete domains of drug and polymer this situation is referred to as microspheres [128].

Drug loaded Hydrogel films have been moulded into contact lenses by Garcia *et al.* [129]. These lenses contained 0.79 $mg.g^{-1}$ of triamcinolone acetonide a corticosteroid used for treatment of ocular conditions. 80% of the drug loaded was released over a 24 hour period and control of the drug diffusion was attained by increasing the swelling capacity of the hydrogel lenses by including methacrylic acid monomer in the lens formulation. A UV photo-polymerisation method produced polymers which swelled to a greater extent. The polymers produced were more flexible. The physical

properties already outlined improved drug loading and release properties from the polymer films prepared.

Polymer monoliths have been used to deliver cyclosporine, an immuno-suppressant drug routinely used to prevent transplant rejection. It is also used in the form of eye drops for the treatment of dry eye syndrome. This drug has been entrapped in a hydrogel polymer by polymerising a mixture of HEMA, EGDMA and the drug. The polymer rod was then coated with EGDMA. Cyclosporine was released from these ocular inserts at a rate of 20 µg a day for a month [69]. Increasing EGDMA content slowed drug release.

1.4.11. Polymer particles used to attenuate drug delivery

1.4.11.1. Microparticle drug delivery vehicles

Polymer microparticles have been generated to encapsulate drug and control its elution from the particle. Dispersion polymerisation was used to prepare particles from the homogenous reaction solution of monomer initiator and stabiliser. Sairam *et al.* generated microparticles by dissolving PVP and initiator (potassium persulfate) in 50 mL of water: methanol (1:1) [130]. The liquid was nitrogen sparged and then EGDMA or NNMBA and 5-fluorouracil were added. At the end of the reaction the particles generated were separated from the solvent via centrifugation and dried. The particles produced were in the size range of 2-3.5 µm. Extended drug delivery from the particles was observed over 14 hours. Drug release was determined to be swelling controlled (non fickian drug release). The nature of the cross-linker i.e. hydrophobic or hydrophilic had a significant impact on the drug release. EGDMA polymers exhibiting slower drug release.

Starch acetate microparticles have been prepared by Touvinen *et al* [131]. A water-in-oil-in water double emulsion was used to prepare the particles. 500 mg of the starch acetate was dissolved in chloroform. Then 5 mL of calcein dye (0.02% w/v) was added and mixed with a high shear mixer. This was then poured into 300 mL solution of PVA in water (0.1% w/v) and stirred for 3 hours. The particles were removed using a sieve (diameter 15 µm) washed and vacuum dried. In this *in vitro*

study cellular uptake of the particles of 8 % was recorded. A single injection of these particles would be able to maintain a therapeutic concentration of drug without any toxic effects.

1.4.11.2. Precipitation polymerisation polymer particle preparation

Precipitation polymerisation was used to synthesis molecularly imprinted drug loaded polymer particles. Tramadol base along with methacrylic acid and solvent (either chloroform or acetonitrile) EGDMA and AIBN were mixed and after time to allow monomer template complexation, the mixture was sonicated at 60 °C for 15 minutes and nitrogen sparged. Once polymerisation was complete particles were washed and separated by centrifugation. These MIP particles demonstrated prolonged release of 45 minutes to release 100% of the drug loaded compared to 5 minutes from NIP particles [87]. The use of precipitation polymerisation has the advantage of not using surfactants or stabilisers which can be difficult to remove after polymerisation [132].

Precipitation polymerisation was used to manufacture nanoparticles. A nanoparticle can be defined as any particle within the size range of 1-100 nm [133]. For the purpose of drug delivery, slow diffusion of the drug from polymers can be achieved by creating highly cross-linked, drug laden polymer nanoparticles. Wang *et al.* has generated molecularly imprinted nanoparticles using a UV initiated precipitation polymerisation [134]. Here a mixture of HEMA, DEAMA, EGDMA and photo initiator was dissolved in 40 mL of acetonitrile: water (3:1). Dexamethasone drug was added and the entire mixture was stirred and exposed to UV light. These nanospheres were being used in a bio-sensing application and showed significantly increased drug uptake compared to non-imprinted polymer particles.

Precipitation polymerisation was used to manufacture mono-disperse molecularly imprinted microspheres. A variety of drugs were used to synthesise the imprinted microspheres. 1 mmol of template drug was mixed with 4-vinylpyridine (12 mmol), EDMA 24 (mmol), AIBN (40 mg) and 10 mL of porogen. This mixture was then photo polymerised for 24 hours after nitrogen sparging for 10 minutes. The particles

were separated using centrifugation and then washed with chloroform [135]. The particles generated were within the 2- 3 μm size range

Nano precipitation is used to fabricate more than 50% of the nanoparticles reported [136]. An organic solution of lipophilic drug and polymer is added to an aqueous solution slowly, as individual drops with constant stirring. The miscibility of the solvent with water is the most critical factor regulating NP formation. The rate of addition and stirring speed control drug loading and particle size to some extent. A second step can be employed where NP in the organic phase once formed are vigorously sonicated or mixed in the aqueous phase and an emulsion formed and either a nano or macro emulsion is formed depending on the emulsified system [137].

Emulsification solvent evaporation can be used to form NP. In this method a polymer is dissolved in a volatile solvent, i.e. dichloromethane or chloroform and emulsified in an aqueous solution. NP were formed by the evaporation of the solvent under reduced pressure. The evaporation of the solvent can take a number of hours which makes this method slower than nano precipitation which forms nanoparticles in milliseconds. Coalescence increases the particle size of the NP formed. Using poly vinyl alcohol (PVA) or sodium dodecyl sulphate (SDS), surfactants can minimise coalescence and produce smaller NP's. This type of NP preparation is not ideal for hydrophilic drugs due to poor loading levels caused by diffusion of drug into the aqueous phase. To overcome this issue double emulsions are used, i.e. water in oil in water (W/O/W). A primary emulsion is formed by sonicating the aqueous phase with dissolved therapeutics and an organic phase. The polymer and organic surfactant act as a stabiliser to create a water in oil (W/O) emulsion [138].

The second emulsion is formed by sonication of the W/O emulsion and the aqueous phase with a hydrophilic stabiliser, this forms a W/O/W emulsion. Sonication during this step determines the size of the NP fabricated, longer sonication times create smaller particles with reduced polydispersity, but this needs to be balanced against the possibility of longer sonication times possibly damaging the drug.

Emulsification-solvent diffusion NP formation begins with the polymer dissolved in a moderately water miscible solvent i.e. benzyl alcohol, which has been pre saturated with water-in-oil-water emulsion. The droplets are then formed from the water polymer saturated solvent using an emulsification method. Droplets formed are dispersed and diluted in a large volume of water containing a stabiliser; diffusion of solvent out of these droplets causes the condensation of material within the droplet causing the formation of the NP (See Figure 1.16). The solvent is extracted in milliseconds instigating a reduction in particle size, particles of approximately 150 nm are produced and the method is highly reproducible and polydispersity of NP fabricated is substantially lower than other methods [139].

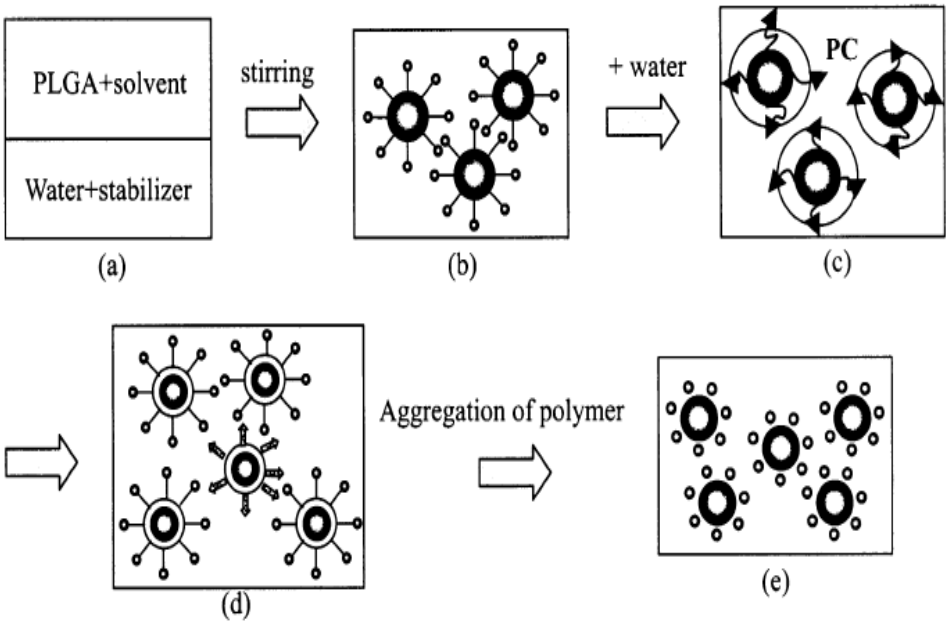


Figure 1.16: **Schematic of the proposed mechanism of nanoparticle formation via emulsification solvent diffusion [139].**

Emulsification salting out is a derivative of the emulsion solvent diffusion where the organic solvent is totally miscible with water. The polymer containing solvent is emulsified with an aqueous phase saturated with salt; this saturated solution prevents the solvent mixing in the aqueous phase. Diluting the droplets in copious amounts of water drops the salt concentration drastically and leads to the extraction of the solvent and precipitation of the NPs. This method is used almost exclusively for lipophilic drugs and the choice of salting out agent impacts particle size of NP

created [140]. Nano-particles can be viewed using a transmission electron microscope and an image of sparfloxacin loaded PLGA nanoparticles is provided in Figure 1.17.

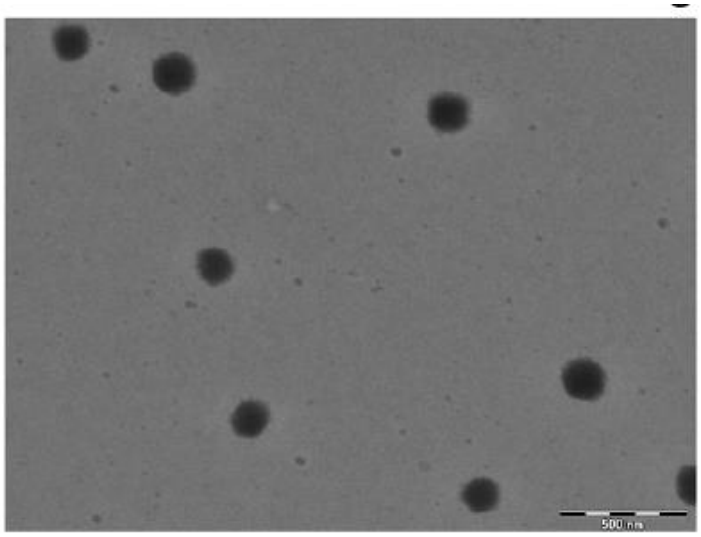


Figure 1.17: Transmission electron micrograph of sparfloxacin loaded PLGA nanoparticles in a PVA suspension [38].

Drug loaded core shell nanoparticles have been created using PLGA and lecithin. The particles consist of a hydrophobic PLGA core surrounded by a lecithin monolayer with the surface coating of hydrophilic PEG. These core shell particles have been used to deliver Doxil a liposomal drug used in the treatment of AIDS. Chan *et al.* achieved controlled release from the core shell particles which required 30 hours to deliver 50% of the drug loaded [141].

1.5. Novel aspects of this study

The novel aspects of this study include the development of a lab scale contact lens manufacturing process, which can manufacture contact lenses which are of commercial quality. This system can be used to drug load and analyse contact lens formulations to produce novel drug delivery vehicles for analysis.

Using these novel devices created the ability to perform formulation trials. The data collected provided the means to determine effect of the monomeric composition and chemical cross-linking on drug release..

Polymer drug laden particles were designed which attenuated drug release due to the amount of chemical cross-linking and physical properties. These novel drug delivery polymer particles have the potential for use as a platform for the delivery of a number of drug substances as the attenuation of release is not based on drug specific interactions.

Kinetic investigations performed using differential scanning calorimetry discovered that there was an impact on the polymerisation reaction. Contact lens monomer polymerisation was altered when drug laden polymer particles were added to the pre-polymerisation monomer mixture. This information highlights a new area worthy of further study to determine the physical effect of this change on the polymers produced

1.6. Thesis outline

This study will investigate the use of contact lenses as medical devices for the controlled delivery of ocular drugs.

Chapter 2 aims to develop a pilot scale manufacturing process and the methods required to assess contact lens physical characteristics, such as, optical clarity, refractive index and physical strength.

Chapter 3 focusses on Drug loading and investigate how drug release could be attenuated by soaking lenses in solutions of drug and diffusion barriers. The impact of polymer formulation and chemical cross-linking on drug release will also be measured.

Chapter 4 concentrates on the synthesis and characterisation of drug-laden polymer particles. The ability of these drug-laden polymer particles to control the release of drug will be studied by performing drug release studies. These drug-laden particles will then be loaded into contact lenses and the efficacy of the drug delivery vehicles prepared will be assessed.

Chapter 5 will assess the impact of adding drug-laden polymer particles to the pre-polymerisation mixture of contact lens monomers. Thermal analysis by Differential Scanning Calorimetry (DSC) will be used to measure the activation energy of polymerisation reactions and determine the impact on the polymerisation kinetics of these monomers.

Chapter 6 details the future work and areas which warrant further investigation.

Chapter 2

Development of a Fabrication Method for Contact Lenses

2. FABRICATION AND DEVELOPMENT OF CONTACT LENSES

2.1. Introduction

As stated in Chapter 1, delivery of drug from contact lenses can result in increased bio-availability of drug delivered to the eye. Bio-availability can increase from 5% to 50% and residence time of the drug in the eye can be raised from 5 minutes to 30 minutes [142]. This characteristic makes contact lenses, ideal for use as an ocular drug delivery vehicle. Combining this characteristic with controlled release of material from the lens would create a novel drug delivery vehicle capable of treating a wide variety of ocular diseases. Commercial contact lenses have been used as drug delivery vehicles, but have suffered from burst release and demonstrated no control over drug delivery [6, 8, 106]. The ability to tailor the chemical cross-linking of the polymeric contact lenses would allow some control over drug release. Formulation control and rationally designing the polymer drug interaction has been proven to increase the polymer matrix attraction for the drug and has enhanced drug loading and release parameters compared to polymer matrix systems prepared without such design [15]. Venkatesh *et al*. demonstrated controlled drug delivery was achieved from polymers cast as thin films [74]. 80% of the ketotifen drug loaded was released after 4 days from the acrylic acid, acrylamide, HEMA and poly ethylene glycol dimethacrylate polymer.

The aim of this Chapter was to develop a reproducible method of fabricating contact lenses, which have similar shape size and geometries to commercial lenses. Measuring drug loading and release characteristics of prototype lenses allows the assessment of their potential as drug delivery vehicles. Assessing their physical properties determined if their visual performance and strength were sufficient for use as contact lenses. This analysis can was used to aid in the design and improvement of these devices. The manufacturing process needs to be robust in order to deliver polymeric devices of reproducible size and shape as well as physical properties such as strength, light transmission and refractive index. The lenses must be fit for use as optical inserts, as well as drug delivery vehicles and so were characterised for refractive index and % light transmittance.

At the centre of these criteria, is a controlled free radical polymerisation. To achieve functional devices, monomers, cross-linkers and initiators must be carefully selected, purified and formulated. The manufactured lenses must then be characterised to ensure that they can function as contact lenses as well as to determine the effect, if any of loading these devices with drug diffusion barriers. The ability to produce commercial quality contact lenses reproducibly will be critical to the subsequent analysis of drug delivery from the manufactured lenses. Early studies used moulds to create polymer sheets [16, 106]. In such studies, the sheets were cut as required but they did not have the same physical geometry and shape as a contact lens, with a defined edge and centre thickness.

The emphasis of the work in this Chapter, was to create the capability to manufacture and to load drug into contact lenses. The equipment and methods required to characterise the contact lenses were designed and developed by the researcher as part of this study. Characterisation of the contact lenses fabricated was then performed to ensure that these lenses were of commercial quality. These polymeric devices could then be modified as required in order to facilitate the delivery of ocular medication over a prolonged period of time. Fabrication of lenses was conducted using wet cast moulding, using commercial lens moulds supplied by Bausch + Lomb. These moulds were used to produce contact lenses which were the approximate size shape and geometry of commercial lenses. Monomers which are used to manufacture commercial lenses were utilised and formulated with cross-linkers and initiator to form prototype contact lenses.

Manufactured lenses were characterised, using a variety of techniques, to determine their suitability for use as contact lenses and drug delivery vehicles. Characterisation techniques were developed to measure characteristics such as refractive index, % light transmission, physical strength and compressibility. These factors affect the ability of the device to be used by the patient. The polymer matrices formed needed to be capable of being loaded and facilitate the release of relevant drug molecules. Such characterisation parameters also needed to be measured to determine the best candidate formulations for further study.

The ability of the polymers to load material was assessed by loading a potential diffusion barrier, vitamin E. Polymers loaded with vitamin E were then characterised so the impact on the polymers could be measured. The ability of the polymer to load this material was used to gauge the size of the reservoir within the lens, as well as, how the formed lenses could be affected by the material loaded. Differential scanning calorimetry (DSC), high performance liquid chromatography (HPLC), % light transmission and refractive index were used to assess the impact of loading material into the polymer matrices formed.

2.2. Materials used in the fabrication of contact lenses

Table 2.1 outlines the monomers, cross-linkers and initiators used in the fabrication of contact lenses. All monomers materials were purchased from Sigma Aldrich Ltd., but required further purification due to the presence of inhibitors. Once purified (As detailed in section 2.3.1) all were stored at 5 °C.

Table 2.1: Materials used in the fabrication of contact lenses.

Reagent	Supplier	Assay
2-Hydroxy-ethyl-methacrylate (HEMA)	Sigma Aldrich	98%
N-vinyl-pyrrolidone (NVP)	Sigma Aldrich	99%
2-(Di-ethyl-amino)-ethyl-methacrylate(DEAMA)	Sigma Aldrich	99%
Ethylene-glycol-dimethacrylate (EGDMA)	Sigma Aldrich	99%
Azobis-*iso*-butryonitrile (AIBN)	Sigma Aldrich	99%
α Tocopherol (Vitamin E)	Sigma Aldrich	99%
Glycerol	Reagecon	N/A
ACUVUE® Lenses	Vistakon	N/a

2.2.1. Manufacturing equipment used in the fabrication of contact lenses

AIBN is scavenged by oxygen reducing the amount of free radicals available to react with the monomer to initiate of the polymerisation reaction [143]. In order to ensure a consistent, complete polymerisation and to exclude oxygen, A novel oxygen depletion chamber was designed and built to specifications so that it could be purged with nitrogen. Suir Precision Engineering Ltd were commissioned to build a 220 mm diameter 65 mm high 316L stainless steel chamber with a threaded lid and two gas tight gate valves, one on the lid and one on the side of the vessel. This stainless steel vessel with stainless steel lid was used to thermally polymerise the monomers into

contact lenses, using polypropylene moulds provided by Bausch & Lomb Ireland Ltd. (Figure 2.1).

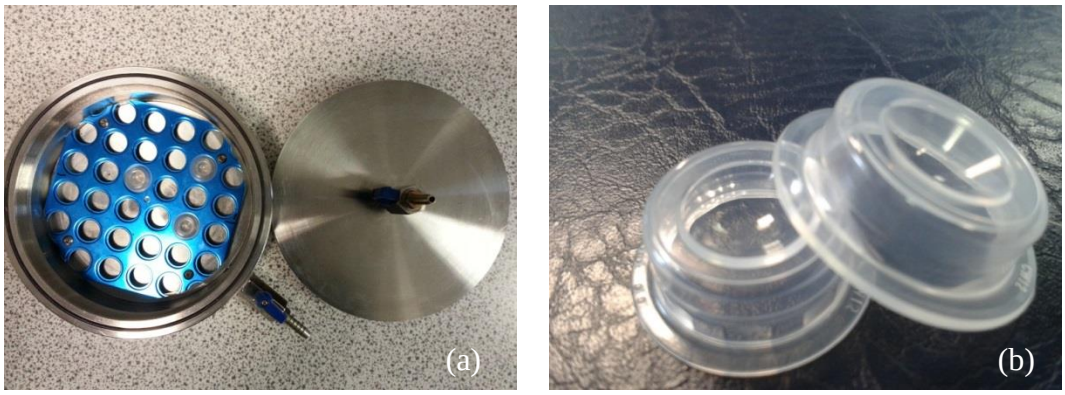


Figure 2.1: Oxygen depletion chamber (a) and polypropylene contact lens moulds used (b).

Also pictured in Figure 2.1 are the polypropylene moulds and support rack (blue aluminium tray) which are used in the manufacture of the contact lenses. The support rack allows the formation of 32 lenses at one time. Polypropylene moulds were placed in the support rack and the monomer was pippeted into the bottom mould and the lid of the mould placed on top.

Wet cast moulding, e.g. forming the contact lenses from commercial contact lens moulds, ensures consistent size geometry and surface area as well as the ability to measure and compare the lenses manufactured to commercial specifications (Figure 2.2).

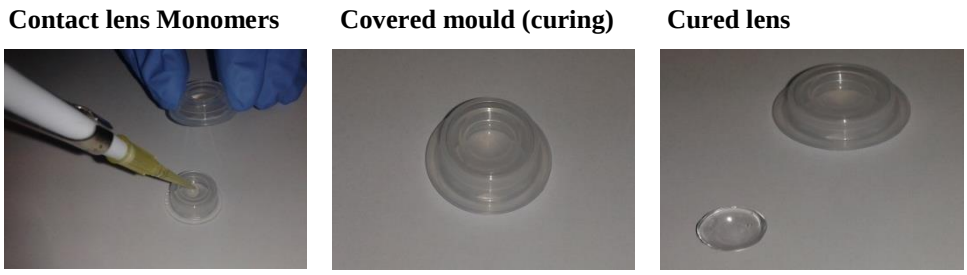


Figure 2.2: **Wet cast moulding process of monomer into cast polymer lens.**

In Figure 2.2 monomer liquid is filled into the concave female mould, which creates the shape of the lenses outer surface. The male mould is placed on top of the female mould and the inside surface of the lens and the edges are created, while any excess

monomer liquid is forced out to the edge of the mould. The removal of the excess monomer is critical to the formation of a comfortable lens for the patient and the physical properties of the lens. Lens moulds are designed so that the cured lenses are removed dry from the mould with a fully formed edge with no need to polish the edge [60]. There is considerable expertise and engineering involved in mould manufacture to ensure that this happens consistently and forms an edge that is comfortable for the patient. The mould can then be placed in an oven for curing or cured by ultraviolet radiation as the polypropylene moulds are transparent to UV light. Wet cast moulding is a continuous process ideal for high volume manufacture of daily disposable lenses. Manufacturing using commercial quality moulds ensures reproducibility and comparability of the results obtained. The impact of loading techniques on the lenses produced can also be assessed as the lens's physical characteristics can be measured pre- and post- loading. The size and shape of the lens will also closely match that of a commercial lens. There may, however, be slight differences in diameter of the lenses produced due to swelling of lenses, as this is unique to each polymer formulation [60].

A separate lid for this chamber was designed and commissioned from Suir Precision Engineering Ltd. to allow UV initiated polymerisation of contact lenses. The lid was made from Nylon (Acetal®) with a UV transmitting polycarbonate window and separate UV diffuser purchased from knight Optical Ltd. The systems are nitrogen purged as required using the pressure valves attached to the lid and vessel (Figure 2.1 and 2.3).

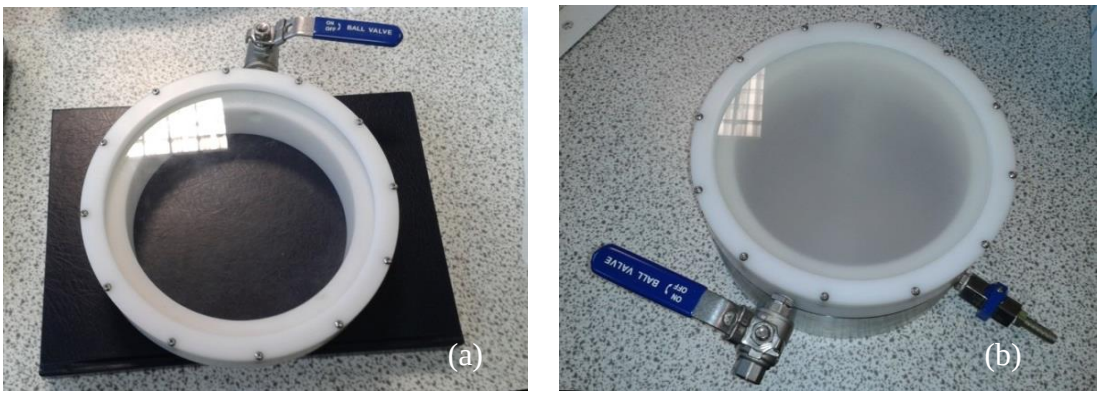


Figure 2.3: **Polycarbonate UV transmissible window (a) and separate UV diffuser (b)**.

The monomers cross-linker and initiator used in all formulations presented in this Chapter are present in commercially available soft contact lenses and had been previously used within the research group, where an initial ratio of monomers to cross-linker and initiator were established and are presented Table 2.2.

Table 2.2: Polymer formulations used in initial trials.

Formulation	Backbone Monomer	Functional Monomer	Cross-linker	Mole Ratio%	AIBN %
A	HEMA	DEAMA	EGDMA	69:29:2	0.05
B	HEMA	NVP	EGDMA	69:27.5:2.5	0.05
C	HEMA	NVP	EGDMA	67.5:27.5:5	0.05
D	HEMA	N/A	N/A	N/A	0.05

Such lenses and constituent monomers have also been previously used for drug loading and release studies [2, 11, 70]. The contact lenses are placed in the chamber nitrogen purged for an hour and then exposed to UV light. The system configuration for lens production uses Phillips Actinic BL PL-S 9W/10/2P 1CT mercury lamps which emit in a wavelength range of 350-400nm (UVA), with a peak wavelength of approximately 380nm.

2.3. Experimental methods

2.3.1. Monomer / Initiator purification

Prior to use, all monomers were vacuum distilled to remove any inhibitors present. In the case of HEMA, phenothiazine was added to inhibit the monomer polymerising during distillation. 250 mL 1-vinylpyrrolidone (NVP) was mixed with approximately 5-10g of magnesium sulphate; vacuum filtered to remove the magnesium sulphate and the resulting filtrate was then vacuum distilled. 2 Diethyl-amino-ethyl-methacrylate (DEAMA) was distilled without any pre-treatment. 200 mL of ethylene-glycol-dimethacrylate (EGDMA) was washed twice with 75 mL of sodium hydroxide (10% (w/v)) solution in water, and then washed with 75 mL of saturated sodium chloride salt solution. The resulting solution was dried by mixing with 4-5 g of magnesium sulphate, vacuum filtered and the resulting solution was vacuum distilled. All monomers were stored in a refrigerator at 5 °C. AIBN was recrystallized from acetone and stored at 5 °C.

2.3.2. Contact lens preparation

Initiator (AIBN) and monomers were weighed into a 22 mL Wheaton vial, then sonicated for 10 minutes. 35 µL of the mixture was pipetted into moulds which were capped and the monomer within the mould was thermally cured in the oxygen depletion chamber after it was purged with nitrogen for two hours and then subjected to the temperature ramp as per Table 2.3. Once this was complete the chamber was removed from the oven and allowed to cool. Lenses were removed from the moulds and washed 3 times with water at 60 °C for 30 minutes.

Table 2.3: **Polymerisation temperature profile, (all steps performed for 1 hour).**

Temperature	Step
50 °C	Polymerisation
70 °C	Polymerisation
80 °C	Polymerisation
112 °C	Annealing

2.3.3. Vitamin E loading and analysis

Lenses were loaded with vitamin E by soaking them in 50 $mg.mL^{-1}$ ethanol solution of vitamin E. Each lens was placed in a micro centrifuge tube and 1 mL of the loading solution was added. Loading was performed over 5 days. Lenses were then removed from the ethanol solution and allowed to dry to remove the ethanol. Prior to the lenses being drug loaded.

Vitamin E samples were analysed using an Agilent 1200 series HPLC fitted with UV detection set at 229 nm and a Zorbax C8 (4.6 X 150 mm 5µm) column. The mobile phase used was 95:5 methanol: water with a flow rate of 1.5 $mL.min^{-1}$. 10 µL of each sample was injected for analysis.

To determine if the vitamin E eluted from the loaded lenses, the lenses were placed in 1 mL of a phosphate buffered saline (PBS pH 7.2) ethanol solution, PBS: Ethanol in a ratio of 90: 10. Lenses were left in this solution for 24 hours, after which the solutions were analysed directly by HPLC. Both of the HPLC methods were developed and optimised specifically for this analysis.

2.3.4. Texture analysis of contact lenses

A stable micro system Texture Analyser XT "TAXT" plus was used to measure mechanical properties of the lenses. A P25 probe was used to compress a swollen lens and the force used was measured. The instrument settings for analysis were developed in conjunction with Stable Micro Systems Ltd. The parameters used were developed and set from trial analysis of commercial lenses. The instrument settings for measurements are detailed in Table 2.4.

Table 2.4: Texture analyser instrument settings.

Test parameter	Setting
Test speed	0.1 $mm.s^{-1}$
Trigger type	Button
Stop plot at	target position
Post speed	0.1 $mm.s^{-1}$
Target mode	Distance
Data acquisition	500 pps
Post-test speed	1.00 $mm.s^{-1}$
Break mode	Off
Trigger force	0.01N

2.3.5. Equilibrium water content analysis of contact lenses

Contact lenses were dried for 24 hours *in vacuo* and then weighed prior to swelling studies. The lenses were swollen for 24 hours at 37 ^{0}C in DIW. Prior to being weighed lenses were dabbed dry on lint free tissue. Equilibrium water content (EWC) was calculated using the equation 2.1 below [144].

$$\%EWC = \frac{Ws - Wd}{Wd} \times 100 \qquad \textbf{Equation 2.1}$$

Where W_s = swollen weight W_d = dry weight

2.3.6. Thermal analysis of contact lens monomers

2.3.6.1. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was used to measure the boiling point of the monomer samples. 5-10 mg of the mixture was weighed into an aluminium pan. The sample was loaded into the furnace equilibrated at 20 ºC, then heated at a rate of 10 $^{o}C.min^{-1}$ to 250 ºC with a nitrogen flow rate of 50 $mL.min^{-1}$. A Q50 TGA, manufactured by Texas Instruments was used.

2.3.6.2. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using two methods. Monomer samples were analysed using method 1. Liquid monomers were pippeted into the hermetic aluminium pan and weighed and subsequently crimped shut. The pan lid was pierced with a pin to ensure no pressure build up in the DSC pan. Contact lenses were analysed using Method 2. Lenses were vacuum dried for 24 hours, then crushed using a mortar and pestle; the ground hydrogel material was then weighed into an aluminium pan (hermetic pan with pierced lid). Table 2.5 details the Q2000 DSC method parameters for analysis of both monomers and polymerised hydrogel lenses.

Table 2.5: DSC method parameters used for the analysis of monomers (method 1) and polymer hydrogels (method 2).

Test Parameter	Setting	Method
Equilibrate	20 °C	1
Ramp	3.00 °C.min^{-1} to 150 °C	1
Equilibrate	0.00 °	2
Ramp	10.00 °C.min^{-1} to 250 °C	2
Mark end of cycle	N/a	2
Ramp	10.00 °C.min^{-1} to 0.00 °C	2
Mark end of cycle	N/a	2
Ramp	10.00 °C.min^{-1} to 250 °C	2
Mark end of cycle	N/a	2

Note nitrogen flow rate for both methods was 50 mL.min^{-1}.

2.3.7. Refractive index of contact lenses

A Bellingham Stanley RFM 340 refractometer with sample illumination light at 589 nm was used to measure the RI of all lenses. The instrument was temperature controlled at 25 ºC for all analysis.

2.3.8. Light transmission of contact lenses

A Shimadzu UV Vis 2401-PC spectrometer set at 800 nm was used to measure the amount of light transmitted through the swollen contact lenses manufactured and commercial lenses. The lenses were suspended in the path of the beam using a film attachment and lens holding rig. The lenses were measured with reference to air.

2.3.9. Gas chromatographic analysis of contact lens solvent extractions

An Agilent 5890 Gas chromatograph (GC) fitted with a flame ionisation detector was used. This analysis quantified the amount of unreacted monomers and oligomers remaining in the lens polymer matrix after washing with water. A Restek GC Column (15 M length 0.53 mm internal diameter 1 µm film thickness) was fitted to the GC and samples were run using the operating conditions as displayed in Table 2.6. Ten washed lenses were extracted in 3 mL of methanol for 16 hours and the methanol solution was then run on the GC. This method was developed specifically for the analysis of extractives from the contact lenses manufactured.

Table 2.6: **GC instrument settings for extractive analysis of contact lenses.**

Parameter	Set point
Injector Temperature	200 °C
Detector Temperature	275 °C
Column Temperature	Initial 50 °C Ramp rate 15 °C.min^{-1} Max 120°C 2nd ramp 25 °C.min^{-1} Max 220 °C
Injection volume	1 µL
Split ratio	Split-less
Column flow	8 mL.min^{-1}

Note: Carrier gas Helium flow rate 8 mL.min^{-1}.

2.4. Results and Discussion

2.4.1. Thermal analysis of monomer systems

To create good quality lenses the polymerisation reaction must be controlled and repeatable as well as converting as much of the monomers present into polymers. To gain an understanding of the polymerisation process, initial samples of the monomers and cross-linkers were analysed by DSC. DSC analysis showed that exothermic reactions occurred for all monomers but with different profiles for each material. Analyses performed on the monomer alone will always be less uniform, as the polymerisation reaction will be more erratic without initiator, the temperature increases and reaches the boiling point of the monomer which will result in the monomer boiling off. The presence of initiator ensures that polymerisation starts

quickly and ensures a high conversion rate from monomer to polymer. The DSC heat flow graph in Figure 2.4 and 2.5 illustrates these points.

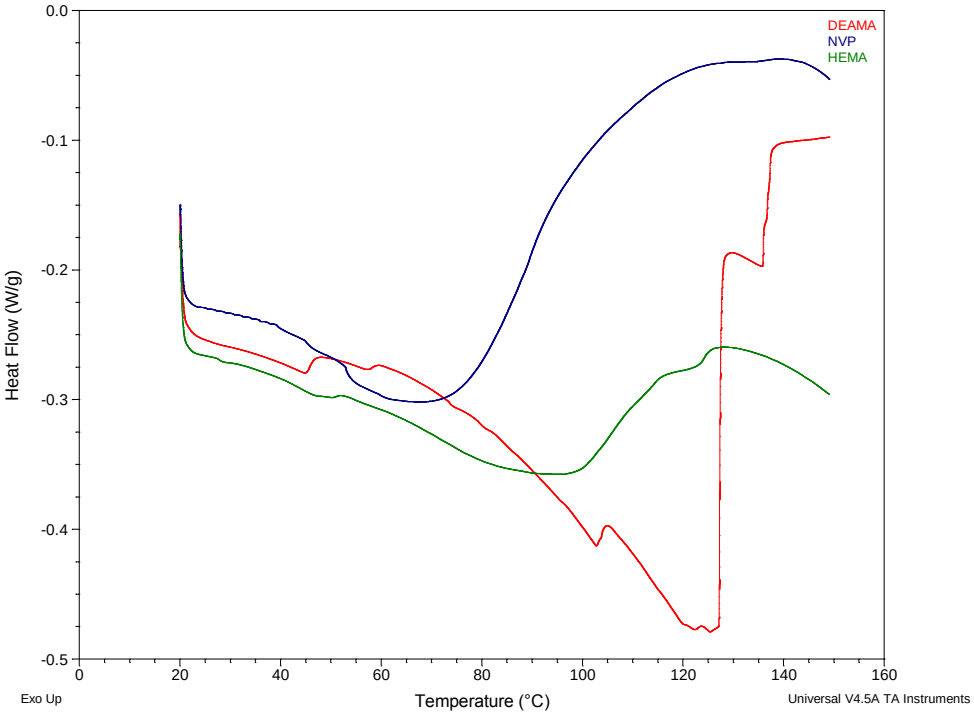


Figure 2.4: **Monomer DSC thermograms with no initiator added.**

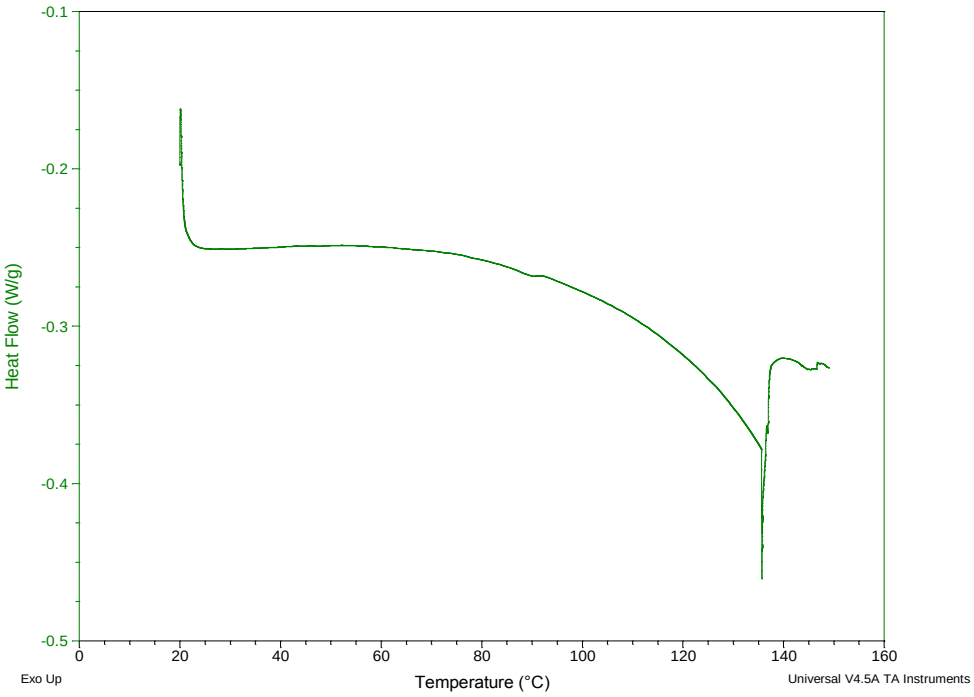


Figure 2.5: EGDMA thermogram with no initiator added.

The evolution of heat from the polymerisation was erratic, especially when compared to the thermogram when the initiator was present in Figure 2.5. There is no clear exothermic peak and it was not possible to determine if any polymerisation occurred or did the monomers present just evaporate into the nitrogen flow.

Three formulations were analysed with initiator to determine a temperature profile for heat curing the monomer mixtures. The presence of initiator increased the rate of polymerisation and lead to DSC thermograms which were easier to interpret. DSC analysis of the monomer initiator mixtures was performed by mixing the materials and pipetting the formulation into a DSC pan for analysis. These profiles were repeatable with the polymerisation reaction exhibiting a large exothermic peak, due to the initiator providing sufficient free radicals to initiate the polymerisation uniformly throughout the mixture. The polymerisation is exothermic as the carbon double bonds in the monomer are broken during the reaction releasing energy [145]. The DSC thermograms of the polymerisation of the monomers are presented in Figure 2.6.

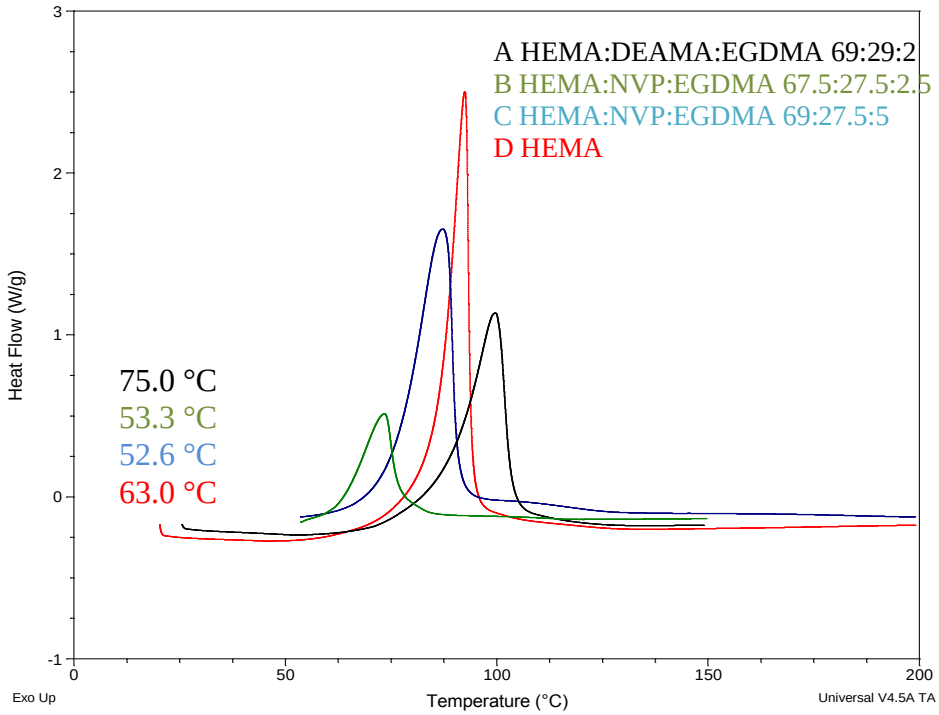


Figure 2.6: **DSC thermogram of the polymerisation reaction of 4 contact lens formulations: HEMA:DEAMA 69:29:2 (A) Black line, HEMA:NVPEGDMA 67.5:27.5:2.5 (B) green line, HEMA:NVP:EGDMA 67.5:27.5:5 (C) blue line and HEMA only formulations. (D) red line. The temperature at which the reaction started is labelled in the appropriate colour.**

Samples were heated at a rate of 3 ºC.min^{-1} and an exothermic reaction was observed. An exothermic reaction is indicative of a free radical polymerisation where the stages of initiation propagation and termination are clearly evident. Initially there is no release of energy until the initiator; AIBN decomposes, releasing free radicals at temperatures of 50 ºC approximately and initiates polymerisation. Polymerisation occurred at temperatures of 52.6 to 75.0 °C as evidenced by a rapid increase in the amount of energy being evolved by the reaction. This identifies the beginning of the propagation stage for all formulations. This increased energy output continues until termination reactions start to occur at temperatures of 85 ºC for the HEMA and HEMA, NVP and EGDMA formulation and 95 °C for the HEMA, DEAMA EGDMA formulation. In the termination stage the amount of energy evolved decreases as the reaction rate decreases. This decrease continues until polymerisation was complete and no further reactions occurred and the heat flow returned to zero. This analysis allowed a temperature ramp to be defined (see Figure 2.6). Analysis of this thermogram provided a temperature profile which ensured complete polymerisation. This analysis recorded an average enthalpy of reaction for HEMA of 57.46 kJ mol^{-1} this is in close agreement with enthalpies of reaction for HEMA in literature of 61.3-61.8 kJ.mol^{-1} [146]. The most likely reason for the difference between the two enthalpies is due to a difference in the amount of initiator used in the experiments. The average enthalpy values for the reaction are displayed in Table 2.7.

Table 2.7: Contact lens formulation monomer reaction enthalpies (n=3).

Formulation	ΔH J.g^{-1}	Standard Deviation J.g^{-1}
A HEMA:DEAMA:EGDMA 69:29:2	391.5	6.6
B HEMA:NVP:EGDMA 67.5:27.5:2.5	455.7	15.3
C HEMA:NVP:EGDMA 69:27.5:5	429.9	22.5
D HEMA	443.0	15.4

Reaction enthalpies vary from 391.5 to 443.0 J.g^{-1} the most likely cause of this variation are the changes in the monomer formulation. Standard deviation varied from 6.6 J.g^{-1} to 22.5 J.g^{-1} a maximum variation of 5 % of the enthalpy of reaction.

