EXTENDED OCULAR DRUG DELIVERY USING HYALURONIC ACID-CONTAINING MODEL SILICONE HYDROGEL MATERIALS

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By

MYRTIDIOTISSA KOROGIANNAKI, Dipl. Ch. E.

A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfillment of the Requirements

For the Degree

Master of Applied Science

McMaster University

MASTER OF APPLIED SCIENCE (2012) McMaster University
(Chemical Engineering Department) Hamilton, Ontario

TITLE: Extended Ocular Drug Delivery using Hyaluronic Acid containing model Silicone Hydrogel Materials
AUTHOR: Myrtidiotissa Korogiannaki, Dipl. Ch. E. (University of Patras, Greece)
SUPERVISOR: Professor H. Sheardown and Professor L. Jones
NUMBER OF PAGES: xvi, 143

ABSTRACT

While eye drops are a well-accepted and convenient method for ocular drug delivery, they exhibit significant limitations such as poor drug bioavailability, low ocular residence time, pulsatile delivery profiles in the tear fluid as well as the need for patient compliance. Silicone hydrogel contact lenses have been proposed as alternative ocular drug delivery systems due to their potential for targeted delivery to the corneal surface and high oxygen permeability. The ability of novel hyaluronic acid (HA)-containing silicone hydrogel materials to release timolol maleate (TM), a β-blocker widely used for glaucoma treatment, or ketotifen fumarate (KF), an anti-histamine administered for ocular allergies was examined. Polyvinylpyrrolidone (PVP) was used as an alternative wetting agent for comparative studies.

The model silicone hydrogels used consisted of a hydrophilic monomer, either 2-hydroxyethyl methacrylate (HEMA) or N, N-dimethylacrylamide (DMA) and a hydrophobic silicone monomer of methacryloxypropyltris (trimethylsiloxy) silane (TRIS). The wetting and the therapeutic agent were added to the polymer mixture during synthesis through direct entrapment. The reaction was performed by UV induced free-radical polymerization and the compositions of focus are pHEMA/TRIS (90/10 wt%) and DMA/TRIS (50/50 wt%). The impact of the wetting agent on the swellability, surface wettability, optical transparency and *in vitro* drug release was studied.

Non-covalent entrapment of the wetting agent into the silicone hydrogels led to materials with releasable wetting agent. Simultaneous drug and wetting agent incorporation resulted in modified silicone hydrogel materials with slightly increased water content and significantly improved surface wettability ($p<0.05$). DMA/TRIS materials exhibited higher surface wettability and swellability compared to pHEMA/TRIS.

In addition, the optical transparency of these materials was not affected by drug loading. However direct entrapment of HA decreased their optical clarity. *In vitro* release of TM and KF showed that TM was released within 4 days for DMA/TRIS and over a 14 day period for pHEMA/TRIS silicone hydrogels. However, KF release lasted 24 days in DMA/TRIS silicone hydrogels and 36 days in pHEMA/TRIS materials. For both therapeutic agents used in the current research, non-covalent entrapment of wetting agent and its MW did not significantly change the release kinetics, however the release rate of TM was slowed and controlled by the release of the HA, due to electrostatic interactions between protonated TM and anionic HA. This relatively low alteration in release kinetics may be attributed to the low amount of wetting agent added as well as to the fact that it is also released through the matrix of the materials along with the drug. Generally, pHEMA/TRIS silicone hydrogels showed a higher and more controlled release profile than DMA/TRIS, due to differences in the degree of swelling as well as different interactions developed between the silicone hydrogel matrix and the therapeutic agent.

The development of silicone hydrogel materials capable of simultaneously releasing a therapeutic and a wetting agent for an extended period of time and in a sustained manner can have a significant potential as extended drug delivery systems for the treatment of front of the eye diseases while also possibly providing comfort during wear.

ACKNOWLEDGEMENTS

This research project would not have been possible without the support and encouragement of many people. First of all, I would like gratefully to thank my family for being constantly there for me. Their love and support were always abundant in my life allowing me to follow my dreams, no matter how far from home I am. I would like to sincerely thank my dad for believing in me and giving me the chance to come to Canada, my mom for always supporting my education and making me believe in my abilities and my brother who despite being younger gives me the wisest advices.

I would like to express my sincere gratitude to my supervisor Dr. H. Sheardown. Heather, I greatly appreciate all your critical insights, thoughtful comments and enthusiastic support throughout my Masters and my thesis writing. Even though I was many times “a stubborn bulldog” you were always abundantly helpful and offered me invaluable guidance. I would also like to thank you for believing in me and giving the chance to continue my PhD in your group. I would also like to thank Dr. Jones, for his guidance and support throughout my Masters.

Moreover, I would like to express my appreciation to Dr. J. Vlachopoulos and Dr. D. Weaver for helping me over my concerns and general academic pursuits, having their door always open for me. Also, I would like to thank my Greek professors who gave me the appropriate education so as to be able to pursuit my endeavors.

I am also thankful to all the members of the Sheardown lab (Laura, Marta, Scott, Ben, Fran, Ivana, Michelle, Vida, Robyn, Marion, Andrea and Holly) for their advices and getting along with all my release samples that are still everywhere in the lab - and especially Giuliano, Lina and Stefan for sharing their knowledge and experience with me,

as well as the summer students Nicole and Steven for their help and Saman Mohammadi for his assistance with the ELISA assay.

I would also like to thank all my friends “new” and “old” for helping me to get adapted to my new life here in Canada. I owe you all so much, especially those that I met in the beginning. ”Giant” across of my desk, Pedram, Coalsie and Ammes, I would like to thank you for all those crazy moments of laugh and happiness!

I would also like to acknowledge NSERC for financial support and 20/20 NSERC Ophthalmic Network for offering me tremendous experience by attending the 20/20 conferences.

I would like to dedicate my work to my dad, to Alex and especially to my mom (cliché but true) who stayed with me, in Hamilton (!), supported me and tolerated my whining throughout my thesis writing, cooked me real food and motivated me every single day; and to my aunt, Anastasia, who was always proud of me but did not have the chance to see who I am today.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

1. Introduction

The physiology and the anatomy of the eye render this organ impermeable to exogenous substances, in order to shield the visual pathway from toxicants. As a result, successful drug delivery of ocular therapeutics is very challenging as the drug has to penetrate the protective barriers without harming or damaging permanently any healthy tissue.

Accounting for approximately 90% of the ophthalmic medications, the most common method of treating diseases of the anterior segment of the eye is instillation of topically administered formulations such as eye drops, emulsions and suspensions. Eye drops can deliver high drug concentrations to the targeted tissue non-invasively, but this requires frequent dosages to achieve therapeutically effective concentrations due to the low drug bioavailability. The latter is a significant drawback for eye drops that limits their applicability, since only 5% of the drug initially instilled gets absorbed by the corneal surface. The short residence time of the drug on the ocular surface due to reflex tearing and tear turn-over in combination with drug absorption by the nasolacrimal duct and other vascularized ocular tissues, lead to surprisingly low drug bioavailability of most topically administered medications and undesired systemic side effects.

Patient compliance with topical solutions is also problematic, especially in the case of chronic diseases like glaucoma that need constant management. Based on recent studies, only 22–33% of patients self-administered their glaucoma medications properly (Robin et al., 2008), while 50% of glaucoma patients discontinued all topical ocular hypotensive therapy within six months (Nordstrom et al., 2005). Therefore, the development of an effective ocular drug delivery device which can overcome all the

disadvantages exhibited by conventional formulations, like eye drops, is critical for the safe and successful treatment of ophthalmic diseases.

During the last decades, researchers have focused on developing therapeutic contact lens materials with improved transcorneal penetration, able to provide controlled or sustained drug delivery for an extended period of time. Although the main use of contact lenses is to correct ametropia, they have been also used for relief of post-surgery ocular pain, promotion of corneal healing, mechanical protection and support (Alvarez-Lorenzo, Hiratani et al., 2006). The introduction of silicone hydrogel contact lenses, in the late 1990's, was the key for designing therapeutic contact lenses of continuous wear (overnight wear as well for up to 30 days) since they provide significantly higher oxygen permeability avoiding undesired hypoxic side effects. There are approximately 125 million contact lens wearers worldwide (Barr, 2005), with this number exponentially increasing every year making contact lenses one of the abundant categories of biomedical devices in clinical use (Karlgard et al., 2004). The use of contact lens materials as a drug delivery devices is a classic example of what has become known in regulatory agencies as a combination device (Kramer, 2005) as they can simultaneously correct vision and deliver medication (bandage lenses) based on patient's need. Because of the popularity of these novel devices, special regulations and procedural approvals have begun to be addressed in the United States (Office of Combination Products, 2006) and European Union regulatory communities.

At the research level, many different material development strategies have been studied in order to design a conventional or silicone contact lens based drug delivery system that can provide controlled or sustained release profiles for extended periods of time. Colloid and nanoparticle laden contact lenses, hydrophobic diffusive barriers

incorporated within the silicone hydrogel matrix, surface coated with drug-loaded layers and molecularly imprinted contact lens materials are some of the different techniques followed recently in order to improve the release kinetics of the drug and hence achieve prolonged release. Even though, these materials exhibit interesting results the clinical trials are fairly limited. Recently, Vistakon Pharmaceuticals, LLC (Philadelphia, PA, U.S.) has completed a multicenter Phase III clinical trial in humans for presoaked the contact lenses in a ketotifen solution, an antihistamine drug used for the treatment of allergic conjunctivitis (ClinicalTrials.gov, 2010) with the results demonstrating the increased bioavailability that contact lens material can exhibit as ocular drug delivery systems.

In addition, comfort during extended or continuous wear of contact lens wear is extremely important, especially in the case of silicone hydrogel contact lenses since they more hydrophobic than conventional lenses and able to reside on the surface of the eye for longer periods of time. It is reported that discomfort is the principle reason of discontinuing contact lens wear, caused mainly due to protein deposition or dry eye symptoms (Fonn, 2007). As a result, different methods have been developed to enhance the surface hydrophilicity of silicone contact lenses, including surface plasma treatment and incorporation of an internal or releasable wetting agent. In previous work, the Sheardown group has developed model silicone hydrogels using hyaluronic acid (HA) as internal non-releasable wetting agent which improved their surface hydrophilicity and water content (van Beek et al. 2012; Weeks et al. 2011). When these materials were used as drug delivery devices of an antibiotic and anti-inflammatory drug, the presence of HA in the matrix led to higher and therapeutically effective levels of drug released for 6 days, suggesting promising properties for HA in ocular drug delivery (Nguyen et al., 2012). Moreover, HA can interact with the ocular mucins when delivered on the surface

of the eye, creating a “artificial mucin” (Pirie et al., 1948) that can cover the surface of the contact lens and counteract with tear film destabilization that occurs during contact lens wear, leading possibly to higher degree of comfort.

Therefore, the aim of this work is to investigate the *in vitro* release of two model ophthalmic drugs, timolol maleate (antiglaucoma) and ketotifen fumarate (antihistamine), under physiological conditions from previously developed model silicone matrices that also contain releasable HA as wetting agent. Important properties for ocular drug delivery systems such as water content, transparency, surface wettability of HA and drug containing materials are determined as well. The novelty of this work is based on the simultaneous release of a therapeutic and wetting agent that leads potentially to higher degree of comfort during the therapy duration. So far, controlled and extended release of a single therapeutic agent or a wetting agent has been reported, whereas no papers have been published concerning a multi-drug loaded contact lens based drug delivery system.