2.4.2. Contact lens mould monomer fill trials

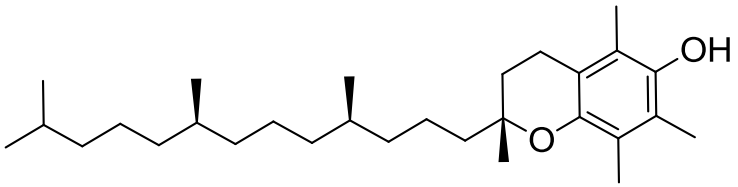
To determine how much of the monomer mixture to fill into the lens moulds, a fill trial was performed. Various volumes of monomer mixtures were pippeted into the lens moulds and visually assessed to ensure enough monomer was present to form a complete lens and leave a small 2-3mm tab of excess monomer, 35 µL was found to be the optimum amount. This fill level was assessed visually by polymerising trial batches. This fill level was chosen as it was sufficient to create full lenses in all of the 32 moulds. The level of excess was also not enough to cause issues with opening the mould after polymerisation. Once cured the excess polymer was separated from the lens as presented in Figure 2.7.

Figure 2.7: **Polymerised excess monomer removed from the contact lens polypropylene mould post polymerisation.**

2.4.3. Vitamin E loading and elution

Vitamin E was loaded into dried lenses by drying the manufactured lenses *in vacuo* for 24 hours prior to being placed in a solution of ethanol with a vitamin E concentration of 50 $mg.mL^{-1}$. The lenses were left to load for five days to ensure equilibrium had been reached as the vitamin E is a hydrophobic material with a relatively high molecular weight (430.71) and it is being loaded into a hydrophilic polymer. The vitamin E loaded lenses were then dried and assayed after insertion into 1 mL of pure ethanol for 24 hours. The solution was then analysed by HPLC. The loading solution concentration was chosen as it had been used in previous studies in the literature to load silicon hydrogel lenses with vitamin E [8]. Vitamin E had not been loaded previously into hydrophilic polymers in any literature reviewed

and warranted investigation as a novel drug attenuation technique the structure of vitamin E is illustrated in figure 2.8.



alpha tocopherol (vitamin E)

Figure 2.8: Vitamin E structure

The polymer with the lowest amount of EGDMA cross-linking monomer in its formulation loaded the most vitamin E. Formulation A loaded 1.77 mg of vitamin E per lens. Increased chemical cross-linking creates a stiffer more structured polymer; this structure will swell to a lesser extent and should be more difficult to penetrate. The amount of vitamin E loaded into the lens decreased with increasing EGDMA content. Formulations B and C differ in the amount of EGDMA cross-linking monomer present (C has double the cross-linking monomer concentration of B). This increased amount of cross-linking monomer should lead a more structured polymer matrix which will be more difficult to load. The quantity loaded may be more reproducible as this structured matrix will be more uniform. A one-way Anova analysis was performed on the vitamin E loading data in Table 2.8 using graph pad prism 6 software with no statistical difference observed, between the result sets. The most likely cause of the high variation observed is the hydrophobic nature of vitamin E and its poor solubility in the hydrophilic polymers used. A possible cause of this is that Vitamin E is more suited to being loaded into less hydrophobic contact lens monomers such as silane [8, 124, 125]. The presence of oligomers in the polymer matrix would also negatively impact vitamin E loading reproducibility Due to the high standard deviation observed it was difficult to draw strong conclusions in relation to the loading data, however, some general observations could be made. As polymers with the highest amount of chemical cross-linking monomer (EGDMA) loaded the smallest amount of vitamin E it appears that chemical cross-linking can impact drug loading. Direct embedding of vitamin E into the lenses prior to

polymerisation would ensure reproducibly loaded lenses. Table 2.8 displays vitamin E loading for the polymer formulations.

Table 2.8: Assay of amount of vitamin E loaded into contact lenses in mg/lens n=3.

Formulation	A	B	C
Vitamin E	1.77	1.56	1.08
Std Dev	0.63	1.06	0.13

Contact lenses were loaded with vitamin E via swelling in an ethanol solution. Contact lenses of formulation A, B and C were vitamin E loaded in batches 3 lenses of each batch were assayed in as in Table 2.8 were, and 3 lenses from each contact lens formulation batch were analysed to measure the elution of vitamin E into an aqueous PBS (pH 7.2) release medium. The lenses were left in the release medium for 72 hours and analysed. There was no vitamin E detected in the release medium. This was important as any impact on drug release due to the presence of vitamin E will be constant as its concentration in the polymer matrix will not change over the time of release. No peaks were detected in the chromatography. This signified that vitamin E was not detectable i.e. any vitamin E that may have been released was below the Limit of Detection (LOD). The LOD was calculated in accordance with ICH guidelines as per equation 2. LOD was determined to be 0.17 µg.mL^{-1}.

$$\boldsymbol{LOD = \frac{3.3\ (\delta)}{\text{Slope of Calibration curve}}} \qquad \textbf{Equation 2.2}$$

Where δ= standard deviation of the area of 10 blank injections

$(\delta) = 0.05\ area\ units$ Slope of graph = 0.99982

$therefore\ Limit\ of\ detection = \ 0.017 = \frac{3.3\ (0.05)}{0.9982}$

2.4.4. Contact lens characterisation

2.4.4.1. Compression force measurement

Analysis performed using the compression test rig allowed the direct comparison of commercial lenses to initial lens formulations. The graphical representation of the force needed to compress a lens is displayed in Figure 2.9.

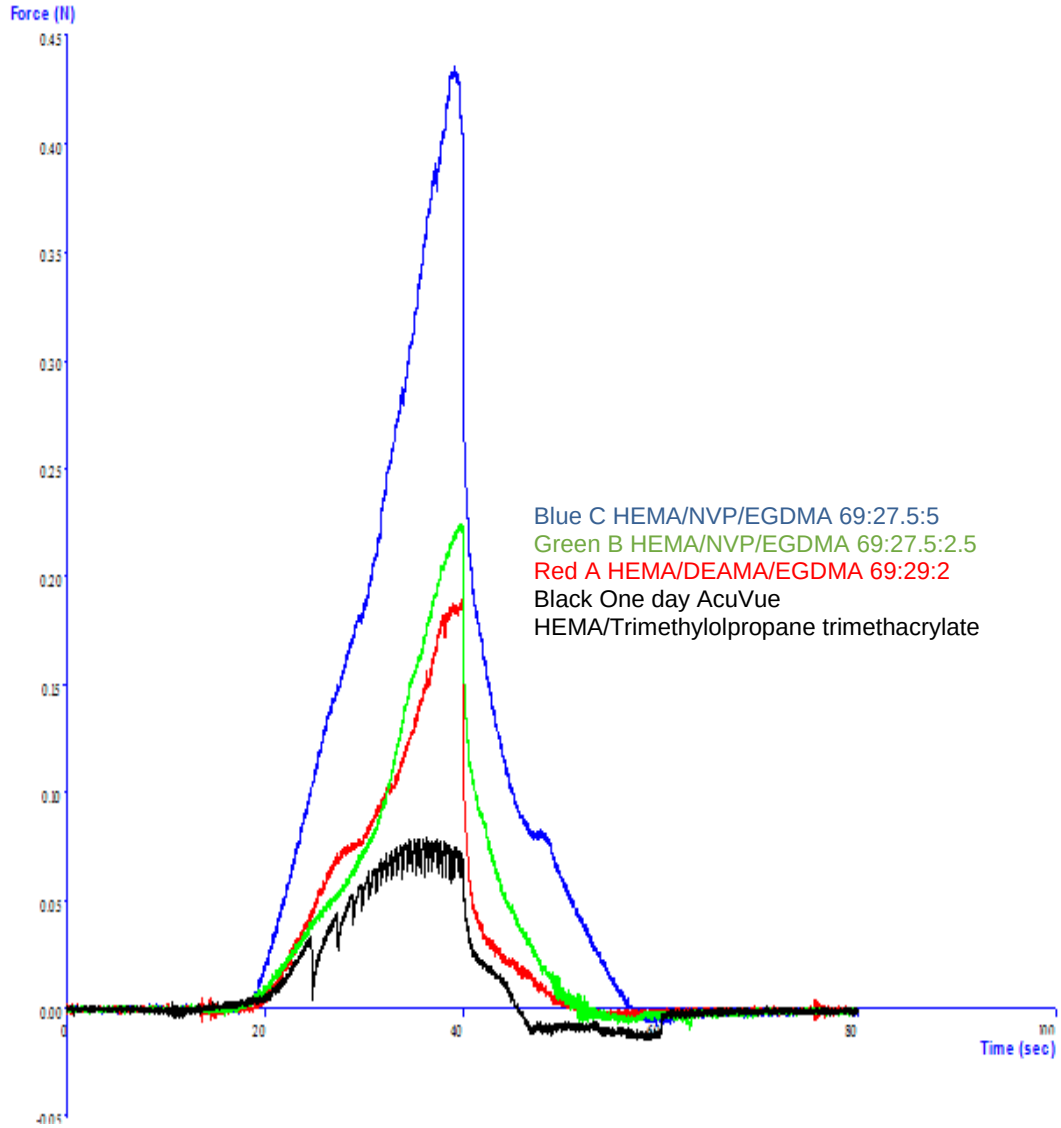


Figure 2.9: **Compression force comparison of in-house synthesised contact lenses to commercial lenses.**

Analysis demonstrated that the three initial formulations were different to commercial lenses, and the difference in compression force could be determined by this analysis. The commercial lens diameter was 14.2 ± 0.2 mm which compares to 12.10 to 13.12 mm for the manufactured lenses. This change in geometry could also be a factor in the compression forces measured. It was also able to clearly illustrate the effect of chemical cross-linking on the contact lenses produced. Formulations which had the highest amount of cross-linker (EGDMA) demonstrated a significantly higher compression force than the other polymer formulations. Formulation C in blue displayed a peak compression force of 0.44 of a Newton (N). This compares to 0.23 N and 0.19 N for formulations B and A, green and red respectively in Figure 2.9. The commercial lens had the lowest compression force 0.08 N. Compression force

increase as chemical cross-linking increases, this is logical as the stiffer polymers are formed with higher amounts of cross-linking monomers. These stiffer polymers will require a greater force to be compressed. Texture analysis allowed different lens formulations to be compared to commercial lenses but this method is not widely used in literature so analysis cannot be readily compared to other research groups. Initial trials were performed using a test rig that was purposely designed and built, which clamped the contact lens between two washers in a plastic assembly. The taxt instrument was used to rupture the lens with a steel probe. The rupture force of the lens and the measureable displacement of the lens would have given valuable information on the elasticity and strength of the lenses. The rupture force was measured, but measurements were not repeatable as the swollen lenses were difficult to clamp and slipped in the test rig. As a result this method of analysis was not continued. An image of the test rig is presented in Figure 2.10.

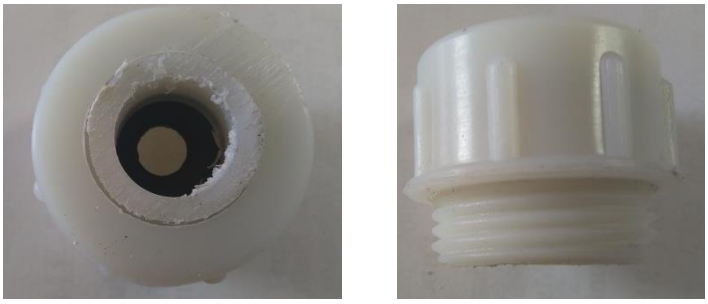


Figure 2.10: **Rupture force test rig top and side elevations.**

2.4.4.2. Equilibrium water content measurement

Swelling analysis was performed on the formulations to determine their hydrophilicity and water uptake. Initial swelling studies established a wide range of results for the equilibrium water content of lenses manufactured in the same batch. This inconsistency could impact all analysis and needed to be addressed. After a review of literature and patents was performed, the addition of a diluent was chosen as the best way to improve swelling reproducibility [97, 147]. The diluent does not interact in the polymerisation reaction and it allows movement of polymer chains and oligomers throughout the matrix. When there was no diluent present these oligomers and monomer units can become trapped in the forming polymer matrix creating a non-uniform polymer. The diluent was removed from the polymer matrix during the

washing stage, as it is water soluble and cannot bond with the polymer chains chemically during polymerisation. Analysis of the trial lenses manufactured was performed by an industrial partner where lens dimension and amount of oligomer present in the lenses was measured. This analysis confirmed the presence of unreacted monomers and oligomers in the trial lenses. Using a diluent allows for greater conversion of monomer to polymer and using diluent for this purpose is standard practice for commercial manufacture of contact lenses and water displaceable diluents are routinely used [147]. Glycerol was chosen due to its widespread use and acceptance in pharmaceutical formulations by regulatory agencies such as the Federal Drug Agency (FDA) [148]. A study was performed to select a suitable concentration of diluent. This study used the 3 formulations with 1 %, 5 %, 10 %, and 15 % (W/V) glycerol added to each formulation. Polymer formulations were prepared as usual and then diluent was added by weight to the prescribed amount. Polymer mixtures produced were sonicated and added to polypropylene moulds and manufactured in the same manner as previously outlined in Section 2.3.2. Once the lenses were removed from the moulds they were washed and dried and swelling studies were then performed. Lenses were soaked in de-ionised water over 24 hours at 25 °C. The equilibrium water content (EWC) method used by Jung *et al*. [149] where lenses where dried the lenses and then soaked them for 24 hours and then calculated the % EWC from the increase in weight after soaking. Deionised water was used in the analysis performed in this chapter to soak the lenses instead of PBS. Initial trials showed that the HEMA lenses reached equilibrium in less than 12 hours, but repeated removal of the lenses from their containers caused damage to the lenses. This method ensured the lenses were hydrated but not impaired so they could be used in further analysis. EWC was calculated and the standard deviation for each formulation and diluent concentration was obtained (Table 2.9).

Table 2.9: **Percentage EWC of cured contact lenses in de-ionised water (n=3).**

Material	% EWC	Standard Deviation (%)
HEMA:DEAMA:EGDAM 69:29:2 A	44.8	4.0
HEMA:NVP:EGDMA 6927.5:2.5 B	35.4	5.1
HEMA:NVP:EGDMA 67.5:27.5:5 C	61.7	1.7
HEMA:DEAMA:EGDAM 69:29:2 A*	24.6	2.8
HEMA:NVP:EGDMA 6927.5:2.5 B*	35.1	1.1
HEMA:NVP:EGDMA 67.5:27.5:5 C*	46.5	1.3
HEMA:DEAMA:EGDAM 69:29:2 A 1%	47.2	10.6
HEMA:DEAMA:EGDAM 69:29:2 A 5%	40.6	3.0
HEMA:DEAMA:EGDAM 69:29:2 A 10%	51.3	7.9
HEMA:DEAMA:EGDAM 69:29:2 A 15%	48.3	6.8
HEMA:NVP:EGDMA 6927.5:2.5 B 1%	58.1	0.8
HEMA:NVP:EGDMA 6927.5:2.5 B 5%	61.5	5.4
HEMA:NVP:EGDMA 6927.5:2.5 B 10%	62.5	2.4
HEMA:NVP:EGDMA 6927.5:2.5 B 15%	68.7	0.7
HEMA:NVP:EGDMA 67.5:27.5:5 C 1%	76.3	3.0
HEMA:NVP:EGDMA 67.5:27.5:5 C 5%	82.5	4.4
HEMA:NVP:EGDMA 67.5:27.5:5 C 10%	76.8	3.6
HEMA:NVP:EGDMA 67.5:27.5:5 C 15%	83.0	2.6

*** Polymer loaded with vitamin E.**

A reproducible % EWC result data indicated that the polymer lenses manufactured were uniform. A % EWC of 60-70 % would be standard for daily disposable lenses of all formulations [60]. Formulations with the lowest standard deviation were chosen for further study as the reproducible swelling is indicative of a controlled polymerisation process. Two glycerol concentrations of formulation B were chosen, this was due to their very low standard deviation and the 10 % change in % EWC by increasing glycerol content from 1 % to 15 %. The 5 and 10 % glycerol concentration for formulation B exhibited a 5.4 and 2.4 % standard deviation. There is a trend in swelling or % EWC of formulation B, which increases as glycerol concentration was, increased EWC ranged from 58.1 to 68.7 %. There is no trend evident in the standard deviation of the samples. The lowest standard deviation was recorded for samples at the extremes of glycerol concentration. Any impact on drug release due to diluent concentration would be observed by comparison of the drug release profile from these two formulations. All future contact lens monomer formulations contained glycerol. Table 2.10 presents the EWC of the selected diluent formulations.

Table 2.10: **Selected diluent formulations.**

Formulation	% EWC	Standard Deviation
HEMA:DEAMA:EGDAM 69:29:2 A 5 %	40.6	3.0
HEMA:NVP:EGDMA 6927.5:2.5 B 1 %	58.1	0.8
HEMA:NVP:EGDMA 69:27.5:2.5 B 15 %	68.7	0.7
HEMA:NVP:EGDMA 67.5:27.5:5 C 15 %	83.0	2.6

2.4.4.3. Differential scanning calorimetry analysis

The glass transition temperature (T_g) provides information on the polymer network produced in the polymerisation process. T_g can be described as the temperature where the polymer changes from an amorphous or glass-like solid to a rubber-like liquid [150]. Table 2.11 details the T_g data obtained.

Table 2.11: **Comparison of vitamin E and its impact on Glass transition temperature (Tg) of contact lens polymers (n=1).**

	T_g °C		
Material	**A**	**B**	**C**
T_g ^{0}C Lenses	122.4	133.1	142.4
T_g ^{0}C Loaded lenses	120.1	132.8	139.2

To observe the T_g a ramp cool cycle using DSC pans with pierced lids were utilised, this allowed water to be removed and reduced pressure build up in the DSC pan which could impact the T_g measured on a subsequent heating ramp. As water is a plasticiser, this measurement was artificially high due to the removal of water. However, the measurement can be used to compare each of the polymer formulations and measure the change in T_g after the addition of vitamin E. Typically the higher the T_g the more rigid the polymer. When the polymers are loaded with vitamin E the T_g decreased for all formulations by 1-2 °C demonstrating the possibility that polymer physical properties can be altered when loaded with vitamin E however this change in Tg is not significant. There was an increase in the T_g as chemical crosslinking increased, T_g increased from 122.4 °C to 142.4 °C. Lens strength and hardness will increase with increasing Tg but the lenses will become increasingly more brittle and uncomfortable for the user. Release of material once loaded will be slower from a more cross-linked polymer matrix [16]. Figure 2.11 illustrates how the glass transition temperature is calculated by the QA software. Glass transition temperature is labelled with an (I).

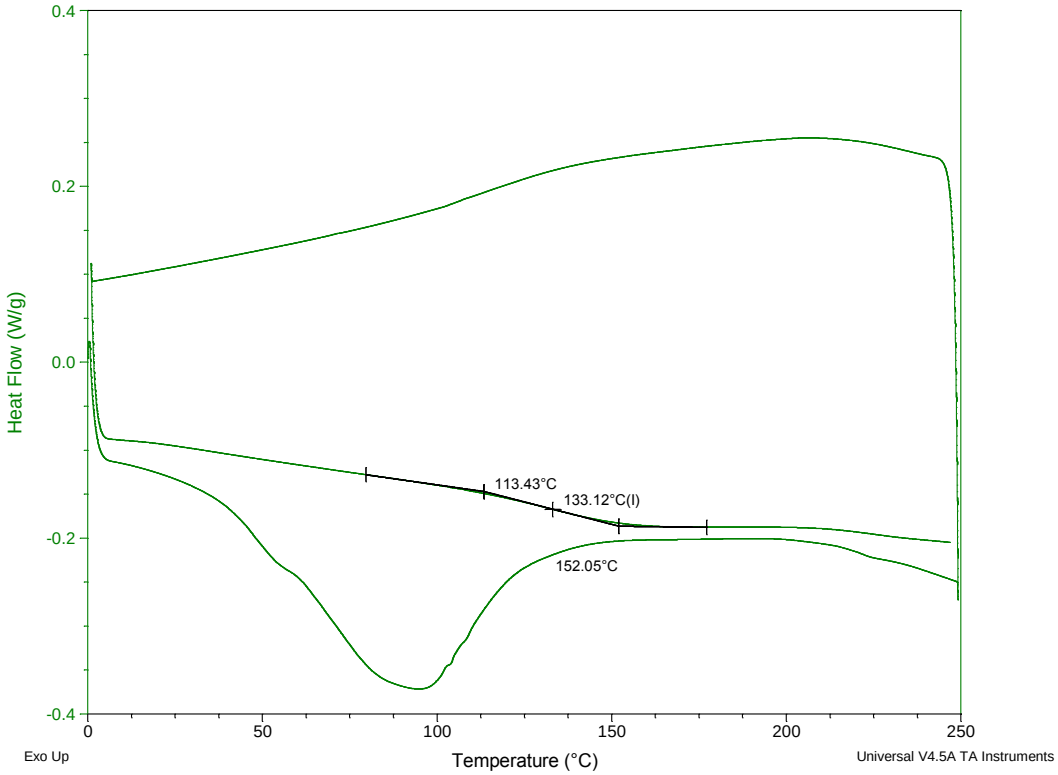


Figure 2.11: Example of glass transition analysis and calculation performed by QA software.

2.4.4.4. Refractive index measurement

Commercial lenses have a refractive index (RI) of 1.3 to 1.45 [60]. Analysis of the lenses prepared in this work demonstrated that the lenses manufactured have a refractive index in the range of commercial lenses (Table 2.12) and are capable of being used as ophthalmic inserts and ocular devices. Also, with the addition of vitamin E, this had no impact on the RI of the hydrogel lens. The refractive index of the vitamin E used was 1.506. RI for all lenses produced was unchanged and as the polymer is HEMA (67-69%), it was logical that RI would not vary across the formulations. This analysis does compare the RI of lenses produced to commercial lenses namely One Day ACUVUE® refractive index = 1.33 (Table 2.12).

Table 2.12: **Refractive Index data for synthesised and ACUVUE® lenses, n=3.**

Material	A	B	C	ACUVUE®
Lenses prior to loading	1.33	1.33	1.33	1.33
Vitamin E loaded lenses	1.33	1.33	1.33	1.33

2.4.4.5. Light transmission measurement

Commercial lenses have approximately 96-98% light transmittance [16]. In order for a lens to be suitable for use optical clarity has to be of this order so the patient can see clearly. Light has to be able to penetrate the polymeric matrix and the amount of light scattered or lost needs to be minimal. The lenses manufactured using the manufacturing method developed produce similar results to commercial lenses. When analysed for % light transmittance results ranged from 96.0 to 97.5% compared to 98% for commercial lenses. From this analysis it can be observed that the lenses made had a similar amount of light transmitted through them when compared to commercial lenses. Hydrogels which are used for contact lenses transmit over 90% of the light from the visible part of the spectrum [60]. When loaded with vitamin E the amount of light transmitted from swollen lenses decreased to 84-89% depending on the polymer formulation. This change was important and shows the possibility of changing contact lens parameters negatively when adding a diffusion barrier. The material loaded has to be matched to the monomer formulation used so the optical properties will not be affected. In this instance, vitamin E is not soluble in the polymer matrices and this impacts the light transmittance of the loaded polymers as there are separate phases in the polymer. No trend was observed for the decrease in % light transmission and amount of vitamin E loaded into the polymer. Formulation C recorded the largest decrease in light transmission and has the lowest amount of vitamin E loaded (C 1.08 $mg.mL^{-1}$) (Table 2.8 p.61). A negative impact appears to be caused by the relative solubility of the vitamin E in the polymer rather than amount of vitamin E present. Commercial lenses were also negatively impacted when vitamin E was loaded. The Drop in % light transmitted is very similar at 13 % compared to 9-13% for the manufactured lenses (see Table 2.13).

Table 2.13: **Percentage of light transmission through synthesised and commercial contact lenses, n=3.**

			% T	
Material	A	B	C	ACUVUE®
Lenses	97.5 ± 0.6	97.0 ± 0.5	96.0 ± 2.5	98 ± 1.8
Vitamin E Loaded Lenses	88.5 ± 1.3	86.1 ± 1.9	83.5 ± 1.7	85 ± 0.8

Note %T= % transmission at 800nm.

2.4.4.6. Gas chromatographic analysis

Lenses were manufactured and washed as per manufacturing procedure with 10 lenses subsequently extracted with methanol for 16 hours. No peaks were present in subsequent analysis of these methanol extracts. The washing procedure in combination with the addition of a diluent appears to be sufficient to remove any unreacted material from the polymers manufactured. Previous GC analysis highlighted the presence of oligomers in the lenses after washing. The addition of diluent allowed for a more uniform polymer matrix to form. Swelling studies demonstrate an increase in the reproducibility of % EWC of polymers fabricated with diluent. GC analysis proved that the addition of a diluent ensured washing was capable of removing any unreacted monomers from the polymer matrix. The Limit of Detection (LOD) was determined by measuring the signal to noise ratio of blank injections. The LOD was calculated at a peak height of 10 which compared to responses of 160 to 27,000 for samples of 1% w/v solutions of monomer in ethanol. Examples of the chromatograms produced are displayed in Figures 2.12 and 2.13.

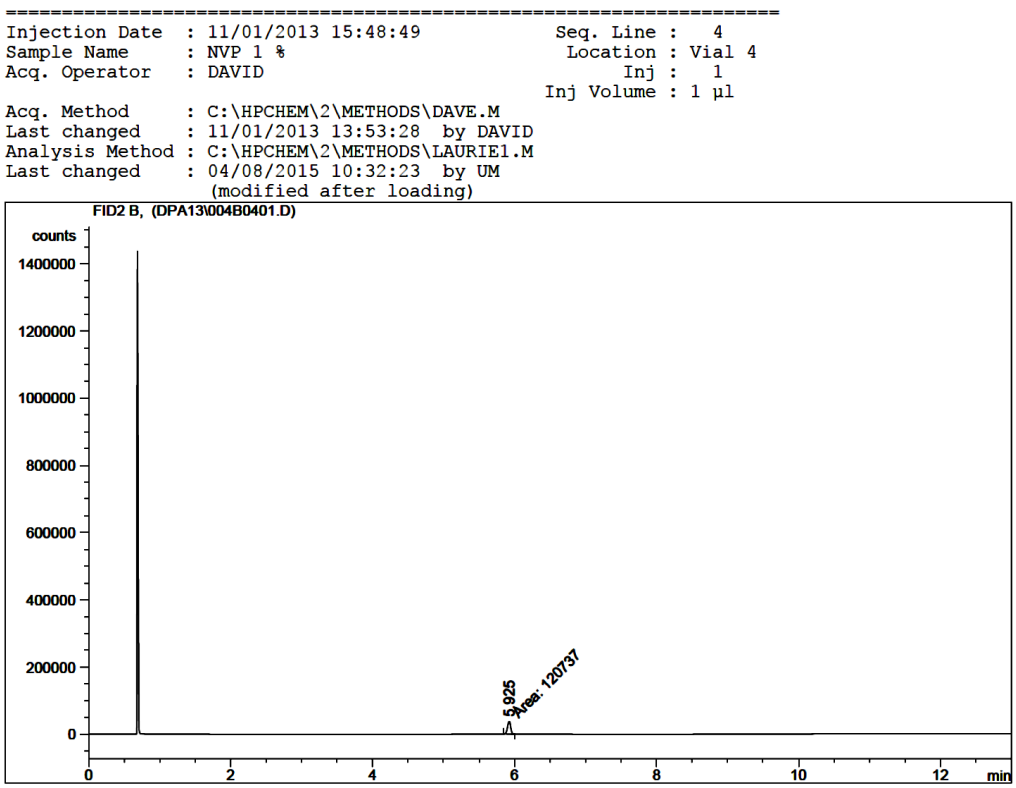


Figure 2.12: Example chromatogram showing the peak and area of a 1% NVP monomer solution.

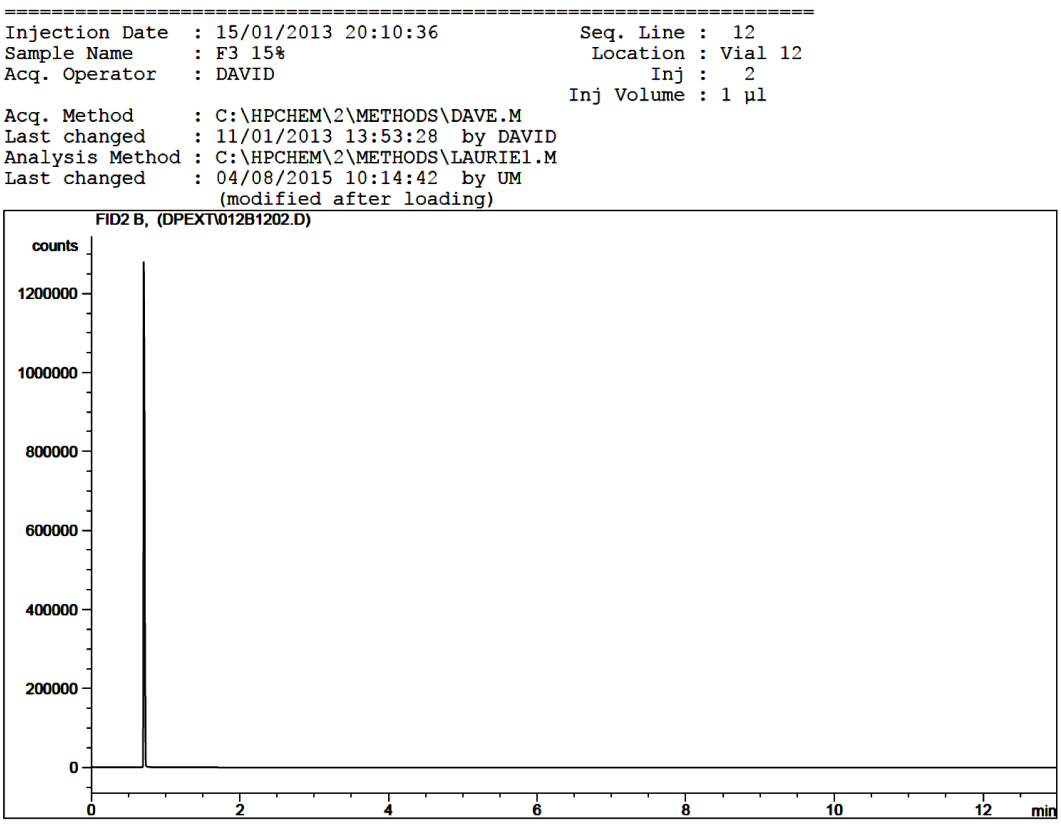


Figure 2.13: Example chromatogram of the extraction of 20 washed lenses illustrating that there were no extractable oligomers remaining after washing.

2.4.4.7. Contact lens size and curvature measurement

The lenses manufactured were analysed by an industrial partner and the physical dimensions are displayed in Table 2.14. The standard commercial lens diameter is 14.2 mm ± 0.200 mm therefore these lenses are all slightly smaller than commercial lenses. The size of the lenses fabricated ranged from 12.10 to 13.12 mm. This may be due to the fact the moulds are designed for a specific polymer formulation with each formulation absorbing different amounts of water. The geometric shapes of the lenses are comparable to commercial lenses. The curvature of a circle is measured by measuring the height of the curve over a fixed length known as a sag measurement. These lenses were also measured using industrial equipment. This data was compared to commercial lenses and was within 0.5mm of the commercial lens (see Table 2.14). The shape and geometry of the lenses are as close to commercial lenses as possible without fabricating polymer formulation specific contact lens moulds, at great expense.

Table 2.14: **Contact lens measurements (n = 10).**

Formulation	Diameter (mm)	Sag (mm)
A	12.10 ± 0.2	3.28 ± 0.1
B	12.65 ± 0.2	3.43 ± 0.1
C	13.12 ± 0.2	3.36 ± 0.1
Commercial lenses	14.20 ± 0.2	3.80 ±0.1

The contact lenses fabricated are compared to a commercial One Day ACUVUE® lens (Figure 2.14).

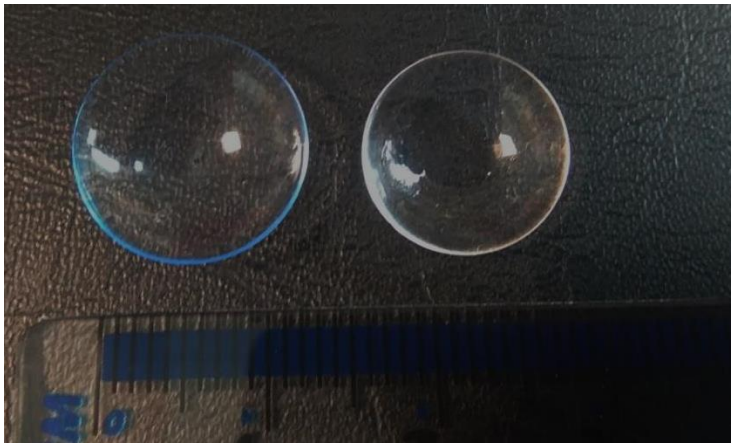


Figure 2.14: **Commercial contact lens on the left compared to fabricated contact lens on the right.**

The lenses are optically clear have the correct refractive index and % light transmission. There is a visual difference, as the commercial lens has a UV light blocking material added and therefore has a slight blue tint.

2.5. Conclusions

Novel manufacturing equipment as well as the methods of manufacturing contact lenses, using thermally initiated polymerisation has been designed and proven to produce contact lenses of commercial quality. An analytical assessment of the lenses found the lenses manufactured had a high degree of optical clarity and the refractive index was identical to commercial lenses. Characterisation methods have been developed so the physical properties of lenses produced can be analysed to ensure the manufacturing process was consistent. The lens manufacturing process and characterisation tests developed have been used successfully. These tests have established that vitamin E loading increases as the amount of cross-linking monomer present in the formulation decreases from 1.77 mg per lens to 1.08 mg per lens. Also

the loaded vitamin E was not released in phosphate buffered saline: ethanol 90:10 over 72 hours.

The contact lenses manufactured were capable of being handled and used in laboratory trials. They also had high visual clarity 96 - 97 % and a refractive index of 1.33 ensuring the lenses produced could be successfully used as contact lenses. Each lens was also consistent in size and shape with diameters 12.10 mm to 13.12 mm which are 15 % smaller than commercial lens diameters of 14.20 mm. Swelling data and inconsistent loading were caused by, or exacerbated by the presence of unreacted monomers in the polymer matrix. The equilibrium of the swollen polymers was assessed during initial trials. The polymers did not continue to increase in weight after 12 hours. This ensured that any variation in the lens ability to swell was most likely due to presence of oligomers. The addition of a diluent allowed the movement of monomers during polymerisation and prevented monomers becoming entrapped in the forming polymer matrix. Glycerol was used as a diluent as it increased the consistency of the lenses in regard to the completeness of polymerisation and ensured reproducible results in equilibrium water content (EWC %) studies (as performed by Jung *et al*.[149] Analysis of manufactured lenses by gas chromatography has determined that unreacted monomers were removed by washing after polymerisation with the presence of diluent. Consistent EWC data was achieved where the standard deviation of replicate samples varied by 3.0 % or less.

Cross-linker concentration had an impact on both loading parameters and physical characteristics. The polymer composition needs to be tailored to the loading process to minimise damage or physical changes to the lenses. Thermal analysis, specifically glass transition measurements, provided information on the loading handling and physical strength of polymers and can be used to screen possible polymer formulations. The (T_g) for ranged from 122.4 °C to 142.4 °C depending on polymer formulation and crosslink density.

The study has revealed that it was possible to manufacture commercially comparable lenses in-house and load these lenses with a possible diffusion attenuator vitamin E. The attenuator was retained in the polymer matrix so subsequent hydrophilic material

can be loaded without loss of the diffusion barrier. It was also possible to assess the effect of different polymer formulations on loading and lens characteristics.

The novel lab scale manufacturing system developed, allows for the future analysis, characterisation and formulation of novel drug delivery prototypes. This manufacturing system allows for direct casting of drug or drug laden particles into the lenses. If commercial lenses were used, drug loading can only occur via soaking. This method of manufacture will allow an opportunity for control over the lens formulation used as well the drug loading method. These drug delivery vehicles can be drug loaded using a variety of drugs as well as loading strategies. The following chapters will use these methods to drug load, manufacture and assess the contact lens drug delivery vehicles prepared.

Chapter 3

Evaluation of Drug Loading and Release Characteristics of Contact Lenses

3. EVALUATION OF DRUG LOADING AND RELEASE CHARACTERISTICS OF CONTACT LENSES

3.1. Introduction

This study sets a baseline for drug loading and release studies. Drug loading via soaking will be used to determine the amount of drug material that can be loaded into lenses and determine if a therapeutic dose of antihistamine can be loaded into contact lenses. While Chapter 2 detailed the manufacture and characterisation of contact lenses, the focus of this Chapter was placed on drug loading and release characteristics of the prototype drug delivery vehicles fabricated.

Drug loading of contact lenses can be performed using a number of techniques i.e. molecular imprinting [16, 151], drug loaded particles [139, 140, 149], and Super Critical Fluids (SCF) [4, 13, 14]. The techniques employed may not only increase drug loading, but also impart control over the subsequent release of drug from the polymer. Drug soaking is used to load drug into polymers in all of these methods. A brief review of the state of the art for these methods is addressed below.

Hiratani *et al*. demonstrated that molecularly imprinted lenses had increased loading capacity .These lenses released a dose of 34.7 µg (imprinted lenses) into the tear film of rabbits versus 21.2 µg for non-imprinted lenses. They also released drug for 180 minutes into the tear flow, which was twice the duration of drug release from non-imprinted lenses [144]. A number of researchers in the field suggest the reason for this increase in drug loading is that drug specific recognition sites are created in the polymer by drug and monomer interactions prior to polymerisation [114, 152-155]. These pre-polymerisation complexes are permanently set into the polymer matrix during polymerisation, creating cavities where drug molecules can be specifically retained, leading to increased drug loading and attenuation of drug release [10, 16, 112].

The use of supercritical fluids to load materials into a polymer matrix has some unique advantages. This method is a variation of drug loading by soaking. Drug loading is assisted by the supercritical fluids ability to swell the hydrogel and thus

aid drug loading. Polymers are swollen significantly by supercritical CO_2, which allows easier penetration of the polymer and increased drug loading [156]. In addition, the supercritical fluid can be tuned to increase solubility of the desired compound. This is achieved by adding solvents (e.g. ethanol) which increase the solubility of the drug in the super critical fluid. Once temperature and pressure are reduced, the swelling dissipates, trapping the loaded material [156]. Braga *et al.* [109] discovered that the operating conditions of supercritical fluid decreased release rate, and suggested that at least one reason for this altered release was due to the drugs position in the polymer matrix. Drug that was deeply embedded into the polymer matrix was released over a longer period of time. This was demonstrated by the controlled release of acetazolamide over a period of 8 hours. The ability to control release over this timeframe presents a real opportunity for daily disposable lenses. Figure 3.1 displays images of supercritical fluid drug loaded lenses [157]. The influence of increasing temperature and pressure on the lenses can be seen by the increased opacity of lenses from A to D.

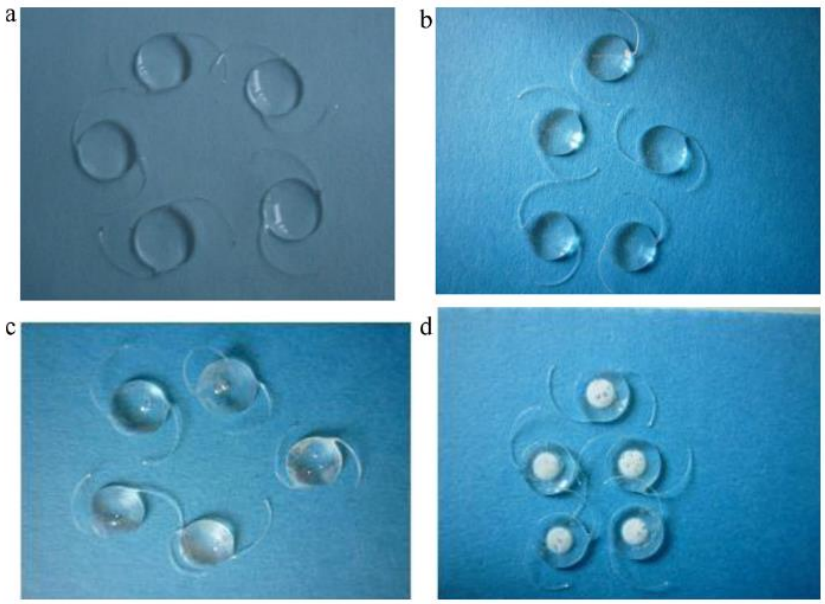


Figure 3.1: Images of supercritical fluid drug loaded lenses [157]. Non impregnated lenses (a), lenses impregnated at 8MPA and 308K for 2 h (b), impregnated at 8 MPA and 333 k for 5 h (c), and impregnated at 20MPA and 333 K for 2 h (d).

In addition to drug loading methods, it may be possible to include a diffusion barrier to control drug release. The diffusion barrier slows down or attenuates drug release

from the polymer by taking up space within the matrix [8]. The presence of bulky barrier materials, such as vitamin E, blocks diffusion of the loaded drug. Delayed or attenuated drug release has been documented, with one example exhibiting reduced drug elution rates, releasing drug at a rate of 6.1 µg per day compared to 10.2 µg per day per day for lenses not loaded with vitamin E [8]. The analysis in section 2.4.2 demonstrated it was possible to load up to 1.8 mg of vitamin E per lens and that the vitamin E once loaded in to the lens did not elute from the lens into PBS.

Experimental work performed in Chapter 2 identified a possible link between chemical cross-linking and amount of vitamin E into loaded into contact lenses. This relationship may also impact drug release and a study to evaluate the effect of chemical cross-linking and formulation on the release of two antihistamine drugs was performed, to determine if chemical cross-linking of the lenses impacted drug release from the lens. It was also observed in literature that increased chemical cross-linking of polymers has been demonstrated to attenuate drug release [130].

A number of polymer formulations with different cross-link densities, monomers and equilibrium water content (EWC) were investigated. Drug loading was performed by soaking. In this study, six formulations were loaded with two antihistamine pharmaceuticals, cetirizine and olopatidine. Antihistamines were chosen as they are routinely used to treat ocular conditions. One such example is allergic conjunctivitis, which is a very debilitating condition that leads to excessive tearing and inflammation of the eyes. 15% of the world's population suffer from allergic reactions, with up to 30 % of the US population reporting some form of allergy, most having an ocular effect which is frequently the most incapacitating of the symptoms [158]. Allergic conjunctivitis and rhinitis symptoms, prevent the wearing of contact lenses, and are induced by pollen, house dust mites, insect and animal dander's. Allergic conjunctivitis and rhinitis are linked to the hyper response of the immune system and can occur separately or in combination. Allergens bind to mast cells or basophils causing the release of cytotoxic compounds [159]. These cytotoxic compounds cause allergic inflammation and widening of the blood vessels, which damage the surrounding tissues. Using contact lenses as a drug delivery vehicle would provide a distinct advantage over current treatments as smaller drug doses

would have to be administered and patient symptoms would be more effectively controlled. Antihistamines are a viable option for use as a model drug for polymer loading and release, as they are quite hydrophilic and are routinely used as ocular pharmaceutical compounds in eye drops (Ref Table 3.3 p.81 for physical properties). Drug loading contact lenses with antihistamines could provide an effective treatment for allergy patients. The optimum formulations were chosen based on swelling studies performed, in Chapter 2, which identified glycerol levels that increased swelling consistency. Glycerol levels from 1 to 15% were added to formulations F1-F5 (see Table 3.2 for formulation components), and a sixth formulation was added which doubled the amount of EGDMA compared to formulation F1.