2. Literature Review

2.1 Silicone Hydrogel Contact Lens Materials

2.1.1 The components, structure and properties of silicone contact lenses

Hydrogels are hydrophilic polymers than can absorb water up to an equilibrium state without dissolving due to crosslinking that creates a three-dimensional network. The presence of a crosslinker in the hydrogel matrix is significant because basic properties of these materials such as definite shape, mechanical strength and transparency are not altered upon hydration. The monomers commonly employed in contact lens materials including 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA) along with N-vinyl pyrrolidone (NVP) and methacrylic acid (MA) determine their physical and chemical properties. However, conventional hydrogel materials are not adequate for extended or continuous wear due to their insufficient oxygen permeability.

An extended wear contact lens should be able to provide adequate hydrophilicity, as well as oxygen permeability (intrinsic to hydrophobic materials such as polysiloxanes and fluoropolymers), mechanical strength in a hydrated state, compatibility with biological tissues, optical transparency and stability arose. Silicone hydrogel materials embody these properties. In silicone hydrogel materials, siloxanes are combined with conventional hydrogel monomers (Sweeney, 2000). However, combining a highly hydrophilic component with a non-miscible highly hydrophobic component like silicone was not an easy process (Sweeney, 2000).

Usually, in silicone hydrogel contact lenses the hydrophilic monomers used include HEMA, NVP and/or N,N-Dimethylacrylamide (DMA) and hydrophobic monomers/macromers of polydimethylsiloxane (PDMS) and/or methacryloxypropyl

tris(trimethylsiloxy) silane (TRIS). The hydrophilic domains permit the diffusion of water through the silicone hydrogel matrix while the silicone phase provides the high oxygen permeability essential for sustaining the health of the eye (Xu, Li, & Sun, 2011). TRIS macromers are usually copolymerized in the silicone hydrogel contact lens network because it is an effective strategy to partially replace the expensive PDMS while maintaining the oxygen permeability (Lai, 1993). Moreover, the presence of TRIS can also reduce the modulus of the final silicone hydrogel matrix by reducing the cross-linking density (Lai, 1993).

In conventional hydrogels the oxygen is delivered to the corneal surface through the water absorbed within hydrophilic domains of the hydrogel. As a result, in order to achieve higher oxygen permeability for a conventional hydrogel the water content should be increased. However, even when conventional contact lenses have 80 wt% EWC there is insufficient oxygen permeability through the aqueous phase. On the other hand, silicone hydrogels even though they have lower EWC due to the incorporation of the hydrophobic silicone they are characterized by higher oxygen permeability than that of conventional contact lenses due to higher solubility of oxygen in the silicone than in the aqueous phase.

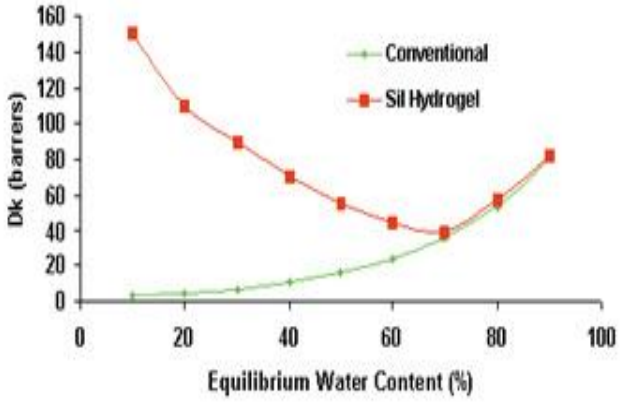

Figure 2.1: Relationship between Oxygen Permeability and Equilibrium Water Content of commercially available silicone and conventional contact lenses (Jones, 2002).

In addition, the diffusion of water through the hydrophilic domains of silicone hydrogels begins when the water content in the lens reaches ~20% (Peppas & Brannon-Peppas, 1990). This provides wettability, on-eye lens movement, and comfort. Ionic permeability through the aqueous phase of the silicone contact lens is significant as it determines the lens' on eye motion (Domschke et al, 1997) while hydraulic flow through the lens allows for the maintenance of a hydrodynamic boundary layer between the contact lens and the corneal surface avoiding hydrophobic binding (Sweeney, 2000).

The silicone hydrophobic phase leads to an increase in the tensile strength and Young's modulus of silicone contact lenses. A suitable Young's modulus of the hydrogel is in the range from 0.5–1.5 MPa for appropriate contact lens design (Nicolson et al., 1998). As it would be expected, the incorporation of silicone into polymer matrix makes these materials significantly stiffer than the corresponding conventional hydrogels.

Therefore, silicone hydrogels are characterized by water permeability as high as conventional hydrogels while on the same time they have significantly higher ion and

oxygen permeability due to connected pores shown in Figure 2.2, since they are designed for continuous wear in order to avoid adverse corneal hypoxic complications.

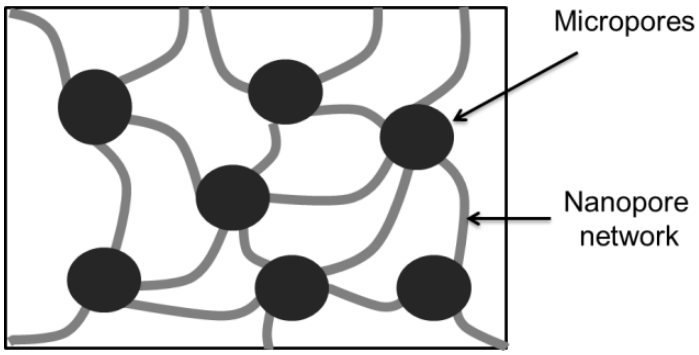

Figure 2.2: Schematic illustration of possible nanopore and micropore interconnected structure of silicone hydrogel contact lens materials.

2.1.2 Surface Treatment and Characteristics of silicone contact lenses

The presence of the hydrophobic siloxane groups however leads to decreased wettability and increased protein and lipid deposition on the lens surface (Grobe, 1999). Therefore, the surface of the silicone hydrogels must be modified in order to make it more wettable and hydrophilic.

Surface modification by plasma treatment or plasma oxidation were the first methods used (Nicolson 2003; Tighe 2004). Bausch & Lomb used the technique of plasma oxidation for the balafilcon A (PureVision) lens, modifying siloxane groups to silicate. Upon plasma oxidization, well distributed hydrophobic glassy islands are present on the surface of the contact lens (Kunzler 1999, López-Alemany et al. 2002) leading to enhanced surface wettability able to support stable tear film without alteration in the oxygen permeability (Sweeney 2000; López-Alemany et al. 2002). Another example of surface plasma treatment is on lotrafilcon A and lotrafilcon B (Focus Day & Night and O_2 Optix) silicone hydrogel contact lenses. These lenses have a high refractive index and

the thin (25 nm) plasma coating of a homogenous hydrocarbon polymer film that is more wettable than the underlying contact lens surface, without affecting the oxygen permeability of the underlying material (Nicolson et al. 1998; González-Méijome et al. 2006).

Johnson & Johnson instead of using plasma treatment to enhance the surface wettability of their silicone hydrogel lenses incorporated a high molecular weight wetting agent, polyvinylpyrrolidone (PVP), into the polymer network of the material during synthesis (Acuvue® Advance™ and Acuvue® Oasys™). PVP is a highly hydrophilic polymer that migrates to the surface and thus improving the lens wettability. This treatment also yields a stable tear film while the oxygen permeability is not affected (Maiden et al. 2002; Steffen & McCabe 2004; McCabe et al. 2006; Jones, 2007). Finally, comfilcon A (Biofinity®) and enfilcon A (AVAIRA™) by CooperVision did not have any surface treatment or internal wetting agent. However, these silicone hydrogel contact lenses contain two silicone-based macromers incorporated into the lens material with other hydrophilic monomers that can provide relative high surface wettability (Jones, 2007).

2.2 The eye and anterior segment

The human eye is one of the most complex organs in the body with an anatomy, physiology and biochemistry that makes it extremely impermeable to foreign substances shielding the visual pathway from toxins. The eye can be divided into two main parts, the anterior and the posterior segment with their complex structure being depicted in Figure 2.3. The anterior segment consists mainly of the cornea, the iris, the ciliary body and the

lens while the posterior segment is comprised by the vitreous humour, the retina, the choroid and the optic nerve.

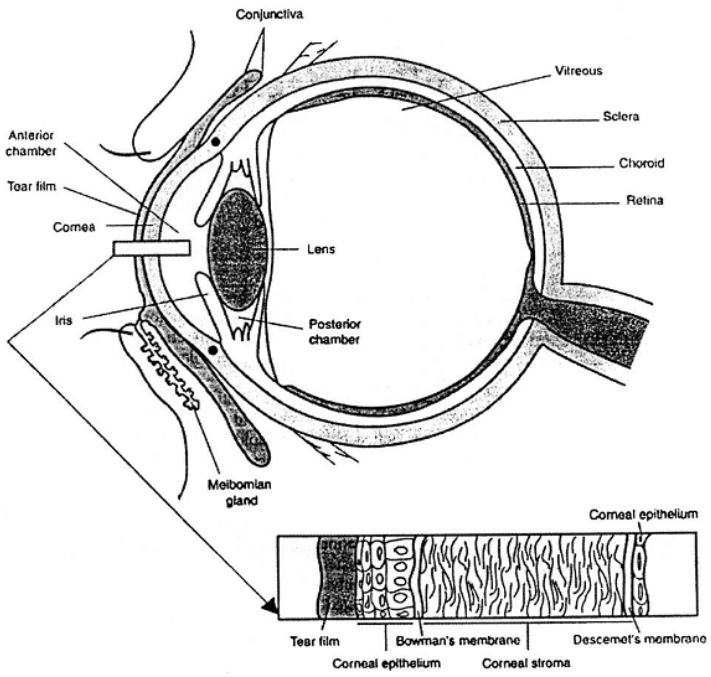

Figure 2.3: Schematic representation of the anatomy of the human eye (Ludwig, 2005).

Contact lens materials can be used as corrective, cosmetic (tinted lenses) or therapeutic devices with the latter aiming to treat diseases correlated with the anterior segment of the eye, like inflammation, infections, allergies, and glaucoma. Of particular relevance to the development of front of the eye drug delivery systems are the interactions developed among the tear film and the corneal, conjunctival and scleral tissues.

2.2.1 Tear Film

The tear film, the interface between the ocular surface and the environment, is responsible for maintaining hydration and an optically smooth surface necessary for vision. It is also responsible for protecting the eye from any injury and infection, as well as providing adequate oxygen. The tear film is comprised by three layers including the lipid, the aqueous and the mucus layer (Davson, 1990) (Figure 2.4). The outermost lipid layer acts as a barrier to tear fluid evaporation and is secreted by the Meibomian glands in the eye lids (Sharma & Ruckenstein, 1985). The aqueous layer, containing dissolved oxygen, nutrients, proteins and antibacterial enzymes, is responsible for spreading the tear film over the ocular surface and promoting osmotic regulation. The mucus layer provides surface integrity maintenance and adequate lubrication upon blinking to the underlying hydrophobic epithelium. The mucins are secreted by the goblet cells while the aqueous layer is secreted by the lacrimal glands which are located above the eye. In humans, the tear film is estimated to be approximately 7 μl in volume and 4-9 μm thick, while the cul-de-sac can contain as much as 30 μl in total if blinking is avoided. The pH of the tears ranges from 6.5 to 7.5.

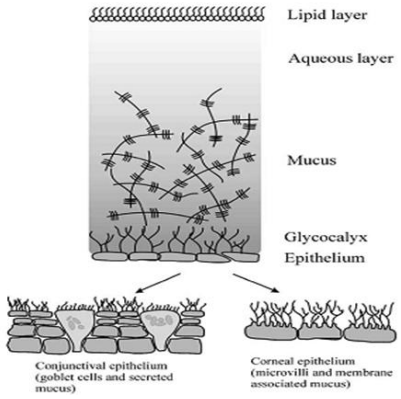

Figure 2.4: Schematic representation of the precorneal tear film (Ludwig, 2005).

2.2.2 The Cornea

The cornea, the outer-most tissue in the eye, is an optically transparent avascular tissue that acts as the primary refractive element of the eye, providing approximately 70% of the optical power (Tripathi,1984). It also acts as a physical and mechanical barrier, limiting the entry of exogenous substances into the eye as well as protecting the sensitive inner ocular sensitive structures from pathogens. In humans, the cornea is approximately 0.52 mm thick in the centre and 0.97mm thick peripherally at the limbus (Tripathi ,1984, Hitzenberger et al., 1994) and has a diameter of approximately 12 mm (Newell, 1986). The cornea consists of five layers: the epithelium, Bowman's membrane, stroma, Descement's membrane and the endothelium layer (Tripathi ,1984). Since the cornea is not vascularized, nutrients are received via diffusion either from the tear film or the aqueous humour, while the oxygen is derived directly from the air.

On the outer side, the corneal epithelium is comprised of 6 lipophilic cellular layers, which act as a barrier to ion transport, restricting the absorption of hydrophilic drugs. The corneal epithelium has unmyelinated nerve endings, making it highly sensitive to touch, temperature and chemicals, an important factor for preventing any corneal injury (Maurice, 1962). It is also responsible for providing a smooth wettable surface. Therefore any inflammation or edema of the corneal epithelium will affect the smoothness of the tear-air interface, reducing the acuity of vision. The tight junctions of the corneal epithelium act as selective barriers for small molecules, delaying the paracellular penetration of drugs from the tear film.

Below the epithelium, Bowman’s membrane is comprised mainly of the cell adhesion protein laminin and irregularly arranged mainly Type I collagen fibres. The latter are attached to and interact with each other (Maurice, 1962). The stroma,

representing more than 90% of the thickness of the cornea (Maurice, 1962), acts as a barrier to the penetration of lipophilic substances. Human stroma is composed mainly of a lamellar arrangement of collagen fibrils; their parallel structural arrangement is important for maintaining normal vision acuity. Fibroblasts (keratocytes) represent 2-3 volume % of total corneal stroma. Below the stroma is Descement's membrane which acts as the basement membrane of the corneal endothelium and is an acellular layer of type VIII collagenous filaments (van der Rest & Garrone, 1991), with a thickness dependent on age. The innermost monolayer of hexagonal-shaped cells, the corneal endothelium, is responsible for corneal hydration, “pumping” significant amounts of water from the stroma to the aqueous humour (O’neal & Polse, 1986). Unlike corneal epithelial cells, corneal endothelial cells do not regenerate which can cause problems with fluid regulation in disease.

2.2.3 The Conjunctiva

The conjunctiva is a thin, vascularized and transparent mucus membrane that lines the inner side of the eyelids and covers the anterior part of the sclera up to the cornea. It is responsible for the lubrication of the eye, since it produces the precorneal tear film, and also contributes to the eye’s immune protection. The tight junctions of the superficial conjunctival epithelium are wider than those of the cornea and, as a result, hydrophilic drugs have higher conjunctival than corneal permeability (Huang et al. 1989; Saha et al. 1996).

2.2.4 The Sclera

The sclera is the opaque protective outer layer of the eye, forming the five-sixths of the posterior part of the globe and generally comprising 95% of the ocular surface area. It contains primarily mucopolysaccharides (glycosaminoglycans), collagen (type I, III, V and VI) and elastin fibers (Marshall et al. 1993). It is responsible for maintaining the shape of the globe, offering resistance to internal and external forces. The irregularity of the orientation of the scleral collagen fibers gives the white color to the eye. The sclera is connected to the cornea anteriorly and to the optical nerve posteriorly (Newell, 1986). The scleral permeability is comparable to that of the corneal stroma (Ghate & Edelhauser, 2006).

2.3 Ocular barriers and pharmacokinetics for the anterior ocular segment

The most important principle in designing a drug delivery system is to understand the pathways that the drug will follow upon administration and its pharmacokinetic profile. Therefore, the target of drug action determines the strategy that should be followed for a successful delivery. Developing a system wherein the drug can penetrate the protective barriers of the eye without harming or permanently damaging any healthy tissues is not trivial (Nanjawade et al., 2007).

The most direct method to treat diseases of the anterior segment is the topical administration (instillation) of ophthalmic medications to the ocular surface. The main pathway used for instillation of ophthalmic therapeutic agents is via the cornea, typically through eyedrops. In addition, besides corneal penetration, non-corneal routes such as the conjunctiva and the scleral have also been reported (Figure 2.5). When a therapeutic

agent is topically administered onto the ocular surface, lacrimal drainage may be significant, leading to systemic absorption and undesired side effects.

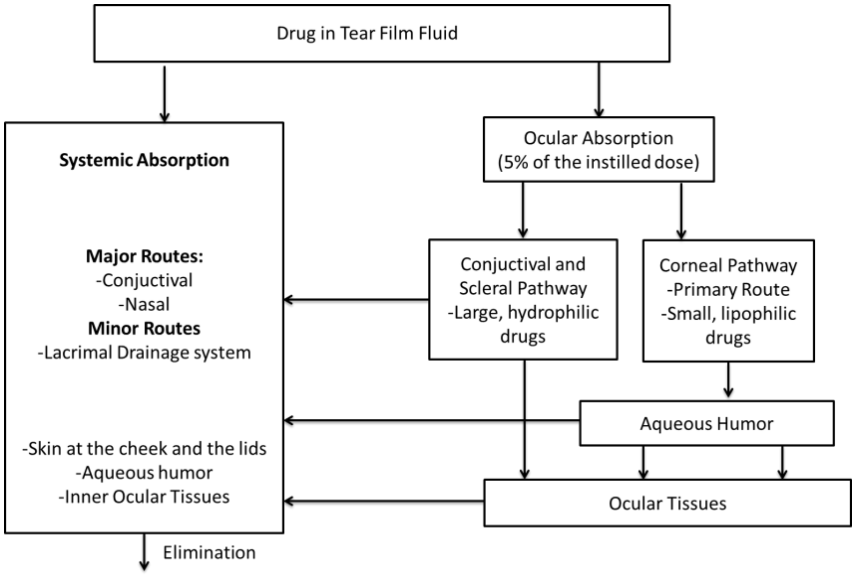

Figure 2.5: Schematic diagram of model ocular absorption for topical ophthalmic drug administration.

The tear film is the first barrier that the drug has to overcome in order to get absorbed by the corneal, conjunctival or scleral surfaces. The tear film is characterized by a rapid restoration time (2-3 minutes) and thus most of the topically administered formulations are depleted within a few seconds or minutes after instillation. In the case of eye irritation upon eye drop administration, increased lacrimal secretion will lead to further dilution of the dosage. Moreover, tear drainage and to some extent the absorption through the eyelids lead to lower drug concentration on the ocular surface available to transport through ocular barriers.

2.3.1 Corneal Barriers to Drug Penetration

The complexity of corneal anatomy makes the cornea, and more specifically the corneal epithelium, the main barrier limiting drug absorption by the ocular surface (Newell, 1986). Two major factors determine transcorneal drug absorption; the ocular contact time and drug solubility in the cornea.

Corneal permeability is always the sum of passive diffusion and active transport with the former being the main mechanism, following either the transcellular or the paracellular pathway. Lipophilic drugs follow the transcellular route while hydrophilic drugs follow the paracellular route (through the tight cell junctions) (Borchardt, 1990). However the presence of enzymes including esterases and proteases capable of metabolizing ophthalmic medication during or after absorption, may impact drug transport (Stratford & Lee, 1986). As a result, inactive ocular prodrugs that undergo enzymatic modification and release the active parent drug in the corneal epithelium have been developed.

The physicochemical properties of a therapeutic agent, including molecular size and shape, hydrophilicity/lipophilicity as well as net charge and degree of ionization determine the route and the rate of diffusion into the cornea. As a result, the lipophilic corneal epithelium acts as a rate limiting barrier for highly hydrophilic therapeutic agents, whereas neither the stroma nor the endothelium restrict the diffusion of these drugs due to their relatively high affinity to water. Partitioning of highly lipophilic drugs between the corneal epithelium and the stroma determines corneal penetration. In addition, it has been suggested that therapeutic agents of molecular dimensions less than 20 nm can diffuse across normal endothelium (Järvinen et al., 1995). Concerning the charge of the drug, negatively charged corneal epithelial mucosa shows increased permeability to

positively charged molecules at physiological pH (Saishin et al., 2003). The corneal endothelial junction complexes are not as connected as tightly as those of corneal epithelium, allowing for the passage of macromolecules between the stroma and the aqueous humour (Sunkara et al., 2003). Based on the above, the permeant (drug) should be amphipathic in nature in order to permeate through these layers (Gaudana et al., 2010).

2.3.2 Conjunctival and Scleral Barriers to Drug Penetration

The conjunctival and scleral layers absorb those drugs that bypass the corneal pathway. Molecules such as inulin (Ahmed, 1985) and carbonic anhydrase inhibitors (Brechue & Maren, 1993) as well as drug carriers such as peptides and proteins, more easily penetrate the conjunctiva and sclera than the cornea. Generally, the drug absorption by the conjunctiva is an order of magnitude higher than that of cornea due to the rich blood flow combined with the large surface area (Ahmed, Gokhale, Shah, & Patton, 1987). On the other hand, the presence of conjunctival blood capillaries and lymphatics cause significant drug loss into the systemic circulation resulting to lower ocular bioavailability (Ghate & Edelhauser, 2006).

Upon instillation, drug permeates the sclera through perivascular spaces, the aqueous media of gel-like or voids within the collagen network. Scleral permeability is higher than that of cornea, where the molecular radius of the drug is the limiting factor to transport and not the drug lipophilicity (Prausnitz & Noonan, 1998). Finally, recent studies indicate that the charge of the drug molecule affects its permeability across the sclera with positively charged molecules exhibiting poor permeability, presumably due to their binding to the negatively charged proteoglycan matrix (Kim et al., 2007). Typically

5% of the instilled drug reaches the anterior chamber, between the cornea and the lens (Schoenwald, 1997).

2.4 Topical Ophthalmic Medication and Restrictions

Topically applied ocular formulations are the most direct and convenient method of ocular drug delivery to the anterior segment of the eye. Upon instillation, they penetrate the cornea and enter the aqueous humour prior to distribution to the surrounding tissues including iris, lens, vitreous and retina. Elimination of the drug from the anterior chamber is achieved via aqueous humour turnover, the blood circulation of the anterior uvea and also via metabolic pathways (Schoenwald, 1990). On the other hand, ophthalmic therapeutic agents that get absorbed by the conjunctiva and the sclera enter the uveal tract and the vitreous humour directly, without passing first from the aqueous humour. Drug elimination from the vitreous humour is achieved either via the anterior or the posterior pathway, penetrating the blood-retina barrier, ending into systematic circulation (Schoenwald, 1990).

Topically administered formulations, such as ophthalmic solutions, suspensions and ointments, represent the 90% of the ophthalmic medication used for treating the anterior of the eye. Approximately 62.5% of the ophthalmic formulations are solutions, while 17.5% are ointments and 9% are suspensions (Saettone, 2002). Compared to other methods of ocular drug administration, topical drug delivery is a non-invasive method that avoids the first pass of metabolism in the liver, is targeted directly to the anterior chamber and delivers a high concentration of drug to the target site. Moreover, it is simple to prepare, filter and sterilize most topical solutions with low cost and without affecting their therapeutic properties. While eye drops, suspensions and ointments can

be effective for treating ocular disease, they exhibit significant drawbacks that limit their applicability.