3.2. Materials used in the evaluation of drug loading and release characteristics of contact lenses

Table 3.1: Materials used in this work.

Material	Supplier	Purity
Cetirizine	*Richemg	99%+
Olopatidine	Tokyo Chemicals Industry	99%+
ά Tocopherol (Vitamin E)	Sigma Aldrich	99%
Ethanol	Lennox	98%
Hydroxy-ethyl-methacrylate (HEMA)	Sigma Aldrich	98%
N-vinyl-pyrrolidone (NVP)	Sigma Aldrich	99%
2-(Di-ethyl-amino)-ethyl-methacrylate (DEAMA)	Sigma Aldrich	99%
Ethyleneglycoldimethacrylate (EGDMA)	Sigma	99%
Azobis-*iso*-butrylonitrile (AIBN)	Sigma Aldrich	99%
Glycerol	Reagecon	N/A
ACUVUE® Lenses	Vistakon	N/A

***Richemg Development Ltd. Dalian China.**

3.3. Experimental methods

3.3.1. Loading contact lenses with vitamin E

Contact lenses were loaded with vitamin E by soaking them in a 50 $mg.mL^{-1}$ solution in ethanol. Each lens was placed in a micro centrifuge tube and 1 mL of the loading solution was added. The lenses were allowed to load for 5 days. This extended drug

loading time was used by other researchers when loading hydrophobic polymers with hydrophilic drugs [8, 124, 125]. In contrast to these studies, this research was loading a hydrophobic material into a hydrophilic hydrogel. The extended loading time would ensure equilibrium in loading vitamin E was reached.

Vitamin E samples were analysed using an Agilent 1200 series high performance liquid chromatography (HPLC) fitted with UV detection set at 229 nm and a Zorbax C8 (4.6 X 150 mm 5 µm) column. The mobile phase used was 95:5 methanol: water with a flow rate of 1.5 $mL.min^{-1}$. 10 µL of each sample was injected for analysis. The limit of detection (LOD) was calculated in accordance with ICH guidelines and determined to be 0.17 $\mu g.mL^{-1}$. The Limit of Quantitation (LOQ) was 0.51 µg $\mu g.mL^{-1}$ and the r^2 for the standard curve generated was 0.99998. The vitamin E HPLC method was developed for this analysis.

3.3.2. HPLC analysis of antihistamines

An Agilent 1200 series HPLC fitted with UV detection set at 254 nm and Waters C18 (4.6 X 250 mm 5µm) column was used for antihistamine analysis. The mobile phase used was 65:35 acetonitrile:water (pH adjusted to 2.7 with *ortho*-phosphoric acid), a flow rate of 0.9 $mL.min^{-1}$, and an injection volume of 40 µL was used to analyse samples. The Limit of Detection (LOD) was calculated as per ICH guidelines for both olopatidine and cetirizine. Cetirizine LOD was calculated as 0.44 $\mu g.mL^{-1}$ and 0.05 $\mu g.mL^{-1}$ for olopatidine. The LOQ for cetirizine was determined to be 1.32 $\mu g.mL^{-1}$ and 0.15 $\mu g.mL^{-1}$ for olopatidine. The square of the correlation coefficient values for the calibration curve of cetirizine was 0.99998 and 0.99879 for olopatidine.

3.3.3. Drug loading and release methods used

3.3.3.1. Loading of contact lenses with antihistamines via soaking

Lenses were loaded with either olopatidine or cetirizine by soaking in a 5 $mg.mL^{-1}$ solution of drug in ultra-pure water. Each lens was placed in a micro centrifuge tube and 1 mL of the loading solution was added. The lenses were allowed to load for 6 days [124]. Dug loading of contact lenses was also performed using 165 $\mu g.mL^{-1}$ and

330 µg.mL^{-1} solutions to load lenses with a target concentration of 40 and 80 µg per lens.

3.3.3.2. Drug release analytical method

The drug loaded contact lenses were rinsed in water (to remove any drug adsorbed onto the surface of lenses), then gently tapped onto low fibre lens tissue and transferred into Eppindorf vials containing 1 mL of phosphate buffered saline (PBS).

After one hour the lenses were then removed from the Eppindorf vial, rinsed in ultra-pure water, tapped on low fibre tissue and transferred to a new Eppindorf vial containing 1 mL of PBS (pH 7.2). This was repeated for each time point selected. Lenses held within the Eppindorf vials were placed in an orbital shaker at 37 °C at 70 RPM for release studies.

3.3.4. Lens characterisation methods

3.3.4.1. Refractive index

A Bellingham Stanley RFM 340 refractometer with sample illumination light at 589 nm was used to measure the RI of all lenses. The refractometer was temperature controlled at 25ºC.

3.3.4.2. Light transmission

A Shimadzu UV Vis 2401-PC spectrometer set at 800 nm was used to measure the amount of light transmitted through the in-house manufactured lenses and control commercial lenses. The lenses were suspended in the path of the beam using a film attachment and lens holding rig. Lenses were measured with reference to air.

3.4. Results and Discussion

The focus of this study was to determine if vitamin E could be used in hydrophilic contact lens polymers to attenuate drug release. The lens formulations employed for drug loading and release studies are detailed in Table 3.2. The formulations were chosen based on the previous swelling studies performed in Chapter 2.

Table 3.2: **Contact lens polymer formulation components and concentration.**

Formulation	Monomer	Monomer	Cross-Linker	Ratio Mole %	Glycerol %
F 1	HEMA	DEAMA	EGDMA	69:29:2	5
F 2	HEMA	NVP	EGDMA	67.5:27.5:5	1
F 3	HEMA	NVP	EGDMA	67.5:27.5:5	15
F 4	HEMA	NVP	EGDMA	69:27.5:2.5	15
F 5	HEMA	NVP	EGDMA	69:27.5:2.5	1
F 6	HEMA	DEAMA	EGDMA	67:29:4	5

Note: All formulations contained 0.05 % mole fraction of AIBN.

3.4.1. Drug choice rationale

The two compounds chosen were cetirizine, as it is a commonly used antihistamine, and olopatidine due to its long term action and efficacy in the treatment of allergic rhinitis [159]. See Figure 3.2 for structures of these compounds and vitamin E.

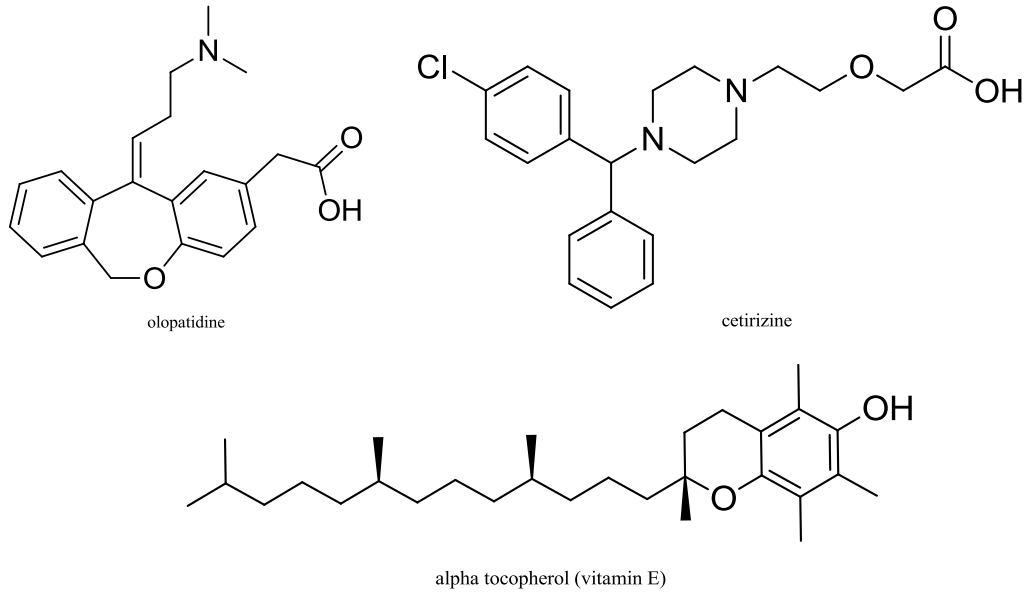


Figure 3.2: **Chemical structures of olopatidine and cetirizine candidate antihistamine drugs.**

The physiochemical properties of the two antihistamines used are detailed in Table 3.3.

Table 3.3: Physical properties of candidate antihistamine drugs [160].

Properties	Olopatidine	Cetirizine	Vitamin E
Water solubility	0.0313 $mg.mL^{-1}$	0.0658 $mg.mL^{-1}$	7.04 X 10^{-6} $mg.ml^{-1}$
pKa acid	3.78	3.6	Not available
LogP	3.99	2.98	10.51
Melting point	248 ^{0}C	112.5 ^{0}C	N/A
Molecular weight	337.41	388.88	430.71

Note: values presented are predicted.

3.4.2. Vitamin E loading of contact lenses

Vitamin E was loaded into lenses as a diffusion barrier that could delay the release of subsequently loaded pharmaceutical compounds. Results indicate that chemical cross-linking is a factor in vitamin E loading (see section 2.4.3). EGDMA (cross-linking monomer) and diluent added prior to polymerisation impact the physical structure of the polymer network and determine how the polymer swells and how materials will diffuse through it. Formulation F1 (Table 3.2) loaded 0.91 mg of vitamin E per lens (lens weight 21 mg) and it had the lowest concentration of cross-linker (Table 3.4). This low level of chemical cross-linker used (EGDMA) may make it easier for material to diffuse into the polymer matrix. The loading of vitamin E into contact lenses conducted in this trial was not reproducible. One possible reason for this is that vitamin E is a very hydrophobic material more suited to loading into silane contact lens monomers. Formulations F5 and F6 loaded 1.27 mg and 2.18 mg per lens, respectively as can be seen in Table 3.4. These results are out of line with the previous analysis conducted in this study. Formulations F1 and F6 differ in the amount of EGDMA present in their formulation. F6 has double the EGDMA of F1 and loaded 68% more vitamin E than formulation F1 loaded (2.18 mg per lens compared 0.91 mg per lens). The more structured polymer formed appears to allow more vitamin E to be loaded. Variation in the amount loaded in replicate lenses is shown by the high standard deviation observed as much as 1.14 mg per lens for F5. To uniformly load vitamin E, directly embedding it in the monomer mixture prior to polymerisation, would be an improvement which would remove vitamin E loading issues. Vitamin E loading results are presented in Table 3.4.

Table 3.4: **Vitamin E loading data obtained from 50:50 ethanol:water assay (n=3).**

Formulation	Vitamin E mg / lens	Std Dev mg / lens
F1	0.91	0.19
F2	0.40	0.18
F3	0.28	0.01
F4	0.26	0.06
F5	1.27	1.14
F6	2.18	0.14

3.4.3. Calculation of therapeutic dose of olopatidine and cetirizine for contact lens drug loading

To load lenses with a therapeutic level of drug, the daily required dose must be defined. The most convenient way of defining this was by calculation from a commercial product, in this case eye drops. Patanol is available as a 0.1 % eye drop containing olopatidine which is administered twice daily. Therefore 100 µg is administered twice a day, equating to 200 µg daily dose. 1-5 % of the active ingredients in eye drops are bio-available, therefore the effective daily dose available from eye drops for corneal absorption is between 2 and 10 µg per day. The bio-availability of drug delivered from contact lenses is 50 % [142]. This implies a daily dose from a contact lens should be 20 µg per day. Making an assumption, that 60-80 % of the drug loaded would actually be released from the contact lens leads to a required drug loading for the lens of 25-33 µg of olopatidine. This assumption was based on the study of Jung *et al.* where 48.25 % of the drug loaded was observed not to be released from the drug loaded polymer particles [93].

3.4.4. Antihistamine loading of contact lenses

Cetirizine and olopatidine were loaded into lenses which had been previously loaded with vitamin E. They were also loaded into polymer lenses that had not been loaded with vitamin E. These polymers were labelled as controls. The analysis was performed in two sets: initially with formulations F1-F4, and then again with formulations F5, F6 and ACUVUE® drug loaded lenses used as a control. Only olopatidine was used in this experiment, as the initial study highlighted that release of this drug from the polymer matrix was more affected by the presence of vitamin E. Drug loading results are listed in Table 3.5.

Table 3.5: Olopatidine, cetirizine assay results from the drug loading of contact lenses via soaking of lenses in drug solutions (n=3).

Formulation	Amount olopatidine Loaded µg / lens	Std Dev Concentration µg /lens olopatidine	Amount cetirizine Loaded µg / lens	Std Dev Concentration µg /lens cetirizine
F1 Vitamin E	1830	190	3000	70
F1 control	1860	90	2920	0.14
F2 Vitamin E	680	80	930	90
F2 control	860	90	920	40
F3 Vitamin E	810	30	910	70
F3 Control	870	120	940	30
F4 Vitamin E	830	80	1000	40
F4 Control	970	50	940	80
F5 Vitamin E	870	30		
F5 Control	950	20		
F6 Vitamin E	410	130		
F6 control	600	90		
ACUVUE® Vitamin E	620	160		
ACUVUE® control	680	0.0		
ACUVUE®	*45.87	0.72		
F5	*41.52	0.83		
F4	*40.64	2.43		
ACUVUE®	**92.12	0.63		
F5	**76.49	1.24		
F4	**76.97	0.30		

*** Therapeutic Dose loaded ** Double therapeutic dose loaded.**

The quantity of drug loaded into vitamin E lenses was consistently lower for olopatidine in comparison to cetirizine. F1 loaded 1170 µg more cetirizine. This is most likely due to the physical properties of the drugs with cetirizine having a lower logP compared to olopatidine (2.99 and 3.99 respectively). The lower logP value indicates that cetirizine is more hydrophilic and therefore more soluble in the hydrophilic polymer matrix. All formulations containing vitamin E were able to support higher drug loading of cetirizine, with the different loading quantities being more pronounced in formulations with the lowest amount of EGDMA (cross-linking monomer) (i.e. F1). Other formulations only varied by 50-200 µg per lens.

With regards to olopatidine loading, the presence of vitamin E in the polymer matrix decreased the drug quantity in the lenses. This was most evident in formulation F2

and F6, where approximately 200 µg less olopatidine was loaded into polymers containing vitamin E. In contrast, control lenses for F2 and F6 contained similar quantities of olopatidine, differing by a maximum of 70 µg per lens or less across all the formulations analysed. One plausible reason for this observation is a physical interaction between vitamin E and olopatidine preventing higher drug quantities being incorporated.

The impact of chemical cross-linking specifically the amount of EGDMA present in the formulation was significant as formulation F1 which has no cross-linking monomer added (EGDMA) loaded 1830-1860 µg of olopatidine more than double the amount loaded into the other formulations which contained EGDMA. FI loaded 2920-3000 µg of cetirizine, which was 3 times the amount loaded into the other polymer formulations which contained EGDMA. Cetirizine drug loading was not observed to be impacted by the presence vitamin E. Cetirizine was loaded into lenses with and without vitamin E, results obtained were within 80 µg of each other which was within the standard deviation of the results generated. The same is true for olopatidine where the largest difference in drug loading was 60 µg which was also well within the standard deviation of the drug loading results. The olopatidine drug loading varied from 1860 µg to 680 µg across formulations F1-F4. A similar change was observed for cetirizine loaded polymers drug loading ranged from 910-3000 µg across formulations F1-F4. The impact of chemical cross-linking was highlighted by the amount of olopatidine loaded by F1 and F6; these formulations are identical with the exception of F6 having twice the amount of EGDMA (cross-linking monomer). Increasing chemical cross-linking negatively impacted the loading of lenses by reducing the amount of drug loaded, from 1800 µg per lens to 400 µg per lens. The physical strength of the lenses was increased by increasing the chemical cross-linking and none of the lenses were damaged by handling during the release study. This decrease in drug loading as chemical cross-linking increases has been observed in previous studies. Hiratani *et al*. noted drug loading of a N,N-diethyl-acrylamide could only be increased for a small concentration range of cross-linker (EGDMA) and outside of this drug loading was negatively impacted [16].

The change in the amount of drug loaded into the lenses may also be due to the physical properties of the two drugs as olopatidine has a lower logP value, it was more affected. This less polar drug was less soluble in the hydrophilic polymer.

3.4.5. Effect of diffusion barrier on drug release

The drug release from the lenses was monitored over a 36 hour period at 37 °C to determine if the presence of vitamin E slowed drug release. Contact lenses were made from formulations F1 to F4 and were then subsequently drug loaded. It was not possible to analyse drug release from lenses made from F1 as they had disintegrated in the drug loading medium (ethanol) to an extent where they could not be tested. Lenses are stored in centrifuge tubes to avoid evaporation and as the lenses can adhere to the centrifuge tube; this can then lead to the lenses being torn when removed from the tube if they do not possess adequate physical strength. Lenses loaded between 910 -3000 µg of cetirizine and 620-1860 µg of olopatidine, which was a sufficient reservoir to enable drug release measurement. Release rates and amounts released varied from each formulation as can be seen from the graphs in Figure 3.3.

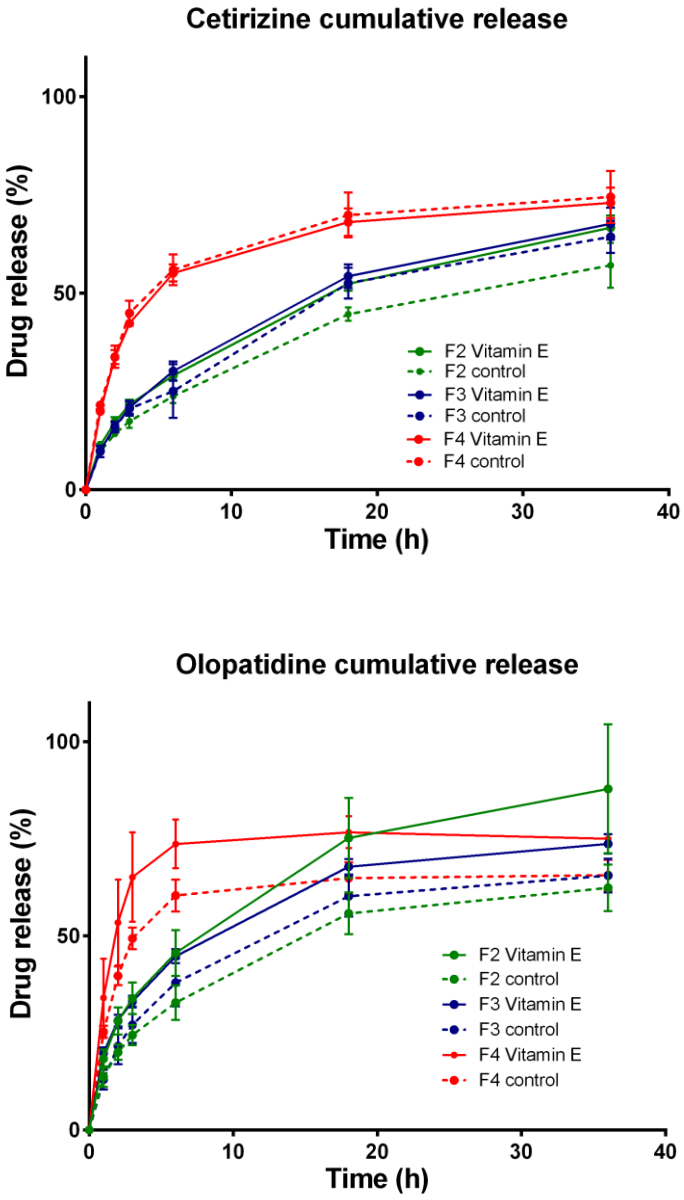


Figure 3.3: Percentage cumulative release at 37 °C from contact lenses drug loaded with cetirizine and olopatidine over 36 hours (n=3).

The presence of vitamin E did not retard the release of drug from the polymer lenses. This was in direct contrast to the drug release from silicon hydrogels observed by Peng *et al* [124]. In Peng's work drug release rates were decreased when vitamin E was present in the polymer formulation. In this work by Peng *et al.* the drug diffusion barrier and the lenses (silane) are both relatively hydrophobic. These silane lenses released 57 µg of timolol. This compares to drug release of between 3000 µg and 680 µg of drug presented in Figure 3.3 from fabricated HEMA lenses. Vitamin E loaded polymers did not display reduced drug release rates, of either drug from

polymer lenses (Figure 3.3). However, variation in the analysis of 3 replicate samples was reduced in polymers which had been loaded with vitamin E, (Figures 3.3 to 3.6). Drug release was more repeatable from these formulations, but drug elution was not delayed. Standard deviation halved in some cases (5.6 % standard deviation to 3.1% with vitamin E loaded lenses). This observation indicates there may be an interaction between vitamin E and the loaded drug which impacts drug elution.

Vitamin E aided cetirizine release from formulation F2 vitamin E loaded lenses it released almost 10% more drug in total. The elution profiles of the control and vitamin E loaded lenses were similar and there was very little variation in replicates analysed for either formulation (Figure 3.4).

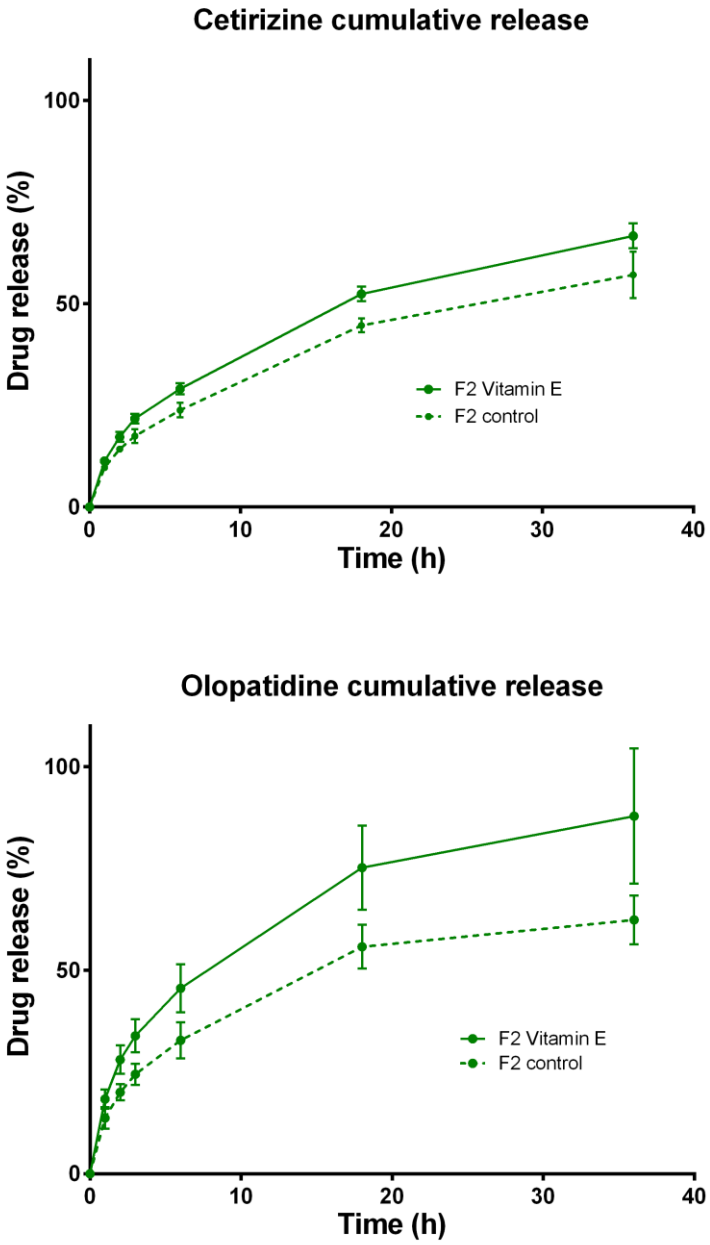


Figure 3.4: Percentage cumulative drug release at 37 °C of cetirizine and olopatidine over 36 hours from formulation F2 contact lenses (n=3).

Formulation F2 loaded with olopatidine and vitamin E released more drug when compared to control lenses; the control lenses released 25.5% less drug over the same time period. However, there was a smaller variation of the replicate control samples analysed versus vitamin E loaded lenses. Formulation 2 exhibited a less repeatable delivery of olopatidine in the presence of vitamin E exhibiting a 22.2% range in replicate drug release results compared to 11.1 % for the control polymer, This is illustrated by the larger variation in the error bars the in Figure 3.4.

Formulation F2, however, released a larger % of drug. This may be due to the olopatidine solubility in the hydrogel matrix.

Cetirizine drug release was not impacted by the presence of vitamin E. A similar quantity of cetirizine was released for control polymer lenses compared to vitamin E loaded lenses (Figure 3.5).

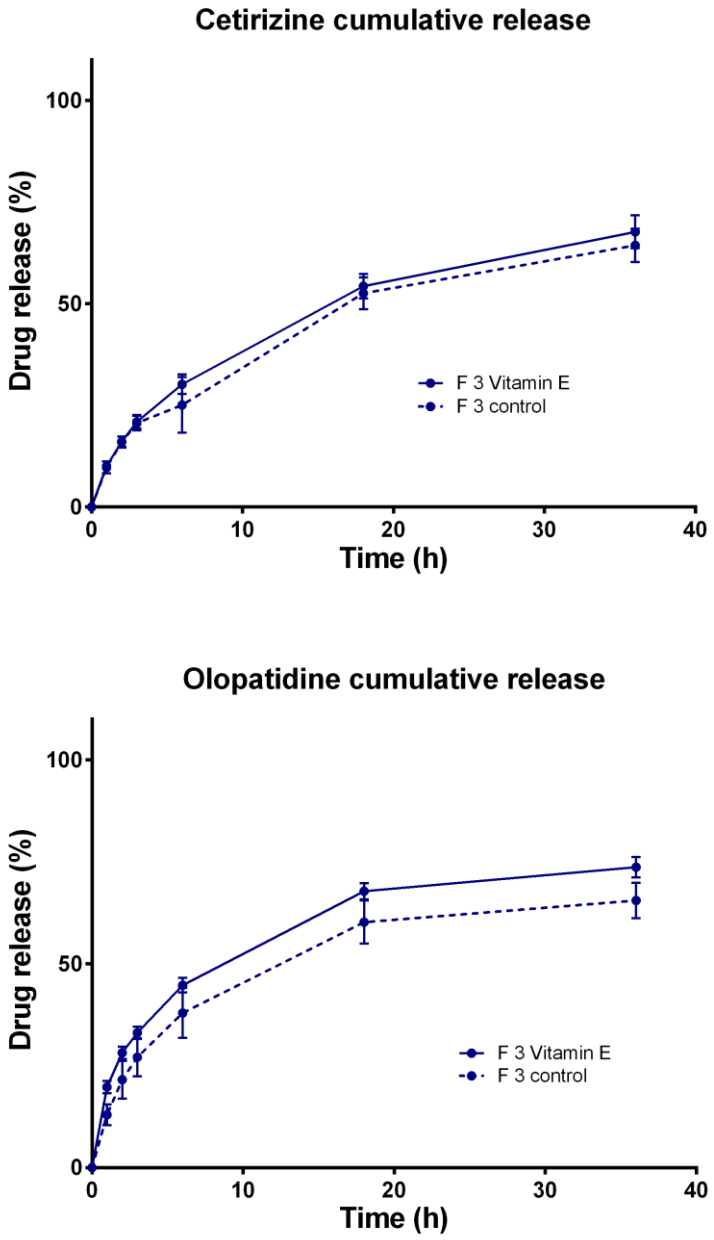


Figure 3.5: **Percentage cumulative drug release at 37 °C of cetirizine and olopatidine over 36 hours from contact lenses manufactured from formulation 3 (n=3).**

Vitamin E may have aided the release of olopatidine from formulation F3 lenses as vitamin E loaded lenses delivered slightly more drug when compared to control lenses. The addition of the nonpolar vitamin E may have an impact on solubility of the olopatidine in the hydrogel matrix. The release profiles presented in Figure 3.6 are very similar to formulations F2, F3 and F4.

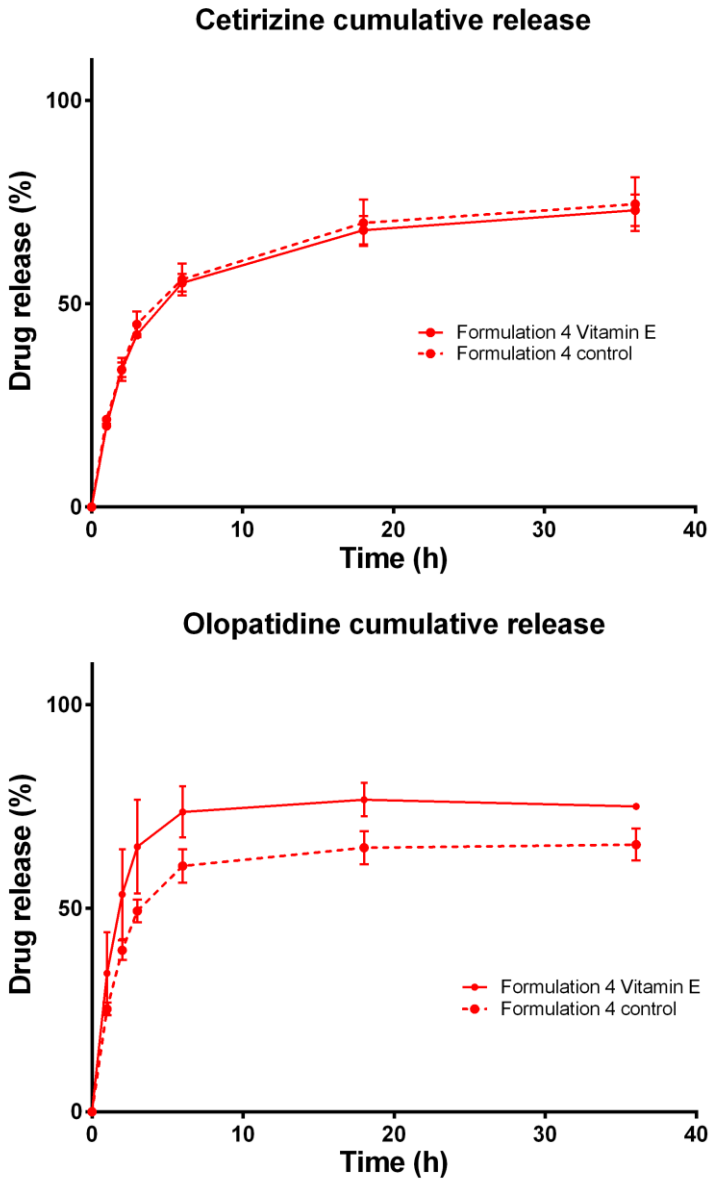


Figure 3.6: Percentage cumulative drug release at 37 °C of cetirizine and olopatidine over 36 hours from contact lenses manufactured with formulation 4 (n=3).

There was an 11.6 % increase in the amount of olopatidine released from vitamin E loaded lenses in formulation F4. For lens formulation F4 the elution profile of cetirizine was not affected by the presence of vitamin E, as can be seen in Figure 3.6.

It was also the only formulation where the presence of vitamin E did not increase the amount of cetirizine released. The most likely reason for this is cetirizine's increased water solubility. Cetirizine is less hydrophobic than olopatidine as described by its lower logP value 2.98 versus 3.99 for olopatidine. As the Lens polymers are relatively hydrophilic it is likely that the cetirizine is more soluble in these polymers than olopatidine.

The amount of olopatidine released from lenses F1- F4 was increased when vitamin E was present in the polymers. There appears to be an impact on drug when vitamin E and olopatidine are loaded in the same polymer as it occurs across all the formulations tested. As olopatidine is a less polar molecule than cetirizine it may be less soluble in the lens polymers. The addition of the hydrophobic vitamin E may decrease further the solubility of olopatidine in the lens polymer.

There was no observable impact on the release of cetirizine when vitamin E is loaded into the polymer lenses. Cetirizine is more hydrophilic than olopatidine and this increased water solubility of cetirizine will most likely be the dominant force in its release from the polymer.

3.5. Drug loading and release of reformulated manufactured lenses and commercial lenses

Following on from preliminary drug loading experiments a further drug loading study was performed loading olopatidine and vitamin E into formulations F5 and F6 (See Table 3.2 p.80 formulation details) and ACUVUE® commercial lenses to compare drug release profiles from the three lens types. This analysis would highlight differences in drug release amount and % drug release as well as comparing the lenses fabricated to commercial lenses.

The release profiles are presented in Figure 3.7. Cetirizine was not used in this study as it drug release profile was not impacted by the presence of vitamin E.

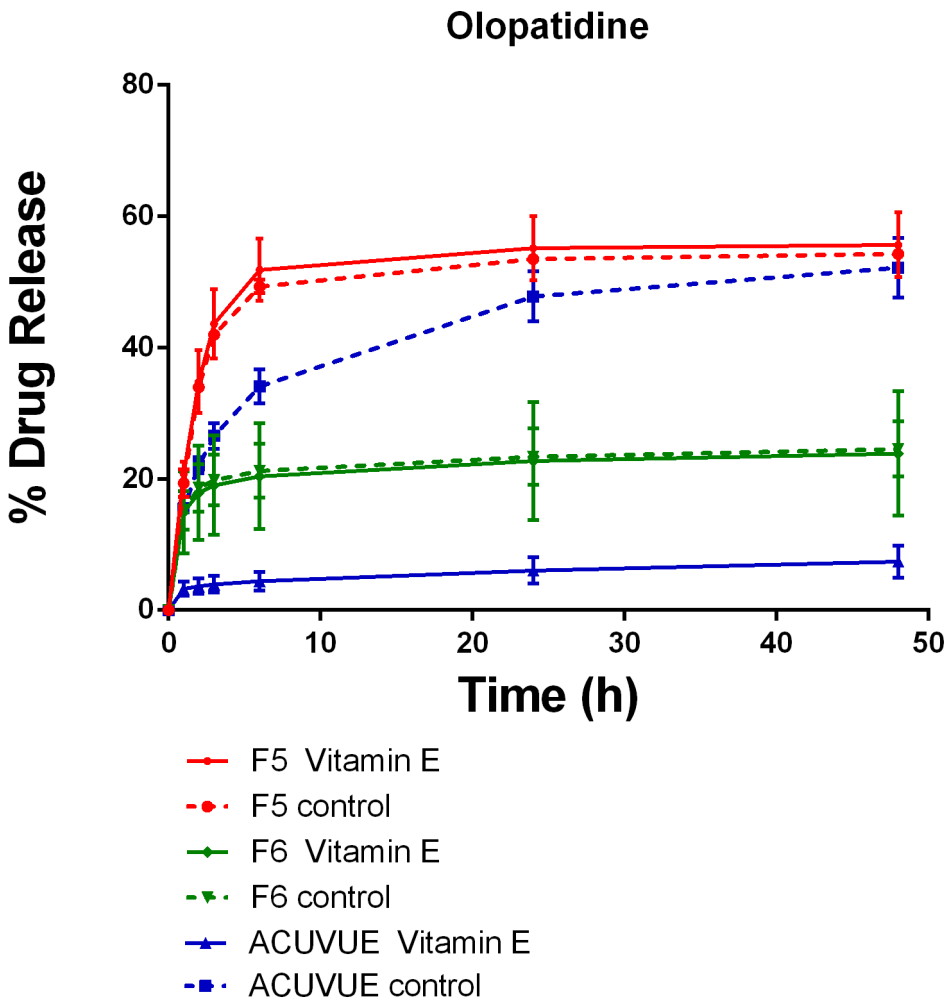


Figure 3.7: Percentage cumulative drug release of olopatidine from contact lenses over 48 hours. Contact lenses synthesised with formulations F5 and F6 are compared to ACUVUE ® control lenses (n=3).

Release of olopatidine from formulation F5 was unaffected by the presence of vitamin E compared to control non vitamin E loaded lenses. The smaller amount of diluent present in formulation F5 (1 %) creates a polymer which swells to a smaller extent and this decrease in swelling may inhibit the ability of materials to diffuse through it. ACUVUE® control lenses loaded very little drug when they were previously loaded with vitamin E. Formulation F5 drug release appears to be unaffected by the presence of vitamin E (Figure 3.8).

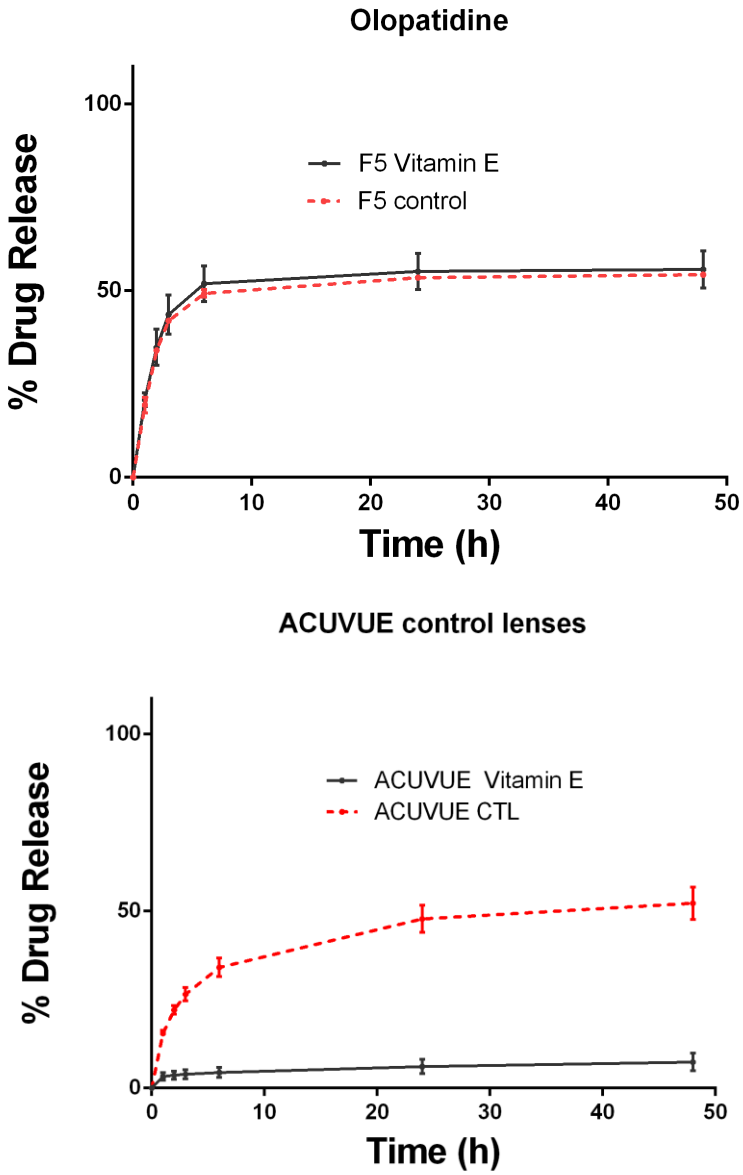


Figure 3.8: Percentage cumulative drug release of olopatidine over 48 hours from contact lenses produced from formulation F5, with a comparison to ACUVUE® control lenses (n=3).

Drug release was not impacted by the presence of vitamin E in the polymer. Vitamin E was successfully loaded into contact lenses as demonstrated by the assay performed on vitamin E loaded lenses in Table 3.4 p.82. The ACUVUE commercial lenses used as a comparison did not release any drug when previously loaded with vitamin E. The formulation of these lenses is different and the polymeric composition of the ACUVUE lens is the most likely cause of the reduced drug release compared to F5. It is possible that the vitamin E loaded into these lenses impaired the ability of the lenses to load olopatidine.

Lenses manufactured were too hard to be properly representative of actual contact lenses, but this formulation can be altered to be more flexible. Formulation F5 released 950 µg of drug from the control lens in 36 hours and 870 µg from vitamin E loaded lenses. This compares to 600 µg (control lens) and 410 µg (vitamin E loaded lens) from formulation F6. F5 lenses were assayed for vitamin E content and, as shown in Table 3.4 p.82, contained 1.27 mg of vitamin E per lens.

The most likely explanation for this reduction in the amount of drug loaded, and subsequently released from the contact lens was the higher concentration of EGDMA present in this formulation (4% EGDMA cross-linking monomer concentration). Increasing chemical cross-links leads to the formation of a more structured polymer. Diffusion of drug through a more structured polymer network will be more difficult. Figure 3.9 illustrates the decrease in drug release from these two polymers. F6 lenses were assayed for vitamin E content and, as shown in Table 3.4 p.82, contained 2.18 mg of vitamin E per lens.

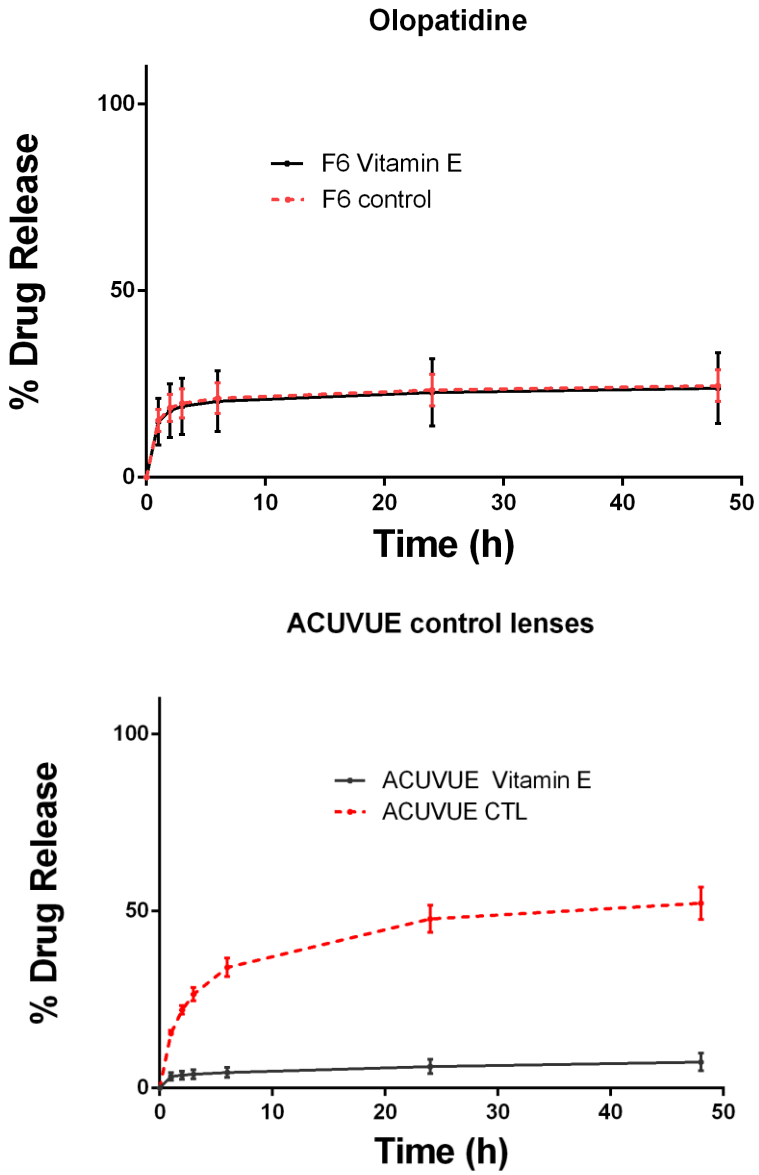


Figure 3.9: Percentage cumulative drug release of olopatidine drug over 48 hours from contact lenses made with formulation F6, with a comparison to ACUVUE® control lenses (n=3).

3.6. Olopatidine release from contact lenses loaded with therapeutic concentrations

Drug loading performed in the previous experiments was not reflective of the dose required per lens. The drug loading solutions were altered so that a relevant daily dose of antihistamine was loaded. Two concentrations were used and lenses were loaded with a therapeutic dose of 40 µg per lens and a double therapeutic dose of 80 µg per lens. A double therapeutic dose was used to determine if the amount of drug

loaded into the lens would impact release rate. Formulations F4 and F5 were used for this analysis as theses lens formulations were the most similar to the commercial lens characteristics.