The main disadvantages of topically administered formulations in ocular drug delivery are the poor bioavailability exhibited by the therapeutic agent upon instillation as well as the undesired systemic side effects. The residence time of topically applied solutions is limited to 3-5 minutes and only 5% of an instilled drop is absorbed into the eye (Xinming et al., 2008), with the rest of it being lost into the nasolacrimal drainage, conjunctival absorption and spillage onto the cheek leading to undesired adverse systemic side effect. Reduced drug bioavailability is also caused by metabolic degradation. Spillage on the cheeks may lead to undesired side effects concerning periocular skin irritation as well as loss of eyelashes.

The short residence time of the instilled eye drop is caused by precorneal tear clearance due to the rapid tear turnover (0.5-2.2 μl/min) (Schoenwald, 1997). Upon administration, reflective blinking spreads the drop over the ocular surface and the conjunctiva becomes the major route of non-corneal absorption due to the relatively small area of the cornea (1 cm^2) compared to that of conjunctiva (17 cm^2) (Hillery et al. 2001; Xinming et al. 2008), vascularization and higher permeability compared to that of the cornea. Moreover, the size of a drop (10-50 μl) is another factor that leads to reduced bioavailability, as it is 5 times greater than that of the eye (7-10μl). The size of the eye drop it is also directly related to the rate of the drug dissolution and potential irritation and discomfort (Schoenwald & Stewart, 1980). A drop with size smaller than 10 μl minimizes the chances of ocular irritation and reflex tearing (Sieg & Robinson, 1977).

The degree of ionization of the drug also plays a significant role in the drug tissue penetration and the ocular irritation (Mitra & Mikkelson, 1988). It has been shown that

reducing the degree of the ionization leads to increased transcorneal permeability. Generally, the eye tolerates hypotonic solutions that improve the tissue permeability, better than hypertonic solutions that cause drug dilution because the drug absorbs water from the cornea and conjunctiva by osmosis (Hillery et al., 2001).

In addition, the pulsatile release profile of topically administered drugs is also a significant drawback. Upon instillation, the rapid increase in drug concentration is followed by a rapid decline resulting in extreme fluctuations in ocular drug levels (Figure 2.6). Therefore, to maintain effective drug concentration eye drops should be instilled several times a day and in case of some antimicrobial and corticosteroids, eye drops should be administered every hour. However, patient incompliance is a major problem in the case of topical administration with recent studies showing that only 24-59% of the patients consistently follow their therapy by self-administering eye drops (Rotchford & Murphy, 1998). Patient compliance is deteriorated in case of chronic and age-related diseases, like glaucoma.

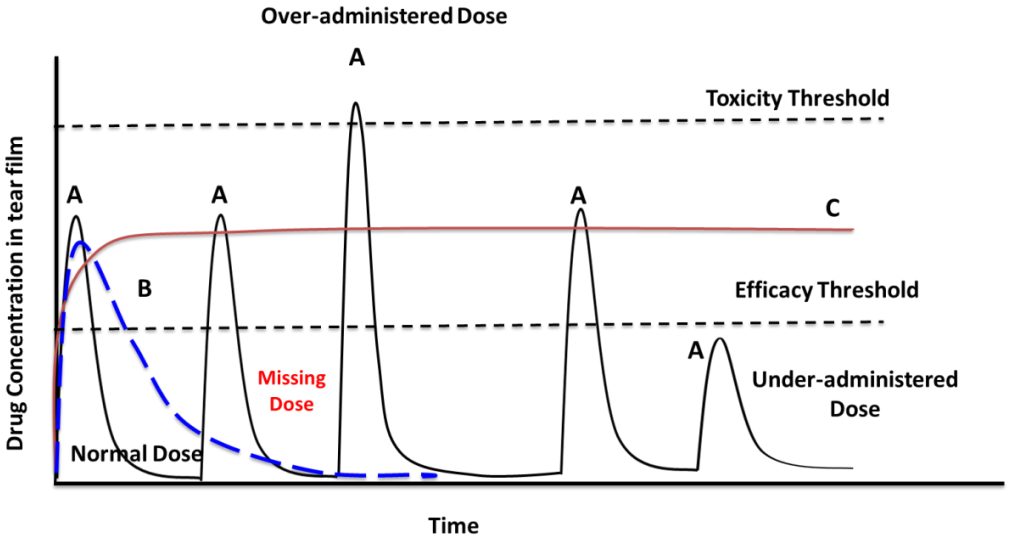

Figure 2.6: Schematic drawing illustrating the therapeutic band of drug delivery profile from (A), eye drops (pulsatile), (B) presoaked contact lenses (burst), (C) controlled and sustained delivery device.

Finally, eye drop formulations contain preservatives in order to prevent pathogen contamination, provide standard sterility, and, sometimes also to stabilize the drug (Kaur & Smitha, 2002). However, preservatives might cause toxicity to ocular tissue as well as eye irritation, thus many research has been done in order to optimize ratio between contamination and toxicity (Fraunfelder, 2006). Typically, preservative-containing eyedrops aim to last for a month while preservative-free containers are for single-use in order to avoid any the risk of contamination. Typically, preservative-free formulations are single dose containers suited for patients with allergies or with significant surgical concerns, since preservative toxicity may interfere with healing.

Even though the eye is an accessible organ to treat topically, all these limiting barriers and protective mechanisms lead to poor drug absorption. As a result, it is thought that extending the contact time of the drug on the surface of the eye should increase its bioavailability. Theoretically, this could be accomplished by increasing the concentration or/and the administration frequency of the therapeutic. However, Liebowitz et al. (Leibowitz & Kupferman, 1976), using topically administered prednisonole acetate showed that increasing the concentration (>1%) did not lead to decreased inflammation. Moreover, more frequent drug instillation did not increase the efficacy of the therapeutic agent due to decreased patient compliance (Leibowitz, 1980).

The main mechanisms that can lead to better bioavailability and duration of therapeutic action of ophthalmic medications involve increased residence time of the therapeutic agent on the ocular surface, controlled and uninterrupted drug delivery and maximization of corneal drug absorption while minimizing precorneal drug loss. Under these conditions, the desired therapeutic concentration can be achieved with a lower dose leading to fewer systemic and ocular side effects.

2.5 Contact Lenses used as Devices for Ocular Drug Delivery

The dosage forms of the topically applied formulations described earlier are clearly no longer sufficient to treat many significant ocular disease of the ocular anterior segment (Hughes & Mitra, 1993). As a result, contact lens materials have been suggested as a novel device for ocular drug delivery, providing targeted and uninterrupted drug release to the site of action.

The main concept of using contact lenses for ocular drug delivery is to provide extended and controlled drug release at concentrations comparably close to that normally administered by eye drops, leading to improved mean retention time (MRT) of drug in the eye (Hiratani, Fujiwara et al., 2005). They also have the potential to provide enhanced compliance, clear vision, greater drug efficacy as well as fewer undesired side effects from system absorption while the patient is able to still wear their refractive correction device during treatment (Lesher & Gunderson, 1993). Similarly to topical formulations, contact lens drug delivery systems can be used to treat ocular diseases such as infection or inflammation, promote wound healing or deliver a drug for a variety of other ocular diseases, like glaucoma, dry eye syndrome or ocular allergies.

The presence of a contact lens in the ocular environment can stabilize the tear film by creating a thin fluid layer called post-lens tear film (PoLTF) between the cornea and the lens. In the case of drug-loaded contact lenses, the therapeutic agent gets released form the contact lens into the PoLTF where it can stay for 30 minutes due to limited mixing and exchange with the rest of the tear film, whereas eye drops have a corneal residence time of approximately 2 minutes before tear turnover (Creech et al., 2001). As a result, the residence time of the drug on the cornea in the case of contact lenses is significantly longer than with an instilled eye drop, leading to enhanced drug

bioavailability. Consequently, the therapeutic effect of the drug is the same with lower concentration without causing any possible permanent damage on the ocular tissues.

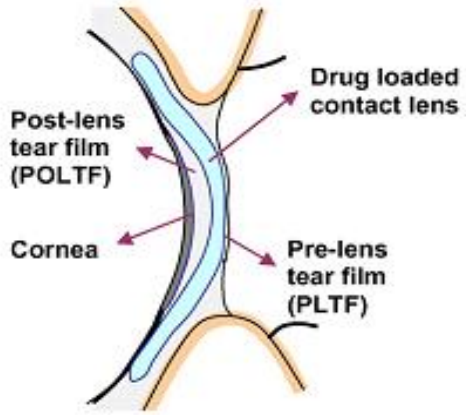

Figure 2.7: The separation of tear film in prelens tear fiml (PLTF) and postlens tear film (PoLTF) upon contact lens application (Kim & Chauhan, 2008)**.**

An ideal contact lens-based ophthalmic drug delivery system would have the capacity to load large amount of drugs and exhibit a zero-order release profile with drug concentration within the tear fluid between the maximum safe concentration (MSC) and the minimum effective concentration (MEC) without influencing properties such as shape retaining, transparency, stability, comfort, biocompatibility, cost effectiveness, and a good shelf-life as well as corneal oxygen permeability giving the ability of overnight wear (silicone hydrogel contact lenses). From a clinical perspective, the challenge is to provide medication conveniently, non-invasively and in therapeutically relevant concentrations for long times with minimal transfer of drug to the systemic circulation (White & Byrne, 2010). Using a mathematical model, Li et al. (Li & Chauhan, 2006) calculated that approximately 50% of a drug released from a contact lens can be absorbed by the corneal surface with the efficiency of ocular drugs delivered by contact lens being 35

higher than that of eye drops. *In vivo* studies are required though to confirm these conclusions.

2.5.1 Conventional Contact Lenses used a drug delivery systems

The first patent suggesting that soft contact lenses can be used to deliver ocular medication was from Otto Wichterle in 1965 (Wichterle, 1965). Based on the content of his patent, medically active substances such as antibiotics can be dissolved in the aqueous phase of the hydrogel in order to provide extended release via diffusion. There are two main approaches to incorporate the therapeutic agent into the lens matrix, via soaking and via direct entrapment. In the first case, the drug loading occurs post hydrogel synthesis while according to the second case the incorporation of the drug into the hydrogels matrix takes place during hydrogel polymerization.

2.5.1.1 Drug loading and release from pre-formed conventional contact lenses

There are two different methods to incorporate drug into a pre-formed contact lens, the post-soaked and the pre-soaked technique. The post-soaked technique involves instillation of topical formulation on the contact lens during wear. As a result, the eye drop gets absorbed by the lens due to the presence of the tear film and then slowly diffuses on the ocular surface as the concentration of the drug in the tear film declines (Lesher & Gunderson, 1993). In this case the contact lenses are used as drug reservoirs and their main application is as protective devices after either photorefractive keratectomy (PRK), corneal injury or serous infective complication, promoting corneal healing (Edwards et al., 2008; Engle et al., 2005; Reynolds et al., 2010). For example,

topical antibiotic solutions are applied on top of the contact lens prophylactically to either prevent any post-surgical infection or increase the rate of healing, while also providing symptomatic pain relief.

According to the pre-soaked technique, the contact lens is soaked in a drug solution of a specific concentration for a period of time (until it reaches an equilibrium) and then is placed on the patient's eye. According to this method, the contact lens acts as a drug reservoir here too, since the therapeutic agent gets adsorbed into the porous hydrogel matrix and through the aqueous domains without developing any significant affinity with the backbone of the polymer matrix. As a result, the driving force of the drug release is diffusion or drug adsorption-desorption from the polymer, with an initial burst release due to the significant concentration difference of the drug between the hydrogel matrix and the release medium followed by a slower release period during the lens wearing time.

These two methods have been used clinically and have the advantage that no further modification to the commercially available contact lens is required in order to be used as drug delivery system (Hillman 1974; Ruben & Watkins 1975; Schultz et al., 1998; Schultz & Mint 2002). The major problem of these techniques is the inadequate loading capacity they exhibit in order to provide therapeutically effective treatment during long periods of time. As a result, further modification of either the drug loading technique or the hydrogel materials is required in order to provide a more sustained release profile.

Recently, dos Santos et al. (dos Santos et al., 2008) designed a pHEMA hydrogel with methacrylated derivative of *β*-cyclodextrin (*β*–CD) incorporated into the material matrix. Cyclodextrins (CDs) are used as "host" molecules as they have hydrophobic internal cavities that can accommodate "guest" drug molecules by developing non-

covalent bonds and other weak interactions (Thatiparti & von Recum, 2009). As a result, the presence of *β*–CD within the pHEMA hydrogel network improved the drug loading and release duration by developing direct binding with the therapeutic agent and by increasing the hydrogel's crosslinking density (dos Santos et al., 2008). Relatively small drugs, such as acetazolamide (MW=222.2 kDa), were found to diffuse deeper within the hydrogel during loading, via soaking, compared to larger drugs, such as hydrocortisone (MW=362.46), leading to greater degree of interaction with *β*–CD. As a result, acetazolamide was released for longer periods (23 days) compared to hydrocortisone which was released for 10 days. It is of interest to mention that the mechanical properties as well as the cytocompatibility of these hydrogels were not altered by the incorporation of *β*–CD.

Xu et al. (Xu Jinku, et al., 2010) photopolymerized HEMA, monomethacrylated *β*-CD (mono-MA-*β*-CD), and trimethylolpropane trimethacrylate to synthesize *β*-CD containing pHEMA hydrogels for sustained release of ophthalmic drugs. Based on their results, the presence of *β*-CD in the hydrogel improved the water content and the tensile strength as well. Puerarin, a drug used for glaucoma treatment, was used as model drug. The *β*-CD content in these hydrogels affected the drug loading and the *in vitro* release rate. In addition, based on *in vivo* results the puerarin-loaded *β*-CD/pHEMA hydrogel contact lenses demonstrated a more sustained drug release as well as better bioavailability in the precorneal area of rabbits when compared to either pHEMA contact lenses or 1% puerarin eye drops respectively, by increasing the drug residence time in rabbit tear fluid.

In another approach, the surface of disposable soft contact lenses was coated with drug-loaded liposomes (Danion et al., 2007; Danion, Brochu, et al., 2007) in order to

provide efficient site-specific delivery. Liposomes are microscopic spherical vesicles (0.01-10 μm) comprised of lipid bilayers with a hydrophobic annulus and a hydrophilic core in the centre (Ebrahim, Peyman, & Lee, 2005) able to accommodate either hydrophilic or hydrophobic therapeutic agents. Moreover, it was found that topical solutions containing drug-loaded liposomes were characterized by higher bioavailability because of the improved residence time of the liposomal formulation on the ocular surface (Barber & Shek, 1986). Taking advantage of this, Danion and coworkers developed contact lens materials coated with a different number of layers in order to find the optimum design for sustained release (Danion et al., 2007). Based on the results, the cumulative mass of the drug loaded into the liposomes was dependent on the thickness of the layer. As a result, the contact lenses coated with 10 layers of levofloxacin-loaded liposomes released about 5 times more drug than corresponding lenses coated with only 2 liposomal layers. However, in both cases a 6-day controlled release profile was observed.

In order to verify the immobilization of the different liposomal layers on the surface of Hioxifilcon B contact lenses Danion and his coworkers (Danion, Brochu, et al., 2007) conducted an XPS study, while AFM imaging revealed that liposomes had a size of 106 and 155 nm depending on the method used for the fabrication of these liposomal layers (Danion, Brochu, et al., 2007). Moreover, the stability of the surface-bound liposomal layers was confirmed by the release kinetics of a fluorescent dye at different temperatures. The multilayer scheme utilized, provided strong interfacial bonding between each layer with a temperature dependent stability. Contact lenses with surface-immobilized liposomes could be stored up to 1 month at 4^{o}C without exhibiting significant release of their content. Further study of these materials exhibited no significant changes

in cell viability or cell growth, while elution assays revealing that no cytotoxic compound leaked from the lenses. Finally histological analyses of reconstructed human corneas and rabbit corneas showed that there is no alteration in the corneal cell and tissue structures (Danion, Doillon et al., 2007).

2.5.1.2 Entrapment of drug within the conventional contact lens during synthesis

In the case of direct entrapment, the drug can be incorporated into the hydrogel during the synthesis assuming that the drug is stable under the polymerization conditions. Based on this technique, the loading concentration depends on the solubility of the drug in the prepolymer solution. As a result, contact lenses loaded with the therapeutic agent during synthesis via direct entrapment, may lead to higher loading capacity, hence a greater mass of drug can be delivered with longer release. However it may also result in a decreased activity of the drug during polymerization and a major loss of drug through lens during packaging, leading to short in period on-eye release. *In vitro* and *in vivo* studies have been conducted with and without (Ende et al., 1998; Colombo et al., 1999; Ward et al., 2001) the use of additional controlled release mechanisms. Using the method of direct entrapment in order to fabricate dexamethasone-loaded pHEMA hydrogels, Kim et al. (Kim & Chauhan, 2008) found that an amount of drug loaded into the hydrogel during synthesis was permanently immobilized within the polymer matrix, due to either physical entrapment in tightly bound regions or chemical entrapment due to reaction.

A wide variety of different techniques have been studied in order to provide an extended release profile by reducing the drug release rate from the hydrogel matrix. Some of the most noticeable are colloid-laden hydrogels such as liposomes (Gulsen et

al., 2005), micro-/nanoemulsions (Li et al., 2007), and surfactant-laden hydrogels (Kapoor & Chauhan 2008; Kapoor et al., 2009). Hydrogels coated with polymer films (Ciolino, Hoare, et al., 2009) were also examined.

Gulsen et al. (Gulsen et al., 2005) designed pHEMA hydrogels that contained dispersed unilammelar dimyristoyl phosphatidylchoxline (DMPC) liposomes loaded with a model hydrophobic drug, lidocaine. An initial burst release was observed, with approximately 15-30% of the drug getting released within the first hours while the remaining drug showed a slower but therapeutically effective release rate for 6-7 days. The control step of the release mechanism was the diffusion of the drug from the liposomes throughout the contact lenses. In order to improve the loading capacity of conventional contact lenses and to control the drug release kinetics as well, pHEMA hydrogels containing oil-surfactant microemulsions laden with the base form of timolol were produced (Li et al., 2007). Despite the enhancement of the loading capacity since more drug (in the base form) could get incorporated in the oil phase, the system exhibited a rapid release in ophthalmic physiological conditions. It is of interest to mention though, that when the same system was immersed in DI water, a sigmoidal release profile was observed with release duration over 60 days. Li et al. (Li et al., 2007) suggested that this system could be useful either for hydrophobic ocular drugs or for other biomedical application such as transdermal drug delivery.

A one-step *in situ* colloid-laden hydrogel was created by surfactant-laden pHEMA hydrogels, by adding the surfactant and the drug into the polymerizing mixture. As a result, micelles were formed during the polymerization process since the surfactant interacted with the hydrophilic chains of the polymer forming the hydrophobic core. When a hydrophobic drug is present in this system, the hydrophobic core of the micelle will be

the place to accommodate it preferentially. Based on this mechanism, aiming to improve the loading and to provide sustain release rate, Kapoor et al. (Kapoor & Chauhan, 2008) prepared Brij surfactant laden pHEMA hydrogels able to release cyclosporine A (CyA), a potent immunosuppressant used for dry eye treatment, at a controlled diffusion rate through the hydrogel matrix for 25 days.

The studied materials were characterized with appropriate mechanical and optical properties for contact lens applications as well as improved water content and wettability due to surfactant entrapment (Kapoor et al. 2009). However, when these surfactant-laden hydrogels were used as vesicles for two other hydrophobic ophthalmic drugs, dexamethasone and dexamethasone-21 acetate, the system did not exhibit the same behavior towards controlled release due to insufficient partitioning inside the surfactant aggregates (Kapoor et al. 2009).

Although the results based on the release profiles and kinetics of colloid-laden hydrogel contact lenses seem appealing, there are some disadvantages with these systems. More specifically, the instability of the colloid-laden hydrogel during preservation, sterilization and packaging as the loaded drug diffuses through the materials is one drawback (Gulsen & Chauhan, 2005). Another drawback is the decreased release rate of these systems, and in some cases, below the therapeutically effective concentrations when the formulations formed are small enough in order to provide appropriate optical properties for ocular applications (Xinming et al., 2008).

Using a different technique than colloid-laden hydrogel contact lenses, Ciolino et al. (Ciolino, Hoare, et al., 2009) produced a prototype contact lens that contained a drug-polymer film coated pHEMA hydrogel able to load a large mass of drug and also release it over a prolonged period. The drug-polymer film consisted of poly(lactic-co-glycolic)

acid (PLGA) which is an FDA approved, biodegradable and biocompatible that has the ability to control drug release kinetics (Herrero-Vanrell & Ramirez, 2000; Jain 2000). Fluorescein and ciprofloxacin were used as models for release. The advantage of this system is that films have a decreased surface area to volume ratio compared to small polymer particles leading to greater drug loading and a slower release rate (Kohane, 2007). The formulation resulted in a steady zero-order release of both drugs for up to 4 weeks with ciprofloxacin exhibiting a therapeutically effective concentration at all time-points. Also, the release rate could be tuned by altering the ratio of PLGA to drug and/or the molecular weight of PLGA.

2.5.1.3 Molecular imprinting technique

In general, molecular imprinting is a technique used to generate templates shaped "cavities" in polymer matrices. Molecularly imprinted polymers (MIPs) have been widely studied in the field of biomedical engineering because of their active sites of specific recognition. As a result, recently the same technique was also studied in ocular drug delivery systems in order to improve the drug loading and sustain release duration. The concept of this technique is the "lock and key" model that is followed by enzymes for substrate recognition. Therefore, hydrogels fabricated with this technique are also known as biomimetic hydrogels (Venkatesh et al., 2007), where the interactions between the functional monomer (M) and the template (T) (therapeutic agent or releasable molecule of interest) represent the "lock and key" model.

The selection of the appropriate functional monomer(s) is crucial as there should an appropriate interaction between the template and functional monomer to efficiently create the "cavities" during and upon the polymerization process (Venkatesh et al.,

2007). These “cavities” specifically interact with the template molecules leading to slower diffusion through the hydrogel matrix, and thus are responsible for prolonging the release duration (Hiratani & Alvarez-lorenzo, 2002). Moreover, the functional monomer(s)/template (M/T) ratio is also important as a low functional M/T ratio leads to the creation of insufficient amount of cavities, hence low affinity with the template, while high M/T ratio results in the formation of a redundant amount of cavities where the functional monomer will not be able to interact with the template (Alvarez-Lorenzo, Yañez et al., 2006).

Therapeutic agents such as timolol maleate (Alvarez-Lorenzo et al., 2002), ketotifen fumarate (Venkatesh et al., 2007) and antibiotics (Alvarez-Lorenzo, Yañez et al., 2006) have exhibited improved release kinetics and release periods using this technique.