Formulations F4 and F5 released a smaller percentage of the loaded drug compared to control commercial lenses. Approximately 65% of the drug loaded was released from F4 and F5. This compares to almost 90% drug release from the ACUVUE® control lenses. Almost exactly the same pattern of drug release was obtained from loading the lenses with double the concentration of olopatidine. Two deductions can be made from this data. Firstly, the reservoir for drug in the lenses is adequate for a daily dose of antihistamine. The drug loading per lens was calculated in section 3.3.2, to be 25-33 µg of drug. The lenses loaded 76 µg of olopatidine 230% of the loading required. Secondly the elution profile of the drug has been slowed down due to the chemical cross-linking and the other polymer components in formulations F4 and F5 when compared to drug released from ACUVUE® lenses. ACUVUE® lenses release almost 65% of the drug after one hour where it takes three time hours to observe that % of drug elution from formulations F4 and F5 (Figure 3.10).

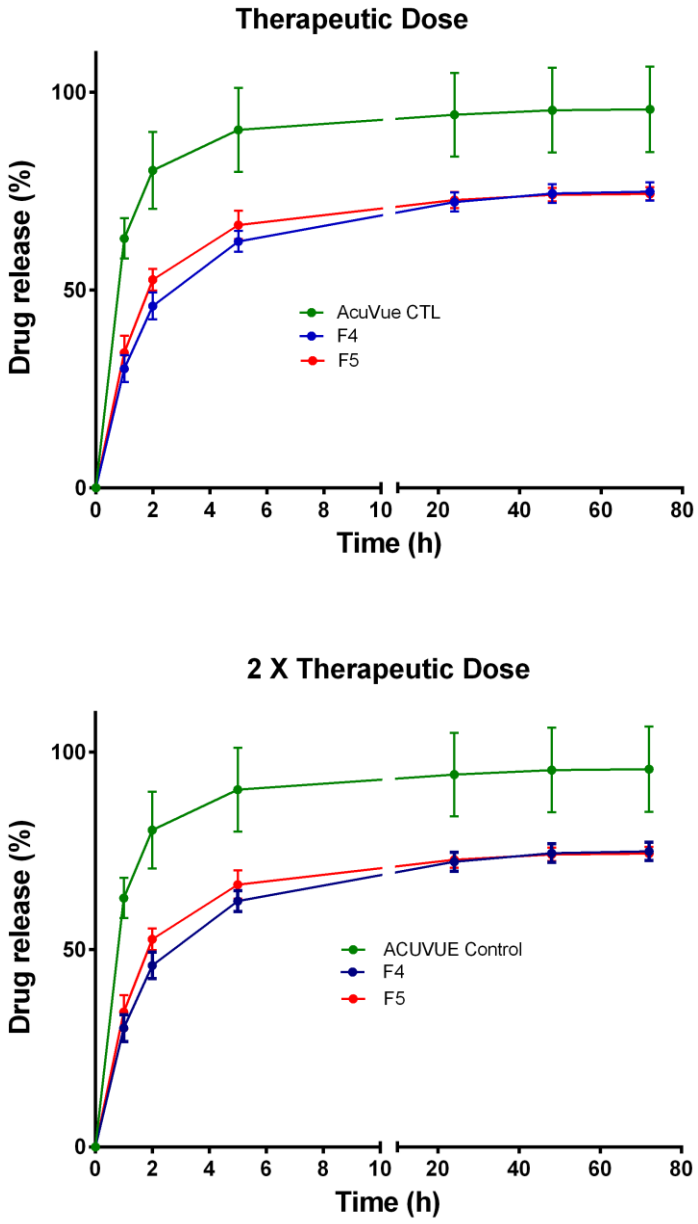


Figure 3.10: Percentage cumulative drug release from olopatidine over 72 hours using two drug loading concentrations (n=3).

This release data indicated that the chemical cross-linking was a factor in controlling drug release; however, this needs to be balanced by the ability of a patient to be able to tolerate wearing the lens. Formulation F2 and F3 have a slower drug release, but the lenses are much harder than commercial lenses. There was a large difference in diluent concentration between formulations F4 (15%), F5 (1%). This change did not impact drug release and elution profiles for both formulations were similar for this level of drug loading of olopatidine. The physical characteristics of the lenses were

unaffected by drug loading and release and refractive index remained unchanged at 1.33 and % light transmission was not changed pre or post drug release. Results ranged from 96 to 97.5%. Doubling the drug loading of the lenses did not affect the drug release and the release profile for both drug loading amounts is similar. 50 -80% of the drug loaded was released in two hours, suggesting the drug was burst released and so unaffected by increased drug loading. If the drug was first order diffusion controlled drug loading would have had an impact on drug release rate.

3.6.1. Pharmaco-kinetic analysis

The drug release profiles were fitted to model dependent, drug release mathematical simulations, to determine the drug release kinetics of olopatidine from the drug loaded polymers analysed. The models used were zero order, first order and Higuchi models, as these represented the closest approximation of the drug delivery from a polymer matrix [161]. The Korsmeyer Peppas drug model is discussed, but could not be used as drug release was too rapid with 60% drug release occurring in the first 3 hours. It is discussed here only to highlight the rapid drug release encountered from the polymeric drug delivery vehicles thus far. It is anticipated that this model will be used in future studies.

3.6.1.1. Zero order

Zero order drug release is defined by Equation 3.1. Release is modelled on pharmaceutical products i.e. tablets which do not dissolve or disintegrate and release drug relatively slowly. This drug model has been used to define drug release from a number of dosage forms including, matrix tablets and osmotic systems. Water soluble drug release is typically controlled by diffusion whereas low-water soluble drug release is controlled by the erosion of the polymer [162]. Zero order drug release is based on the principle that the rate of reaction is not impacted by the concentration of drug [163].

$$\mathbf{Q_0 - Q_t = K_0\, t} \qquad \textbf{Equation 3.1}$$

Where Q_0 = initial drug concentration at time zero, Q_t = amount of drug present at time t

K_0 = Zero order release constant in (sec^{-1}), and t = time in seconds.

3.6.1.2. First order

First order absorption and or elimination of drugs are expressed in equation 3.2. This drug delivery model has been used to describe the release of water soluble drugs from porous matrices [161]. The reaction rate is based only on the concentration of one reactant. Reaction rate for all other reactants in the system will be zero order [163].

$$\textbf{Log C = log } C_0 \textbf{ - } \frac{Kt}{2.303} \qquad \textbf{Equation 3.2}$$

Where C = drug concentration at time t, C_0 = initial concentration, K = First order rate constant (sec^{-1}), and t = time in seconds

3.6.1.3. Higuchi

The Higuchi mathematical model has been used to model release from planar geometric and porous drug delivery systems [164]. The mathematical model is based on six hypotheses

(1) The initial drug concentration in the matrix is much higher than drug solubility.
(2) Drug diffusion takes place in one direction edge effects must be negligible.
(3) Drug particles are much smaller than system thickness.
(4) Matrix swelling and dissolution are negligible.
(5) Drug diffusivity is constant.
(6) Perfect sink conditions are always attained.

For drug delivery from contact lenses, hypothesis (1) that the initial drug concentration in the matrix is much higher than drug solubility is not clearly defined. It is possible that in the case of drug loaded contact lenses the drug may be loaded solely in the water which is present in the swollen hydrogel and therefore not be present in the polymer. The Higuchi model was used as it appears to fulfil some of the mathematical model criteria (See Equation 3.3) and as a comparison for information only. This model is also used for transdermal systems [161].

$$f_t = Q = A\sqrt{D\,(2C - Cs)Cs\,t} \qquad \textbf{Equation 3.3}$$

Where Q = amount of drug released in time t per unit area A; C = Initial concentration; Cs = Drug solubility in the matrix and D = diffusion coefficient, a measure of how the drug will diffuse through the polymer matrix in this case.

3.6.1.4. Korsmeyer Peppas

The Korsmeyer Peppas drug release model describes the drug release from polymeric systems. The equation

$$\frac{M_t}{M_\infty} = K_{kp}t^h \qquad \textbf{Equation 3.4}$$

The main advantage of this drug release model is that it outputs n values. The n obtained characterises the type of drug release mechanism. Values of $0.45 \leq n$ correlate to a Fickian diffusion mechanism, with $0.45 < n < 0.89$ illustrating non Fickian diffusion mechanisms. Other release mechanisms can also be described. This model was not used on the drug release profiles obtained in this work as in all cases 60% of the drug had been released by the first or second hour and more than 4 time points are required to provide an accurate assessment of drug delivery kinetics [161].

The data from the drug delivery modelling is presented in Table 3.6. The low r^2 values obtained illustrate that there was poor correlation of the drug released with any of the three drug delivery models. The conclusion reached was that the drug delivery is burst release in nature and this appears to correlate with the drug delivery profiles (see Figure 3.10) where approximately 45-65% of the drug is released in 3 hours. The loading of drug using soaking does not create controlled delivery of drug from the contact lenses and another means of controlling drug delivery is required.

Table 3.6: Release rate constants and drug loading amount calculated from contact lens drug delivery data.

Lens Formulation	Model	K value (sec^{-1})	r^2	Loading (µg)
ACUVUE®	Zero order	0.93	0.21	40-50
F5	Zero order	1.27	0.37	40-50
F4	Zero order	1.13	0.31	40-50
ACUVUE®	Zero order	0.89	0.21	70-90
F5	Zero order	0.94	0.36	70-90
F4	Zero order	0.86	0.30	70-90
ACUVUE®	First order	$4.8X10^{-3}$	0.26	40-50
F5	First order	0.01	0.35	40-50
F4	First order	0.02	0.24	40-50
ACUVUE®	First order	$4.6X10^{-3}$	0.29	70-90
F5	First order	0.01	0.34	70-90
F4	First order	0.03	0.29	70-90

Kinetic evaluation was performed using first and zero order drug delivery models as these were the only ones which complied with the drug release obtained from the lenses or complied with the drug delivery model. Figures 3.11 and 3.12 are examples of the first and zero order drug release graphs used to calculate the K values detailed in Table 3.6.

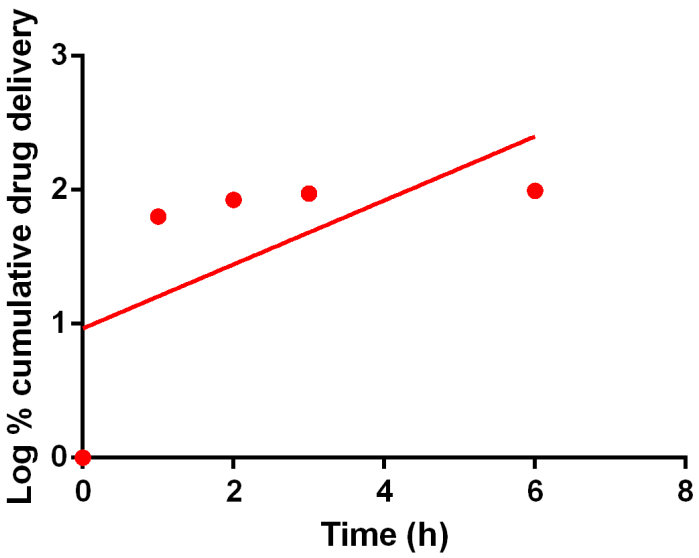


Figure 3.11: First order drug release graph from ACUVUE® drug loaded lens (n=3) (40 µg).

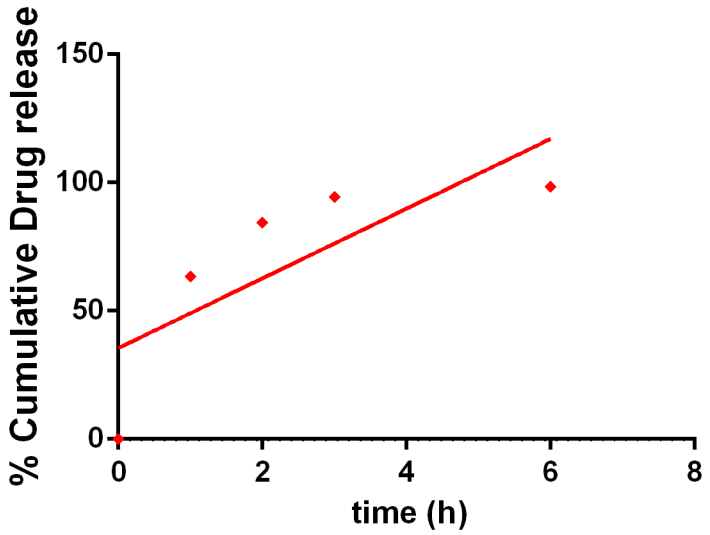


Figure 3.12: Zero order drug release graph of One DAY AACUVUE® drug loaded lenses (n=3) (40 µg).

K values are calculated from the slope of the line generated by the release graph. First order release kinetics derive the K value from the slope of the line multiplied by -2.303. This value is then divided by 3600 so the units of K are in Sec^{-1}. For the zero order release graph, the slope of the line generated is the K value this is also divided by 3600 so K is in Sec^{-1}.

3.7. Statistical analysis of contact lens drug delivery results

Statistical analysis was performed on the drug elution data from all of the studies carried out in this Chapter using Graph Pad Prism 6 software. Two types of statistical analysis were performed. When comparing drug and vitamin E loaded polymers to drug loaded only polymers (control polymers), a Student's t test with Walsh's correction was used with a confidence level of 95%. This analysis determined there was no statistical difference between vitamin E loaded polymers and control polymers and between the different polymer formulations analysed with the exception of formulation F4 where there was a significant difference between the % cumulative olopatidine released from the vitamin E loaded polymer versus the control polymer. The P value obtained was 0.02 this compared with p values of 0.104 and 0.06 for formulations F2 and F3 respectively. The P values obtained for cetirizine loaded polymers was 0.21 0.37 and 0.073 for formulations F2, F3 and F4.

The statistical analysis illustrated that there was an interaction between vitamin E and olopatidine release for formulation F4.

When comparing drug loaded (soaking) formulations F4 and F5 to drug loaded commercial lenses (control data), a one way Anova analysis, was performed using Dunnett's multiple comparison test. The total amount of drug released from each formulation was compared and a statistical difference was observed at a 95% confidence interval between the formulations F4 and F5 and the drug loaded commercial lenses. This data illustrates that there is a difference in the drug release between the fabricated and drug loaded lenses and the commercial lenses. The drug release was burst release from all contact lenses so although there was evidence of some attenuation of drug release from the lenses manufactured it was not sufficient and another method drug loading the lenses was required.

3.8. Conclusions

Three factors emerged from the results observed in this work. The first being that vitamin E has a greater impact on the release of olopatidine than cetirizine from polymer matrices, as can be seen from the data presented in Section 3.4. Faster release of olopatidine from the polymer lenses was observed with vitamin E. This was most likely due to olopatidine being less soluble in the polymer formulations used. 3000 µg of cetirizine was loaded into formulation F1 compared to 1860 µg of olopatidine in the same polymer. This relationship may hold promise that, the olopatidine elution from the polymers can be mediated by the presence of other materials in the polymer matrix. Vitamin E is not very soluble in the HEMA polymer used in this study. Vitamin E is not solubilised in the polymer matrix. It is merely trapped in the matrix and retained only because it is not soluble in the PBS release media. Other options for use as a diffusion barrier should be chosen or the monomers used replaced with monomers with increased solubility of vitamin E.

Secondly the glycerol content impacted on the reproducibility of drug released from the polymer formulation as seen by the reduction in standard deviation of formulations with the same components but a larger concentration of glycerol. In

order to achieve consistent loading and release of drug from a polymer matrix, the polymer matrix formed must be uniform. Diluent level has been identified as a critical formulation parameter.

Finally, chemical cross-links were a factor in controlling drug release as can be seen from the decreased loading and release in Formulation 4. Doubling the amount of EGDMA cross-linking monomer in the formulation not only decreased drug loading, it also decreased the amount of material released from the polymer. The more structured polymer matrix formed will swell less and be more rigid. This causes the reduction in loading and release (see Section 2.4.5). However, chemical cross-linking can only be increased to a point where it is comfortable for the patient so its use is restricted even though it impacted release. It did not impart control over drug release and drug was still released quickly over the first three time points for all formulations tested.

A statistical analysis of the release data was performed. No statistical difference of the drug release data was observed between formulations with vitamin E and controls, for both antihistamines used.

Drug delivery modelling has shown there was no discernible control over the release of drug from the lenses. Although there was a difference between the drug release from commercial lenses and in-house formulations there was no evidence of controlled drug release. Vitamin E did not reduce drug release rate from the polymer formulations tested. This study has demonstrated that loading diffusion barriers, i.e. vitamin E at a concentration of approximately 2 mg per lens, can impact drug release rate. However, not in the way envisaged closer matching the diffusion barrier to the polymer matrix may provide the desired impact on drug release rates.

Drug release studies have been developed and tested. Replicate analysis has shown that consistent results can be obtained and a baseline for therapeutic drug release has been established.

The next Chapter will focus on the use of drug loaded polymer particles to control the release of drug. Drug loaded polymer particles will be formed by polymerising monomer in the presence of cetirizine drug and solvent. The drug loaded polymer particles will have entrapped cetirizine within the forming polymer matrix. Drug loaded particles can then be cast into contact lenses to deliver drug to the eye. The novel drug delivery vehicles can then be used to treat ocular conditions such as dry eye hay fever.

Chapter 4

Attenuation of Drug Release from Contact Lenses using Drug loaded polymer Particles

4. ATTENUATION OF DRUG RELEASE FROM CONTACT LENSES USING DRUG LOADED POLYMER PARTICLES

Molecularly imprinted polymers (MIPs), have demonstrated controlled release of drug and comfort agents from drug loaded polymer particles. Studies by Tieppo *et al* [155] have produced controlled release of non-steroidal anti-inflammatory drugs ketotifen and diclofenac. The tear stabilising / comfort agent hydroxy-propyl methylcellulose has also been molecularly imprinted to control release [11]. These imprinted polymer particles have attenuated the release of drugs and have achieved zero order release in some cases. Smart polymers which exhibit triggered release depending on stimuli (e.g. temperature, pH and polarity) have been used to develop targeted drug release from polymer matrices. The common link in almost all of these controlled drug delivery techniques was the preparation of drug loaded polymer micro-particles (MPs). Drug loaded polymer MPs have been investigated as possible drug delivery vehicles by a number of research groups [36, 69, 95, 130, 165, 166].

Cetirizine is a popular over the counter medication used for treating ocular conditions such as hay fever and allergic reaction. From a review of the literature, it is seen that drug release from contact lenses has concentrated primarily drugs for the treatment of glaucoma, such as timolol maleate or dexamethasone used post ocular surgery. Delivering an antihistamine drug in a controlled manner via contact lenses is novel and has a number of key advantages. It has the ability to treat both hay fever and other allergic conditions affecting the eyes. Antihistamines, when released from contact lenses, have the potential to increase the comfort of all contact lens wearers and prevent the symptoms of allergic reactions. This option provides a large financial benefit as there are in excess of 300 million contact lens wearers worldwide [167].

Using antihistamines brings with it some major challenges, not least controlling the release of such a hydrophilic drug (e.g. cetirizine). This thesis will focus on daily disposable contact lenses, which are comprised of hydrophilic polymers and swell to 60-80 % by weight in water. Also, a small amount of drug must be released over an 8 to 12 hour period ideally. The concentration of drug required for a therapeutic effect has been calculated by Jung *et al.* [149] using instillation or eye drop drug delivery

as a guide. Here the daily dose of timolol was calculated to be 2.5 µg per day. This is a fraction of the 125 µg delivered by the eye drops. The same approach was used to estimate the amount of antihistamine (cetirizine) required for a therapeutic dose. This was calculated to be 10 µg per day. Assuming a 50% loss of drug from the lenses and the possibility that not all the drug would be released from the lens, a drug loading value of approximately 25-33 µg per lens was used in the work reported in this Chapter.

The focus of this Chapter is to engineer novel drug loaded polymer particles which attenuate the release of a relatively hydrophilic drug, cetirizine (pKa acid 3.6) (pKa base 7.79). The drug loaded polymer particles prepared were added to a contact lens pre-polymerisation mixture of initiator, diluent and monomers and the particles and monomer mixture were cured in moulds to form drug loaded contact lenses. These novel ocular inserts displayed the ability to deliver 5.84 µg of drug into the eye over a period of 24 hours. Control over drug release was obtained by altering the types of monomer and cross-linker (hydrophobic or hydrophilic) used in the drug loaded polymer particles. In this research novel polymer particles, which attenuate drug release due to chemical cross-linking, swelling characteristics and chemical properties of the cross-linking monomers used, were developed. As the interactions within these polymer particles are not specific, these drug delivery vehicles may provide a platform for the delivery of a variety of ocular pharmaceuticals, creating a new class of drug delivery device.

4.1. Materials used in this study

Table 4.1: Materials used in this work.

Reagent	Supplier	Assay
2-Hydroxy-ethyl-methacrylate (HEMA)	Sigma Aldrich	98%
N-vinyl-pyrrolidone NVP	Sigma Aldrich	99%
2-(Di-ethyl-amino)-ethyl-methacrylate (DEAMA)	Sigma Aldrich	99%
Ethylene-glycol-dimethacrylate (EGDMA)	Sigma Aldrich	99%
TPO 2,2-Dimethoxy-1,2-diphenylethan-1-one	Bausch & Lomb Gift	99%
N,N methylene Bis-acrylamide (NNMBA)	Sigma Aldrich	99%

N,N methylene Bis-acrylamide was used as received, all other monomers were purified by vacuum distillation. AIBN initiator was recrystallized from acetone (source Acros) and TPO was used as received.

4.2. Experimental methods

4.2.1. HPLC drug release analysis

An Agilent 1200 series HPLC with UV detection at 254 nm and Waters C18 (4.6 X 250 mm 5µm) column was used for antihistamine analysis. The mobile phase used was 65:35 Acetonitrile: Water (pH adjusted to 2.7 with *ortho*-phosphoric acid). A flow rate of 0.9 $mL.min^{-1}$, and an injection volume of 40 µL were used to analyse samples. The method used is an adaptation of the method developed by Walily *et al* [168]. The LOD was calculated as per ICH guidelines for cetirizine. Cetirizine LOD was calculated as 0.44 $\mu g.mL^{-1}$. The LOQ for cetirizine was determined to be 1.32 $\mu g.mL^{-1}$. The square of the correlation coefficient values for the calibration curve of cetirizine was 0.99998.

4.2.2. Particle synthesis of drug laden polymer particles

Drug loaded polymer particles were prepared in a 50 mL round bottom flask. Cetirizine (2 mmol), HEMA (0.5 mmol), NVP (5 mmol) and DEAEMA (0.5 mmol), cross-linker EGDMA (20 mmol), and photoinitiator 2,2-dimethoxy-1,2-diphenylethan-1-one (TPO 651) (2 wt. % of total monomers) were dissolved in a mixture of 30 mL acetonitrile and 10 mL water. The solution was purged with nitrogen gas for 10 min and was subsequently sealed and irradiated by UV light (365 nm) at 4 °C for 1 hour with stirring. The resulting suspension was filtered and washed with 10 mL of water and 3 x 10 mL aliquots of acetonitrile, filtered and dried in a vacuum oven at 60 °C overnight. This method was an adaptation of a method by Wang *et al* [134].

4.2.3. Contact lens characterisation methods

4.2.4. Scanning electron microscope analysis of drug laden polymer particles

Samples were first gold coated using an Emitech sputter coater unit and then imaged using a Hitachi S-2460 N Scanning electron microscope set at 20-25 Kv and a magnification of 6000.

4.2.5. Particle size analysis of drug laden polymer particles

Particle size distribution was measured using a laser light scattering particle size analyser (Mastersizer 2000 Malvern instruments UK). Powder samples were pre-mixed with a spatula to break up agglomerates and then analysed using the Malvern Hydro 2000S water dispersion attachment. Sample was added until an obscuration of 10% was achieved (Approximately 50mg of particles were dispersed in 100 ml of DI water) with a pre-measurement period of 900 seconds was used. Sonication was performed to further disaggregate particles prior to measurement. The sample compartment was cleaned using sonication and DI water flushes between sample measurements.

4.2.6. Drug release from drug laden polymer particles

Drug release analysis for particles was performed by weighing 20 mg of particles into a micro-centrifuge tube. 1mL of release media either PBS artificial lachrymal

fluid [169] (6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, 0.084 g/L$CaCl_2$ _$2H_2O$, pH 8), or 50:50 H_2O: Ethanol was added to each micro-centrifuge tube and they were placed in a 37 °C orbital incubator at 70 RPM. After an hour the samples are removed from the incubator placed in a centrifuge and rotated at 1500 RPM for 10 minutes. The supernatant liquid was filtered and analysed by HPLC. 1 mL of release media was then added to the micro-centrifuge tube and the process was repeated. Drug release from contact lenses was performed in the same manner except there was no need to centrifuge and filter the release media.

4.2.7. Differential scanning calorimetry analysis of drug laden polymer particles

An indium calibrated Q2000 DSC was used perform dynamic scanning with the nitrogen feed set to 50 $mL.min^{-1}$ with samples using a heating rate of 10 °C per minute up to 300 °C. All samples were prepared and run in triplicate. Sample weights analysed were within the 3-5 mg range.

4.2.8. Swelling ratio analysis of drug laden polymer particles

The swelling ratio of polymer particles was measured using an adaptation of the method used by Malaekeh-Nikouei *et al* [170]. 20 mg of drug loaded polymer particles were weighed into 1 mL centrifuge tubes and 1 mL of PBS was added. The centrifuge tubes were placed in a 37 °C orbital incubator at 70 RPM for 48 hours. The supernatant liquid was removed by pipette and the centrifuge tubes were reweighed. The weight of the wet polymer particles was then divided by the dry weight to yield the swelling ratio as in equation 4.1.

$$\textbf{Swelling ratio} = \frac{\boldsymbol{Wet\ weight\ of\ polymer}}{\boldsymbol{dry\ weight\ of\ polymer}} \qquad \textbf{Equation 4.1}$$

4.3. Results and Discussion

The following section summarises the results obtained from the development of polymer drug micro-particles. This section breaks the experimental analysis into a number of stages outlining how the particles were formulated, optimised and characterised. Results of drug release trials from contact lenses loaded with drug loaded polymer micro-particles is also outlined.

4.3.1. Initial drug loaded polymer particle preparation

The method chosen to produce polymer drug particles was a UV initiated polymerisation, specifically a variation of the method developed by Wang *et al* [134]. The chemical structure of the monomers and drug template molecule to be entrapped by the monomers are shown in Figure 4.1.

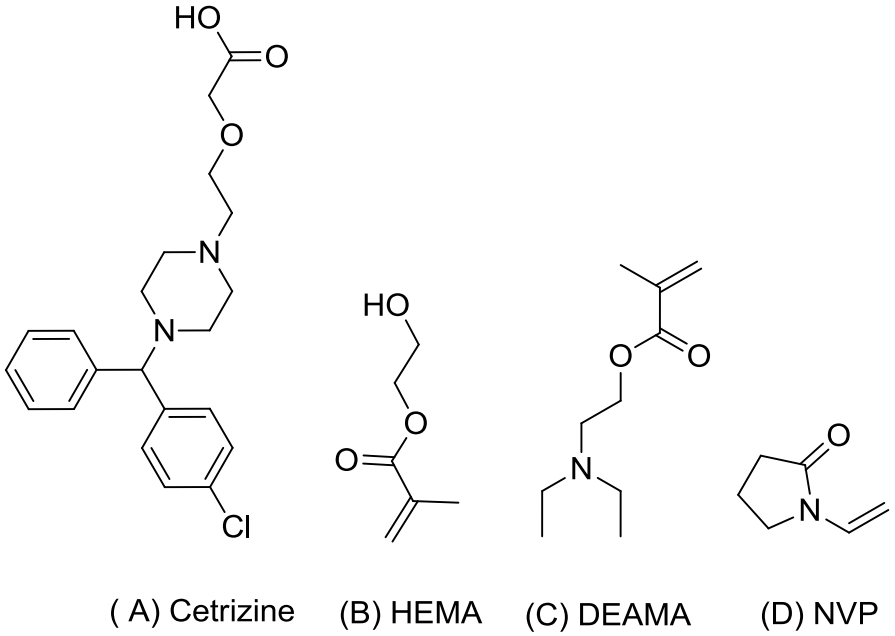


Figure 4.1: Chemical structures of Drug (A) and monomers (B-D) used in the preparation process.

Some of the possible hydrogen bonding and molecular interactions between cetirizine, HEMA and DEAMA are illustrated in Figures 4.2.

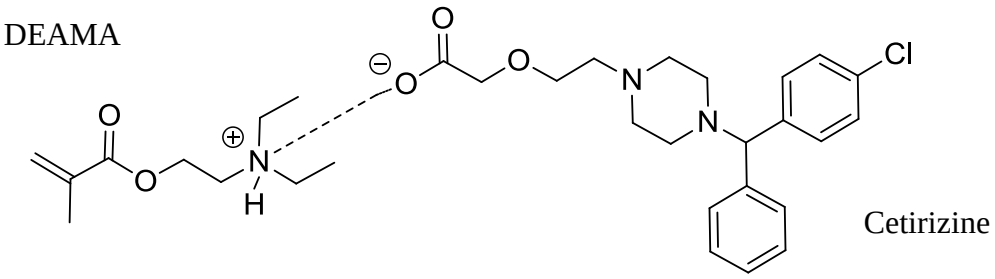


Figure 4.2: Possible molecular interactions between cetirizine and DEAMA.

An electrostatic interaction between the DEAMA or NVP molecules and the cetirizine molecule was the most likely interaction between the monomers and drug

during the polymerisation [134]. These interactions could lead to a level of molecular orientation and assembly during the polymerisation process.

4.3.2. Drug release from initial drug loaded polymer particles

The particles were manufactured (as per section 4.2.2) dried and then analysed for drug loading using a 50:50 mixture of water and Ethanol. Initial trials using acid and base to degrade the particles to assay the drug loading of the polymer particles, proved unsuccessful. An assay was developed which used water and ethanol as a release medium. Ethanol can act as a plasticizer or swelling agent [171]. When polymer particles are soaked in ethanol solutions the polymers swell to a greater extent facilitating drug release of drug from the drug loaded polymer particles. As Cetirizine is readily soluble in water, a combination of both solvents was used. This method proved to be more effective for measuring drug loading of the particles than using deionised water as an extraction medium. The drug loading of these particles was both erratic and very low (see Table 4.2).

Table 4.2: Drug loading assay of initial 20 mg of polymer drug loaded microparticles (n=3).

	Replicate 1	Replicate 2	Replicate 3
Total amount of drug released mg	0.94	0.02	0.02
Drug loading of polymer mg/g	47.10	0.99	0.85

Results indicated that the hydrophobic nature of the EGDMA cross-linking monomer has a negative impact with regard to loading or release of the hydrophilic drug (Cetirizine). The high amount of EGDMA relative to HEMA, DEAMA co-monomers, creates a highly cross-linked hydrophobic polymer matrix, the hydrophilic cetirizine may not become entrapped in the forming polymer particles as cetirizine and EGDMA interactions may be limited. These relatively highly cross-linked polymer drug particles formed will swell to a very small extent [172]. As cetirizine has a molecular weight of 388.88 this molecule may be able not pass through the hydrogel formed. It is also possible that cetirizine was not uniformly encapsulated within the polymer particles during polymer particle formation due to

the hydrophilic nature of the drug. In that case any cetirizine present on the surface of the particles would be washed away during separation and washing. Figure 4.3 presents the drug release as a graph.

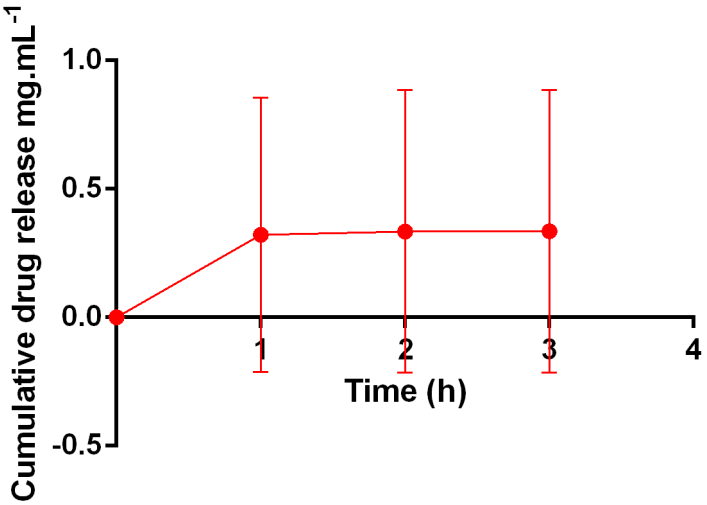
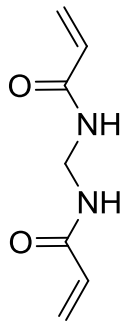


Figure 4.3: Cumulative drug release from EGDMA:HEMA:DEAMA drug loaded polymer particles.

Drug release was not consistent illustrated by large error bars in Figure 4.3. To drug load a contact lens a high drug loading in the polymer ensures that only a small amount of drug loaded polymer would be required. To simplify future drug loading of contact lenses methods of increasing the amount of drug released from the polymer particles was investigated. Two approaches were considered to overcome the low level of drug released from the particles. Firstly N, N methylene bis-acrylamide (NNMBA) cross-linking monomer was used to replace EGDMA in the monomer mixture due to its more hydrophilic properties. Secondly the ratio of EGDMA used was reduced, to determine the impact of increased chemical cross-linking on the amount of drug released. This would still yield a relatively highly cross-linked polymer but it would be more likely to swell in aqueous media and thus release the drug. The inclusion of a hydrophilic monomer would increase the affinity of the ensuing polymer for hydrophilic materials which could also impact drug release rates. The particles created should swell to a much greater degree in aqueous media when prepared using NNMBA. The structure of NNMBA is presented in Figure 4.4.



NNMBA

Figure 4.4: Structure of NNMBA crosslinking monomer,

George *et al.* observed an increase in polymer particle swelling when EGDMA was replaced with NNMBA [173]. This increased swelling allowed for increased drug diffusion from polymer drug particles fabricated by Sairam *et al* [130]. Reducing the amount of EGDMA present would reduce the chemical cross-linking of the particles as well as change the amount the polymer particles swelled in aqueous media.

4.4. Drug loading optimisation of polymer particles

To modify the drug loading properties, (NNMBA) was added to the pre-polymerisation monomer mixture replacing EGDMA entirely. NNMBA was chosen to address the poor drug release from drug loaded polymers which used EGDMA as the cross-linking monomer, as well as decrease the variation observed in replicate analysis of EGDMA drug loaded polymer particles (ref sect 4.3.2). Using NNMBA to cross-linker would alter the physical properties of the polymer particles produced and as it is less hydrophobic than EGDMA and the polymer particles produced would swell more in aqueous environments Sairam *et al*.[130] and Babu *et al* [174] both observed increased swelling from polymer particles containing NMMBA. Table 4.3 summarises the polymer composition utilised.

Table 4.3: Monomers used to synthesis cetirizine drug particles, using UV light.

Material	Function	Concentration mmol
Cetirizine	Drug	2
HEMA	Monomer	0.5
DEAMA	Monomer	0.5
NNMBA	Cross-linking Monomer	20

***Note Initiator TPO 2 wt%.**

The particles were synthesised in the same manner as section 4.2.2 and drug release experiments were performed to determine the effect of cross-link monomer properties on amount of drug released from the particles. Drug release from the particles was measured over time with results summarised in Figure 4.5.

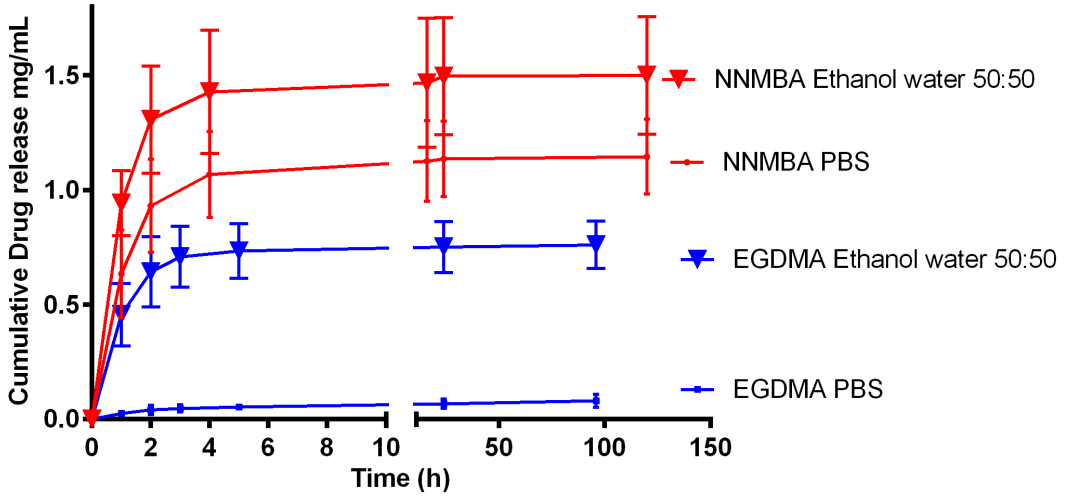


Figure 4.5: Comparison of drug release from NMBA drug loaded polymer particles in red vs EGDMA drug loaded polymer particles in blue (n=3).

Release data in Figure 4.5 is shown as mg/mL to illustrate the change in amount of drug release due to the change in formulation. If % drug release was used it would not be possible to observe the increase in amount of drug released from NNMBA polymer particles compared to EGDMA particles. There was an appreciable increase in the amount of drug released from drug loaded polymer particles which used NNMBA as the cross-linking monomer. This occurs for release into PBS as well as

into ethanol water 50:50 extraction media. NNMBA produced particles in which a larger amount of cetirizine was released. This was possibly due to the increased hydrophilic nature of the cross-linking monomer. This allowed the particles to swell more in an aqueous media allowing them to release more of the drug also it is possible that less cross-linking occurred with NNMBA cross-linker than EGDMA. The drug loaded polymer particle released 81.43 to 29.13 $mg.g^{-1}$ of cetirizine ($mg.g^{-1}$ represents mg of cetirizine released per g of polymer particles). This represented an increase in amount of drug released as well as being more reproducible than EGDMA cross-linked particles. (As calculated in section 3.2 the therapeutic dose of antihistamine was 25 to 33 µg per lens). This analysis proved that using NNMBA had a large impact on the amount of drug released from the particles. Significantly more cetirizine was released, from the NNMBA particles into PBS, than was released from EGDMA drug loaded polymers into PBS (Figure 4.5).

4.4.1. Drug loaded polymer particles prepared using a mixture of cross-link monomers

To further investigate the impact of cross-link monomer on drug release, a number of monomer compositions were prepared where EGDMA (hydrophobic) and NNMBA (hydrophilic) cross-link monomers were mixed in varying amounts as detailed in Table 4.4.

Table 4.4: Monomer composition of pre-polymerisation mixtures for drug loaded polymer particle synthesis.

Polymer concentration	NNMBA (mmol)	EGDMA (mmol)	HEMA (mmol)	DEAMA (mmol)
A	20	-	0.25	0.25
B*	10	5*	5.25	0.25
C	10	7.5	2.75	0.25
D	10	6.5	3.75	0.25

Note: DEAMA TPO and cetirizine concentrations were unchanged * illustrates that particles were not produced.

The HEMA concentration was increased to ensure the total monomer concentration was constant at 4% (w/v) for all experiments. Drug release analysis was performed on samples in both PBS and water:ethanol (50:50) extraction media. The total

amount of drug released over 24 hours was recorded in $mg.mL^{-1}$ from the 20mg of particles used in the release study.

The effect of reducing EGDMA concentration on the amount of drug released from the polymer drug particles was determined (see Table 4.5). Three concentrations of EGDMA were used to manufacture drug loaded polymer particles to determine if reducing EGDMA concentration would favourably impact the amount of drug released from the drug loaded polymer particles.

Table 4.5: Impact of EGDMA concentration on cetirizine release from polymer drug particles (n=3).

Cetirizine drug release $mg.g^{-1}$	**EGDMA (7.5 mmol)**	**EGDMA (6.5 mmol)**	**EGDMA (0 mmol)**
PBS Cumulative drug release ($mg.g^{-1}$)	0.000	0.009	1.14
Standard deviation ($mg.g^{-1}$)	0.000	0.0002	0.16
Water: Ethanol ($mg.g^{-1}$)	0.0301	0.134	1.499
Standard deviation ($mg.g^{-1}$)	0.004	0.02	0.26

Note: Drug loading is in mg of cetirizine per g of drug loaded polymer cetirizine particles.

No drug release was observed into PBS from polymer drug particles prepared with a 7.5 mmol concentration of EGDMA. The amount released increased when water ethanol extraction was used. Drug loaded polymer particles containing a 6.5 mmol concentrations of EGDMA released 0.009 $mg.g^{-1}$ of cetirizine per g of drug loaded polymer particle into PBS (drug release was measured in $mg.g^{-1}$ of cetirizine per gramme of drug loaded polymer to illustrate the respective drug loading of each formulation). This compared to 1.14 mg released when NNMBA was used as the cross-linking monomer (listed as 0 mmol EGDMA in Table 4.5). Reducing the concentration of EGDMA does increase the amount of drug released from the drug loaded polymer particles, but a far larger amount of the drug was loaded when NNMBA was used as the cross-link monomer.

Due to the very low quantities of drug released from EGDMA particles into aqueous media, future studies used NNMBA as the main cross-linking monomer due to its ability to release drug into an aqueous environment Figure 4.5 p.115. The drug release from the 6.5 mmol concentration EGDMA particles was miniscule. This may be due to the chemical cross-linking of the polymer drug particles or the reduced contact with the aqueous medium of these particles due to the hydrophobic nature of EGDMA.

4.4.2. Formulating of polymer drug micro-particles using NNMBA as a cross-linking monomer

The impact of the monomers HEMA, DEAMA and NVP monomers on the drug release from particles was also assessed. Various monomer types and concentrations were formulated as part of the drug loaded polymer particle synthesis and the drug release from the resultant particles was measured. These monomers were chosen for two reasons; firstly all were routinely used in the manufacture of contact lenses so they were safe for use in the ocular environment, secondly as they had the ability to either hydrogen bond or ionically interact with cetirizine as shown in Figures 4.2 p. 111. The basic concentrations and ratios used in the original particle synthesis were unchanged. The monomers used and their concentrations are defined in Table 4.6.

Table 4.6: Monomer composition of polymer drug particle pre-polymerisation mixtures.

Formulation	NNMBA (mmol)	HEMA (mmol)	DEAMA (mmol)	NVP (mmol)
A	20	0.25	-	0.25
B	20	0.5	-	-
C	20	-	0.5	-
D	20	-	-	0.5
E	20	-	0.25	0.25

Drug release was measured in both PBS and water: ethanol (50:50). The overall monomer, drug and initiator concentrations were kept constant. The amount of drug released was presented as mg of cetirizine per gramme of drug particles. Table 4.7 outlines the amount of drug released into both extraction medium used.

Table 4.7: Cetirizine release from polymer drug particles (n=3).

Formulation	A NNMBA H EMA NVP	B NNMBA HEMA	C NNMBA DEAMA	D NNMBA NVP	E NNMBA DEAMA NVP
PBS media ($mg.g^{-1}$)	81.43	53.64	43.74	57.20	29.13
Standard deviation ($mg.g^{-1}$)	5.63	1.46	7.80	6.28	2.29
50:50 water: ethanol ($mg.g^{-1}$)	77.75	54.13	41.86	80.29	27.71
Standard deviation ($mg.g^{-1}$)	0.23	5.50	8.8	13.06	3.24

Note: Drug loading is in mg of cetirizine per g of drug loaded polymer cetirizine particles.