2.5.2 Silicone hydrogels used as drug delivery systems

The use of contact lens materials aims to provide targeted drug delivery by extending the residence time of the drug onto the corneal surface for prolonged periods of time. The most recent generation of contact lenses, silicone-based contact lens materials are characterized by increased oxygen permeability eliminating the clinical hypoxic signs associated with extended wear of soft/conventional contact lenses. Hence, they are the most suitable candidates for extended ocular drug delivery in that their wear for prolonged periods of time without removal (overnight use).

Literature on the use of silicone contact lenses used as ocular devices for drug delivery is limited compared to conventional contact lenses. Most of the studies are based on loading the therapeutic agent into commercially available contact lenses post

synthesis, via soaking. Drug-soaked contact lenses exhibit an initial burst release followed by a sub-therapeutic dosing (Ciolino, Dohlman, & Kohane, 2009) in most of the cases, suggesting that further modification of either the material or additional technologies of the loading process need to be performed in order to provide a sustained release profile. Karlgard et al. (Karlgard et al., 2003) showed that the amount of drug incorporated into the contact lens is directly proportional to the water content of the material with commercially available contact lenses of high water content providing the highest drug uptake and release drug concentration.

In general, silicone hydrogels exhibit longer release duration than conventional/soft contact lenses when the drug is incorporated into the hydrogel matrix by soaking (Karlgard et al., 2003). When compared with conventional contact lenses, silicone hydrogel contact lenses that are characterized by lower water content exhibited lower loading capacity due to the presence of the hydrophobic silicone domains. However, the interactions developed between the therapeutic agent and the silicone domains are versatile and lead to longer release duration. Even though the water content of silicone hydrogels is a major factor concerning the loading capacity and consequently the release profile, the drug-lens interactions are also affected by the ionicity, porosity, surface treatment and surface morphology of the lens material.

For instance, Balafilcon A were the only silicone hydrogel contact lenses that exhibited a sustained release profile compared to other commercially available silicone contact lenses, when ciprofloxacin-HCl or ketotifen fumarate were loaded via soaking into the lens matrix (Hui et al., 2008; Soluri et al., 2012). Balafilcon A contact lenses are charged (FDA group III) and relatively more porous than the other uncharged silicone hydrogel contact lens materials (Kunzler 1999; López-Alemany et al., 2002). As a result,

these factors in combination with the charge of the drug, in the case of ciprofloxacin-HCl, resulted in higher cumulative mass of drug released. The release duration though was still short compared to the wear duration, to provide extended release suggesting that the composition of commercially available contact lenses is not adequate for prolonged drug delivery without any further modification.

Recently, *in vivo* release of ketotifen fumarate from silicone hydrogels lenses demonstrated an initial burst release of the drug within the first 5 hours (Xu et al. 2011), however the residence time in the pre-corneal area as well as the smooth drug concentration profile led to higher bioavailability compared to traditionally applied eye drops instilled four time per day.

Kim et al. (Kim, Conway et al., 2008) developed new silicone hydrogel materials comprised of a hydrophilic DMA and NVP phase and a hydrophobic TRIS and/or a siloxane-based macromer. These materials were able to provide an extended *in vitro* release from 10 days for up to three months. Altering the ratio of hydrophilic/hydrophobic domains, the thickness as well as the hydrophilicity of the drug, the release profile could be tuned, without deteriorating ion permeability, degree of swelling, mechanical and optical properties. In order to improve the drug loading capacity of the contact lenses, the silicone hydrogels were soaked in ethanol-drug solution.

During the last couple of years a new approach of achieving sustained release from silicone hydrogel contact lenses was developed, by incorporating biocompatible diffusion barriers of vitamin E aggregates into the matrix of these materials (Kim et al., 2010; Peng & Kim, 2010; Peng & Chauhan, 2011). The release duration exhibited a minimum 2-fold increase due to the presence of the diffusion barriers in the materials matrix. These vitamin E loaded silicone hydrogels are able to maintain adequate oxygen

and ion permeability as well as optical transparency, allowing their use as extended wear therapeutic contact lenses. The impact of vitamin E as a diffusion barrier was more noticeable in hydrophilic therapeutic agents, such as timolol maleate, dexamethasone 21-disodium phosphate and fluconazole due to the absence of affinity between the diffusion barrier and the therapeutic agent. An *in vivo* study in spontaneously glaucomatous beagle dogs showed that daily silicone-based contact lenses loaded with vitamin E were sufficient to lower the IOP (Peng, Ben-shlomo et al., 2012). In a similar study, extended non-interrupted wear of silicone-based contact lenses for up to four days led to more significant lowering of IOP than either daily wear of the same contact lenses or eye drops (Peng, Burke et al., 2012).

In addition, when hydrophobic drugs like cyclosporine A were examined, the presence of vitamin E increased the loading capacity of the contact lens providing a system of extended zero-order release profile with therapeutically effective concentrations being released (Peng & Chauhan, 2011). Partitioning between the hydrophobic drug and vitamin E occurred when the drug diffusion was accomplished through the viscous vitamin E aggregates.

Model silicone hydrogels crosslinked with an internal wetting agent were studied as well, for the *in vitro* uptake and release kinetics of the fluoroquinolone antibiotic ciprofloxacin-HCl and the anti-inflammatory steroid drug dexamethasone phosphate (Nguyen et al., 2012). Hyaluronic acid, a highly hydrophilic glycosaminoglycan widely used in ophthalmic applications, was used as an internal non-releasable wetting agent able to alter the ratio of the hydrophilic/hydrophobic domains within the matrix in order to control the drug release kinetics. Based on the results of this study, in both cases, the presence of hyaluronic acid increased the total amount of drug released and resulted in

an extended release of up to 7 days as well, providing therapeutically significant drug concentrations.

The technique of molecular imprinting was also applied in silicone contact lenses aiming to extend the release of a wetting agent (White et al., 2011) and of an ocular antibiotic drug (Hui et al., 2012). The release of the macromolecular wetting agent hydroxypropyl methylcellulose (HPMC) was controlled and prolonged when acrylic acid (AA) was used as functional monomer in molecular imprinted Lotrafilcon B (Air Optix) silicone hydrogel contact lenses (White et al., 2011). Under physiological flow rates, a constant and therapeutically effective rate of HPMC *in vitro* release was monitored for over a period of 60 days, with the ratio of M/T controlling the release rates here as well, without however altering significantly the optical and mechanical properties of the studied silicone hydrogels. Finally, Hui et al. (Hui et al., 2012) designed model molecularly imprinted silicone hydrogels containing HEMA and TRIS monomers for ciprofloxacin-HCl release, while the functional monomers used here were either acetic or acrylic acid. The release duration (3 to 14 days) varied based on the drug loading concentrations and the M/T ratio. Higher M/T ratio led to longer release periods, whereas lower M/T ratio resulted in shorter release duration but higher and therapeutically relevant drug concentration release.

Concluding, with the advent of nanomaterials much progress has been made in exploiting contact lenses as potential ocular drug delivery devices in order to replace the well-established eye drops. However, contact lens materials are mainly used in research level while the results of the clinical experiments are fairly limited. Further development of contact lens materials and optimization of drug loading techniques may bring to market novel drug delivery systems able to achieve sustained and extended or

continuous drug release. The use of contact lenses as versatile drug delivery devices will potentially provide to the clinicians alternative options in the treatment of ocular disorders, based on the needs of patients and the conditions of the disease.

2.6 Hyaluronic Acid

Hyaluronic acid (HA) is a linear non-sulfated glycosaminoglycan composed of alternating units of the saccharides D-glucuronic acid and D-N-acetyl glucosamine (Figure 2.8). Each disaccharide dimer of HA has an approximate MW of 450 Da and they whole polymer can consist of 2,000 to 25,000 dimers depending on the deriving tissue. It was first discovered in 1934 by Meyer and his coworkers in the vitreous humor, while 3 year later the same polymer was found to be component of the cell coat of *Streptococcus* bacteria (Meyer & Palmer, 1934; Kendall et al., 1937). In addition, HA is also present in knee cartilage, in synovial fluid in joints, inside the umbilical cord and a major component of the extracellular matrix of soft connective tissues (Weissmann & Meyer, 1954; Stuart & Linn, 1985; Lapcik Jr. et al., 1998). It is also referred as hyaluronan because *in vivo* it is negatively charged and not in the protonated acid form. Commercially, HA is available with a MW up to 5 million Da (Milas et al., 2001).

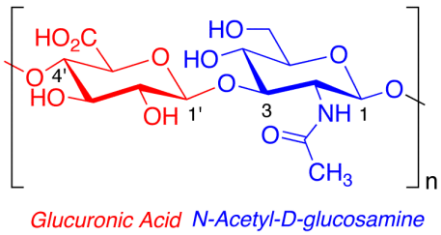

Figure 2.8: Chemical structure of hyaluronic acid

In aqueous solution, HA undergoes a transition from Newtonian to non-Newtonian characteristics when molecular weight, concentration, or shear rate are increased (Gribbon et al., 2000). The random coil structure of HA is responsible for the unique water-retention properties and viscoelasticity (Hargittai et al., 2008). The viscoelastic properties of HA in aqueous solution are pH-dependent, while increasing the molecular weight and the concentration of HA, the solution becomes more viscoelastic (Gibbs et al., 2004).

2.6.1 Medical applications

The versatile properties of HA, such as water-solubility, biocompatibility, non-immunogenicity, biodegradability and viscoelasticity make HA a suitable biomaterial for numerous cosmetic, medical and pharmaceutical applications. Its anionic nature in pH above 3 allows the development of electrostatic interactions between the polymer and positively charged therapeutic agents, developing complexes without the need of a crosslinker. Moreover, several physicochemical properties of HA depend on the MW of the polymer, as a result HA can be used in a wide range of applications. Commercial preparations containing HA have also been used as a viscosupplement in treating osteoarthritis, in surgery to prevent post-operative adhesions, and has been also examined as a biomaterial in wound healing, angiogenesis, tissue engineering, and drug delivery (Brown & Jones, 2005a). HA formulations have been also studied in cancer therapy because this glycosaminoglycan specifically binds to the CD44 receptor which is overexpressed in several types of cancers (Catterall et al., 1999).

2.6.2 Hyaluronic acid and ophthalmic applications

In the eye, HA is a component of vitreous humor, lacrimal gland, conjunctiva, corneal epithelium and tear film (Bourlais et al., 1998). Balazs and his coworkers (Graue et al., 1980) were the first to study the medical applications of HA, by creating a novel non-inflammatory preparations of HA on a commercial scale able to replace vitreous and aqueous humor in ocular surgery and to protect the corneal endothelium from any damage during surgery as well. As a result, HA started to be widely used in the intraocular surgery and mainly during cataract surgery in order to maintain the shape of the anterior chamber (intraocular injection) and to protect cornea and conjunctiva during cataract extraction in human. Topically applied HA during cataract surgery aims to protect ocular cells from damage (Balazs & Denlinger, 1993), while also due to its therapeutic properties, HA can encourage corneal wound healing by promoting the growth of corneal epithelial cells (Tani et al., 2002; Gomes at al., 2004). Healon® (Abbott Medical Optics) contains 1% sodium hyaluronate and initially was designed for vitreous replacement, however nowadays is also used for corneal endothelial protection during cataract surgery and in corneal transplant surgeries in order to provide better graft transparency (Polack, 1986). HA can also help suppress inflammation (Laurent et al., 1996).