The largest amount of drug was released from HEMA NVP particles 81.4 $mg.g^{-1}$ of cetirizine per gramme of polymer drug particles. The amount of drug released for all of the other samples ranged from 57 to 30 $mg.g^{-1}$. Variation in drug release was increased for release into ethanol water for HEMA and NVP polymer particles where the standard deviation almost doubled for release into this medium (D and E). These loading figures equate to 81.43 to 29.13 μg of cetirizine per mg of polymer. Loading 0.5 to1 mg into a contact lens of 20 mg leads to a therapeutic drug loading. There was an impact on amount of drug released depending on the monomers used to synthesise the polymer drug particles. Using a mixture of HEMA and NVP the amount of drug released was increased and variation as measured by the standard deviation of replicate samples was 5.63 $mg.g^{-1}$. Drug release from the particles demonstrated that the changes in monomers have created polymer particles which were capable of delivering appreciable amounts of cetirizine reproducibly. The % cumulative drug release from each of the drug loaded polymer particle formulations was also plotted against time and the drug release profile was consistent, i.e. the drug release profiles overlapped for almost all the polymer formulations tested. The drug release from these drug loaded polymer particles was expressed % cumulative drug delivery so it would be easier to compare changes in % drug release with time and

assess changes in the rate of drug delivery, drug release profiles are displayed in Figure 4.6.

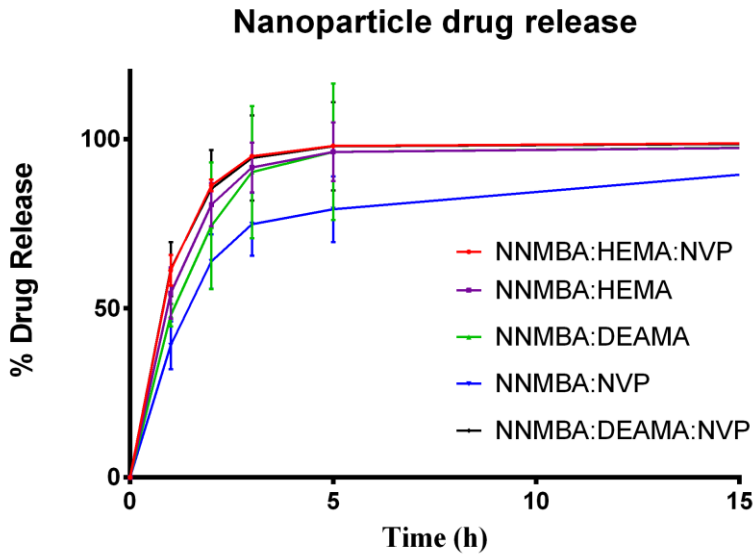


Figure 4.6: % Cumulative drug release profiles of polymer drug particles NNMBA:HEMA:NVP (red line), NNMBA:HEMA (purple line), NNMBA:DEAMA (green line), NNMBA:NVP (blue line) and NNMBA:DEAMA:NVP (black line).

The drug release from the NVP monomer NNMBA polymer drug particles may be slower. After one hour 69 % cumulative drug release was recorded from the DEAMA particles compared to 47 % cumulative drug release from the NVP. After 2 hours 98 % cumulative drug release was recorded from DEAMA particles compared to 72 % from the NVP particles. This demonstrates that the monomers used to polymerise and entrap the drug may have an impact on drug release rate even though they are present at low concentrations in this case 0.5 mmol, compared to 20 mmol of NNMBA cross-linking monomer. Reducing the amount of EGDMA present would reduce the chemical cross-linking of the polymer produced. As less cross-linking will occur due to the lower amount of EGDMA present in the formulation. There was, however, very little drug released from these particles. This analysis proved that using NNMBA as cross-linking monomer had a large impact on the amount of drug released from the particles most likely due to the polymer particle swelling more in an aqueous environment. The concentration of cross-linker for both EGDMA and NNMBA particles was constant at 20 mmol and the monomers

themselves are equally unsaturated. This change in drug release, strongly suggests that the hydrophilic properties of the NNMBA particles produced were the dominant factor in the increase of drug released. Sairam, M., *et al.* also observed attenuated drug release characteristics from EGDMA drug loaded polymer particles compared to particles synthesised using NNMBA [130]. Decreased swelling of the polymer particles containing EGDMA was attributed as attenuating the drug release.

When NNNMBA cross-linking monomer was used to prepare drug loaded polymer particles, higher concentrations of cetirizine were released. However, the drug release profile was not altered significantly, illustrating that using a more hydrophilic cross-linking monomer allowed more drug to be released in total, but it did not appear to control or attenuate the rate at which drug was released, as seen by the similar drug release profiles in Figure 4.6. Analysis performed in this Chapter further demonstrated that physical characteristics of the polymer particle, i.e. hydrophilicity and swelling impacted the amount of cetirizine released. The swelling ratio of the EGDMA and NNMBA particles synthesised were measured. The EGDMA particles had a swelling ratio of 11.1 with a standard deviation of 0.98 versus a swelling ratio of 13.0 and standard deviation of 1.36 for the NNMBA particles. This analysis supports the findings in the literature by Sairam *et al.*[130] regarding increased swelling characteristic of NNMBA versus EGDMA in PBS. This analysis was performed after the particles were suspended in PBS for 48 hours. One measurement was performed as the drug particles have to be centrifuged to separate them from PBS. Multiple measurements would suffer from polymer loss as well as possible compaction of the polymer. Therefore the polymer particles were swollen for 48 hours to ensure they reached equilibrium before they were analysed. The drug release from the polymers prepared were then compared to both First and Zero order drug delivery kinetics and the results are detailed in Table 4.8.

Table 4.8: Release rate constants and linearity results from Zero order and First order drug delivery model analysis of polymer particle formulations A to E drug release.

Formulation	Model	r^2	K value (sec^{-1})
A NNMBA:HEMA:NVP	Zero order	0.72	4.8 $X10^3$
B NNMBA HEMA	Zero order	0.74	4.8 $X10^3$
C NNMBA:DEAMA	Zero order	0.72	4.7 $X10^3$
D NNMBA:NVP	Zero order	0.81	4.5 $X10^3$
E NNMBA:DEAMA:NVP	Zero order	0.75	4.8 $X10^3$
A NNMBA:HEMA:NVP	First order	0.50	0.31
B NNMBA HEMA	First order	0.50	0.31
C NNMBA:DEAMA	First order	0.50	0.31
D NNMBA:NVP	First order	0.53	0.31
E NNMBA:DEAMA:NVP	First order	0.50	0.31

Release rate constants (K) for both zero order and first order drug delivery models are consistent across all the formulations analysed. The observed drug release was compared to first order drug delivery kinetics and a linear regression of the line produced from a plot of the log of % cumulative drug delivery versus time was obtained. For zero order drug delivery % cumulative drug delivery was plotted against time. The maximum r^2 observed for both drug delivery models was 0.81, illustrating that the drug release from these polymer particles is neither zero order nor first order. 50 to 60 % of the drug was released from all formulations analysed. Drug release was rapid and typical of unattenuated drug release. Changing the monomer composition of the drug loaded polymer particles will not achieve the controlled drug release required.

4.5. Preliminary findings from drug loaded polymer particle analysis

The amount of drug released from 20 mmol EGDMA polymer drug particles prepared directly using the method described by Wang was not reproducible [86]. An adaptation of the pre-polymerisation mixture was required. Initial drug particle synthesis successfully generated particles, but the drug loading of the particles was not sufficient or reproducible varying from 47 mg of drug per g of polymer to 0.85 mg of drug per g of polymer particles. To increase drug loading of polymer particles, the level and type of cross-linker present was varied. Finally the co-monomers used

in the formation of the particles were varied. The analysis demonstrated that reproducible drug release was possible from polymer drug particles when NNMBA was used as the cross-linking monomer. EGDMA particles were swollen to a lesser extent than the NNMBA particles. This change in the hydrophilicity of the particles facilitated drug release in a reproducible manner. Changing the monomer used, also impacted the amount of drug which was released from the drug loaded polymer particles. From this study a formulation of HEMA and NVP, using NNMBA produced consistent drug release. The ratio of the monomers was the same as the original particles produced by Wang *et al.* (0.25 mmol, 0.25 mmol and 20 mmol) [134]. Drug release was also sufficiently high. These particles were loaded into a contact lens with a dry weight of 18-22 mg per lens. Drug loading needs to be high, so the amount of particles loaded into the lens is minimised, to ensure drug loading of the lenses is a feasible process. With a drug release of 81.4 mg per g of polymer each lens would require approximately 30 - 40 µg of drug per lens using the Jung *et al.* calculation method for therapeutic dose [149]. The HEMA:NVP particles released 81.4 mg per g, or 81.4 µg per mg, therefore approximately 0.5 mg of particles was required per lens in a 1 in 40 ratio of particles to lens material. It was feasible to load that amount of particles into the lens as previously 1 mg of vitamin E was successfully loaded per lens (Chapter 2).

4.6. Drug release optimisation from drug loaded polymer microparticles

Achieving consistent drug release allowed experiments to be performed to determine if the drug release could be delayed / controlled. Drug loaded polymer particles manufactured, released cetirizine but the drug release profile was not controlled. As a burst release profile was obtained this demonstrated that the drug-laden polymer particles produced did not attenuate drug elution. The main objective of all further experiments was to attenuate drug release from the particles. 16 batches of polymer particles were synthesised as previously described in section 4.2.2 and drug release and particle size analysis was then performed.

4.6.1. The effect of total monomer concentration on drug release

The total monomeric formulation was varied to obtain a range of total monomer concentrations. This was achieved by altering the amount of the ACN:water reaction solvent used to alter the total monomer concentration without affecting the ratio monomer to cross-linker. These drug laden polymer particles were analysed to determine any impact on the rate of drug release from the particles synthesised. Table 4.9 illustrates the impact on both drug release and particle size of changing the total concentration of monomer (w/v).

Table 4.9: Monomer concentration impact on particles size and drug release from polymer drug particles (n=3).

Total monomer concentration (%w/v)	Drug release (mg)	Standard deviation (mg)	Drug release after 1 hour (%)	Standard deviation (%)	Drug released after 2 hours (%)	Standard deviation (%)	Particle size µm d(0.1)	Particle size µm d(0.5)	Particle size µm d(0.9)
1%	0.50	0.02	43.10	0.99	71.18	3.52	0.603	2.105	14.306
2%	0.60	0.04	52.07	2.51	75.34	4.65	0.768	2.366	5.616
4%	1.66	0.11	56.33	1.90	81.11	4.90	0.834	2.508	8.026
6%	0.79	0.03	46.16	1.98	71.30	3.35	1.558	5.346	14.604
8%	1.37	0.24	29.97	5.54	63.36	10.27	1.718	6.954	30.359
10%	1.39	0.11	40.05	4.97	68.14	3.58	1.453	5.387	17.622

Note: Monomer ratio 20:1, solvent ratio 3:1 all remain parameters were not varied. Particle size analysis performed by Malvern Mastersizer.

The amount of drug released from the three replicate samples varied as seen by the standard deviation of 0.02 to 0.24 mg across all the samples tested after 1 hour. As the concentration of monomer increased there was an increase in amount the total amount of drug released from the polymer particles from 0.5 to 1.39 mg of drug from 1 to 10 % monomer concentration. Total drug release in expressed in mg was used to measure the amount of drug released from formulations as their composition was varied as % drug release values normalise any change in the total amount of drug released from drug loaded polymer particles. As the % monomer present increases it becomes more likely for the forming polymer particles to entrap drug within them. This increase appeared to stop at 8 % monomer concentration as there was very little change in the amount of drug released, i.e. 1.37 to 1.39 mg of drug, suggesting that release had reached its maximum.

The 4 % total monomer concentration formulation in Table 4.9 released 1.66 mg of cetirizine, which was the highest amount of drug released in this study. The drug release profile is also expressed using % cumulative drug release per hour. This normalises the total amount of drug released for each formulation and allows the % drug release per hour to be compared across each formulation. The lowest % release after 1 hour was obtained for the 8 % (w/v) monomer concentration. Approximately 30 % of the drug was released compared to a range of 40 % to 56 % for the other monomer concentrations. After 2, hours the drug release was still lower for the 8 % and 10 % (w/v) monomer concentrations with 63 % and 68 % release compared to 71 % to 81 % for the lower monomer concentrations. An increase in drug encapsulation was observed by Mehta *et al.* during polymer encapsulation of proteins when the concentration of monomer increased [175, 176].

After the 4 % total monomer concentration there was a marked change in the particle size distribution at the 10% distribution volume, where particle size increased from (0.6 to 0.8 µm) to (1.43 – 1.71 µm).

There was some influence on particle size from the total monomer concentration but only when a large change in total monomer concentration occurred. Bao *et al.* observed an increase in particle size of nanoparticles as monomer concentration

increased, particle size increased from 103 to 215 nm [177]. Bao *et al.* postulated that solubility induced changes arising from the nucleation of the polymer particles was responsible for the changes in particle size.

The particle size of 90 % of the distribution varied from 5.6 to 30.4 µm. Lower concentrations of monomer in the reaction media will create a disperse mixture of monomer, drug and initiator leading to smaller particle size for low % monomer concentration polymerisation reactions. Sajjad *et al.* observed that control over particle size of nano-latexes could be achieved by controlling the concentration of hydrophilic monomers [178].

4.6.2. Impact of chemical cross-linking on drug release

The ratio of cross-linker to monomers used to manufacture the nanoparticles was varied to ascertain the effect on both particle morphology and drug release. The impact of varying the relative concentration of cross-linker to monomer was investigated by varying the cross-linker to monomer ratio from 5:1 to 30:1. The amount of drug released and % drug released over 1 and 2 hours as well as particle size data is listed in Table 4.10.

Table 4.10: Impact of cross-linker monomer ratio on particle size and drug release from polymer drug particles (n=3).

Ratio of cross-linker to monomer	Drug release (mg)	Standard deviation (mg)	Drug release after 1 hour (%)	Standard deviation (%)	Drug released after 2 hours (%)	Standard deviation (%)	Particle size µm d (0.1)	Particle size µm d(0.5)	Particle size µm d(0.9)
5:1	0.60	0.09	50.99	5.25	74.04.	10.86	1.482	5.027	12.215
10:1	0.81	0.04	47.00	1.55	73.64	5.36	1.234	4.332	11.546
15:1	0.86	0.13	41.70	11.87	66.76	15.37	1.283	4.182	10.275
20:1	1.66	0.11	56.33	1.90	81.11	4.90	0.834	2.508	8.026
25:1	0.714	0.05	50.04	3.22	75.32	5.30	1.037	3.373	9.129
30:1	0.802	0.05	45.23	5.8	68.81	5.05	1.245	3.753	9.804

Note % total monomer concentration 4%, 3:1 solvent ratio were parameters were not varied. Particle size analysis performed by Malvern Mastersizer.

The amount of drug released over 1 hour varied from 0.6 to 0.86 mg over the cross-linker to monomer ratios with the exception of the 20:1 ratio. The 20:1 cross-linker to monomer ratio particles released double the amount of drug compared to the other 5 cross-linker ratios analysed (1.66 mg). There was no trend observed in % drug release and it was relatively constant varying from 41.7 to 56.3 % after 1 hour and 67 to 74 % after 2 hours. Indicating there was no trend in drug release rate drug as the ratio of cross-linker to monomer was increased from 5:1 to 30:1. This data suggests that the ratio of monomer to cross-linker had little impact on the drug release profile of the particles produced. Hiratani *et al.* observed a decrease in swelling of hydrogels as EGDMA concentration of molecularly imprinted polymers increased, however the drug loading of the polymers did not appear to be effected [16].

The lowest particle size distribution was observed for the 20:1 cross-linker to monomer ratio. The particle size as illustrated by the d (0.9) µm. The lowest particle size was obtained at a cross-linking monomer ratio of 20:1. Babu *et al.* observed that the particle size of nifedipine microspheres decreased as chemical cross-linking of the microspheres increased [179]. In the case of these particles no such trend was observed.

4.6.3. Polymerisation solvent composition effect on drug release

The ratio of the components of the solvent mixture i.e. acetonitrile and water were altered. The impact on drug release from the drug laden particles produced was analysed and the results are detailed in Table 4.11.

Table 4.11: Comparison of acetonitrile water solvent ratios and drug release from polymer drug particles (n=3).

Solvent ratio Acetonitrile:H_2O	Drug release (mg)	Standard deviation (mg)	Drug release after 1 hour (%)	Standard deviation (%)	Drug released after 2 hours (%)	Standard deviation (%)	Particle size µm d (0.1)	Particle size µm d(0.5)	Particle size µm d(0.9)
***1:1**									
***2:1**									
3:1	1.66	0.11	56.33	1.90	81.11	4.90	0.834	2.508	8.026
4:1	1.19	0.01	47.95	2.5	73.63	3.12	1.032	3.304	9.517
6:1	1.04	0.01	48.72	2.92	74.04	0.38	0.908	2.842	8.146
10:1	1.66	0.30	41.45	7.07	69.13	11.33	1.155	5.024	21.674

Note: Cross-linker to monomer ratio 20:1, 4% total monomer concentration parameters were not varied.* No particles were formed from synthesis. Particle size analysis performed by Malvern Mastersizer.

The initial two solvent ratios (Acetonitrile:water) tested 1:1 and 2:1 did not produce particles and a space filling gel was obtained. It was not possible to filter the gel so it was not practical for further analysis. The highest amount of drug released was from the 3:1 acetonitrile: water solvent ratio. Solvent ratios of 4:1 and 6:1 both released approximately 1 mg of cetirizine when the acetonitrile concentration was increased to a 10:1 ratio the amount of drug released from the particles rises to 1.66 mg. Figure 4.7 presents the % cumulative drug release from the polymers prepared.

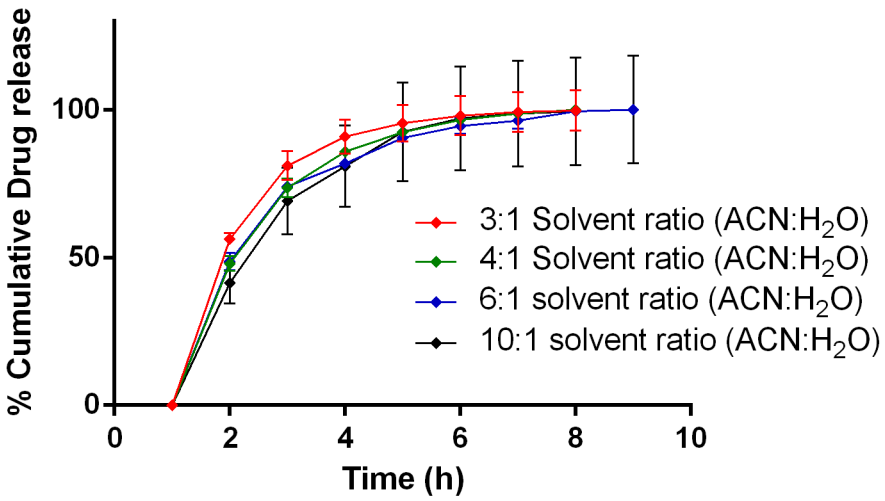


Figure 4.7: % Cumulative drug release performed at 37 °C from drug loaded polymer particles prepared with varying acetonitrile to water solvent ratios.

There was no significant change observed in the drug release profile when the acetonitrile:water solvent ratio was altered. There was, however, a difference in the total amount of drug released. For example, both the 3:1 and 10:1 solvent ratios released 1.66 mg of cetirizine in 24 hours, compared to 1.19 mg and 1.04 mg for the 4:1 and 6:1 ratios, respectively. The standard deviation for the replicate analysis of all the samples was low. The 3 solvent ratios analysed released 41-48% of the drug within 1 hour and 69-74% after 2 hours. Changing the solvent ratio did not impact the rate of drug release and the release profile of all the drug loaded polymer particle formulations was unchanged. The difference in total amount drug released from the particles may have been caused by the solvent composition during polymer particle synthesis.

The particle size distribution was not significantly impacted by the solvent composition of the reaction media until a 10:1 solvent composition was used (particle size data is shown in Table 4.11). When a 10:1 acetonitrile:water solvent ratio was used there was an increase in particle size of the particles formed, the particles size increased from 3 to 5 µm and 9 to 21 µm at the 50 and 90 % distributions for the 10:1 acetonitrile:water solvent ratio formulation as illustrated in Figure 4.8.

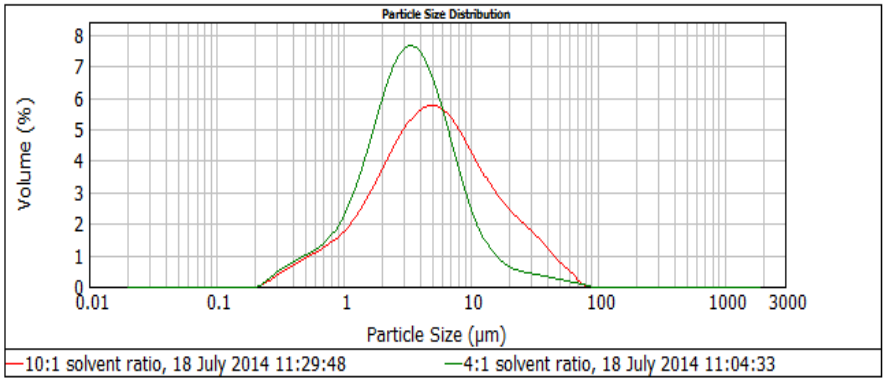


Figure 4.8: Malvern particle size particle distribution data for drug loaded polymer particles prepared in 10:1 acetonitrile:water (RED line) and 4: acetonitrile:water (green line)

The 3:1 acetonitrile:water solvent composition produced the particles with the lowest particle size in this study. The most likely cause of the change in particle size is the higher water content causing an increase in the particle size due to swelling. Changes in polymer solvent interactions can have an impact on the degree of swelling [180]. The composition of the polymerisation solvent may influence the physical characteristics of the drug/polymer particles formed Table 4.11. The polarity of the solvent will decrease as more acetonitrile is to the solvent mixture this may impact the solubility of the forming polymer drug particles [181].

4.6.4. The impact of hydrophobic and hydrophilic cross-linking monomers on release.

Hydrophobic monomers such as EGDMA have the ability to decrease the drug release rate from polymer particles they produce. This impact on drug release has been observed by a number of researchers [16, 93, 130, 182, 183]. These drug-laden polymer particle materials, however, did not release enough cetirizine to be feasible

for drug release (0.009 mg of cetirizine released from 20 mg of polymer particles (ref Table 4.5 p.117). The possibility mixing of two cross-linking monomers to provide polymer drug particles with a reduced drug release rate and sufficient drug loaded polymer particles was examined.

NNMBA is a hydrophilic molecule so adding hydrophobic monomer in the form of cross-linking monomers such as EGDMA, should impact the ability of the nanoparticles formed ability to swell and restrict or decrease the rate of drug elution from polymer particles. To ascertain the impact of EGDMA on drug release, batches of drug loaded polymer particles were prepared where the EGDMA to NNMBA ratios were varied. The levels of each cross-linker were varied in relation to each other so that two sets of particles were formed. A High ratio of EGDMA to NNMBA was used (9.9:0.1, 9.5:0.5, 9:1 and 8:2). Particles prepared using only EGDMA cross-linker are highly hydrophobic, and have been previously shown to be less than ideal chemical environments for releasing hydrophilic drugs as demonstrated by the erratic drug release profile (ref Table 4.2 p.112) obtained previously. Table 4.12 details the results from the drug release of these particles into PBS.

Table 4.12: Quantity of drug released and comparison of drug release profile to drug delivery models for high EGDMA content drug loaded polymer particles (n=3).

EGDMA:NNMBA (mmol)	**9.9:0.1**	**9.5:0.5**	**9:1**	**8:2**
Drug released $mg.g^{-1}$	0.20	0.39	0.41	0.18
Standard deviation $mg.g^{-1}$	0.0085	0.12	0.02	0.03
First order linearity	0.9871	0.9209	0.9440	0.9590
Zero order linearity	0.9911	0.9817	0.9993	0.9913

Note drug release is expressed as mg of cetirizine per g of polymer particles to allow comparison of polymer drug loading across formulations.

A mixture of the two cross-link monomers (EGDMA, NNMBA) produced drug loaded polymer particles which were observed to release a higher amount of drug, than drug loaded polymer particles which were cross-linked with EGDMA alone. Drug release from these particles had increased and was much less varied than results generated for EGDMA only particles. Drug release recorded from EGDMA cross-

linked particles was 0.2-0.4 $mg.g^{-1}$. Drug loaded polymer particles manufactured with a high concentration of NNMBA released 30 - 81 $mg.g^{-1}$ of cetirizine. Figures 4.9 and 4.10 display examples of the zero order and first order drug delivery graphs.

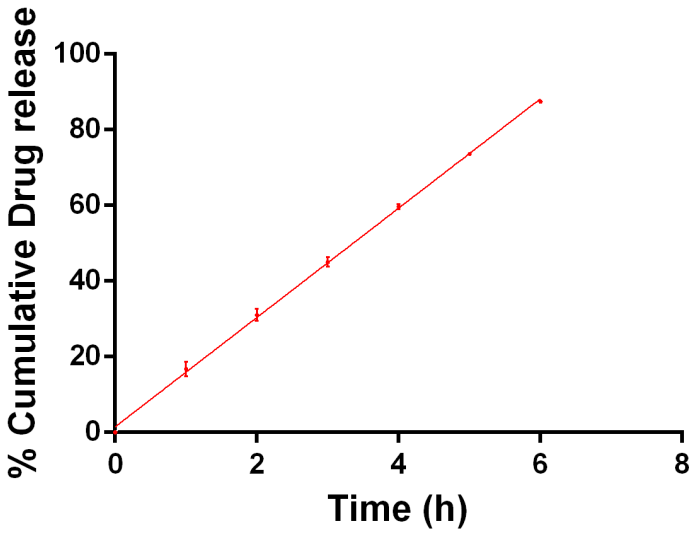


Figure 4.9: Zero order drug delivery of 9:1 EGDMA:NNMBA drug loaded polymer particles performed at 37 °C.

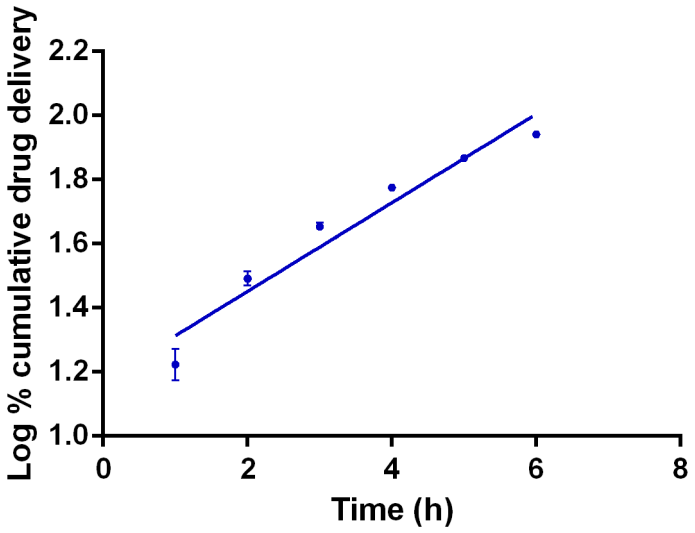


Figure 4.10: First order drug delivery graph of 9:1 EGDMA:NNMBA drug loaded polymer particles performed at 37 °C.

A linear regression of the line is used to compare how well the drug release fits to the drug delivery model. There was a change in drug release rate, illustrated by the

higher correlation for zero order drug release. Correlation to zero order drug release was consistent for all High EGDMA concentration particles ranging from 0.981 to 0.999. The % cumulative drug release over time was also decreased results presented in Table 4.13.

Table 4.13: % cumulative drug release versus time from 9:1 EGDMA: NNMBA polymer drug particles (n=3).

Time (h)	% Drug released
0	0.00
1	16.79
2	31.07
3	45.08
4	59.67
5	73.64
6	87.49
24	100.00

Particle size analysis was performed (Table 4.14) to determine if increasing the EGDMA ratio affected the particle size of the polymer particles produced. 4 hours was required to release 59.7% of the drug from EGDMA,NNMBA polymer particles. This was in contrast to the drug release from NNMBA only polymer drug particles, where drug release was at 50-60% after 1 hour. The release from these 9:1 EGDMA: NNMBA particles was not burst release. The drug release was attenuated or slowed due to the physical properties i.e. reduced swelling that the high proportion of EGDMA had on polymerised drug/polymer particles. The % drug release over time from the 9:1 EGDMA: NNMBA particles results are presented in Table 4.14.

Table 4.14: Particle size analysis of polymer drug particles prepared using increasing EGDMA concentration (n=3).

EGDMA:NNMBA ratio	Particle size d(0.1) (µm)	Particle size d(0.5) (µm)	Particle size d(0.9) (µm)
8: 2	1.38	6.53	26.70
9: 1	1.42	5.60	16.34
9.9: 1	1.62	6.92	18.09
9.5: 0.5	1.46	6.29	17.12

When the ratio of EGDMA to NNMBA was varied, there was no significant change particle size of the drug loaded polymer particles produced and no trend could be discerned. The chemical cross-linking was maintained as the ratio of cross-linking monomer to HEMA or HEMA/NVP was constant at 20:1, therefore only the hydrophobic property of the particles was changing as the ratio of EGDMA monomer was increased.

The physical properties of the hydrophobic EGDMA in the drug loaded polymer particles attenuated drug release. The hydrophobic polymer which surrounds the drug will swell to a lesser extent in aqueous media, which decreases the interaction between the drug and extraction medium PBS. As the drug release was rate was slower, four hours was required to release 59.6% of the drug, this allowed Korsmeyer Peppas analysis of drug release. This model can only be performed on the initial 60% of drug release and a minimum of four release points are required [162]. This was the first time that a drug release profile met these conditions. Previously this method could not be used as more than 60% of the drug was released in under four hours from previous drug loaded polymer particle formulations. A value for N was obtained; The N value characterizes the type of release mechanism of the drug. The analysis generated a value of N. N = 0.92 which equated to non-Fickian drug release or non-diffusion controlled drug release [184]. This was a very successful outcome, indicating that particles with a controlled drug release profile had been created. The Korsmeyer Peppas power law equation listed in Equation 4.1.

$$\frac{M_t}{M_\infty} = K\,t^n \qquad \textbf{Equation 4.1}$$

Where Mt and M∞ are the concentration at time t and infinity respectively. K is the equation constant. t= time and N a value which characterises the mechanism drug release which is also the slope of the line generated in Figure 4.11. The data obtained from this analysis is listed in Table 4.15.

Table 4.15: Korsmeyer Peppas kinetic data derived from Figure 4.11.

N	K	r^2	Transport mechanism
0.92	-1.784	0.9997	Non Fickian

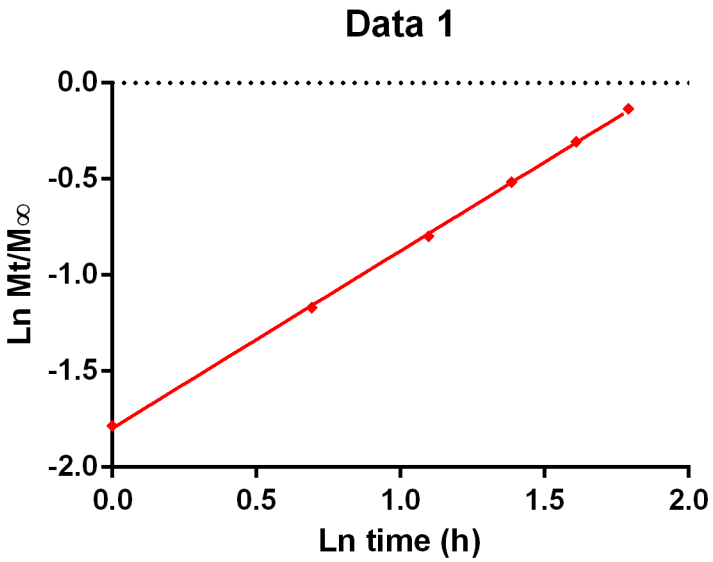


Figure 4.11: Drug release from EGDMA: NNMBA polymer drug particles modelled using the Korsmeyer Peppas drug delivery model (n=3).

The control over drug release was dependent on the type of cross-linker used not the concentration of cross-linking monomer in the particles produced. As the total cross-linking monomer (EGDMA and NNMBA) concentration of all the particles produced was kept constant at 20 mmol. The polymer formulation of the particle was 18 mmol NNMBA: EGDMA 2 mmol, 0.5 mmol HEMA, and 0.5 mmol of NVP. The drug release was controlled by the physical properties and hydrophobic character of the polymer of the drug loaded polymer particles. The hydrophobic nature of the EGDMA monomer used has been successful in attenuating drug release from a

number of polymer drug delivery systems. The reduced swelling of the EGDMA particles is the most probable cause of the attenuated drug release [16, 93, 144, 149].

There was an issue as these particles released a relatively small amount of drug, 0.2-0.4 mg of drug per g of polymer drug particles. As each lens has a dry weight of approximately 20 mg, to obtain a therapeutic dose of approximately 40 μg per lens this would require 200 mg of drug/polymer particles which was not possible. Drug loading of the particles needed to be increased to make using these particles feasible for incorporation into contact lenses.

4.6.4.1. Impact of mixing NNMBA and EGDMA monomers on the rate and amount of drug released from drug loaded polymer particles

A range of EGDMA concentrations was added to predominantly NNMBA pre-polymerisation polymer particle synthesis formulations. The addition of this hydrophobic cross-linking monomer could restrict swelling of the polymer particles formed, possibly restricting drug diffusion. However, due to its relatively low concentration in the final polymer formed, any effect on the polymer produced should be limited. EGDMA only formulations released relatively small concentrations of cetirizine when analysed. A mixture of the two cross-linking monomers may provide a combination of attenuated drug release with a higher total amount of drug released over time. An example of the first order drug delivery plot for a 1.5 mmol concentration drug loaded polymer particles is illustrated in Figure 4.12.

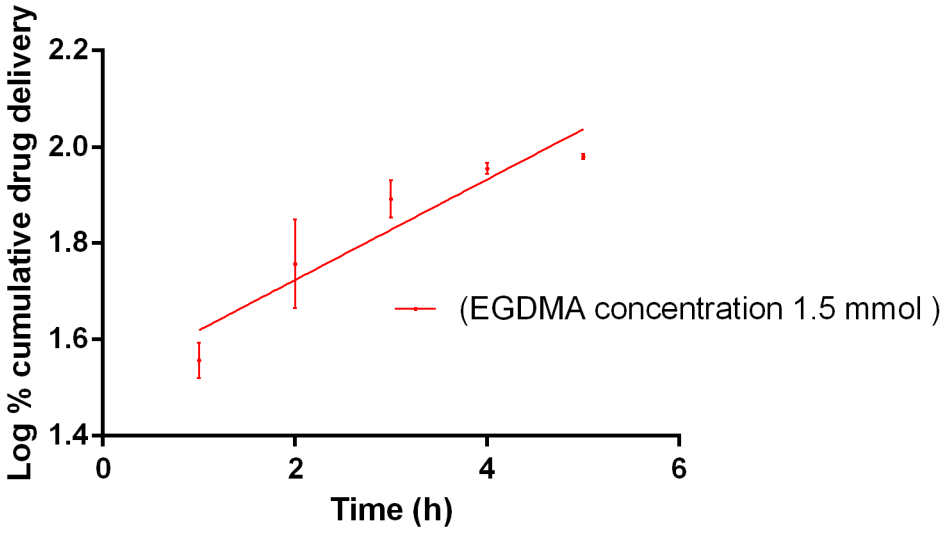


Figure 4.12: Example of First order drug release from EGDMA drug loaded polymer particles listed in Table 4.16

The polymer particles formed are hydrophilic in nature due to the large % of NNMBA. The aim of this experiment was to ascertain whether it was possible to obtain attenuated drug release. The observed drug release and its correlation to first and zero order drug delivery models are presented in Table 4.16.

Table 4.16: Quantity of drug released and comparison of drug release profile to drug delivery models for low EGDMA content drug loaded polymer particles (n=3).

EGDMA Conc (mmol)	**1.5**	**1.8**	**4**	**6**	**8**
Drug loaded of polymer particles mg.g^{-1}	176.95	185.34	190.39	168.04	157.93
Standard deviation	45.3	2.75	29.64	8.30	9.25
First order linearity	0.8151	0.792	0.859	0.841	0.801
Zero order linearity	0.915	0.874	0.945	0.9387	0.909

The high NNMBA content polymer drug particles released a large amount of cetirizine (168-190 $mg.g^{-1}$). Drug release was very high due to the fact that these formulations formed space filling gels not particles. Space filling gels cannot be filtered due to their physical properties. Therefore, all the residual cetirizine was retained in the gel and not washed off as would occur when drug loaded polymer particles were washed during filtering. The gels obtained were dried in a vacuum oven, dried gel were then used in the release experiments that followed. Drug release was not controlled as illustrated by the very poor correlations to first order and zero order drug delivery models, correlation varied from 0.8 to 0.9.

To determine the impact on drug release due to the gels not being washed a portion of the 18.5 mmol NNMBA to 1.5 mmol EGDMA particles were washed using a 1 x 10 mL of water followed by 3 x 10 mL portions of acetonitrile. After the washing step the particle and washing liquor were centrifuged at 1500 RPM and the supernatant solvent removed. Centrifugation removed the supernatant liquid without the use of additional solvents which would have occurred if other techniques such as soxhlet extraction was used to wash the gels. As no additional solvents were added to the gels drug loss would be reduced. The particles were then vacuum dried. Table 4.17 compares the % drug release from the 18.5 mmol NNMBA: 1.5 mmol EGDMA (total cross-link monomer concentration 20 mmol) drug loaded polymer particle produced and then vacuum dried. The drug release from these particles was compared to 20mmol NNMBA particles for reference.

Table 4.17: Comparison of % drug release of centrifuged space filling gel and vacuum dried gels 18.5:1.5 EGDMA:NNMBA polymer drug particles gels and drug loaded polymer particles prepared with no EGDMA (n=3).

Time (h)	1.5 NNMBA (mmol) (gel) Drug released (%)	1.5 NNMBA (mmol) (gel) centrifuged Drug released (%)	20 NNMBA (mmol) (polymer particle) Drug release (%)
0	0	0	0
1	26.18	36.47	56.33
2	58.01	66.29	81.11
3	78.14	82.51	90.90
4	90.15	90.88	95.44
5	95.48	95.68	98.04
6	98.44	98.32	99.25
24	100	100	99.75

Washing the particles reduced the drug concentration in the polymer particles formed by half from 180 to 80 $mg.g^{-1}$. This brings the amount of drug release in line with previous release from NNMBA drug loaded polymer particles which released approximately 80 mg of drug per gramme of particles. The gels produced could not be filtered to as the gel formed could pass through the filter. The reason for the high amount of drug released for these particles was that they could not be washed as normal due to the fact they were gels.

The washed centrifuged and dried polymer particles were then analysed to determine their drug release characteristics. The drug was rapidly released from the gels as shown by the very high release rate where 58.01 to 66.29 % of the drug was released in 2 hours from these dried polymer gels prepared from the 1.5 mmol EGDMA particles. The % drug release recorded in the first hour for these particles was 26 to 36 % which, compared to 56 % for drug loaded polymer particles with no EGDMA present. Figure 4.13 presents an overlay of the % cumulative drug delivery from all three polymer particles discussed.

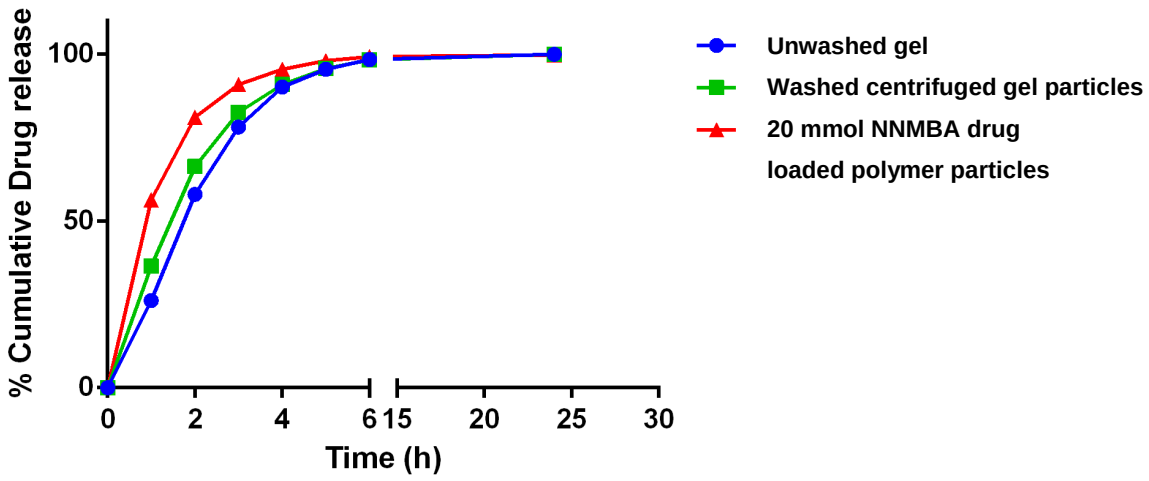


Figure 4.13: Comparison of % cumulative drug delivery from unwashed gels (blue line), washed centrifuged gel particles (green line) and 18:2 NNMBA:EGDMA drug loaded polymer particles.

This data demonstrates that although the drug was still released rapidly the addition of EGDMA to the polymer may have had an impact, reducing the drug release rate specifically in the first and second hour. The addition of the EGDMA had possibly reduced the amount the polymer particle swelled by thus slowing the release of drug. A repeat of the synthesis of the 18:2 EGDMA:NNMBA was performed and two additional particle batches were prepared and all 3 batches of the particles were then analysed to determine their drug release characteristics Table 4.18.

Table 4.18: Quantity of drug released and comparison of drug release profile to drug delivery models for replicate preparations of 9:1 EGDMA:NNMBA polymer drug particles (n=3).

EGDMA Conc mmol	18:2 Original	Repeat 1	Repeat 2
Drug released over 24hr ($mg.g^{-1}$)	0.48	0.15	0.021
Standard deviation ($mg.g^{-1}$)	0.07	0.006	0.0006
First order linearity	0.913	0.936	0.956
Zero order linearity	0.999	0.997	0.996

Drug release in mg of cetirizine per g of drug loaded polymer particles varied from 0.021 $mg.g^{-1}$ to 0.48 $mg.g^{-1}$. The drug release was still zero order, correlation to zero order release was 0.996 or greater for all 3 drug loaded polymer particle batches

produced. Drug release was not concentration dependant which was corroborated by the zero order drug release correlation observed (0.996 to 0.999). The % drug release versus time for the particles in question is displayed in Table 4.19.

Table 4.19: % Drug release from replicate preparations of 18:2 EGDMA: NNMBA drug loaded polymer particles (n=3).

Time (h)	Original drug released (%)	Repeat 1 drug released (%)	Repeat 2 drug released (%)
0	0	0	0
1	13.74	18.10	13.96
2	30.01	33.04	22.10
3	45.88	47.98	38.90
4	60.25	62.92	53.82
5	74.24	76.23	66.88
6	87.68	88.44	80.09
24	100.00	100.00	91.81

The % drug release ranged from 13.7 to 18.1 % in the first hour. The largest variation in % drug release occurred at the two hour time point where % drug released ranged from 22.1 % to 33.04%. Although drug release from the replicate batches exhibit variation in % drug release per hour they release a lower % of drug per hour than previous formulations analysed. This formulation required 4 hours to release 60 % of the drug, a significant decrease in drug release rate per hour. Previous formulations, comprised of only NNMBA cross-linking monomer, exhibited 95 % drug release after three hours (see Table 4.17 p.141).

4.7. Increasing the quantity of drug released from drug loaded polymer microparticles

To increase amount of drug released from the polymer drug particles the solvent ratios were altered using the following ratios of acetonitrile to water, 1:1, 5:1, 7:1 and 8:1. For all solvent compositions the pre-polymerisation mixture of monomers was soluble. Once the polymer forms it will at some point fall out of solution. When it precipitates will be dependent on its solubility in the reaction media. However, only

the 1:1 solvent ratio generated data as particles generated from the other solvent ratios did not release any observable amount of drug Table 4.20.

Table 4.20: Quantity of drug released and comparison of drug release profile to drug delivery models for a preparation of 18:2, EGDMA: NNMBA polymer drug particles manufactured with a 1:1 acetonitrile water reaction solvent mixture (n=3).

EGDMA:NNMBA 18:2 Conc (mmol)	**Acetonitrile : water (50:50)**
Drug released over 24hr ($mg.g^{-1}$)	4.13
Std Dev	0.36
Zero	0.995
First	0.972

Using a solvent composition of acetonitrile:water 1:1 allowed a greater level of drug to be released from the particles formed. The amount of drug released from the particles over 24 hours had increased from 0.021 to 0.48 $mg.g^{-1}$ to 4.13 $mg.g^{-1}$ mg of cetirizine per g of drug loaded polymer. The amount of drug released increased 20 fold for the particles manufactured in the 1:1 acetonitrile water solvent. The most likely cause of the increased loading is the change in solvent composition. It appears to have impacted the drug loaded polymer particle formation and increased the amount of drug released.

Drug release from the particles prepared with the 1:1 acetonitrile:water solvent maintained a zero order drug release as supported by the 0.995 for the zero order release drug release linear regression of the % drug release illustrated in Figure 4.14.

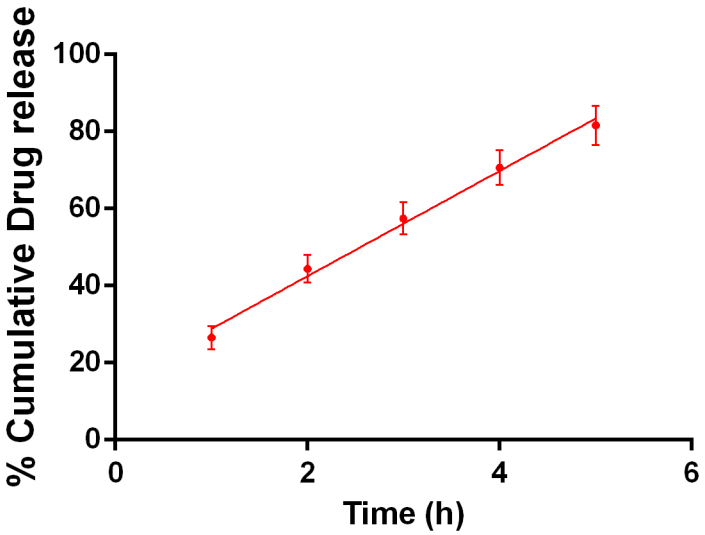


Figure 4.14: Zero order drug release from 18:2, EGDMA: NNMBA drug loaded polymer particles produced using a 1:1 acetonitrile water reaction media (n=3).

The % cumulative drug release results are presented in Table 4.21 for comparison.

Table 4.21: % Drug release versus time from 18:2, EGDMA: NNMBA drug loaded polymer particles produced using a 1:1 acetonitrile water reaction media (n=3).

Time (h)	% drug released
0	0
1	26.51
2	44.40
3	57.48
4	70.66
5	81.62
6	90.99
24	100.00

The % cumulative drug released within the first hour was almost double the amount released from the first set of 18:2 EGDMA: NNMBA particles where 1 hour release ranged from 13-18 %. Although the initial amount released has increased the release was still attenuated. There was 9 times the amount of drug released from these particles compared to the highest drug release amount from drug loaded polymer particles prepared with the 3:1 solvent ratio (0.48 $mg.g^{-1}$). This increased drug release improves the ability of using these particles to drug load contact lenses.

Three replicate batches of polymer drug particles were then prepared using the same preparation method to ensure that increased drug release was repeatable Table 4.22.

Table 4.22: Quantity of drug released and comparison of drug release profile to drug delivery models for replicate preparation of 18:2 EGDMA: NNMBA polymer drug particles manufactured with a 1:1 acetonitrile:water reaction solvent mixture (n=3).

Drug laden particles	Original	Replicate 1	Replicate 2	Replicate 3
Cetirizine (mg g^{-1})	4.13	5.47	1.38	5.25
Standard deviation	0.36	0.9	0.08	0.60

All three batches displayed an increase in drug release; however, batch B had a much smaller increase. Compared to the first batch prepared replicates A and C showed a 20 % increase where replicate B released just over a quarter of the drug released from the initial 1:1 Acetonitrile water prepared particles. This solvent mixture may have increased drug loading, but the solvent itself may not be ideal. The solvent system may not generate stable polymer growth [185].

The order of drug release rate was not impacted by the change in solvent composition used to prepare the polymer drug microparticles and correlates strongly to zero order drug release. Figure 4.15 illustrates the zero order drug release obtained from these drug loaded polymer particles.

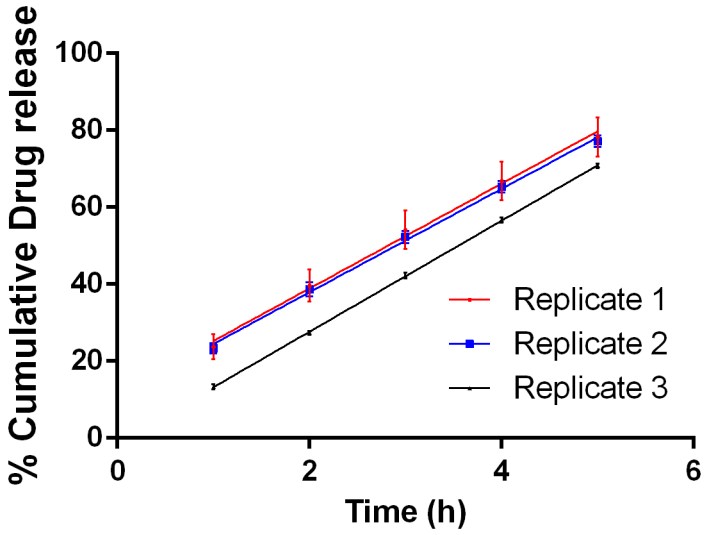


Figure 4.15: Zero order drug delivery graph of three replicate 18:2 EGDMA:NNMBA drug loaded polymer particles listed in Table 4.22.

Table 4.23 details the correlation of drug release from these drug loaded polymer particles to zero order, first order and Korsmeyer Peppas drug delivery models.

Table 4.23: Comparison of drug release to drug delivery models for a preparation of 18:2 EGDMA: NNMBA polymer drug particles manufactured with a 1:1 acetonitrile:water reaction solvent mixture (n=3).

Drug release model Linearity	Replicate 1	Replicate 2	Replicate 3
Zero order	0.994	0.995	0.987
First order	0.947	0.934	0.927
Korsmeyer Peppas	0.996	0.999	0.998

Drug delivery graphs comparing the drug release from the polymers to zero order first order and Korsmeyer Peppas drug delivery model are presented in Figures 4.14, 4.15 and 4.16.

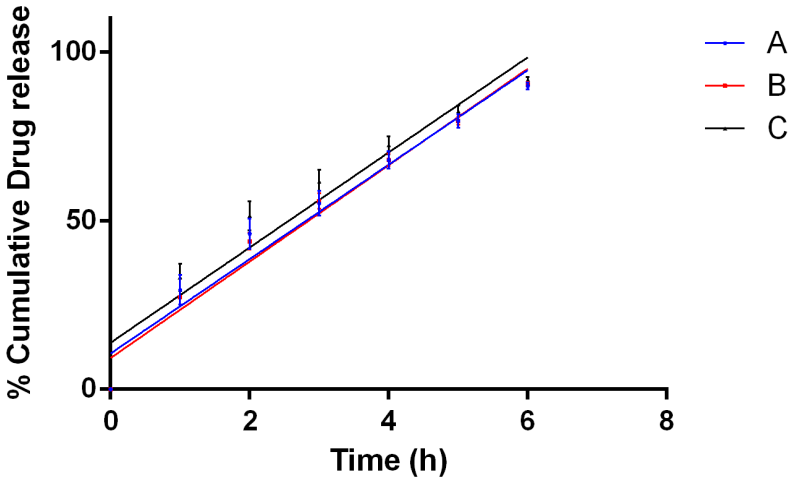


Figure 4.16: Zero order drug delivery graph of three replicate (A,B and C) 18:2 EGDMA:NNMBA drug loaded polymer particles.

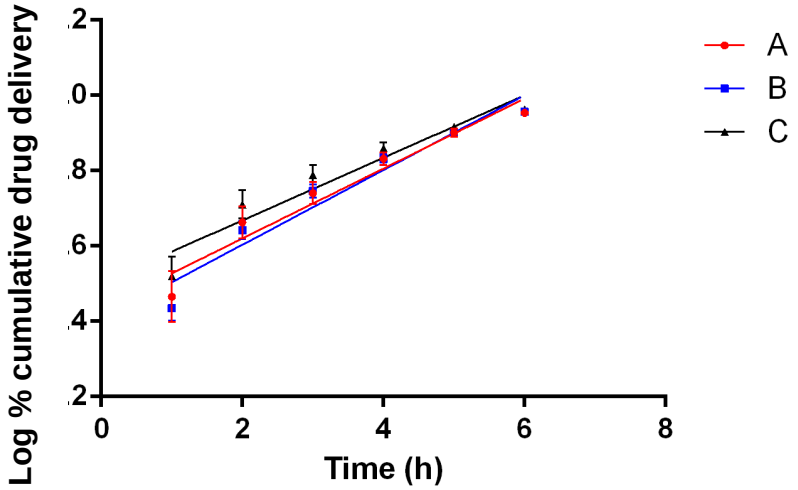


Figure 4.17: First order drug delivery graph of three replicate (A,B and C) 18:2 EGDMA:NNMBA drug loaded polymer particles.

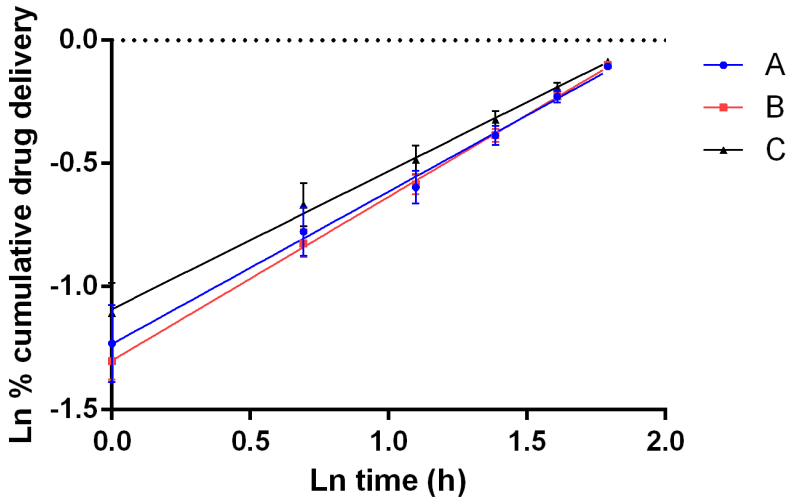


Figure 4.18: Korsmeyer Peppas drug delivery graph of three replicate (A,B and C) 18:2 EGDMA:NNMBA drug loaded polymer particles.

Polymer particles still demonstrated zero order drug release. The % drug released per hour is presented in Table 4.24.

Table 4.24: % Drug release from 3 replicate 18:2 EGDMA: NNMBA drug loaded polymer particle batches produced using a 1:1 acetonitrile:water reaction media (n=3).

Time (h)	% Drug released A	% Drug released B	% Drug released C
0	0	0	0
1	29.44	27.26	33.22
2	46.14	43.91	51.43
3	55.14	55.77	61.59
4	68.00	68.01	72.39
5	79.63	79.65	82.45
6	89.95	90.48	91.83
24	100	100	100

The % drug released after 1 hour was now 30 % compared to 13-18 % drug release after one hour recorded (ref Table 4.19). The most likely cause of this change in % drug release was the (1:1) acetonitrile:water solvent ratio used to prepare the drug loaded polymer particles. An increased the amount of drug was released from the drug loaded polymer particles but there was also an increase in the drug release rate as seen by the higher % drug release per hour. Changing in polymerisation solvent composition impacts the formation precipitation of polymer particles [186]. Therefore changing the of the solvent may have impacted the polymer particles formed and affected their ability to attenuate drug release. This was not an ideal event but the increase in amount of drug released was required to make drug delivery from contact lenses feasible. The % drug released from the 3 drug-laden polymer particle preparations varied between 27 and 33 %. The major factor was the change in % drug released at the 4 hour time point. Here percentage drug released ranged from 68-72 %, which compared to 54 to 63 % from the initial preparations using a 3:1 acetonitrile: solvent ratio. This change was significant as it was no longer possible to use the Korsmeyer Peppas drug release model as % drug released was above 60%. It was still zero order in nature but drug release rate had increased. The

particles released approximately 80 µg of cetirizine drug from 20 mg of polymer particles.

4.8. Drug loading and release from contact lenses loaded with drug loaded polymer microparticles

The final stage of the process was to introduce the polymer drug particles into contact lenses. This was performed using two methods of curing the lenses. Firstly thermal curing and secondly UV curing. These methods have been proven to produce contact lenses of commercial quality. The drug laden polymer particles were direct cast with the contact lens monomers to produce the drug loaded contact lenses. Drug release was performed using an artificial tear solution to more accurately reflect the chemical composition of lachrymal fluid. A number of drug loaded polymer particle loaded lenses and drug soaked lenses were prepared. The lenses were manufactured using both thermal curing and UV curing; secondly drug loading via soaking and direct casting lenses with drug laden polymer particles was also performed. Finally, a combination of polymer drug particle loading and pure drug was performed to determine if the release rate was increased with increasing concentration of drug. Drug loading was set at a therapeutic level of 40 µg per lens for direct cast lenses. Lenses loaded with drug loaded polymer particles had a loading per lens of 1.22 µg of cetirizine per lens not at a therapeutic level but set at a feasible level of loading drug loaded polymer particles per lens Table 4.25.

Table 4.25: Comparison of quantity of drug released and drug release rate to drug delivery models for drug loaded contact lenses (n=3).

Drug release model	Zero Order	First Order	Higuchi	Average drug release in µg over 24 (h)	STD DEV (µg)
Material	r^2	r^2	r^2		
Heat cured particle loaded lenses	0.950	0.870	0.998	5.10	0.31
Heat cured Cetirizine loaded lenses	0.960	0.890	0.996	13.64	0.26
UV cured Particle loaded lenses	0.950	0.860	0.989	5.84	0.53
UV cured Cetirizine loaded lenses	0.970	0.890	0.997	17.43	0.98
Heat cured particle loaded lenses + 6 mg CET	0.951	0.860	0.990	27.89	0.06
UV cured particle loaded lenses + 6 mg CET	0.960	0.860	0.992	31.88	0.41
Control (commercial lenses drug soaked)	0.877	0.551	0.820	32.08	0.88

The drug release rate from the drug loaded polymer particles changed when they were direct cast into lenses. The correlation to zero order release dropped from 0.99 to 0.97 and 0.95 in some cases. There are many possible explanations for this increase in drug release rate. The two most probable causes are firstly the mixture of drug laden polymer particles and contact lens monomers; require sonication to remove oxygen from the monomers prior to polymerisation. This could cause drug loss from the particles to the lens monomer mixture. Secondly, drug molecules could be removed from the particles due to the drug solubility in the contact lens pre-polymerisation monomer mixture. The particles were also suspended in a hydrophilic polymer matrix and the physical forces encountered could cause the polymer particles to be stretched and pulled in a swelling polymer matrix.

55mg of drug loaded polymer particles were added into 3.7g of monomer mixture which equated to approximately 1.22 µg of drug per lens. However, 5 µg of drug was released. This increase was due to the solubility of the drug in the lens monomer mixture. Swelling of the drug loaded polymer particles in the HEMA:NVP:EGDMA monomer mixture could occur, either before polymerisation or after polymerisation when the particles were suspended in the polymer matrix. This effect was in contrast to results of Malaekeh-Nikouei B., *et al*, [170], Peng *et al* [7]. and Jung, H.J. and A. Chauhan[149]. This work reported that there was no increase in amount of drug delivered when the particles were loaded into contact lenses. There was a difference in the drug used by Peng *et al* [7], as they used esters of hydrophilic drugs or hydrophobic drugs such as prednisolone or dexamethasone. In this work diffusion barriers namely vitamin E to attenuate release. These changes could prevent the early loss of drug to the lens monomer mixture.

The drug-laden polymer particles released (0.8-2 %) of the drug which was entrapped into the particles into PBS. When assayed in 50:50 ethanol water the lenses with drug loaded polymer particles “direct cast” into them released 646.57 ± 17 µg of drug per lens. The amount of drug released from the drug loaded lenses into PBS from the three loading methods trialled was consistent. This indicated that the drug particles can release more drug if the solubility of the extraction media changes, i.e. during sonication and mixing of the particles when they were in a suspension or

solution with the contact lens monomers. Figure 4.19 provides a comparison of the drug release profiles from the three drug loaded contact lenses.

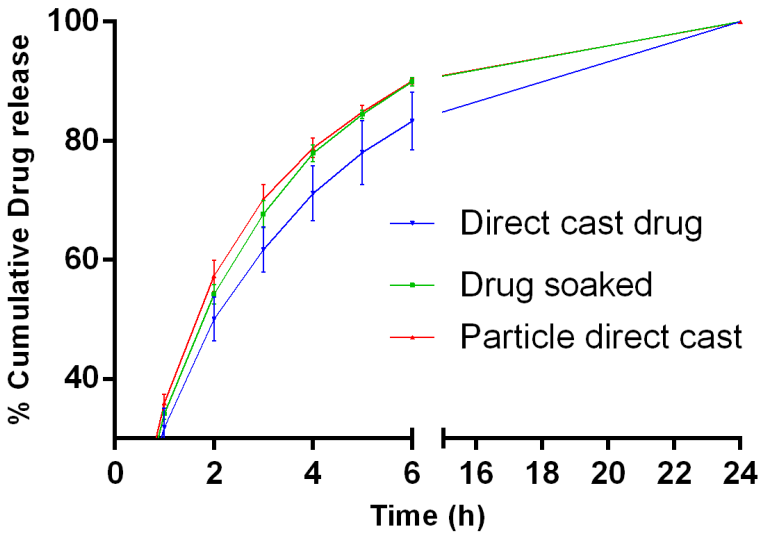


Figure 4.19: Comparison of % cumulative drug release from contact lenses made from HEMA:NVP:EGDMA which were drug loaded by direct casting with cetirizine drug, polymer drug particles and drug soaking (n=6).

The drug soaked lenses and the particle loaded lenses had similar drug release profiles. The direct cast cetirizine lenses follow the same release profile but release the drug at a slightly slower rate. The drug release from the drug soaked lenses and particle loaded lenses was consistent with drug being released from the lens polymer matrix and no significant attenuation of drug was observed. The drug loaded polymer particles produced appear to release the drug into the lens polymer matrix during direct casting. Drug loading the contact lenses in this manner will not be possible without this issue being resolved.

One possible method of reducing drug loss from the drug-laden particles to the contact lens monomers is using a different chemical form of the drug. Hyun *et al.* used the oily base of the timolol molecule for encapsulation of the drug in PGT nanoparticles [149]. Using this form of the drug reduces any loss to the contact lens monomers during fabrication of the lenses, as the drug was less hydrophilic and will be less soluble in the hydrophilic monomer i.e. HEMA.

The formulation that provided zero order release of cetirizine from drug loaded polymer particles with a drug loading of 4.13 $mg.g^{-1}$ was: EGDMA:NNMBA:HEMA:NVP 18:2:0.5:0.5 mmol). Using a solvent ratio acetonitrile:water of 1:1 (40 mL).

4.9. Characterisation of drug loaded polymer particle loaded contact lenses

4.9.1. Refractive index

Refractive index analysis was performed on the contact lenses loaded with drug and drug-laden polymer particles Table 4.26.

Table 4.26: Refractive index analysis of drug loaded contact lenses and comparison to commercial lenses (ACUVUE ®) (n=3).

Lens type manufacture and drug loading method	Refractive index
Control 1 day ACUVUE ©	1.3326
UV cured no drug	1.3297
Heat cured no drug	1.3272
Heat cured particles direct cast	1.3304
Heat cured Cetirizine drug direct cast	1.3302
UV cured Cetirizine drug direct cast	1.3309
Heat cured Cetirizine drug and polymer drug particles direct cast	1.3308
FUV cured Cetirizine drug and polymer drug particles direct cast	1.3308

The refractive index did not change significantly with the presence of particles or drug. The lenses were visually different and the particles could be seen by eye. The % of light transmitted was a more useful measurement of the impact of adding drug particles to the contact lenses see section 4.9.2.

4.9.2. Light intensity

The contact lenses loaded with cetirizine and polymer drug particles were analysed by UV to determine the impact on the amount of light transmitted through the contact lenses when the drug particles or cetirizine was present. Lenses with a

combination of drug and drug particles were also analysed. A commercial daily disposable lens (ACUVUE®) was used as a control, Table 4.27.

Table 4.27: % light transmission measured from contact lenses at 800 nm (n=3).

Lens type and drug loading method	% transmission
ACUVUE	90.51
UV cured lens no drug	83.15
Heat cured lens no drug	83.33
Heat cured Lens with Cetirizine direct cast	83.47
UV cured lens with Cetirizine direct cast	91.81
Heat cured lens with particles direct cast	39.07
UV cured lens with particles direct cast	54.93
Heat cured lens with a Combination of polymer drug particles and cetirizine	37.30
UV cured lens with a Combination of polymer drug particles and cetirizine	55.89

There was an impact on % light transmission from the drug-laden polymer particles. There was between 44 to 46 % reduction in transmitted light for heat cured lenses with drug loaded polymer particles present. There was a drop for UV cured lenses with drug loaded polymer particles present but it was reduced, 27 to 28 % decrease in the light transmitted. The method of curing the lenses and also the initiator used has had an impact on the amount of light transmitted through the lens. TPO was used as the initiator for UV cured lenses. This initiator when decomposed creates very reactive phosphonyl free radicals as well as acyl free radicals [187]. This compares to AIBN the initiator a symmetrical molecule used in heat cured lenses which splits into two identical free radicals leading to different polymerisation reactions [145]. Garcia *et al.* observed microstructural changes in the polymers manufactured by UV, where pores were created in the polymers [129]. The change is not in the chemistry of the reaction but the rate of polymerisation, the UV reaction is faster and this is why it is used in industrial applications. The difference in the % transmission could be due to the phosphonyl radicals causing reactions between the drug loaded polymer particles and the contact lens monomers. This could reduce the opacity of the particles in the lenses formed as the particles are bonded to the polymer matrix of the

contact lens. The phosphonyl radical as mentioned earlier could possibly react with any unsaturated sections or oligomers in the drug loaded polymer particles, Figure 4.20

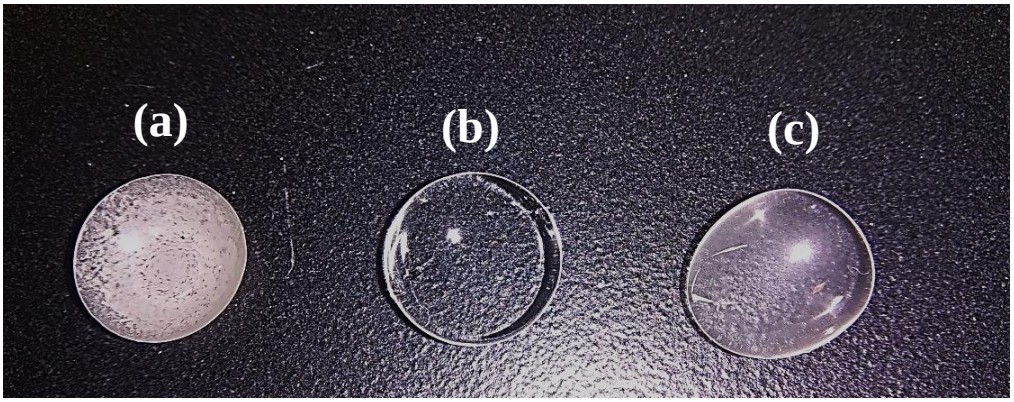


Figure 4.20: Contact Lens images, heat cured drug loaded polymer particle loaded lenses (a), heat cured control lens (b), UV cured drug loaded polymer particle loaded lens (c).

4.9.3. Equilibrium water content

Equilibrium Water Content or EWC analysis was performed on all drug loaded lenses. Lenses were soaked in DI water over 24 hours then removed prior to analysis. This analysis measured the impact on the swelling characteristics of the lenses when: either cetirizine or drug-laden polymer particles, were present during polymerisation (direct casting), Table 4.28.

Table 4.28: Comparison of equilibrium water content of drug loaded lenses manufactured by heat and UV curing processes (n=3).

Lens curing and drug loading method	EWC (%)	Standard Deviation (%)
UV blanks	113.86	4.11
Heat cured lenses with cetirizine	35.46	7.50
Heat cured lenses with particles	41.03	14.02
Heat cured lenses with particles and cetirizine	47.41	9.74
UV cured lenses with cetirizine	37.39	11.52
UV cured with particles and cetirizine	34.16	7.79
UV cured with particles	32.20	13.06

Equilibrium water content of polymer particles loaded lenses was reduced. UV cured lenses were swollen by almost double the amount that heat cured AIBN initiated

lenses were % EWC of 60 to 70 % reference section 2.4.3.2 The method of curing the monomers had an impact on the properties of the lenses produced. The UV curing process produces more flexible polymers which can swell more in water [129].

4.9.4. Scanning electron microscopy of drug laden polymer particles

Scanning electron microscopy was used to image the drug-laden polymer particles produced using the modified Wang method [134]. Both the EGDMA: NNMBA: HEMA: NVP and NNMBA: HEMA: NVP particles were imaged using this technique. The purpose of this analysis was to determine if there was any difference in particle morphology caused by the change in particle composition, Figure 4.21.

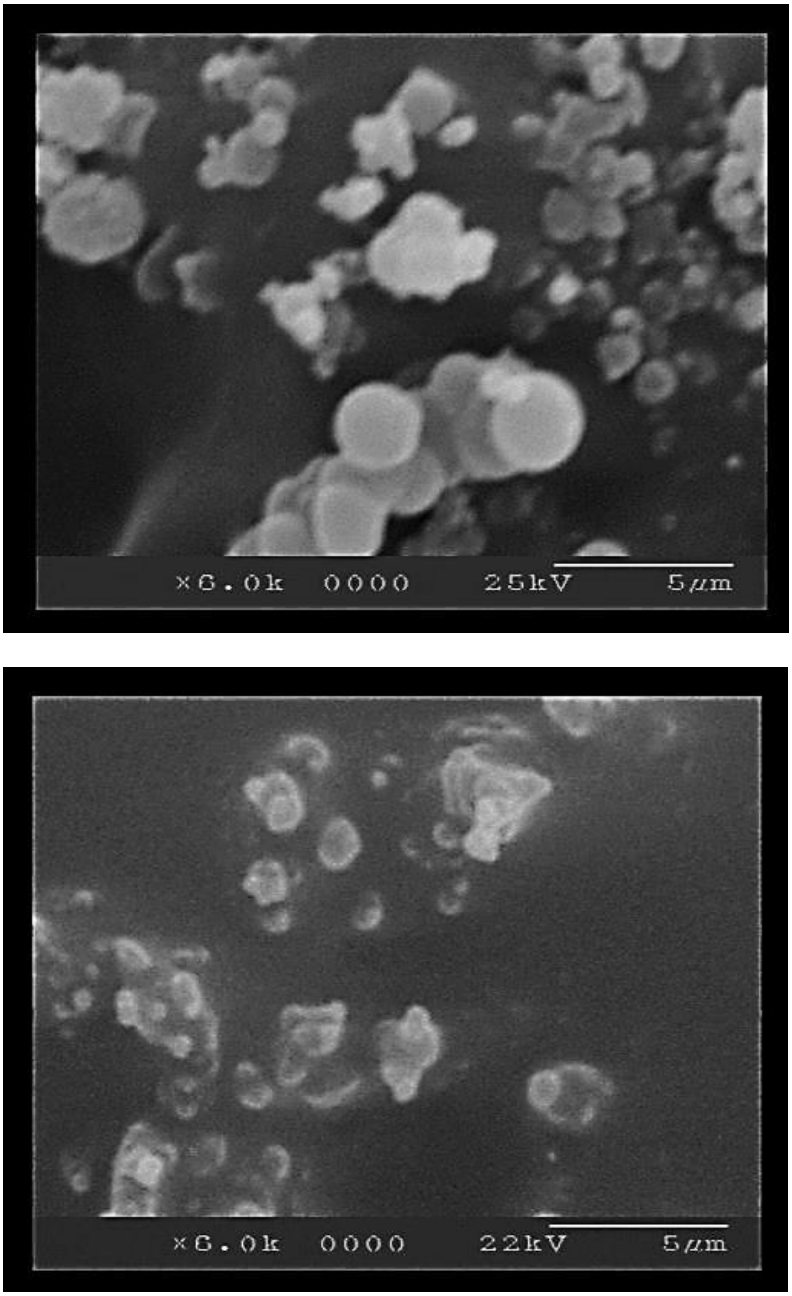


Figure 4.21: EGDMA: NNMBA polymer drug particles imaged by scanning electron microscope using 6.0K magnification. Top, EGDMA:NNMBA:HEMA: NVP 18:2:0.5:0.5 and bottom, NNMBA:HEMA:NVP (20: 0.5: 0.5).

The Scanning electron microscope images demonstrated a change as the high concentration EGDMA particles appear to be spherical particles. Whereas the NNMBA drug loaded polymer particles are rougher and are not as clearly spherical as the mixture of EGDMA: NNMBA particles. These more evenly circular shaped particles may be responsible for increased effectiveness for completely entrapping the drug during polymerisation and release. This may play some role in the improved

drug release attenuation exhibited by the EGDMA:NNMBA:HEMA:NVP drug-laden polymer particles.

4.9.5. Differential scanning calorimetry of drug laden polymer particles and cetirizine

Differential Scanning Calorimetry (DSC) analysis of the drug-laden polymer particles was performed in duplicate to determine if the drug was absorbed onto the surface of the polymer particles, or incorporated into a core shell. The DSC thermograms clearly show the cetirizine melt at 230.10 °C. This endothermic melt was not present in either the EGDMA: NNMBA:HEMA: NVP or NNMBA: HEMA: NVP polymer drug particles as shown in Figure 4.22.

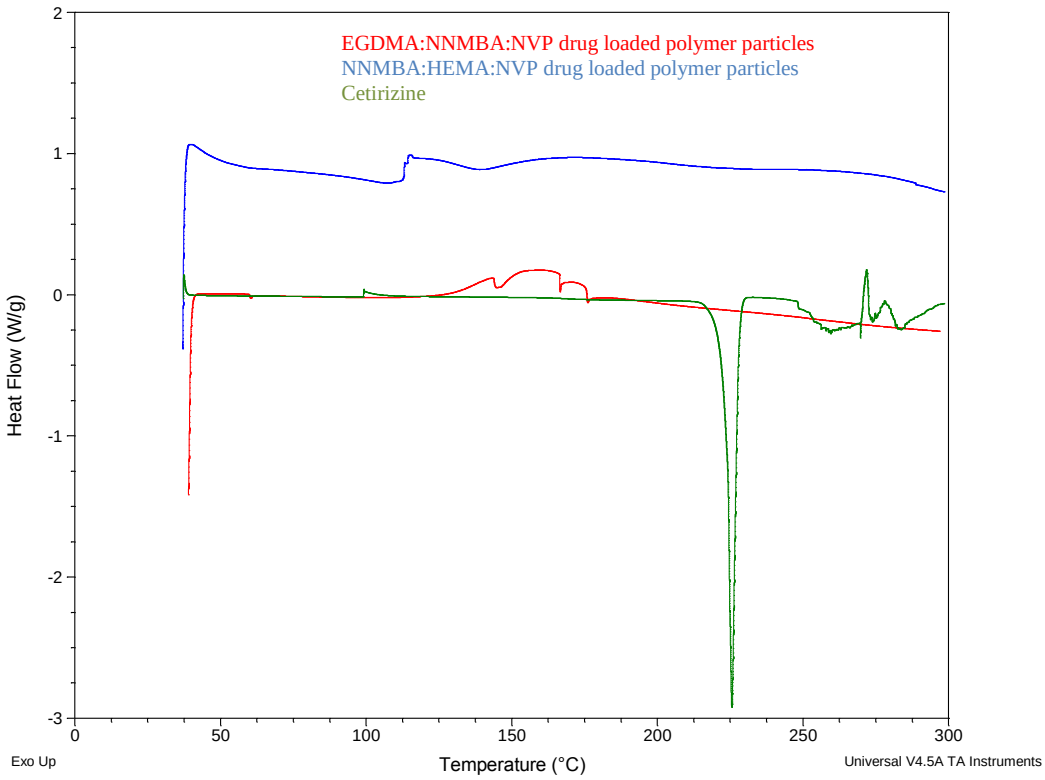


Figure 4.22: Overlay of DSC thermograms from Cetirizine HCl (green line), drug-laden polymer particles EGDMA: NNMBA:HEMA:NVP, 18:2:0.5:0.5 (red line) and NNMBA:HEMA:NVP 20: 0.5: 0.5 (Blue line) (n=2).

As drug loaded polymer particles have no endotherm present in the 230 °C region the cetirizine present in the particles must either be protected by the polymer or the concentration of the drug present in the drug loaded polymer particles was not

sufficient for the melt to be measured. The maximum amount of cetirizine present in the drug loaded polymer particles was 4.13 mg of cetirizine per gramme of polymer. DSC analysis was performed on samples weighing 4-9 mg. This means that there is 4.13 µg of cetirizine in every mg of drug loaded polymer analysed. The cetirizine melt peak when integrated absorbed 125.9 J.g, when divided by 1000 this is 0.1259 J.g the amount of energy that would be absorbed at the concentration of drug present in the drug laden polymer particle. DSC analysis may not be sensitive enough to measure energy changes of this magnitude in drug laden polymer particles. The cetirizine drug present may also be present in the amorphous state and in the drug loaded polymer particles and amorphous drug particles will not exhibit a melting peak [188].

This means that the drug will be uniformly dispersed throughout the polymer particles and protected within the polymer. HPLC analysis confirmed the presence of cetirizine in both drug loaded polymer particles used. The NNMBA:HEMA:NVP particles assayed in 50:50 ethanol water had a % drug loading of 3.13 %. The EGDMA:NNMBA particles assayed in the same manner had a % drug loading of 7.85% ref section 4.9.6. The level of drug loading in these particles is relatively low. As the HPLC analysis is very sensitive with a LOD of 0.17 $\mu g.mL^{-1}$ this analysis the best chosen to identify if the drug was present in the polymer particles as FTIR analysis may not be sensitive enough to identify the presence of the cetirizine in the polymer particles.

4.9.6. Particle drug loading assay

The amount of drug loaded in the NNMBA: HEMA:NVP, (20: 0.5: 0.5 mmol) and EGDMA: EGDMA: HEMA: NVP (18:2:0.5:0.5 mmol) particles was determined by extracting the drug into a 50:50 mixture of water:ethanol. Samples were analysing by HPLC. % drug loading was calculated by dividing actual loading by the theoretical loading. Theoretical loading was estimated as the entire amount of drug added i.e. 1 g being present in the total amount polymer created assuming no losses due to polymerisation. A theoretical drug loading of 35.7 % was calculated for both drug loaded polymer formulations Table 4.29.

Table 4.29: Comparison of polymer drug particle compositions EGDMA: NNMBA: HEMA: NVP vs NNMBA: HEMA: NVP for drug loading efficacy.

Efficiency of Loading	9:1 (EGDMA:NNMBA:HEMA :NVP)	10:0.25:0.25 (NNMBA:HEMA:NVP)
% Drug loading	3.13	7.85

There was a decrease in both % drug loading when the more hydrophobic EGDMA cross-linking monomer was used. This would be expected as the monomer was relatively hydrophobic. The presence of the NNMBA cross-linking monomer increases the amount of drug loaded. Increasing the % drug loading was critical as the smaller amount of particles required will make achieving a therapeutic dose in the lens a more feasible prospect. As expected the NNMBA (20 mmol) load more drug than the EGDMA particles as already observed in release these NNMBA particles release more drug as well.

4.10. Conclusions

Changes in particle size of the different drug loaded polymer formulations did not correlate with a change in the % drug release of cetirizine from the drug loaded polymer particles. There was a difference in particle morphology of the EGDMA: NNMBA particles which were more spherical in shape than NNMBA particles when examined by SEM.

Altering the total monomer concentration, solvent ratio, and cross-link ratio, did not impact the drug release from hydrophilic (20:0.5:0.5 mmol) NNMBA: HEMA: NVP and cetirizine polymer loaded particles. This data would suggest that composition of the polymer particles were less likely to impact drug release than the physical properties of the cross-linker used. EGDMA is hydrophobic and does not swell in aqueous media to the same extent as NNMBA. This reduction in swelling appears to be the dominant factor in attenuating drug release. As the solvent mixture was varied the pH of the solution may also have changed and increased the amount of drug loaded into the polymer particles.

Changing the solvent composition of the polymerisation reaction media increased drug the amount of drug released from the hydrophobic EGDMA: NNMBA: HEMA: NVP particles. Increasing the ratio of water to acetonitrile, (ACN) from 3:1 ACN: H2O to 1:1 increased the solubility of the drug in the polymerisation reaction media. This demonstrates that a higher total amount of drug release was possible and that these drug loaded polymer particles that deliver a therapeutic dose from a lens are possible.

Drug release analysis demonstrates that the drug loading of the polymer has been successful and that the drug release attenuation observed from the particles was due to the physical properties of the cross-linking monomers and swelling characteristics which were engineered into the particles by using a combination monomers with different hydrophobic and hydrophilic characteristics. Novel drug loaded polymer particles were synthesised which can attenuate the release of the therapeutically relevant hydrophilic drug cetirizine. Control over drug release was obtained by using both a hydrophobic and hydrophilic cross-linking monomers. The combination of EGDMA and NNMBA has led to a particle which can load an appreciable amount of cetirizine and control its release. Drug release can be attenuated by both chemical cross-linking and the reduced diffusion caused by the presence of the hydrophobic EGDMA cross-linking monomer. The hydrophobic and highly cross-linked particles appear to control drug release via the amount of swelling the polymer undergoes. Chemical cross-linking impacts release but the effect of hydrophobicity and swelling was greater.

Combining NNMBA and EGDMA has produced novel polymer particles with the required chemical crosslinking to release drug in a controlled manner. Relatively highly cross-linked drug-laden polymer particles with the ability to attenuate drug release have been synthesised. Wang *et al.* achieved release from similar polymer particles using molecular imprinting and the chemical cross-linking of the particles using HEMA, EGDMA and DEAMA monomers [134]. In this work drug release was attenuated without using molecular imprinting. This simpler one stage process is less costly as it does not require the drug to be removed and then reloaded into the polymer particles.UV cured particles and lens monomers by direct casting produced

lenses which allowed 15% more transmission of light than similar lenses thermally cured. The method of curing lenses with drug loaded polymer particles had an observable impact on the lenses manufactured.

The swelling characteristics or Equilibrium Water Content or EWC % of lenses was impacted by directly casting the lenses with either cetirizine drug alone or cetirizine which has been entrapped in polymer particles. The presence of either material inhibits the ability of a lens to swell in water. The impact observed was significant with EWC dropped from 110 % to less than 40 % when particles or drug were added. This reduced swelling of the lenses was the most likely cause of the attenuated drug release from these lenses.

The optimum results achieved from drug release occurred from UV cured direct cast lenses loaded with EGDMA:NNMBA:HEMA:NVP (18:2:0.5:0.5) drug loaded polymer particles which released 5.84 µg of cetirizine over 24 hours.

Use of a novel commercial quality lab scale contact lens manufacturing system allowed contact lens formulation to be varied and drug loaded polymer particles to be added to contact lenses via direct casting. This enabled the manufacture of innovative drug delivery vehicles. Furthermore, the drug loaded polymer produced were unique as they used the hydrophobic properties of the cross-linking monomer to attenuate drug release. This allows the possibility of using this type of drug loaded polymer particle as a platform to deliver a number of pharmaceutical agents. This is possible as the factor controlling release i.e. cross-link monomer chemical properties is not specific to the drug used.

Drug loaded polymer particles were loaded into the contact lenses by direct casting where the contact lens monomers and drug particles were polymerised together to form drug loaded lenses. Drug loaded particles could impact the polymerisation of the contact lenses. The impact of adding these particles on the polymerisation of the lenses was required and investigated in Chapter 5.

Chapter 5

Thermal Investigation of the Impact of Drug loaded polymer Particle Loading on Polymerisation Kinetics

5. THERMAL INVESTIGATION OF THE IMPACT OF DRUG LOADED POLYMER PARTICLE LOADING ON POLYMERISATION KINETICS

HEMA monomer is polymerised via free radical polymerisation in which chain growth, cross-linking and co-polymerisation occur. These reactions occur as there is always a small amount of the HEMA di-ester impurity present as EGDMA in the HEMA monomer [189]. Reaction kinetics are dependent on temperature, concentration of initiator azobis-iso-butyronitrile (AIBN), monomers (HEMA and NVP) and amount of crosslinker present (EGDMA).The "Trommsdorf effect" or auto-acceleration is a component of the polymerisation reaction of HEMA monomer [145]. This phenomenon regulates the reaction rate of vinyl monomers. Typically reaction rates decrease with time, because as conversion increases the concentration of initiator and monomer declines. This auto acceleration effect is exhibited by many monomer polymerisations for example styrene's, vinyl acetates, and methyl methacrylates [145]. This type of kinetic reaction was first observed by Trommsdorf and Norrish–Smith [190].

The aim of this study was to measure the kinetic parameters of the auto accelerated polymerisation reaction of soft contact lens (SCL) formulations (methacrylate monomers and cross-linkers). The analysis would assess the impact of adding drug-laden microparticles on the activation energy of the polymerisation of a number of contact lens monomer compositions. The methods used were established by Ozawa-Doyle [191] and are used in literature, however, this was the first time they have been used to measure the effects of adding drug-laden polymer particles on the polymerisation of contact lens monomers. In this case an antihistamine was loaded in polymer particles in order to mediate its release. This commonly used ocular pharmaceutical was present in a therapeutically relevant concentration (30 to 40 µg) in the contact lens monomer composition. Both scanning (dynamic heating) and isothermal mechanistic or isoconversional DSC methods were used to measure the activation energy of polymerisation reactions and both methods were assessed during the study. The monomer compositions analysed were assessed to discover the impact of adding the drug loaded polymer particles and choose the best candidate for incorporating polymer drug particles for future drug release studies.

5.1. Materials used in this study

Table 5.1: Materials used in this work.

Material	Grade	Source
2-hydroxyethyl-methacrylate (HEMA)	99%	Sigma
N-vinylpyrrolidone (NVP)	99%	Sigma
and ethylene-glycol-dimethacrylate (EGDMA)	99%	Sigma
Azobis-Iso-butyronitrile AIBN	99%	Acros
Cetirizine	99%	Sinoright
2, 4, 6-trimethylbenzoylphenyl phosphinate (TPO)	99%	Gift Bausch & Lomb IRL Ltd.
Acetonitrile	99.9%	Sigma

All used monomers were purified by vacuum distillation, AIBN initiator was recrystallized from acetone (source Acros) and TPO was used as received. Table 5.2 outlines the relative concentrations monomers used in the monomer mixtures.

Table 5.2: Monomer and initiator (AIBN) concentration of the polymers analysed in this study.