HA solutions were found to have positive effects in ocular drug delivery studies. Taking advantage of its viscoelastic and mucoadhesive properties, studies starting from the late 1980s showed that HA is capable of prolonging the precorneal residence time hence increase the bioavailability of numerous drugs in topically administered solutions, such as pilocarpine (Saettone, Giannaccini et al., 1991), tropicamide (Herrero-Vanrell, Fernandez-Carballido et al., 2000), timolol (Bucolo et al. 1998), tobramycin (Gandolfi et

al., 1992). HA is mucoadhesive because it interacts with glycans of mucin layer on the ocular surface, leading to increase in mucin layer's thickness (Saettone, Monti et al., 1994). Moreover, when HA is topically applied its low shear thinning behavior provides low resistance to blinking, and thus patient compliance in HA-containing topical solutions is improved. A commercially available topical solution that contains HA is Hyalcrom NF launched by Bausch & Lomb for the treatment of allergic conjunctivitis.

In addition, HA has been used as a topically administered artificial tear solution for more than 20 years (Aragona et al., 2002) as well as a topical solution for the treatment of ocular discomfort and dry eye syndrome (Aragona et al., 2002; Brignole, Pisella et al., 2005). HA ophthalmic solutions are highly viscous, but change in temperature, pH whereas shear rate lower the viscosity (Lapcik Jr et al., 1998). When HA is applied on the ocular surface or when the eye is open HA is viscous and covers the surface of eye without draining, hence the tear break-up time is improved (Scott et al., 1991). However, during blinking due to increase in the shear rate, HA becomes less viscous and spreads across the ocular surface evenly providing comfort and effective lubrication. This mechanism in combination with the hydrophilic nature of HA were the fundamentals for developing artificial tear formulations. Commercially available HA eye drop formulations are VISMED®, AQuify™ (Ciba Vision) and Blink Contacts Lubricating Eye Drops (Abbott Medical Optics).

Another ophthalmic product that contains HA is the multi-purpose solution Biotrue™ (Bausch & Lomb) where the polymer acts as lens conditioning agent (Scheuer et al., 2010). Scheuer et al. (Scheuer et al., 2010) examined the *in vitro* retention of HA on the surface of commercially available conventional and silicone hydrogel contact lenses. Based on the results, the more the hydrogen bonding contributions to the

interaction between the lens and the hyaluronan molecules, the more hyaluronan was retained. As a result, Group I lenses such as Focus Night & Day, Acuvue Oasys, O_2 Optix and Acuvue Advance retained the most HA. When a contact lens is applied on the eye the tear film gets disrupted by the increased surface tension at the boundary of the lens, leading to ocular dryness triggered by contact lens wear, which is also known as contact-lens induced dry eye (CLIDE) (Glasson et al., 2006). During lens wear, HA may cover the surface of the contact lens with an “artificial mucin", stabilizing the tear film during contact lens wear. Since HA is a hygroscopic polymer, it retains the water molecules close to the ocular surface and reduces dehydration, providing a useful treatment of CLIDE (Nakamura et al., 1993). Treatment of CLIDE does not cause loss of vision, however patients with CLIDE significantly decrease or stop contact lens use because of these symptoms (Young et al., 2002).

Recently, the Sheardown lab developed conventional and silicone hydrogels containing HA as an internal wetting agent (van Beek at al., 2008; Weeks et al., 2011); Andrea Weeks et al., 2012). HA was crosslinked in the polymer matrix using either a dendrimer-based method or photocrosslinking. Based on the results, the presence of HA in the hydrogel’s network led to materials with higher water content, improved surface wettability as well as hydrophilicity leading to lower protein adsorption (lysozyme, albumin and the larger protein IgG) on the hydrogel surface. As a result, model pHEMA based and silicone hydrogel contact lenses with increased lens wettability and comfort were developed.

Conventional contact lenses loaded with releasable HA were also designed (Fagnola et al., 2009; Ali & Byrne, 2009). Fagnola et al. (Fagnola et al., 2009) observed a 5-day release from methafilcon 1B contact lenses, while Ali et al. (Ali & Byrne, 2009)

used the technique of molecular imprinting to develop nelfilcon contact lenses capable of releasing in a controlled manner high molecular weight HA, useful for dry eye treatment.

Finally, corneal shields manufactured from a hyaluronic acid derivative were examined as drug delivery systems for *in vitro* and *in vivo* release of methylprednisolone (Bucolo, Mangiafico et al., 1996). Hyaluronic acid-derived corneal shields exhibited sustained *in vivo* release for 48 hours ensuring effective levels of the non-inflammatory drug into the rabbit aqueous humor. Moreover, increased drug penetration in the aqueous humor and residence time in rabbit tear fluid improved the drug bioavailability leading to reduced primary signs of ocular inflammation.

3. Hypothesis and Objectives of the Present Research

Silicone hydrogel contact lenses can be potentially used as extended ocular drug delivery systems due to their increased oxygen permeability and potential for targeted corneal release. Different techniques have been used in order to develop a system that can control the release kinetics of the therapeutic agent through the silicone hydrogel. The hypothesis of the present research is that improving the hydrophilicity of the polymer domains within a silicone hydrogel network would allow for tailoring of the release kinetics of a therapeutic agent, providing controlled or sustained and extended release duration of at least two weeks. It is also hypothesized that the hydrophilicity of silicone hydrogels can be enhanced through the incorporation of a releasable wetting agent, such as HA, that due to its relatively high MW would be released for prolonged periods of time. Furthermore, electrostatic interactions between the anionic polymer HA (pKa=3) and the positively charged drug TM (pKa=9.2) will be developed under the release conditions (pH=7) thus the release kinetics of HA will determine the release profile of TM. PVP is used for comparative studies as an alternative non-charged wetting agent. The incorporation of a wetting agent (PVP, Hydraclear™) into the silicone hydrogel matrix (Acuvue® Advance™and Acuvue® Oasys™) is a method used in commercial contact lenses to improve the surface characteristics as well as the degree of lubrication of the contact lens materials.

Therefore, the objective of this study is to develop an HA-containing silicone hydrogel drug delivery system able to provide controlled/sustained and extended drug release. *In vitro* drug release study will be conducted, in PBS and under physiological conditions, in order to examine the release profile of two model ocular drugs, timolol maleate and ketotifen fumarate, from these materials mimicking sink conditions. The

incorporation of therapeutic and wetting agent into the silicone hydrogel matrix will be accomplished through the method of direct entrapment, assuming that the releasable molecules are not affected during the polymerization process. In addition, the impact of releasable HA on the bulk and the surface properties of these materials will be determined. Any alteration of bulk properties will be observed through measurement of the water content and the transparency while surface wettability will be determined by measuring the contact angles using the captive air bubble technique.

4. Materials and Methods

2-Hydroxyethyl methacrylate (HEMA, 97%), N,N-dimethyl acrylamide (DMA, 99%), ethylene glycol dimethacrylate (EGDMA, 98%), inhibitor remover for hydroquinone and monomethyl ether hydroquinone removal, polyvinylpyrrolidone (PVP, 10kDa) and the model drugs timolol maleate (TM, ≥98%), and ketotifen fumarate (KF) were purchased from Sigma Aldrich (Oakville, ON, Canada). Methacryloxypropyl-tris-(trimethylsiloxy) silane (TRIS, ≥95%) was purchased from Gelest (Morrisville, PA, USA). The photoinitiator 1-hydroxy-cyclohexyl-phenyl-ketone (Irgacure® 184) was purchased from CIBA (Mississauga, ON, Canada) and hyaluronic acid (HA) (Sodium Hyaluronate) of various molecular weights was purchased from LifeCore Biomedical (Chaska, MN, USA). As release medium, phosphate buffered saline solution (PBS) 10x was used and purchased from Bioshop® Canada Inc. (Burlington, ON, Canada).

4.1 Preparation of pHEMA/TRIS and DMA/TRIS hydrogels

Both pHEMA/TRIS (90:10) and DMA/TRIS (50:50) hydrogels were prepared following the same method. Initially, the monomers HEMA, DMA and TRIS as well as the crosslinker EGDMA were purified in order to remove the polymerization inhibitor 4-methoxyphenol hydroquinone (MEHQ), by passing each of the above chemicals through a column packed with Aldrich inhibitor remover. Since the chemicals used for the silicone hydrogels are light-sensitive, the vials containing the purified monomers and crosslinker, as well as those used for the polymer mixture preparation were covered with aluminum foil and low light conditions were maintained during the entire procedure for the preparation of the materials in order to avoid any unwanted photopolymerization. In all

cases, wt% and mol% are based on the total amount of the monomers. For 3 g of monomers mixture, the hydrophilic monomer HEMA (90 wt%) or DMA (50 wt%), the hydrophobic TRIS (10 wt% for pHEMA/TRIS hydrogels and 50 wt% for DMA/TRIS hydrogels) and EDGMA (5 wt%) were mixed together and left to stir for 5 minutes. The photoinitiator, Irgacure® 184 (0.028 wt%), was added to the mixture under constant stirring for 10 minutes and in the case of the pHEMA/TRIS polymers, the polymer mixture was purged into nitrogen for 2 minutes, in order to avoid any reaction of the monomers with atmospheric oxygen. The solution was then placed into a custom designed acrylic mold as shown in Figure 4.1 and schematically in Figure 4.2 using a syringe. These molds were comprised of two UV-permeable acrylic plates (Plexiglass® G-UVT kindly donated by Arkema Inc., (Philadelphia, PA, USA), two polyester sheets to avoid adhesion of the silicone hydrogels on the acrylic plates and a Teflon® spacer to adjust the thickness of the materials. The polyester sheets were changed after the polymerization of each sample and the Teflon® spacer used in current research had a thickness of 1 mm. The mold containing the mixture was placed in a UV chamber (Cure Zone 2 Con-trol-cure, Chicago IL, USA) for 10 minutes for polymerization at a wavelength of 365 nm.

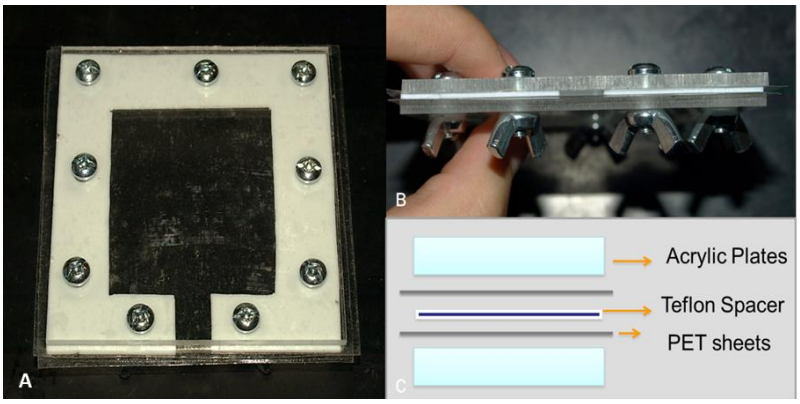

Figure 4.1: (A, B) Acrylic mold used for the preparation of silicone hydrogels, (C) Schematic representation of the acrylic mold components.