Formulation	Monomer	Monomer	Cross-linker	Mole Ratio%	AIBN (concentration) wt %
A	HEMA	N/A	N/A	1	0.002
B	HEMA	NVP	N/A	7:3	0.0006
C	HEMA	NVP	EGDMA	7:3:0.25	0.0006
D	HEMA	NVP	EGDMA	7:3:0.5	0.0006

5.1.1. Experimental methods

5.1.2. Sample preparation for differential scanning calorimetry measurement of drug loaded polymer microparticles

Samples were prepared by weighing the separate components into glass scintillation vials and then sonicating to dissolve the solid AIBN and remove dissolved oxygen from the liquids. The AIBN concentration was maintained at 0.002 weight % for HEMA: AIBN mixtures. All other samples were prepared as lens formulations with the exception of a HEMA, NVP, AIBN formulation which was prepared to ascertain the impact of NVP on the polymerisation of HEMA (see Table 5.2 for composition). The AIBN concentration was kept constant at 0.0006 weight % for these samples. The analysis was then repeated with the same formulations, adding a therapeutic concentration of drug-laden polymer particles to the monomer mixtures (1.5% (w/v)).

5.1.3. Polymer drug particle preparation

To prepare drug loaded particles, cetirizine (2 mmol), HEMA (5 mmol) NVP (5 mmol), EGDMA (20 mmol), and photoinitiator TPO (2 wt.% of total monomers) were dissolved in a mixture of 30 mL acetonitrile and 10 mL water in a 50 mL round bottom flask. The solution was purged with nitrogen gas for 10 min and was subsequently sealed and irradiated by UV light (365 nm) at 4 °C for 1 hour with stirring. The resulting suspension was filtered and washed with 10 mL of water and 3 x 10 mL aliquots of acetonitrile filtered and dried in a vacuum oven at 60 °C overnight. This method is an adaptation of a method used by *Wang et al* [134].

5.1.4. Dynamic mechanical analysis of contact lenses

A DMA Q 800 was used to analyse the contact lenses using a custom deigned stress strain rig developed by SEAM (South Eastern Applied Materials) research group. A preload force of 0.04 N was applied to the lens to remove any air trapped under the lens and then the lens had a strain applied in the range from 0.02 N to 0.10N at $0.02N.min^{-1}$. The thickness of the lens was measured and the displacement measured relative to this value. The stress was calculated from the area used to apply the force to the lens.

5.1.5. Differential scanning calorimetry analysis of drug loaded polymer microparticles

Sample weights analysed were accurately weighed to approximately 10 mg. Dynamic scanning was performed with the nitrogen feed set to 50 $mL.min^{-1}$ and samples were analysed using heating rates of 5, 10, 15 and 20 °C per minute to 220 °C. The samples were then cooled and heated at 20 °C per minute to 210 °C to ascertain if any further polymerisation was possible. For isothermal analysis the pans were held at 65 °C , 75 °C, 85 °C, 90 °C and 95 °C and loaded into the DSC using an auto sampler when the cell was within 2 degrees of the required temperature the pans were then held isothermally at that temperature for 180 minutes. The samples were then cooled and heated at 20 °C per minute to 210 °C to ascertain if any further polymerisation was possible. All samples were prepared and run in triplicate.

5.1.6. Preparation of differential scanning calorimetry data for statistical analysis

The peak temperature for each polymerisation reaction (T_p) was recorded in triplicate for each monomer mixture over four heating rates. The three T_p values for each monomer mixture at each heating rate were averaged and the average T_p values used to derive the slope and subsequently the activation energy were then used for statistical analysis using GraphPad prism® software. Analysis of covariance was performed on the data to determine whether the change in slope of the lines was statistically significant.

5.2. Results and Discussion

5.2.1. Procedure and calculation of kinetic data for activation energy analysis

Thermal analysis was performed using DSC to measure the impact of loading drug loaded polymer particles on the polymerisation kinetics of contact lens monomers. Key kinetic parameters such as enthalpy, reaction rate and activation energy were recorded for a number of contact lens formulations. The peak from the polymerisation was obtained from DSC analysis and the total heat of reaction or ΔH_T was calculated from the area under the exothermic peak on the DSC thermogram, when ($d\Delta H_t/dt$) was plotted against time. The rate of heat released is directly proportional to the reaction or propagation rate of the polymerisation and can be converted into an overall reaction rate ($d\alpha/dt$) or change in conversion with respect to time as well as a fraction of conversion α using Equation 5.1. Where α is the conversion of monomer to polymer.

$$\frac{d\propto}{dt} = \frac{1}{\Delta H_T}\frac{D\Delta H_t}{dt} \qquad \textbf{Equation 5.1}$$

ΔH_T is the total heat released from the polymerisation and ΔH_t is the heat released before a time t. In isothermal mode ΔH_T is a combination of the main peak plus any residual exothermic output during the subsequent heating ramp [192].

As carbon double bonds were consumed during the polymerisation heat was evolved. The DSC thermograms describe the amount and rate of energy released from the free radical polymerisation. In the initial stages the AIBN initiator decomposes and initiates the polymerisation reactions. Free radicals are propagated and the reaction

rapidly increases in rate until it reaches a maximum and eventually reaction rate decreases due to the falling amount of carbon double bonds and various termination reactions. Also diffusion through the polymer matrix becomes a limiting factor, especially as the chemical cross-linking of the polymer matrix increased [145]. The kinetic parameters were obtained from the exothermic peak produced by the polymerisation reaction as presented in Figure 5.1.

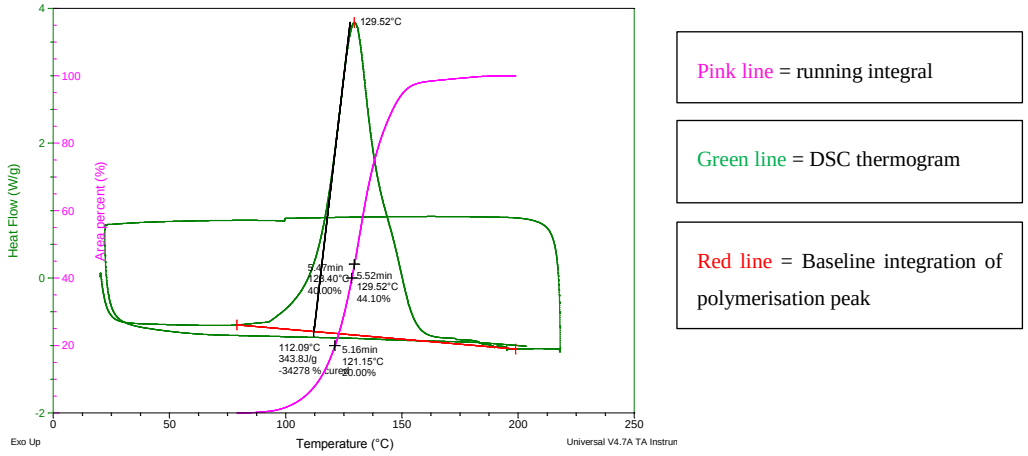


Figure 5.1: Illustration of how kinetic parameters are obtained from a DSC thermogram using an example thermogram from the polymerisation reaction of HEMA monomer and AIBN initiator.

The reaction was measured by DSC displaying the heat evolved during polymerisation (green line).The peak was integrated to measure the enthalpy of the reaction and from this the T_p and enthalpy of the polymerisation reaction was obtained (Red line). A running integral was also obtained from the DSC (pink line) and from this the conversion at maximum temperature and reaction rate was obtained from the slope of the linear part of the line. This measure is dimensionless taking the total heat output as one and showing fraction converted at maximum temperature.

Isothermal analysis was initially performed on the samples, at low temperature i.e. 65 °C. The exothermic peak observed was bell shaped and easily interpreted. Analysis at higher temperature yielded peaks with shoulders and eventually two peaks began to appear at temperatures in excess of 85°C as seen in Figure 5.2 diagram.

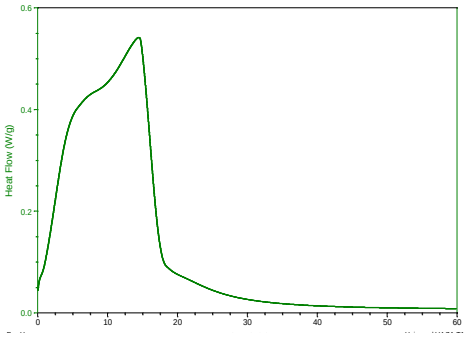


Figure 5.2: Isothermal DSC thermogram of HEMA, NVP and EGDMA polymerisations: isothermal run at 85 °C.

Peak splitting was exacerbated by increasing the initial temperature so higher isothermal runs were impacted more as seen by Huang *et al* [192]. These isothermal thermograms were not ideal and would not allow for predictions of reaction rate using the Kamal equation, see Equation 5.2 [193]. The high initial temperatures used in isothermal runs cause the polymerisation reaction to progress rapidly, which causes an immediate exothermic reaction [145]. Dynamic scanning thermal analysis was also performed and examples of the thermograms are displayed in Figure5.3.

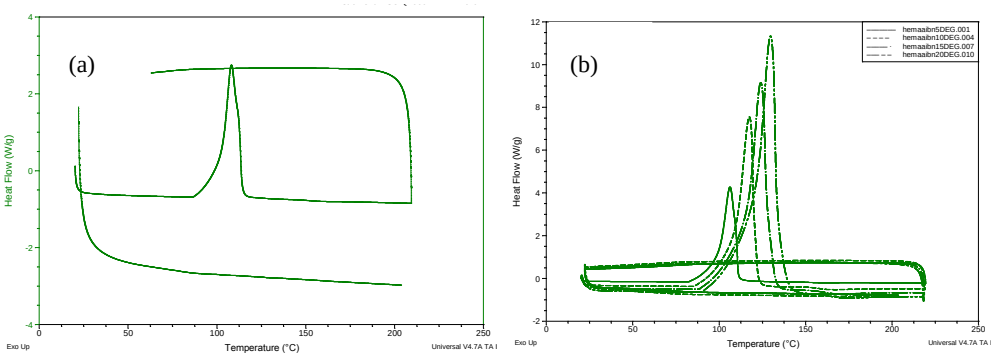


Figure 5.3: Dynamic run at a 5 °C.min heating rate (a); overlay dynamic heating rate runs at 5, 10, 15 and 20 °C heating rates (b).

In diagrams (a) and (b) of Figure 5.3 the thermograms of a 5 °C.min^{-1} dynamic heating rate runs well as an overlay of 5,10,15 and 20 °C.min^{-1} heating rate analysis are show respectively. From these figures it can be seen that the dynamic scanning method provides a thermogram from which the kinetic data of the polymerisation reaction can be reproducibly obtained this was observed in this work and by Huang *et al.* [192]. During a dynamic run the polymerisation reaction has more time to proceed through the distinct phases of initiation and propagation. Temperature rises

over a period of at least 4-5 minutes from 20 °C to 106 °C as the reaction proceeds through its own kinetic pathway.

$$\frac{d\propto}{dt} = k \propto^{m} (1-\propto)^{n} \qquad \textbf{Equation 5.2}$$

This method of analysis can measure the activation energy without using precise kinetic predictions as required, for example by the Kamal equation. This makes the analysis more robust and allows measurement of the activation energy even when non-ideal or non-gaussian exothermic peaks are obtained. This kinetic analysis method requires only that the maximum rate (α_p) is independent of heating rate (Φ), first described by Ozawa-Doyle [194, 195]. Their work expressed a relationship between the heating rate and temperature at maximum rate (T_p). The Arrhenius relationship, described by Ozawa-Doyle (Equation 5.3), allows for the calculation of activation energy.

$$E \approx \frac{-R}{1.052} \frac{d\, ln\Phi}{d(1/T_p)} \qquad \textbf{Equation 5.3}$$

The activation energy of the polymerisation was obtained by plotting the natural log of the heating rate (Ln Φ) against the reciprocal of temperature at maximum rate ($1/T_p$) (Figure 5.4).

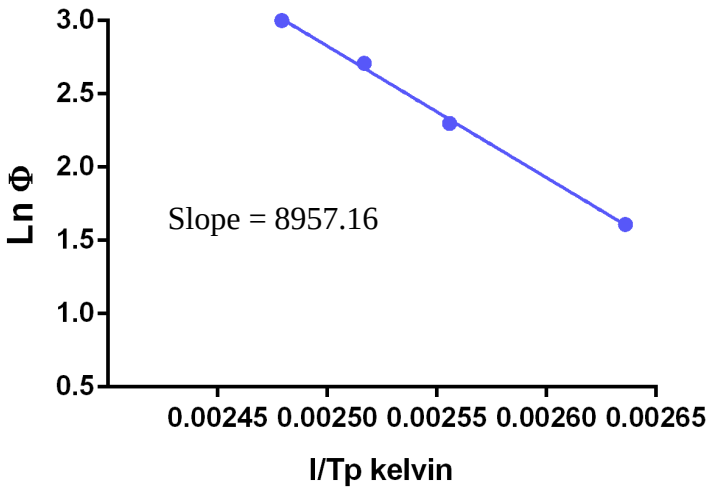


Figure 5.4: Plot of the natural log of heating rate Ln Φ versus reciprocal of Tp for the polymerisation of HEMA with AIB initiator.

Activation energy can then be calculated by using the slope of this graph (obtained from the equation of the line (y=mx + c)) which provides the change in Tp or maximum temperature of polymerisation as heating rate changes. The other parameters in Equation 5.3 are the gas constant and 1.052. using the formula to find

the activation energy requires the slope of the line to be multiplied by the gas constant and divided by 1.052. an example of the calculations are presented.

$$E \approx \frac{-8.314}{1.052}\ 8957.16$$

This calculation generates an activation energy of 70.79 KJ.mol^{-1}.Using a scanning run or heat ramp to analyse the polymerisation reaction provides the data needed to measure the activation energy of the polymerisation without using precise reaction rates. As in the isothermal analysis the exothermic peak can display non-ideal behaviour and shouldering and peak-splitting. As only an overall reaction rate is used in the activation energy calculation, this phenomenon has little impact on the analysis. The lowering of the scan rate can impact the analysis, as there was insufficient energy being applied to adequately drive the polymerisation reaction. Indeed at 1°C per minute dynamic analysis was halted due to erratic polymerisation, this was also noted by Huang *et al* [192].

Dynamic scanning analysis also exhibited peak shoulders on thermograms. The most likely cause was considered to be the presence of the HEMA di-ester EGDMA which is always present in HEMA [189]. So cross-linking polymerisation also occurs to an extent depending on the concentration present. As the scanning rate increases unreacted vinyl moieties which were partially reacted due to their lack of mobility or unreacted monomers may become trapped in the forming polymer matrix. As the heating rate increases more energy is available, improving the probability of reactions occurring, especially reactions where these vinyl groups or trapped monomers are able to position themselves favourably. A zip propagation mechanism allowing for polymerisation of these oligomers i.e. unreacted vinyl groups. Polymerisation could occur due to the position of the vinyl pendants or trapped monomers in relation to the polymer chains or oligomers in the polymer matrix. This was the most likely cause of a shoulder or second peak [145]. This was observed in a number of thermograms, but became more apparent at high scan rates (i.e. 20 °C/min) with EGDMA content (Figures 5.5).

Figure 5.5: Polymerisation exothermic peak of HEMA:NVP:EGDMA, 7:3:0.25 without shoulder observed at a heating rate 20 °C per minute (a). Polymerisation exothermic peak of HEMA: NVP: 7:3 with shoulder at a heating rate of 20 °C per minute (b).

This shoulder was most prominent when no EGDMA was added and at high scan or heating rates. As NVP is not a methacrylate it will not experience the gel effect or auto-acceleration. As there are two distinct kinetic reactions occurring this explains the shoulder produced in the thermogram. At higher concentrations of EGDMA this shoulder was not observed, either due to the, increased density of the polymer; or the faster polymerisation due to the presence of more C=C bonds in the monomer mixture; or the NVP monomer units are possibly more effectively co-polymerised due to the increased level of cross-linker present.

5.2.2. Results of thermal analysis

The results of dynamic or heat ramp DSC analysis is detailed in Tables 5.3 to 5.7. Table 5.7 details the activation energy for the polymerisation of the monomer mixture, both with and without drug-laden polymer particles. All samples were analysed in triplicate. The enthalpy of the reaction conversion at max temperature

and reaction rates are presented in these tables. Table 5.3 below details the DSC analysis of HEMA: AIBN polymerisation.

Table 5.3: DSC results of HEMA: AIBN polymerisation with and without drug-laden polymer particles (n=3).

Heating rate	**5 °C.min^{-1}**	**10 °C.min^{-1}**	**15 °C.min^{-1}**	**20 °C.min^{-1}**
HEMA: AIBN				
ΔH (J.g^{-1})	489.6 ± 24.4	500.2 ± 4.05	495.3 ± 5.34	507.1 ± 3.32
T_p*Kelvin	379.4 ± 0.07	391.3 ± 0.15	397.3 ± 0.23	403.3 ± 0.60
Conversion at max temp α	0.50	0.54	0.55	0.55
K (min^{-1})**	0.29	0.42	0.51	0.61
HEMA with Drug loaded polymer particles				
ΔH (J.g^{-1})	493.2 ± 9.1	513.4 ± 1.9	506.2 ± 14.1	498.4 ± 6.4
T_p *Kelvin	376.7 ± 1.9	391.1 ± 0.2	398.1 ± 1.3	404.3 ± 0.7
Conversion at max temp α	0.69	0.54	0.57	0.59
K (min^{-1})**	0.20	0.19	0.49	0.59

Note * Temperature at max conversion rate ** Reaction rate .

The amount of heat evolved from the polymerisation reaction or change in enthalpy of HEMA:AIBN ranged from 489.6 to 507.1 J.g^{-1}. This compares to a range of 493.2 to 513.4 J.g^{-1} for HEMA:AIBN with drug loaded polymer particles added over the same heating rates.

Temperature at maximum conversion (T_p) increased as the heating ramp rate changes as would be expected, as increasing temperature increases reaction rate. This occurs for both monomer compositions (with and without polymer drug particles).The T_p measured for HEMA: AIBN monomer mixture ranged from 379.4 to 403.3 K compared to 376.7 to 404.3 K when particles were added.

Conversion at max temperature for the HEMA:AIBN polymerisation ranged from 0.50 -0.55 compared to 0.54 - 0.69 for the HEMA:AIBN with polymer drug particles added. The highest conversion at maximum temperature was observed at the lowest heating rate (5 °C.min^{-1}). When drug loaded polymer particles were present conversion at maximum temperature increased by 0.19 change when compared to the HEMA:AIBN monomer heated at the same rate. The addition of polymer particles impacts the activation energy, its impact was more pronounced at lower heating rates. This was due to the fact that at higher heating rates more energy will be available for the reactive species to overcome the activation energy of the polymerisation. At low heating rates the polymerisation was more susceptible to changes, as the temperature ramp is increased the heating rate becomes a more dominant kinetic factor.

Reaction rate increased as the DSC ramp heating rate increased for both HEMA: AIBN and HEMA: AIBN with polymer drug particles added. Reaction rate was lower for HEMA: AIBN with particles at the 5 and 10 °C.min^{-1} ramp rates, but once the ramp rate of 15 °C.min^{-1} or above was used the reaction rates are almost identical for each heating rate with and without drug particles. The reaction rate differed by 0.20 min^{-1} once the heating rate was greater than 10 °C.min^{-1}. As the DSC heating rate was increased the dominant kinetic factor became the heating rate applied to the polymerisation. Reaction rate was lowest for monomer mixtures with drug loaded polymer particles added and this impact was observed at its greatest extent at low heating rates. Any negative impact on reaction rate will be greater at low temperature as the system will have less energy, so any barrier to reaction will be more difficult to surmount as there is a smaller amount of energy available. Table 5.4 presents the DSC analysis HEMA:NVP monomers.

Table 5.4: DSC results of HEMA, AIBN and NVP polymerisations with and without drug-laden polymer particles (n=3).

Heating rate	5 °C.min^{-1}	10 °C.min^{-1}	15 °C.min^{-1}	20 °C.min^{-1}
HEMA:NVP				
ΔH (J.g^{-1})	445.0 ± 10.0	444.4 ± 11.1	410.6 ± 62.1	443.1 ± 1.1
T_p*Kelvin	388.5 ± 0.6	400.0 ± 0.1	405.5 ± 0.1	407.8 ± 0.2
Conversion at max temp α	0.61	0.62	0.52	0.42
K (min^{-1})**	0.18	0.26	0.33	0.39
HEMA:NVP with Drug loaded polymer particles				
ΔH (J.g^{-1})	341.6 ± 25.5	357.3 ± 15.4	338.8 ± 15.2	315.7 ± 1.6
T_p*Kelvin	391.8 ± 1.0	401.0 ± 1.0	404.1 ± 1.2	406.3 ± 0.7
Conversion at max temp α	0.59	0.58	0.58	0.45
K (min^{-1})**	0.14	0.20	0.36	0.34

Note * Temperature at max conversion rate ** Reaction rate.

The change in enthalpy for the polymerisation reaction for HEMA:NVP has a range of 410.6 to 445.0 J.g^{-1}. This represents a 50 J.g^{-1}drop compared to the HEMA:AIBN monomer composition heated at the same rate (ref Table 5.3 p.173). These results established a reduction in heat evolved from the polymerisation when NVP was added. When drug-laden polymer particles are added the range in enthalpy change is 315.7 to 357.3 J.g^{-1}. This illustrated yet another decrease in energy output from the reaction of approximately 100 J.g^{-1}. The polymerisation of the monomer mixture of HEMA: NVP evolved less heat than HEMA monomer alone. When polymer drug particles were added to HEMA: NVP monomer there was also a significant drop in heat evolved.

Temperature at maximum conversion increased as heating ramp rate increase. When HEMA: NVP was compared to HEMA: NVP with added particles, over the 5, 10, 15

and 20 °C.min^{-1} heating rates, there was no significant change in the T_p value with and without drug loaded polymer particles.

Conversion at maximum temperature ranges between 0.61 and 0.42 for HEMA: NVP and 0.59 and 0.45 for HEMA: NVP with drug loaded polymer particles added. The key trend was a drop in conversion at the highest heating rate of 20 °C.min^{-1} for both monomer mixtures i.e. with and without drug this did not occur for the HEMA: AIBN monomer composition. The polymerisation reaction is also diffusion controlled. At faster reaction rates the polymer will become viscous sooner thus slowing the overall reaction rate. This may result in lower conversion due to decreased diffusion in the more rigid polymer formed.

Reaction rate increased with increasing heating rate as observed for HEMA: AIBN. Reaction rates were however lower for HEMA: NVP ranging from 0.14 to 0.39 min^{-1} compared to HEMA: AIBN which were 0.19 to 0.61 min^{-1}. Reaction rate increases as heating rate increases except for HEMA: NVP with drug loaded polymer particles added. Here the highest heating rate does not have the fastest reaction rate, but the difference between the 15 and 20 °C.min^{-1} heating rates was 0.02 min^{-1}. Here an increase in temperature has not caused an increase in reaction rate. The thermograms exhibited split exothermic peaks see Figure 5.5. This peak splitting could cause the reduction in both conversion and maximum reaction rate measured. Table 5.5 presents the DSC analysis HEMA: NVP: EGDMA (0.25 mol %) monomers.

Table 5.5: DSC results of HEMA NVP and EGDMA (0.25 mole %) polymerisations using AIBN initiator with and without drug-laden polymer particles (n=3).

Heating rate	5 °C.min^{-1}	10 °C.min^{-1}	15 °C.min^{-1}	20 °C.min^{-1}
HEMA:NVP: EGDMA (0.25 mole%)				
ΔH (J.g^{-1})	484.6 ± 32.0	477.4 ± 20.4	476.3 ± 4.5	471.9 ± 5.5
T_p*Kelvin	380.8 ± 0.1	390.0 ± 0.1	396.7 ± 0.1	402.0 ± 0.1
Conversion at max temp α	0.49	0.50	0.49	0.49
K (min^{-1})	0.22	0.47	0.63	0.80
HEMA:NVP: EGDMA (0.25 mole%) with Drug loaded polymer particles				
ΔH (J.g^{-1})	352.5 ± 9.6	349.6 ± 3.7	359.2 ± 15.6	344.4 ± 11.1
T_p Kelvin	384.4 ± 1.1	394.3 ± 4.1	399.6 ± 1.0	402.6 ± 0.8
Conversion at max temp α	0.47	0.46	0.48	0.44
K (min^{-1})	0.22	0.38	0.53	0.65

Note * Temperature at max conversion rate ** Reaction rate.

The change in enthalpy for the HEMA: NVP: EGDMA monomer mixtures over the various heating rates ranged from 476.27 to 484.63 J.g^{-1}. This compares with the HEMA: AIBN change in enthalpy, but it would be expected to be higher as the presence of EGDMA should increase the heat evolved as it has double the concentration of double bonds present. The most likely cause of this drop in heat evolved from the polymerisation was the NVP monomer producing the same effect as it did when added to the HEMA monomer which caused a 50 J.g^{-1}drop in heat evolved from the polymerisation of HEMA. The change in enthalpy for the HEMA: NVP: EGDMA and drug loaded polymer particle monomer mixture ranged from 344.4 to 359 J.g^{-1} a decrease of approximately 120 J.g^{-1}when compared to the monomer mixture without particles. A similar drop was observed for HEMA: NVP and drug particle monomer mixtures (100 J.g^{-1}decrease). The trend observed was that addition of drug loaded polymer particles reduced the energy released from the

polymerisation reaction. The particles have an impact on the activation energy thus reducing the amount of monomer converted into polymer and therefore reducing the amount of energy released.

T_p increased with the heating rate for both monomer mixtures (with and without drug loaded polymer particles). T_p ranged from 380.0 to 402.0 K for HEMA: NVP: EGDMA and 384.4 to 402.7 K for HEMA: NVP: EGDMA with drug loaded polymer particles added.

Conversion at maximum temperature ranged from 0.49 to 0.51 for HEMA: NVP: EGDMA which compares to a range of 0.44 to 0.48 for HEMA: NVP: EGDMA and drug loaded polymer particles.

Reaction rate for both monomer mixtures increased with heating rate. There was, however, a greater increase in reaction rate for HEMA:NVP:EGDMA when compared to HEMA: NVP: EGDMA with drug loaded polymer particles. HEMA: NVP: EGDMA ranged from 0.22 to 0.80 min^{-1} compared to 0.22 to 0.65 min^{-1} when drug loaded polymer particles were present. There was a decrease in polymerisation reaction rate when drug loaded polymer particles were added to the monomer mixture and this was observed to a greater extent at higher heating rates with the largest change being observed at a 20 °C.min^{-1} ramp heating rate. The trends observed are that addition of the drug loaded polymer particles decreases reaction rate across all the DSC heating rates. The addition of EGDMA to the monomer mixture reduced peak splitting observed in HEMA: NVP polymerisations, suggesting that the polymerisation reaction had changed due to the presence of the EGDMA. Table 5.6 presents the DSC analysis HEMA: NVP: EGDMA (0.50 mol %) monomers.

Table 5.6: DSC results of HEMA NVP and (EGDMA 0.50 mole %) polymerisations with and without drug-laden polymer particles (n=3).

Heating rate	5 °C.min^{-1}	10 °C.min^{-1}	15 °C.min^{-1}	20 °C.min^{-1}
HEMA NVP EGDMA (0.50 mole %)				
ΔH (J.g^{-1})	487.5 ± 7.9	495.3 ± 4.05	483.0 ± 11.8	476.9 ± 4.6
T_p*°C	376.8 ± 0.0	385.7 ± 2.2	392.7 ± 0.2	397.9 ± 0.2
Conversion at max temp α	0.47	0.48	0.49	0.49
Rp (J.g.min)**	0.30	0.54	0.77	1.0
HEMA NVP EGDMA (0.50 mole %)				
ΔH (J.g^{-1})	386.2 ± 10.3	332.5 ± 28.6	483.7 ± 2.5	347.6 ± 10.4
T_p*°C	380.7 ± 4.41	386.7 ± 4.2	392.8 ± 0.1	397.4 ± 0.8
Max conversion	0.47	0.46	0.48	0.44
Rp(J.g.min)**	0.25	0.44	0.65	0.65

Note ΔH = energy released from polymerisation. T_p =Temperature at max conversion rate α = conversion at maximum temperature. K= Reaction rate.

Enthalpy change for the polymerisation reaction of HEMA: NVP: EGDMA X2 ranged from 476.9 to 495.3 J.g^{-1}, compared to 332.5 to 483.7 J.g^{-1} when no drug loaded polymer particles were present in the monomer mixture. The energy evolved from the polymerisation reaction was reduced when polymer drug particles are added as occurred for both HEMA: NVP and HEMA: NVP: EGDMA monomer mixtures. The higher EGDMA content will cause the polymer to be more cross-linked and this increased rigidity will reduce the amount of monomers which can react as they will not be able to diffuse through the polymer matrix formed [145].

T_p ranged from 376.8 to 397.9 K for HEMA: NVP: EGDMA monomer mixture and 385.1 to 402.7 K when polymer coated drug particles were added. The temperature at maximum conversion increased when the drug loaded polymer particles were present in the monomer mixture.

Conversion at max temperature for HEMA: NVP: EGDMA monomer mixture ranged from 0.47 to 0.49 compared to 0.44 to 0.48 when drug loaded polymer particles are present in the monomer mixture. This conversion at T_p was essentially constant whether drug loaded polymer particles are present in the monomer mixture or not. The higher EGDMA content will cause the polymer to be more cross-linked and this increased rigidity will reduce the amount of monomers which can react as they will not be able to diffuse through the polymer matrix formed [196]. The reaction rate, and time to reach a conversion of 0.5 will remain constant there will however be a lower amount of monomer converted to polymer overall and more oligomers trapped in the polymer matrix.

Reaction rate increased for both monomer mixtures (with and without polymer coated drug particles). Reaction rates for monomer mixtures without drug particles ranged from 0.3 to 1.0 min^{-1} compared to 0.25 to 0.65 min^{-1} when polymer coated polymer particles were present. A trend was evident across all monomer compositions that adding the drug particles reduced reaction rate with the exception of the single monomer composition of HEMA: AIBN. HEMA:AIBN reaction rates were unaffected. The most likely cause of this trend was drug-laden microparticles causing interference to the free radical polymerisation. Any interference in the free radical initiation interactions would decrease the likelihood of polymerisation reactions occurring, between two radical species. Impacting the activation energy due to the effect of the microparticles on the pre-exponential factor of the Arrhenius equation [197].

The observed peak splitting at higher heating rates was however not observed when EGDMA was added to the monomer mixture as illustrated by Figure 5.6.

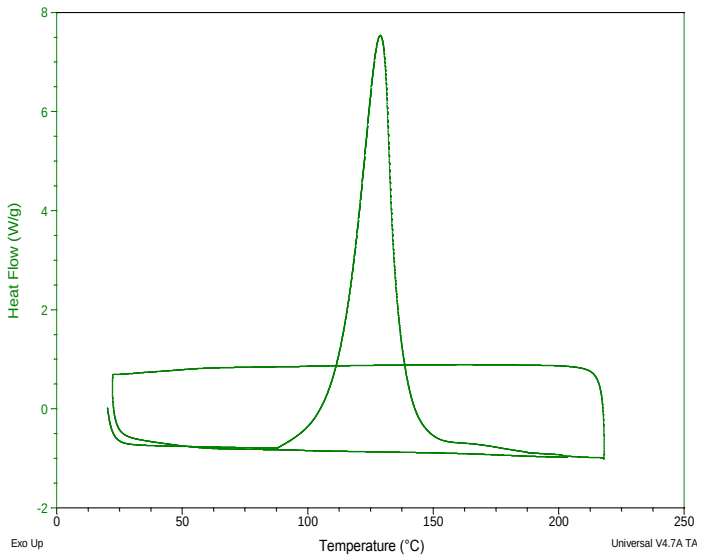


Figure 5.6: DSC analysis of HEMA: NVP: EGDMA (D) monomer mixture at a heating rate of 20°C per minute.

The bell shaped peaks produced show a more ordered polymerisation reaction, compared to the HEMA: NVP polymerisation, with an almost constant conversion at max temperature of (0.47-0.49). Reaction rates increase as DSC heating rates were increased from 0.30 J.g.min, 0.54 J.g.min, 0.77 J.g.min, and 1.0 J.g.min for heating rates of 5 °C, 10 °C, 15 °C and 20 °C respectively.

Table 5.7 outlines the activation energy for the polymerisation reactions for all monomer compositions with and without polymer drug microparticles. The linearity (r^2) of the activation energy graphs were all within the range of 0.964-0.998 displaying strong correlation to the Ozawa-Doyle mathematical model.

Table 5.7: Activation energy measurements for the polymerisation of various monomer compositions with AIBN initiator.

Monomer composition	HEMA	HEMA + Particles	HEMA: NVP	HEMA: NVP + particles	HEMA: NVP: EGDMA (0.25 mole %)	HEMA: NVP: EGDMA (0.25 mole %) + particles	HEMA: NVP: EGDMA (0.50 mole %)	HEMA: NVP: EGDMA (0.50 mole %) + particles
Activation Energy ($kJ.mol^{-1}$)	70.79 ±1.14	60.71 ± 1.08	86.22 ± 1.94	104.04 ± 6.39	79.12 ± 2.82	91.62 ± 2.25	77.80 ± 0.16	97.99 ± 1.16
r^2	0.998	0.996	0.984	0.964	0.996	0.996	0.997	0.984

Note: Activation energy calculated by Huang *et al.* for HEMA:AIBN was 57.2 $kJ.mol^{-1}$ [192]. Mole % refers to EGDMA only.

The lowest activation energy was observed for HEMA monomer compositions with and without polymer coated drug particles present (70.79 and 60.71 $kJ.mol^{-1}$ respectively). Once NVP was added to the monomer composition an increase in activation energy was observed (HEMA: NVP activation energy 86.22 $Kj.mol^{-1}$). This increase in activation energy coincides with a drop in the enthalpy change for the polymerisation reaction. The HEMA: NVP monomer composition exhibits a decrease in the heat evolved from the polymerisation reaction compared to HEMA alone and has higher activation energy. This was logical as the higher activation energy would limit the amount of successful monomer and free radical reactions that occur and therefore exhibit a smaller energy release from the polymerisation. Once drug loaded polymer particles are added to the HEMA: NVP monomer composition, there was an even larger decrease in heat evolved from the polymerisation reaction. Also higher activation energy was observed, 104.04 $KJ.mol^{-1}$ increased from 86.22 $KJ.mol^{-1}$. This trend of increased activation energy was observed for all monomer compositions once polymer drug particles are added, with the exception of the HEMA only monomer composition where there was a decrease in activation energy recorded.

A decrease in the activation energy has been documented by Achilias *et al.* when nano-composites were added to a HEMA AIBN mixture [146]. The cause of this reduction in activation energy was considered to be increased reaction at polymer junctions. Bianchi *et al.* also observed that activation energy increased as crosslink density increases [198]. This was observed over a range of 1 to 4% w/v concentration. The difference in EGDMA cross-linking monomer concentration of the samples analysed in this study was 0.5 mole % and was likely to be too minute a change to impact activation energy resulted in a statistically significant change being observed.

5.2.3. Statistical analysis of activation energy data

Analysis of covariance was performed on the slope of the line created by graphing the natural log of the heating rate versus the reciprocal of T_p. This analysis determined the statistical significance of the change in slope of these lines and hence the change in activation energies as they are proportional. Statistical analysis was

performed to study whether differences in activation energy could be established as real changes or merely experimental variation, a P value of < 0.05 was required for a change to be considered significant, see Table 5.8.

Table 5.8: **Analysis of covariance of the slopes of the activation energy lines (analysed in pairs).**

Formulation	P value
HEMA AIBN vs HEMA AIBN + drug	0.0328
No EGDMA vs no EGDMA with drug	0.0878
0.25 mmol EGDMA with drug vs no drug	0.0844
0.50 mmol EGDMA with drug vs no drug	0.0775

Statistical analysis demonstrated that there was a statistically significant difference in the slopes for the HEMA: AIBN formulation only, when drug-laden microparticles were added. The other formulations displayed no statistical difference in slope and therefore activation energy.

5.3. Dynamic mechanical analysis of contact lenses

The lenses were examined by Dynamic Mechanical Analysis (DMA) to determine if there had been an impact on the physical properties of the contact lenses caused by the presence of the drug-laden polymer particles (See Figure 5.7). The test performed was uniaxial in loading ensuring and the results obtained are Young's modulus of elasticity E.

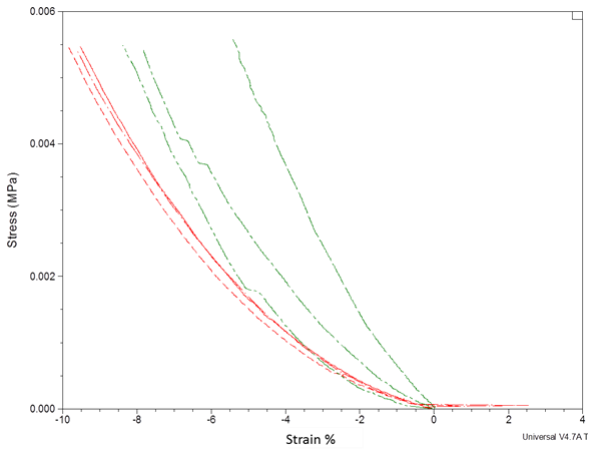


Figure 5.7: Dynamic mechanical analysis of heat cured lenses (red line) lenses cured with particles (green line) heat cured lenses with no drug loaded polymer particles (n=3).

The analysis demonstrates that there is a physical difference in the lenses when the drug loaded polymer particles were present during polymerisation the average of Young's modulus or elastic modulus is a measure of the stiffness of an elastic material [199]. Table 5.9 presents the results of this analysis.

Table 5.9: Young's Modulus calculated from DMA analysis of contact lenses (n=3).

Lens type	Young's Modulus E (MPa)	Std deviation (MPa)
Heat cured no drug	0.098	0.018
Heat cured with drug-laden polymer particles	0.080	0.001

The elasticity of the lenses has been increased by the presence of the drug loaded polymer particles and the polymer particles appear to act as a plasticiser. Heat cured lenses with drug loaded polymer particles have a smaller Young's modulus, this means the material is softer and more elastic. Drug loaded polymer particles could possibly be used to change the physical characteristics of polymers by making them more elastic. The ability to increase elasticity of contact lens polymers is of critical importance to the contact lens industry. Using polymer particles could provide a novel method of altering contact lens physical properties and make them more comfortable for the wearer. Young's modulus (E) was calculated from the initial linear part of the line generated in Figure 5.7.

5.4. Summary

Enthalpy change or heat evolved from the polymerisation reaction decreases when polymer loaded drug particles were added to monomer mixtures i.e. when there was more than one monomer present. For single monomer compositions there was no decrease in heat evolved from the polymerisation recorded when the particles were added to the pre-polymerisation monomer mixture. Also the highest amount of energy released per gramme was observed when the HEMA: AIBN monomer mixture was polymerised.

T_p is a critical measurement for calculating activation energy. The Ozawa-Doyle method plots the natural log of the heating rate vs the reciprocal of T_p. The activation

energy for the reaction was derived from slope of this line. The trend observed was that activation energy was increased by the addition of NVP or drug loaded polymer particles to HEMA monomer. This change in activation energy was also indicated by an increase in the T_p measured at the lowest heating rate (5 °C.min^{-1}). The opposite effect was demonstrated when there was an increase in activation energy. This initial point in the line has shown to be most sensitive for observing changes in activation energy. Higher DSC heating rates display very little change when the composition of the pre-polymerisation composition was altered. The sensitivity of this analysis would be improved by slower DSC heating rates.

Conversion at maximum temperature or α was consistent and unaffected by the addition of drug-laden polymer particles. As the (α) or amount converted is based on the DSC thermogram which measures the heat evolved from the reaction. Any reduction in monomer conversion will not be recorded only a change conversion rate will be observed.

The highest reaction rate was measured for monomer compositions with the highest concentration of carbon double bonds, these being the monomer compositions with the highest concentration of EGDMA. As EGDMA is the di-ester of HEMA it contains two polymerisable double bonds per molecule compared to one for each HEMA molecule.

Reaction rate increased as the DSC heating rate increased with one exception which was observed at the 15 °C.min^{-1} heating rate for HEMA: NVP monomer composition. The split in the exothermic polymerisation peak observed for the polymerisation reaction was the most likely cause of this anomaly which would make it more difficult to accurately measure the T_p for the polymerisation due to the presence of two peaks. Reaction rate decreased for all multiple monomer compositions when polymer drug particles are added to the monomer mixture and the decrease in activation energy coincides with a decrease in reaction rate. The HEMA: AIBN reaction rate also decreased when drug loaded polymer particles were added to the monomer mixture. The decrease in reaction rate occurred at the 5 and 15 °C.min^{-1} heating rates. In spite of the decrease in activation energy which occurred

for the polymerisation of HEMA: AIBN and polymer coated drug particle lower activation energy should lead to faster reaction rates. The variables in the Arrhenius equation are temperature activation energy and the collision coefficient. At lower temperature and heating rates less molecular collisions occur. The addition of the drug loaded polymer particles will reduce the number of successful HEMA monomer collisions. Drug-laden polymer particles will collide/interact with some of the free radicals formed from the decomposition of AIBN and negatively affect reaction rate as they will decrease the A or exponential factor in the Arrhenius equation. Increasing heating rate counteracts this phenomenon and increased reaction rates result. At higher heating rates, there was an increase in reaction rate for HEMA: AIBN monomer mixtures where drug particles were added.

The HEMA: NVP polymerisation reaction has the highest activation energy recorded at 104.04 $kJ.mol^{-1}$. This higher activation energy, was responsible for both the slower reaction rate, and decreased enthalpy change both of which were observed for the polymerisation of this monomer composition. There was also a higher activation energy recorded for all HEMA and NVP monomer compositions once polymer coated drug particles were added. The HEMA: AIBN monomer composition, however, exhibited a decrease in activation energy of 10.08 $KJ.mol^{-1}$ when polymer particles are added and this difference was found to be statistically significant. In this case the addition of the particles appears to reduce the activation energy but not increase the rate of the polymerisation reaction. In all other cases activation energy increased when particles were added and reaction rate decreased.

The activation energy measured was within range of data presented in literature of 57.2 and 89 $kJ.mol^{-1}$ [146, 192]. NVP was calculated to have an activation energy of 46 $kJ.mol^{-1}$ [200]. Adding NVP should have reduced the overall activation energy so the co-polymerisation activation energy was not easily predicted as there are two distinct kinetic reactions occurring (HEMA auto-accelerated and NVP nth order kinetics). HEMA: NVP monomer compositions exhibit a split in the polymerisation peak, which was not present when EGDMA was added to HEMA: NVP monomer mixtures.

The addition of the drug loaded polymer particles decreased the ability of the monomer units to react in copolymerisation reactions as evidenced by an increase in activation energy. A possible mechanism for this increase in activation energy would be that these microparticles were absorbing energy from the environment and decreasing the likely hood of two reactive species meeting and reacting, thus increasing the activation energy. In single monomer i.e. HEMA: AIBN polymerisations the opposite effect was observed and activation energy has been documented to decrease. In this case the particles may catalyse the free radical polymerisation providing sites or reactive surfaces for the free radicals to attach to and increase the possibility of encountering other reactive species and thus decrease activation energy. This may be more likely to occur in a homogenous polymerisation and so will only be observed in a single monomer polymerisations described in this study and Achilias [146].

5.5. Conclusion

The addition of drug-laden polymer particles to contact lens forming monomer mixtures impacts the polymerisation process. The heat evolved from the polymerisation reaction was decreased from monomer mixtures containing NVP and drug loaded polymer particles. The activation energy of single monomer polymerisations was decreased and an increase in activation was observed for more complex co-polymerisation reactions. The DMA analysis has shown that the physical characteristics of cured lenses can be changed by the presence of polymer microparticles during the lens curing process.

This novel investigation determined for the first time the impact of adding drug loaded polymer particles on the polymerisation reaction of contact lenses monomers. It determined that simple binary mixtures of monomer and initiator exhibited a drop in activation energy and more complex multiple monomer mixtures with cross-linker added exhibited and increase in activation energy. This information contributes to the scientific knowledge on the processing of contact lenses and highlights an area of further study as polymer particles could be used to influence polymerisation reactions.

The experiment research performed in this study identified a number of areas worthy of further investigation and these will be outlined in Chapter 6.

Chapter 6

Future Work

6. FUTURE WORK

This thesis has focussed on the manufacture, characterisation, drug loading and drug release from contact lenses. Future work envisaged would build on and develop the polymeric methods and characterisation techniques, toward producing novel drug delivery vehicles with controlled release characteristics. Wet cast moulding has successfully produced contact lenses, which are comparable to commercial lenses. These lenses can be characterised to determine the impact of drug loading on their physical properties. DMA analysis, refractive index and light transmission have been used to measure and compare lenses produced and confirm the aptitude of the lenses manufactured for use as ophthalmic devices. Characterisation methods measured the impact of polymer formulation composition and chemical cross-linking on drug loading. Drug release analysis allowed the assessment of how all these factors impacted drug release and physical properties.

Building on the work outlined in the preceding Chapters, the focus of future studies would be on drug loading techniques; for example, using drug-laden particles synthesised in Chapter 4 to load drug onto the surface of the lenses. The loading of these particles onto the surface of the lenses has the potential to become a novel drug delivery technique. This Chapter will outline drug loading and delivery methods which could be exploited to create controlled drug delivery from the contact lens drug delivery vehicles manufactured.

6.1. Drug loading methodologies

6.1.1. Printing drug loaded polymer particles onto contact lenses

Direct casting drug loaded particles into the contact lens monomers suffered from two issues, firstly the drug loaded polymer particles reduced the amount of light transmitted through the lens and secondly, drug release was attenuated sufficiently. To overcome these issues the printing of a drug loaded polymer particles was explored. Printing of inks onto contact lenses for cosmetic effects, such as increasing the size of the iris or changing eye colour, has been performed for some time. For

example, Bausch & Lomb manufacture the Naturelle® contact lens, an image of this lens is presented in Figure 6.1.

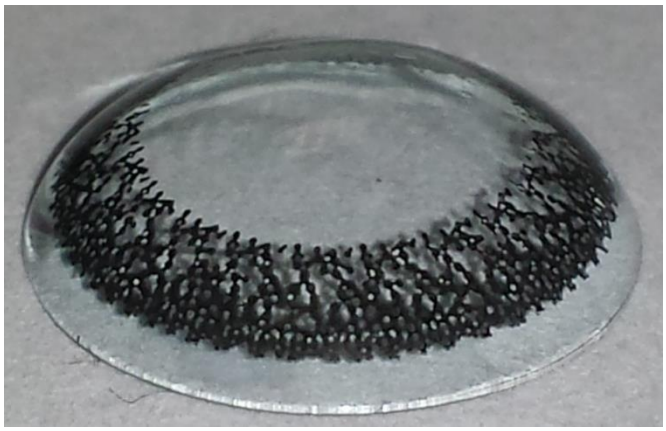


Figure 6.1: Naturelle contact lens from Bausch + Lomb with artificial iris printed on the lens.

This lens is used for cosmetic purposes, as it gives the eye a larger, rounder appearance. The ink is printed onto the surface of the lens in the limbal region around the iris so there is no impact on vision. Printing drug onto one surface of contact lenses provides a possible method of increasing bioavailability above the current 50% [7]. Drug loading the lens in this manner would overcome the issues optical clarity and loss of drug from the polymer particles to the lens monomers during polymerisation.

Drug loading via printing onto the lens would have some major advantages. As the printed drug would be present on the side of the lens closest to the cornea, drug diffusion will happen predominantly into the tear film underneath the lens where the tear flow is reduced. Also the drug can be printed on the edge of the contact lens where it does not interfere with the patient's vision.

However, printing a thin coating of drug-polymer mixture onto the surface of the lens will leave a small amount of material to create a drug reservoir. For this drug loading technique high bioavailability will be a prerequisite. Daily doses of antihistamine were calculated earlier in Chapter 3 and determined to be approximately 2-10 µg. Bioavailability needs to be high to make loading these small amounts of drug a feasible option. High bioavailability is possible from this system

as the entire amount of drug eluted from the drug loaded side of the printed lens would be accessible for corneal absorption. At present there is a limited amount of literature on printing drug technologies. The absence of literature on printing drug onto contact lenses makes this a novel technique for drug loading contact lenses and warrants further investigation.

Drug printing onto polymer substrates has been performed by Scoutaris *et al.* where an ink jet printer was used to print a micro-dot formulation of Felodipine and PVP in, 100 µm diameter drops, onto hydrophobic substrates [201]. Also Pardeike *et al.* used nano-suspensions composed of a Tween 20 water suspension of folic acid and water to ink-jet print drug onto edible substrates [202]. Ciolino *et al* have demonstrated release of a therapeutic dose of Latanoprost, up to 30 days [203]. This method of spin coating has to be combined with removing a central aperture of the coating from the lens and then lathing the lens to the correct thickness for use. This is a lengthy process, not suited to high volume manufacturing. Printing the drug onto the lens surface would shorten this process.

Initial trials were performed where the drug loaded polymer particles which were developed in Chapter 4 have been printed onto glass and contact lenses. In a variety of ink and monomer formulations. Drug release has been observed but further experimentation and formulation is required to produce novel drug delivery devices. This area has great potential and the ability to use both hydrophobic contact lens monomers and hydrophobic printing materials provides another opportunity to further attenuate drug release. Formulation of the ink and monomer mixture was focussed on firstly attenuating drug release and subsequently adhering the drug loaded film to the lens securely. Experimental analysis is not yet at a stage where analytical results are can be reported.

6.1.2. Drug-laden particle synthesis optimisation

The drug-laden polymer particles have been prepared using UV photo-polymerisation to fabricate the particles. The method used was an adaptation of a method used by Wang [134]. Zero order drug release was obtained from these particles but this attenuation was not as evident when the drug loaded polymer

particles were loaded into contact lenses. Also the particles loaded into the contact lenses had a negative impact on the optical clarity of the contact lenses.

The use of other methods of synthesis of the drug loaded polymer particles could be used to improve particle size distribution. Nano-sized particles will have a smaller impact on the % of light transmitted through the lens. As particles less than 100 nm have shown no decrease in % transmittance of light at 800 nm by Itoh *et al* [166]. Secondly, creating particles with the drug incorporated within a core of polymer may be beneficial for the attenuation of drug release. These smaller drug loaded polymer particles may have the ability to load a therapeutic dose of drug without impacting the optical properties of the contact lenses [93, 127, 149, 204]. Changing the method of forming the particles to a suspension polymerisation method, could create nano sized polymer particles which can be loaded in a therapeutic dose without impacting the optical properties of the contact lenses. Suspension or emulsification polymerisation has successfully generated, controlled release particles for a number of research groups and drug delivery vehicles [38, 205, 206]. Suspension or emulsification nanoparticle synthesis has produced nanometre sized particles. Reduced particle size of drug loaded polymer particles may allow for the generation of more effective control of drug release [139, 149].

Generation of polymer particles via suspension and or emulsification polymerisation will focus on creating nano sized polymer particles which could be loaded into contact lenses without impacting the optical clarity of the contact lens.

6.1.3. Loading diffusion barriers into contact lenses to control drug release

Diffusion barriers have been used to impregnate the contact lens matrix. Here the material remains in the polymer matrix. Vitamin E is a very hydrophobic material so does not release into aqueous media therefore any drug loaded into the polymer matrix must diffuse around this material which extends the release of the material. Vitamin E has been successfully used as a diffusion barrier, but has only been reported for use with silicone contact lenses [124].

Vitamin E was not suited for use with hydrophilic polymers, as demonstrated in Chapter 3, where vitamin E was observed to increase the release rate of material from polymer lenses, and to negatively impact the % light transmission from the lenses. Using Silane monomers would improve the solubility of vitamin E in the polymers produced and reduce the impact of these issues. Silane monomers are hydrophobic and have been shown to load vitamin E reproducibly [125].

Although vitamin E may not be suitable for hydrophilic polymers, other compounds may be more compatible with these hydrophilic polymers. One such option is PVA; this polymer has been used as a diffusion barrier. The material has been co-polymerised with NVP and was released over a period of hours demonstrating its ability to be loaded into contact lenses [79]. Any drug material which was loaded with both PVA and drug would create a polymer where the two materials would have to compete regarding diffusion through the polymer. This could result in the attenuation of release of one or both of the materials. PVA along with other long chain polymers should be investigated to determine if it is possible to find a more compatible diffusion barrier which will attenuate release of drug from contact lenses.

Future studies will focus on using novel diffusion barriers to attenuate drug release. Screening other polymer materials may provide an alternative diffusion barrier which is compatible with hydrophilic contact lens monomers.

6.1.4. Application of micro-fluidic devices for measuring drug release from contact lenses

Drug release from polymers, and specifically contact lenses, has been studied for decades using *in-vitro* drug release experiments. In the majority of these experiments the drug loaded lenses are placed in a release medium and then removed after a period of time and drug released into the fixed volume of the media measured [15-17, 74, 144, 202]. This is not representative of the environment the lens will be used in as there is no ocular tear flow. It is possible to more accurately model tear flow using a microfluidic device. Ali *et al* [114], and Tieppo *et al* [155], have both performed analysis using a microfluidic device. Ali *et al.* demonstrated that drug release was both slower and more constant under ocular tear flow [114]. There are

two reasons for increased drug release when infinite sink conditions are present: firstly, there is a larger volume of liquid, which creates a maximum driving force for diffusion and secondly, stirring impacts on boundary layers, which appears to impact drug release [155]. Under ocular tear flow Tieppo *et al*. demonstrated the ability to measure hourly drug release over 48 hours from contact lenses in an ocular tear flow of 3 $\mu L.min^{-1}$. Future analysis using microfluidic devices will build on this research. The schematic of a microfluidic device is displayed in Figure 6.2.

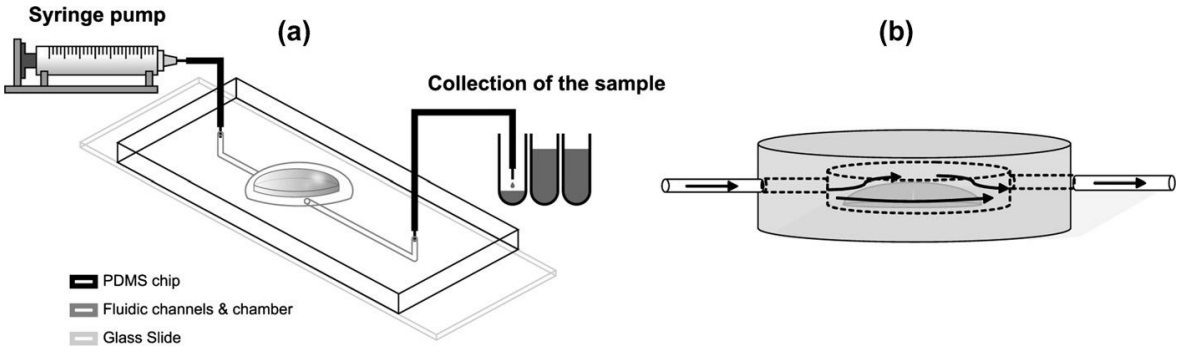


Figure 6.2: Micro Fluidic device for measuring drug elution from contact lenses under ocular tear flow [155] (a). Front view of microfluidic device (b) side view of microfluidic device.

A 3D printed microfluidic device was designed in-house, for the purpose of performing drug release experiments Figure 6.3.


a


b


c

Figure 6.3: Pictures of 3D printed micro-fluidic device lid and top section (a), top lens mount with micro channels to allow extraction media flow under lens, and finally (b) assembled device with extraction media flow tube attached (c).

The device has an outside diameter of 25 mm and is 70 mm in length. The dome (ringed in red) in Figure 6.3 (b) has a radius of 9.49 mm and is modelled on the dimensions of a contact lens. A schematic with all the dimensions of the device is attached in appendix 2. This device will allow the modelling of drug release from medical devices as it would occur in the eye. Contact lenses can be placed in a laminar flow of tear fluid as occurs in the eye.

Measuring drug release from contact lenses has been attempted using this device. Due to fabrication issues the device could not maintain a seal and leaked. Future work will involve the device being redesigned to overcome these issues. Measuring

drug delivery in this manner matches the ocular environment and the laminar tear flow that the contact lens will experience in the eye.

6.1.5. Characterisation method development for analysis of contact lenses

There is potential to develop novel analytical techniques for the characterisation of contact lenses. Two possible options to be investigated are: solid state nuclear magnetic resonance (SSNMR) and solution phase atomic force microscopy (AFM). Rheological analysis could also be developed to enable comparison of lens physical strength to both commercial lenses and analysis performed in literature.

Solution NMR has been used to analyse pre-polymerisation complexes of drug and monomer [24, 207-209]. However, solution NMR cannot be used to measure solid samples. Analysis of polymerised drug loaded polymers by SSNMR could determine the potential of this technique to measure the impact of drug loading polymers. Method development for the analysis of contact lenses using SSNMR could be performed. This would be a novel characterisation technique for drug loaded polymers. This technique could discern the interactions between drug and polymer and be used as a screening tool to identify candidate drug and monomer materials worthy of study.

Solution phase atomic force microscopy (AFM) can be used to measure the surface morphology of polymers in a wet state. This technique could provide information on the surface morphology of contact lenses, adhesive force, charge and force dissipation on a molecular level. Solution phase (AFM) allows contact lenses to be analysed in a hydrated state. This ensures the forces analysed are representative of the lens as it would be in the ocular environment rather than dry as would be the case of standard AFM analysis [210]. The potential of this technique to characterise drug loaded contact lenses requires further investigation.

The friction force of swollen, contact lenses can be measured by a rheometer equipped with Peltier plates or a solid torsion kit. From this analysis, the total friction F, and coefficient of friction, μ can be determined [211]. This method of analysis has a number of advantages over texture analysis. Firstly, it can be performed at a controlled 37 ºC, as the surface temperature of the Peltier plate can be tightly controlled. Secondly the method is highly sensitive, which makes it capable of

measuring the torque of low friction gels such as contact lenses. Also, these measurements are quoted regularly in the literature, making it possible to compare the results obtained. This analysis has been used by Yanez *et al*. [4] to measure the release of poly-vinyl-pyrrolidone (PVP) from polymers (HEMA) hydrogels and was sensitive enough to measure differences in rheology in from different polymer formulations. Using this technique would allow comparison of the contact lenses developed in this study to commercial lenses.

6.1.6. Summary

The main drug loading methods proposed for use in controlling release from the contact lenses manufactured are summarised and the state of the art for each of these methods have been discussed.

Characterisation methods for contact lenses could be further developed. Rheology, solution phase atomic force microscopy (AFM) and solid SSNMR could be used to characterise drug loaded polymers.

Drug loading of contact lenses could be further executed using drug laden nanoparticles. Nanoparticles would allow a number of different drug loading techniques to be employed from direct casting of nanoparticles (NP) in polymer lenses to the printing of NP onto the surface of contact lenses. Printing would be a novel method of drug loading contact lenses. This method facilitates the addition of drug to one side of the lens only. Addition to one side of the lens should further decrease the amount of drug loss from the lens allowing for the µg amounts of drug loading onto the surface of the lens.

Both of these drug loading methods; printing drug onto the surface of the lens and directly casting drug loaded polymer particles into the lenses can be coupled with the use of a diffusion barrier. This would potentially provide increased control over drug delivery. Previous studies performed in Chapter 3 illustrated that vitamin E was not suitable for use with HEMA polymer formulations, but there are other alternatives, such as PVA and other long chain polymers, which can possibly delay drug release from the contact lens and warrant further investigation. This technique would be

novel as the use of a diffusion barrier within drug loaded polymer particles to attenuate drug release has not been published to date.

Drug release analysis also needs to be measured in a more appropriate manner. The use of a microfluidic device for measuring drug release under ocular conditions will allow for a more realistic measurement of drug elution from contact lenses. The lenses could be analysed in a liquid volume, which match the volumes encountered in the tear film of the eye. The mixing of the release media would be reduced due to the laminar flow of lachrymal fluid across the lens, causing boundary layers to form which would reduce mixing effects as occur in larger volumes of liquid. This improved ocular drug release modelling would provide drug elution profiles in conditions that occur in the eye.

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APPENDIX 1

RESEARCH OUTPUTS

Conference proceedings

1. Phelan, D., Fitzhenry, L., Coffey, A. and McLoughlin, P. "Development of Contact Lenses for Ocular Drug Delivery" Bioengineering Ireland, **2013.**
2. Phelan, D., Fitzhenry, L., Coffey, A. and McLoughlin, P. "Development of Contact Lenses for Ocular Drug Delivery" Eurotech 2013, Lyon, France, **2013.**
3. Phelan, D., Fitzhenry, L., Coffey, A. and McLoughlin, P. "Development of Contact Lenses for Ocular Drug Delivery" GSS-MIP, Belfast, **2013**.

Publications in progress

1. Phelan, D., Fitzhenry, L., Coffey, A. and McLoughlin, P. "Attenuation of drug release from contact lenses using drug loaded polymer particles" (2015).
2. Phelan, D., Fitzhenry, L., Coffey, A. and McLoughlin, P." Thermal investigation of the impact of drug loaded polymer particle loading on polymerisation kinetics" (2015).

Bioengineering ... in Ireland[19] 18th-19th January 2013

MEDICAL DEVICE, MANUFACTURE, CHARACTERISATION AND CONTROLLED DRUG DELIVERY

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INTRODUCTION

Approximately 90% of all ophthalmic drug solutions are applied as eye drops [1]. This delivery method has major disadvantages, for example; only 1-3% of an eye drop reaches the intra-ocular tissue, while the rest is lost to tear drainage [2]. Controlling drug release so a therapeutic dose can be maintained for a prolonged period is almost uniquely provided by surgical implants, and as such, any opportunity to provide controlled drug delivery without the need for surgery would be universally welcomed. Drug delivery from contact lenses is one viable option. The focus of this study is to manufacture and characterise contact lenses and by using smart polymer technologies, control the loading and elution of therapeutically relevant material from the contact lenses.

To achieve this goal a manufacturing process for contact lenses was developed. A stainless steel chamber provided an oxygen-free environment for thermal and UV curing of the lenses. The manufactured lenses were loaded *via* soaking in ethanol.Vitamin E was loaded into contact lenses as a diffusion attenuator, its release / retention and effect on the polymer formulation was determined [3].

MATERIALS AND METHODS

Materials

Hydroxyl ethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), N-vinyl-pyrrolidone, azobisisobutylonitrile (AIBN), phosphate buffered saline (PBS) and Vitamin E were purchased from Sigma Aldrich. Polypropylene contact lens moulds were gifted by Bausch & Lombe Waterford.

Methods

- Differential scanning calorimetry (DSC): samples were analysed using a four cycle heating/cooling programme.
- Fourier Transform Infra-red (FTIR): samples were dried and ground to a fine powder prior to being mixed with KBr to make a disc.
- % Transmittance: Samples were measured using a Shimadzu 2401 at 600nm.
- Texture analysis: Measures physical properties of contact lenses.
- Refractive Index (RI): Samples were measured at 25ºC using a RFM 340 refractometer.

RESULTS TABLE 1

TEST	*A*	*B*	*C*	*Ref lens*
RI PP	1.332	1.332	1.332	1.335
RI LP	1.334	1.334	1.334	n/a
T_g °C PP	142	131	132	n/a
% T PP	91	92	92	97
% T LP	83	89	82	n/a
mg/lens E	0.33	0.65	0.88	n/a

%T = % transmittance
LP = loaded polymer
A, B, C Polymer formulations
PP = pure polymer
T_g= glass transition

DISCUSSION

Vitamin E was loaded into all lenses to varying degrees. The effect of this can be seen as the percentage transmittance decreased and the R.I. increased. Vitamin E is insoluble in water and was retained in the contact lens after loading. All lenses were characterised using the above methods, as well as FTIR and solid state NMR, to ensure polymers were completely cured. Differential scanning calorimetry data was collected to determine the effect of loading vitamin E on the polymer used and material retention/elution for polymer formulations, the addition of some Silane monomers could increase the solubility of the vitamin E in the polymer formulation and increase optical clarity.

Characterisation of the polymer lenses demonstrates that the critical lens properties were comparable to commercial lenses. No release of vitamin E was observed from the contact lenses over 48 hours in 90:10 PBS ethanol release media. These devices can be used for drug loading and elution studies which will determine the effect of vitamin E drug diffusion.

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Journals:
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DEVELOPMENT OF CONTACT LENSES FOR OCULAR DRUG DELIVERY

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Abstract

This study focussed on the development of a manufacturing and characterisation process for the production of contact lenses with controlled release properties. Three lens compositions with varying degrees of cross-linking (CL) density were studied, A (2.7% w/w), B (3.8% w/w) and C (7.8% w/w) The formulations were studied to determine the effect of CL density on the loading of Vitamin E. Vitamin E was loaded into the three polymer compositions with increased capacity observed with decreasing cross-link density, (1.8, 1.6 and 1.1 mg of Vitamin E loaded per lens for A, B and C, respectively). Critically Vitamin E was not released from the loaded lenses.

Introduction

Approximately 90 % of all ophthalmic drug solutions are applied as eye drops [1]. This delivery method has major disadvantages, for example: only 1-7 % of an eye drop reaches the intra-ocular tissue, while the rest is lost to tear drainage [2]. Controlling drug release to maintain a therapeutic dose for a prolonged period is almost uniquely provided by surgical implants, and as such, any opportunity to provide controlled drug delivery without the need for surgery would be universally welcomed. Drug delivery from contact lenses is a promising option.

The hydrogel contact lens was invented by Otto Wichterle, a large molecule chemist in Czechoslovakia [3]. Not only did Wichterle discover a novel material, Polymacon, he also developed spin-casting as a method of high volume manufacture for lenses, though the materials and monomers have changed and developed over time [4]. The aim from the beginning has been to produce high quality optics that are comfortable and easy for the patient to use. Polymethylmethacrylate (PMMA) was the initial material used due to its high optical clarity [5]. Poly hydroxyethylmethacrylate (HEMA) was then introduced and its high water content made it more comfortable, introducing the term "soft contact lenses" (SCL). Silane monomers and macromers were later introduced to increase oxygen permeability and increase wear time [4].

Studies on using contact lenses to deliver drugs to the eye began in 1965 [6]. The main barriers to providing a commercially viable contact lens as an ophthalmic drug delivery vehicle have yet to be overcome. The obstacles to drug delivery from a contact lens include: controlling drug elution, increasing the dose available from the lens, while ensuring the device will still operate as a contact lens during and after drug elution. The starting point for drug elution is loading the contact lens with the desired drug. There are a number of drug loading methods: soaking, molecularly imprinting approaches, inclusion complexes, surfactant carrier and direct embedding. Of the options listed above, only molecular imprinting offers a degree of control over the rate the drug is released. Using supercritical fluids to load drug into contact lenses is effective but very difficult to incorporate into a commercial manufacturing process [6]. All of these methods have disadvantages, either high burst release characteristics, negative impact on lens physical properties or inadequate loading capacity. Due to the small reservoir available in the contact lens even the addition of a diffusion barrier which can be used to control release, can have a negative effect as the loading capacity of the contact lens will be reduced. To date no drug eluting contact lens has been marketed.

Many studies use strips or polymer films for drug loading and release experiments. As the size and geometry of the lenses affect loading and release, this study used contact lenses prepared to industry specification. Not only can drug release be more accurately calculated but physical effects of loading and processing the lenses can be assessed for impact on the physical characteristics of the lens. The limited capacity within the lens for drug loading has to be increased to provide prolonged drug delivery. Drug release from SCL's can be characterised as burst release and requires attenuation. The goal is to control the release of material from the contact lens so a therapeutic dose is delivered for a prolonged period. To achieve this, a combination of approaches mentioned above may be required.

To develop the process and characterisation techniques for future drug loading studies initial lens design experiments and characterisation were performed. Vitamin E was loaded into the contact lenses manufactured in-house, as it has been used as a diffusion barrier by other researchers [7].The effect Vitamin E had on the three lens formulations was determined. The ability of the polymers to load Vitamin E and the capacity of the hydrogels was also investigated.

Experimental Procedure

Materials

Hydroxyethylmethacrylate (HEMA), N-Vinylpyrrolidone (NVP), ethyleneglycoldimethacrylate (EGDMA) and diethylaminomethacrylacte (DEAMA) were the monomers used with azobis-iso-butrylonitrile AIBN as the initiator (all purchased from Sigma Aldrich). Polypropylene contact lens moulds were provided by Bausch + lomb.

Lens manufacturing methodology

Polypropylene moulds were filled with the monomeric formulations (Table 1) using a pipette and were placed in a stainless steel polymerisation chamber and purged with nitrogen for 2 hours. The chamber was then placed in an oven and heated through a temperature profile as determined from DSC analysis of the monomeric formulations. The lenses were removed from the moulds by swelling with deionised water, then dried *in vacuo* prior to use.

Table 1: Monomer compositions used.

Material (g)	*A*	*B*	*C*
HEMA	2.25	2.32	2.20
NVP	--	0.77	0.77
DEAMA	1.32	--	--
EGDMA	0.10	0.124	0.25
AIBN	0.0027	0.0022	0.0022

Lens Characterisation Methodologies

I Thermal techniques

The effect of loading Vitamin E into the lenses was determined by differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA). Using, a Q2000 DSC and Q50 TGA, both TA instruments. Approximately 10 mg, of the ground lenses was analysed under 50 $mL.min^{-1}$ of nitrogen and a multiple heating cooling cycle method was used to remove any water absorbed prior to DSC analysis, during sample preparation. TGA samples of approximately 5-7 mg of the ground contact lenses were analysed using 5 $°C.min^{-1}$ ramp from room temperature to 550 ^{0}C under 50 $mL.min^{-1}$ nitrogen.

II Mechanical Testing

A stable micro system Texture Analyser xt plus was used to compare the mechanical properties of the lenses pre and post-loading. A P25 probe was used to compress a swollen lens and the force used was measured.

III Swelling

Contact lenses were dried for 24 hours *in vacuo* and accurately weighed prior to swelling studies. The lenses were swollen for 24 hours at 25 ^{0}C in DIW. Prior to weighing the lenses were dabbed dry on lint free tissue. Equilibrium water content (EWC) was calculated using the equation below.

W_s = swollen weight W_d = dry weight

$$EWC\% = \frac{Ws-Wd}{Wd} x100$$

IV Light transmittance

% transmittance was measured using a Shimadzu U.V. Vis 2401-PC set at 800nm. The lenses were suspended in the path of the beam and measured with reference to air.

V Refractive Index

The refractive Index was measured at 25°C using a RFM Bellingham Stanley 3480 refractometer, a Memmert water bath and Ismatec ms-reglo peristaltic pump.

Vitamin E loading method

I Loading procedure

Lenses were loaded with Vitamin E by soaking in a 50 $mg.mL^{-1}$ solution of Vitamin E in ethanol. Each lens was placed in a micro centrifuge tube; 1 mL of the loading solution was added and the lenses were allowed to equilibrate over 5 days.

II Loading Assay

The loading assay was performed by placing the drug loaded lenses in absolute ethanol for 24 hours and analysing the amount of Vitamin E released by HPLC. An Agilent HPLC 1200 with U.V. detection at 229 nm was employed, using a Zorbax column S8 C8 5µm 4.6x150 mm and methanol-water (95:5) as the mobile phase.

III Release Assay

Vitamin E loaded lenses were placed in micro centrifuge tubes with 1 ml of phosphate buffered saline: ethanol (90:10). The samples were directly analysed using the same HPLC method as previously outlined.

Results and Discussion

The manufacturing process was developed by performing DSC analysis on the monomer compositions to create a temperature profile for polymerisation in an oxygen depleted environment. This process delivered lenses of consistent shape and geometry that closely matched commercial lenses. The formulations used were chosen as the lenses produced had appropriate physical characteristics, (Criteria: lens integrity i.e. ability to be handled and adhesion to polymers or glass). In this work the cross-linker density was modified so that its effect on the loading of Vitamin E and also the contact lens physical characteristic could be explored. This preliminary work was a necessary prerequisite to assess these contact lenses as potential platforms for the delivery of hydrophilic drugs.

Thermal techniques, TGA and DSC, showed that loading material into the polymer matrix did have an impact on polymer properties. The effect was limited; the thermal traces of Vitamin E loaded polymers were similar to traces of the polymers before they were loaded (Figure 1&2). Glass transition temperature (T_g) is a temperature below which polymer segments do not have the energy to rearrange or rotate, materials in this state are brittle or glassy [8]. T_g is also dependant on heating rates applied as well as the degree of polymerisation and the amount of plasticiser present. As water is a

plasticiser a 3 cycle heating, cooling method was employed. The water loss from the samples occurred over a wide temperature range and a clearer T_g was obtained when the sample was dried in a previous cycle. There is a definite trend in T_g of the three formulations prior to loading. T_g increases as CL density increases across the formulations (Table 2).

Table 2: Glass transition temperatures.

Material	*A*	*B*	*C*
T_g °C Loaded lenses	120.1	132.8	139.2
T_g °C Lenses only	122.4	133.1	142.4

There is a definite downward trend in T_g for polymer formulations post loading due to the plasticising effect of Vitamin E on the polymer matrices. The change in T_g, for formulation B, was small and within instrument variability at 0.3 °C. The Formulation with the highest CL density showed the largest drop in T_g after loading Vitamin E. TGA data also displayed a trend, where the thermal traces for each loaded polymer formulation showed a faster drop in weight than polymer formulations with no added Vitamin E. This is due to the loss of the thermally less stable Vitamin E from the polymer matrix as it would be expected to degrade and cause a loss in weight from the polymer in the experiment.

Swelling

There is a pronounced effect on the equilibrium water content (EWC) with polymer formulation after soaking in ethanol. All polymer formulations have reduced EWC values ranging from 10-15 % after soaking (Table 3).

Table 3: Equilibrium water content data.

Material	*A*	*B*	*C*
EWC % loaded	24.6	35.1	46.5
SE	1.6	0.8	0.6
EWC % Lens only	35.4	44.8	61.7
SE	2.9	2.3	1.0

SE = Standard error

The difference in swelling could be explained by loading of Vitamin E, but there is also the possibility of ethanol remaining in the polymer matrix. An additional drying step was employed to remove any residual ethanol after soaking. Lenses were dried at 60 °C for five hours in a vacuum oven prior to use in the swelling experiment. Standard error for replicate swelling samples from each formulation is high for the polymers prior to loading. This indicates inhomogeneity in polymerisation and there may be an amount of un-polymerised monomers trapped in the polymer matrix. This would impact loading and swelling characteristics of the polymers.

RI/% Transmittance

Optical properties are affected by loading material into the lens particularly if such materials are not soluble in the monomeric formulation. Refractive index does not seem to be as easily affected based on this study. Data is detailed in Tables 4 and 5.

Table 4: %Light Transmission at 800nm analysis of contact lenses.

Material	*A*	*B*	*C*
% T loaded lens	88.5	86.1	83.5
% T lens only	97.5	97.0	96.0

Table 5: Refractive index analysis of contact lenses.

Material	*A*	*B*	*C*
Loaded lenses	1.333	1.332	1.333
Lenses only	1.333	1.333	1.333

The amount of material loaded did have an impact on the % light transmission as the material with the lowest loading of Vitamin E had the lowest % transmission. The solubility of the material to be loaded in the formulation is a critical parameter. The refractive index (RI) for saline was measured at 1.333 and the (RI) for an AcuVue lens was 1.336 so there is little impact on optical clarity after loading Vitamin E into the polymer formulations with values obtained, comparable to commercial lenses. The ability to assess the effect, on optical clarity is critical to delivering a functioning device to the patient. Adding silane monomers to the formulation should increase the solubility of the Vitamin E and increase the optical clarity of the loaded lens.

Vitamin E Loading

Vitamin E was successfully loaded into the three contact lens polymer formulations. The trend in loading showed an increase in amount of Vitamin loaded as the cross-linker concentration decreased. Formulation A facilitated the greatest uptake of Vitamin E, but upon soaking in ethanol these lenses showed signs of damage, as cracking and breaking was evident in all lenses. The formulation appeared to swell to such an extent that it could not maintain integrity. Not all lenses loaded reproducibly as can be seen from the high SE (Table 6), with the exception of C, which loaded the same amount across the lenses analysed. This formulation has the highest amount of cross-linker and is therefore the most structured polymer of the three. Loading 1 mg of Vitamin E per lens equates to 5% loading. In other studies Vitamin E loading ranged from 10-27% and did impact subsequent drug release at these concentrations in silane contact lens formulations[9].

Table 6: Vitamin E loading concentrations (mg / lens).

Material	*A*	*B*	*C*
Vitamin E	1.77	1.55	1.08
SE	0.36	0.61	0.07

SE = Standard error

Vitamin E Release

No Vitamin E was released from the lenses over 24 hours. This is due to the poor water solubility of Vitamin E. The addition of 10% ethanol to the release media ensured that any Vitamin E released would be solubilised and be available for analysis by HPLC. The

lenses were placed in 1 ml of release media for one hour prior to being transferred into another micro centrifuge tube. This was performed to remove any Vitamin E solution left on the surface of the lenses after the evaporation of the Vitamin E ethanol solution. This result is critical for the use of Vitamin E as a diffusion barrier. The material is present in the lens and will not decrease over time so it should provide a consistent barrier to release of any additional material to the lens.

Mechanical Testing

The loaded lenses all displayed a decrease in the force required to deform the contact lenses of the loaded polymers versus polymer with no Vitamin E. A number of formulation A lenses were damaged, during the loading process but approximately 50% of these lenses were intact and available for analysis. A, B and C lenses exhibited a reduction in tensile strength and more closely matched the force profile of a commercial lens after loading. Figure 3 is an example of the force profile for the compressed lenses.

Conclusions & Future Work

In this research we set out to manufacture and load lenses as well as develop a suite of characterisation tests for the analysis of lenses produced. The lens manufacturing process and characterisation tests developed have been used successfully. These tests have established that loading increases as cross-linker density decreases for the formulations used. Also the loaded Vitamin E was not released in phosphate buffered saline over 24 hours. The contact lenses manufactured were capable of being handled and used in laboratory trials. Each lens was consistent in size and shape and successfully loaded Vitamin E. The analysis was performed on single lenses and demonstrated that assay of single lenses was possible. Swelling data and inconsistent loading could be caused by or exacerbated by the presence of unreacted monomers in the polymer matrix. Addition of a diluent should allow the movement of monomers during polymerisation and prevent monomers becoming entrapped in the forming polymer matrix. Glycerine is a viable option for use as a diluent. This will increase the consistency of the lenses in regard to completeness of polymerisation and ensure more reproducible results. Ethanol was not a compatible solvent for use with formulation A and this solvent damaged the lenses after soaking. The drug loading process and the polymer formulation must be compatible or the resulting devices will be unusable as contact lenses. Cross-linker concentration has an impact on both loading parameters and physical characteristics. Cross-link density needs to be tailored to the loading process to minimise damage or physical changes in the lenses.

This study has revealed that it is possible to manufacture lenses in-house and load these lenses with a diffusion attenuator Vitamin E. The attenuator is retained in the polymer matrix so subsequent hydrophilic material can be loaded without loss of the diffusion barrier. It is also possible to tailor polymer composition in order to control lens characteristics. This initial research demonstrates that it may be possible to formulate drug delivery lens prototypes using a variety of drugs as well as loading techniques.

Acknowledgements

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3. Goodlaw, E., *A personal perspective on the history of contact lenses.* International Contact Lens Clinic, 2000. **27**(4): p. 139-145.
4. Nicolson, P.C. and J. Vogt, *Soft contact lens polymers: an evolution.* Biomaterials, 2001. **22**(24): p. 3273-3283.
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Key words

Drug delivery, Hydrogel, Contact lenses, DSC and texture analysis.

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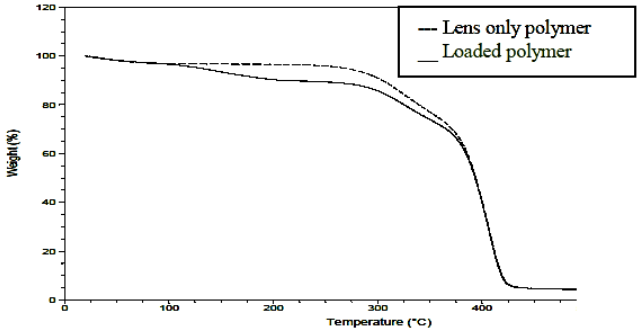

Figure 1: Thermo gravimetric analysis of polymers, pre and post loading of Vitamin E.

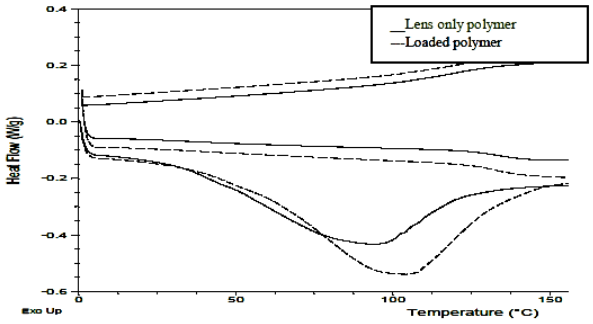

Figure 2: Differential scanning calorimetry traces of polymers pre & post Vitamin E loading.

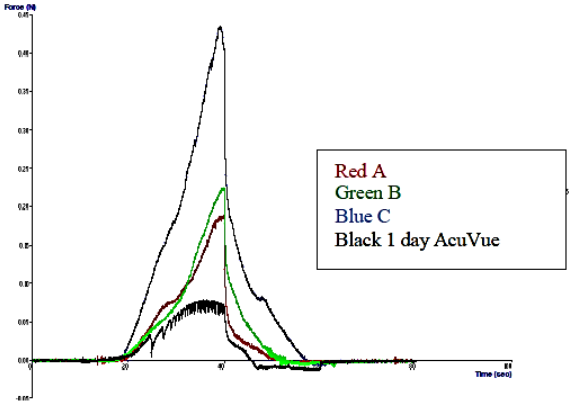

Figure 3: Example of data from Textural analysis of contact lenses.

DEVELOPMENT OF MOLECULARLY IMPRINTED CONTACT LENSES FOR OCULAR DRUG DELIVERY

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Ocular diseases are treated using eye drops, ointments and gels, which account for 90% of the ocular drug delivery market [1]. These drug delivery methods, however, are not very effective, with 95% of the delivered dose lost by tear drainage and blinking [2]. Furthermore, therapeutic dose levels are only present for a short period of time after use [3]. This leads to issues with side effects and poor disease management. Contact lenses have the potential to be used as medical devices that can maintain the therapeutic dose more efficiently, so as to more effectively manage these diseases.

The objective of this research is to produce novel medical devices for ocular drug delivery. To this end, the research focusses on state of the art techniques and applications of controlled drug release from contact lenses. Lens design, in-house lens manufacture, material characterisation, and drug-elution optimisation were used to manufacture and load contact lenses with pharmaceutically relevant materials.

It has been successfully demonstrated that it was possible to manufacture custom lenses in-house and to load them with the chosen drug, thus preparing ocular devices to commercial standards. These prototype drug delivery devices are being investigated to establish their potential for use in the treatment of ocular diseases. To achieve greater control of drug delivery, molecular imprinting of template molecules has been performed to determine the impact on loading and release of drugs from polymer matrices, as molecular recognition in the polymer matrix of the contact lens has the potential to increase drug loading and to attenuate release from the polymer matrix [4].

Two pharmaceutically relevant compounds have been used as template molecules. 2-hydroxyethyl methacrylate (HEMA) was used as the backbone monomer, while ethylene glycol dimethacrylate (EGDMA) and N-vinyl-pyrrolidone (NVP) were used as the cross-linking and functional monomers, respectively. The use of a suitable diluent was investigated to aid polymerisation and to provide the required physical characteristics for the contact lens.

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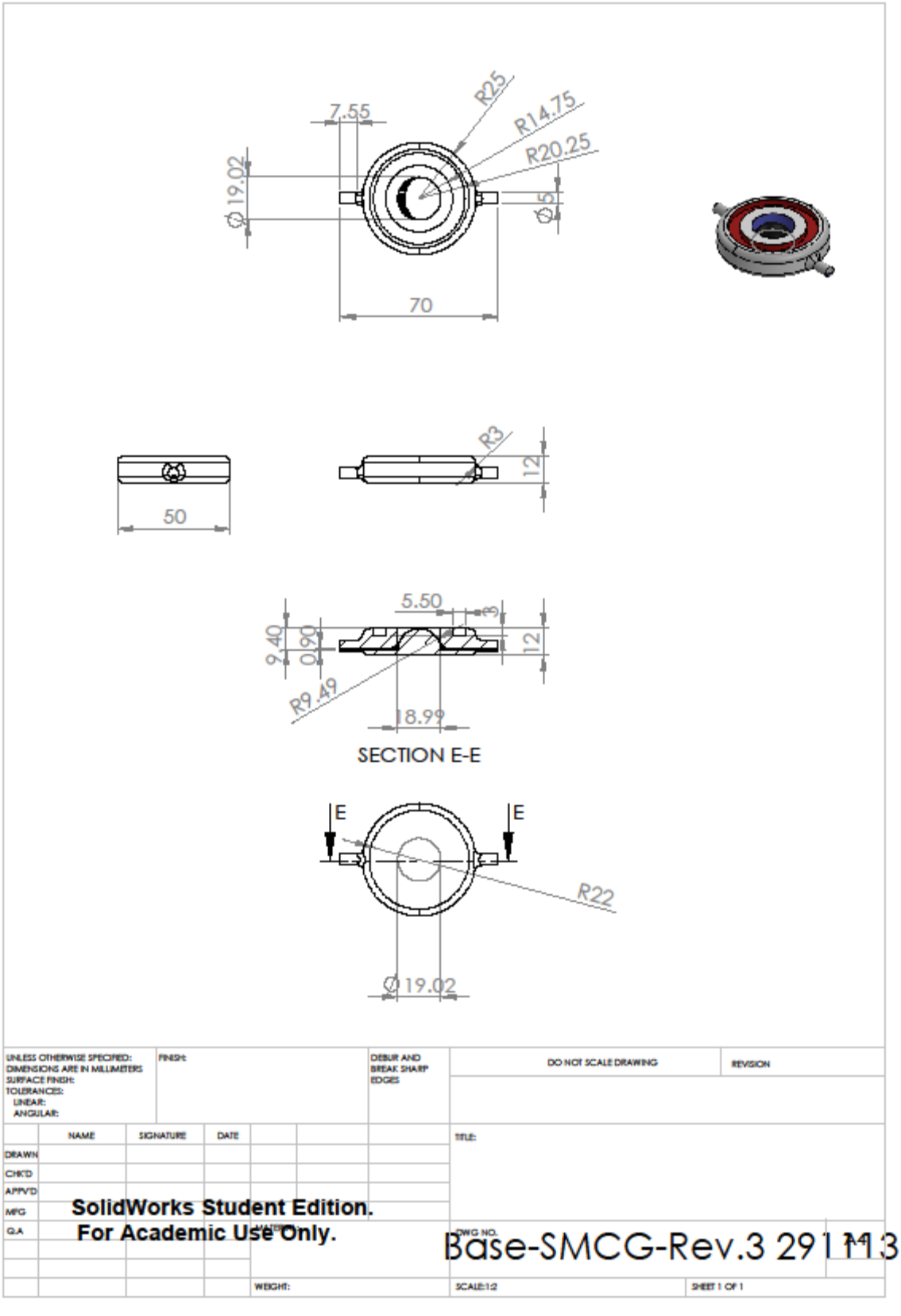
APPENDIX 2

SCHEMATIC OF

MICROFLUIDIC DEVICE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18.99
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LINEAR:
ANGULAR:
FINISH:
DEBUR AND BREAK SHARP EDGES
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APP'V'D
MFG
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