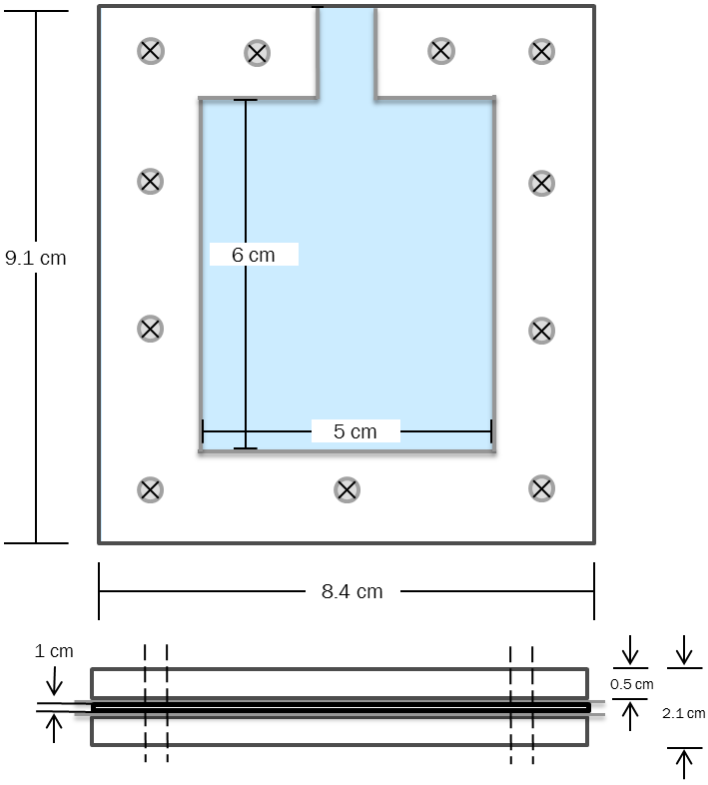

Figure 4.2: Schematic diagram of the mold used for the polymerization of silicone hydrogel materials in the current research.

Following polymerization, the formed silicone hydrogels were removed from the acrylic mold, placed in aluminum mold, weighed and then placed in the fumehood overnight, at room temperature to ensure that the reaction was complete. Finally, the materials were soaked in water (pHEMA/TRIS hydrogels for 3 hours and DMA/TRIS hydrogels for 1.5 hours) to remove unreacted components prior to punching into round discs of 7.94 mm (5/16”) and 5.56 mm (7/32”) in diameter. The discs were placed in a 48 well plate to dry overnight at 37^{o}C and stored until use. The concentrations of the constituents used for these silicone hydrogel materials are shown on the table below (Table 4.1).

Table 4.1: Concentration in wt% and mol% of the silicone hydrogels components based on hydrophilic:hydrophobic monomer ratio.

Components	**pHEMA/TRIS**		**DMA/TRIS**	
	wt%	mol%	wt%	mol%
HEMA	90	96.7	-	-
DMA	-	-	50	81
TRIS	10	3.3	50	19
EDMA	5	3.5	5	4
Irgacure® 184	0.028	0.16	0.028	0.18

4.2 Preparation of drug and/or wetting agent loaded hydrogels

For the preparation of drug and/or wetting agent loaded materials, the procedure followed was pretty similar as above. Initially, the drug (timolol maleate 0.5 or 2 wt% and ketotifen fumarate 0.5,1 or 2 wt%) was dissolved into the hydrophilic monomer (HEMA or DMA) under stirring conditions for 15-20 minutes. The wetting agent (HA 0.1, 0.5 or 2 wt%, 7.5, 132 or 910 kDa, and PVP 0.5, 1 or 10 wt%, 10 kDa) was then added in the monomer/drug mixture for 30 minutes under vigorous stirring to ensure uniform dispersion. TRIS, EGDMA and Irgacure® 184 were then added to the polymer mixture, following the same method as above. Similar to the control polymers, the UV curing time was 10 minutes and the drug and/or wetting agent loaded silicone hydrogels were punched into discs of 7.94 mm (5/16”) and 5.56 mm (7/32”) in diameter, and then placed into a 37^{o}C oven overnight to dry. The punched discs were stored in 48 well plates until further use. Tables A4.1-A4.3 of the appendix show the samples prepared for both the pHEMA/TRIS and the DMA/TRIS materials.

4.3 Bulk characterization

4.3.1 Swelling Study – Equilibrium Water Content

In order to assess the effect/impact of the direct entrapment of the wetting agent and the drug, the equilibrium water content was determined. The punched discs (5.56 mm in diameter) were placed in a 48 well plate in a 37^{o}C oven overnight to dry. The mass of the dried discs (n=4) was measured (dry mass) and then the same discs were soaked 1 ml of MilliQ water for 48 hours at ambient temperature to reach equilibrium. The swollen discs were then individually removed from the well plate and gently blotted with a Kimwipe to remove the excess of water from the discs surfaces and weighed again (wet mass). The EWC calculated using the following equation:

$$EWC\ (\%) = \frac{Mass_{wet} - Mass_{dry}}{Mass_{wet}} \cdot 100\% \qquad (1)$$

The results were then plotted in order to determine any change and relationship between the different wetting agents, their molecular weight and the different ratios of loading. Statistical significance of the results was assessed by a t-test.

4.3.2 Optical Transparency Study

The optical transparency of the drug loaded discs was determined by measuring the light transmittance (%), using UV-vis Spectrophotometer (Spectramax Plus 384, Molecular Devices, Corp, Sunnyvale, CA, USA). The same discs used to measure the EWC (n=4) were then used for the transparency study. After weighing, the blotted pHEMA/TRIS and DMA/TRIS discs (5.56 nm in diameter) were placed in Costar® 96 well UV-plates (VWR) with 100 μl MilliQ water and the light transmittance was measured at a wavelength range of 400-750 nm.

4.4 Surface Characterization – Surface Contact Angle Measurement

The surface wettability was determined by the air captive bubble technique. The silicone hydrogel discs, after reaching their EWC, were removed from the well plates, gently blotted with Kimwipes® in order to remove any excess of wetting agent or drug on their surface and finally immersed into a chamber filled with deionized water. The contact angle (θ) between the air bubble and their surface was measured using a Ramé-Hart NRL 100-00 Contact Angle Goniometer, providing relative information concerning the surface hydrophilicity and hydrophobicity. The air bubble (approximately 25μl) was dispensed manually when the air from the tip of a U-shaped, stainless steel capillary that was tightly fastened to a 1-mL syringe (Figure 4.3) was in direct contact with the disc. The capillary was then retracted from the chamber and the static contact angle was measured visually with the help of a microscope. The absence of camera in the goniometer apparatus did not allow the measurement of receding and advancing contact angles, thus no comparisons with commercially available contact lens materials could be made. For a given silicone hydrogel material, three discs were studied every time (n=3) while the contact.

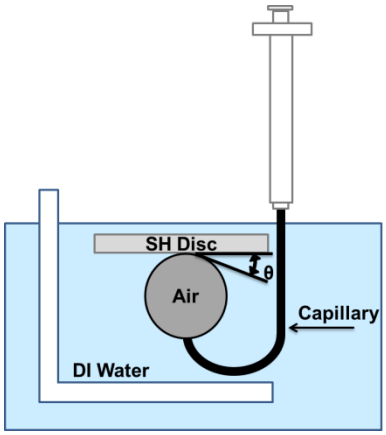

Figure 4.3: Schematic illustration of captive bubble technique used for contact anlge measurements of the surface for silicone hydrogels.

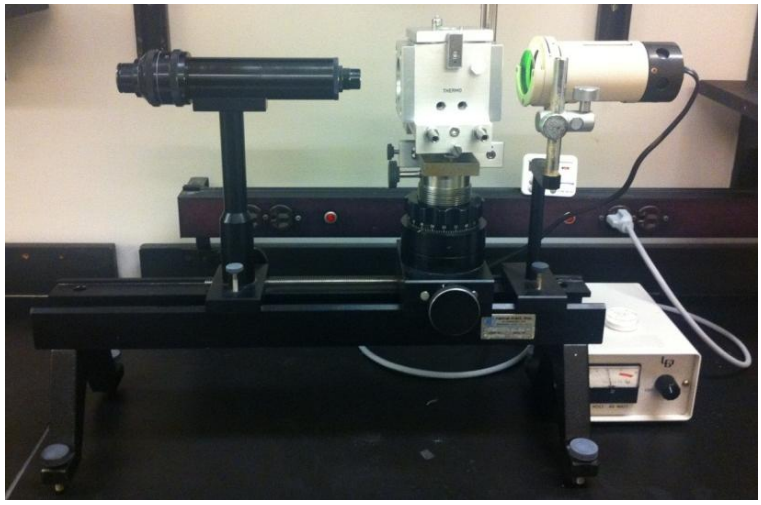

Figure 4.4: Goniometer apparatus (Ramé-Hart NRL 100-00 Contact Angle Goniometer) used for the measurement of the contact angle.

4.5 Therapeutic and Wetting Agent Release Study

For the drug release experiments, discs (7.94 mm or 5/13" in diameter and 1 mm thickness) with and without drug were weighed and then placed horizontally into eppendorf tubes in 1 ml of PBS (pH=7.4) in order to have both side exposed to the release medium, similar to (Figure 4.5). The eppendorf tubes were incubated in an orbital shaker (VWR International, West Chester, PA, USA) and the conditions set during the drug release experiment were 37^{o}C at 90 rpm. For each sample, four discs were used (n=4) for all the drug release experiments.

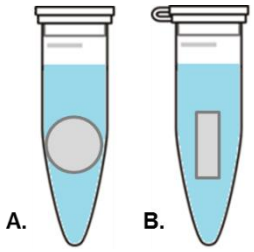

Figure 4.5: Schematic illustration of the silicone hydrogel disc placed in the eppendorf tubes with 1 ml PBS for the release study.

In order to measure the dynamic drug concentration, 200 μl of the releasate solutions were pipetted into a Costar® 96 well UV-plate and the absorbance was measured with a UV-vis spectrophotometer (Spectramax Plus 384, Molecular Devices, Corp, Sunnyvale, CA, USA) at the wavelength of 295 nm for timolol maleate and 300 nm for ketotifen fumarate. The absorbance of the samples was measured in triplicate (200 μl/well) and the eppendorf tubes were filled with fresh PBS (pH=7.4) every time there was a measurement in order to maintain sink conditions, mimicking tear turnover and replenish of tear film. The measured absorbance was converted into concentration based on a calibration curve of the absorbance of each drug in PBS. For both drugs the calibration curve covered the range of 1.5 – 100 μg/ml with R^2=0.9998 for timolol maleate and R^2=0.9996 for ketotifen fumarate. Finally, the concentration was normalized by dividing the concentration of each sample released by their dry mass (measured just before placing them into the eppendorf tubes). The standard deviation of each release graph is based on the normalized cumulative mass of each disc.

For HA release study, the release procedure followed was the same as above. However, as HA does not have a chromophore the dynamic drug concentration cannot be detected through UV-vis spectrophotometric technique, therefore an ELISA (Enzyme-Linked Immunosorbent Assay) was conducted to quantify the concentration (Echelon Inc, Salt Lake City, UT, USA). HA ELISA assay is a competitive ELISA assay in which the colorimetric signal is inversely proportional to the HA concentration. The protocol followed was provided by the company in the ELISA kit. The HA ELISA kits used were purchased through Cedarlane, Missisauga, ON, Canada. The calibration curve covered the range 50 -1600 ng/ml with an average R^2 (non-linear regression)=0.99. The HA standards were diluted using the Diluent provided by the HA ELISA kit. Initially, the

standards and the samples were mixed with the HA Detector and then placed in the ELISA plate reader for competitive binding, at 4^{o}C for 30 minutes. The ELISA plate reader was already coated with antigen. Before and after the enzyme-linked secondary antibody addition (incubation at 37^{o}C for 30 minutes), the plates were washed using 1X Wash Concentrate Buffer. The enzyme/substrate system is inversely proportional to the amount of HA present in the sample colorometric assay. It comprises of alkaline phosphatase/pNPP phosphatase substrate, therefore colorimetric detection is used to detect the HA detector bound to the plate. The standards and the samples were measured at 405 nm. A 4 Parameter Logistic (4PL) nonlinear regression model was utilized for curve-fitting for standards analysis in order to extrapolate relative sample values.

It is important to clarify that the accurate term for the studied materials is silane-based hydrogels and not silicone hydrogels. However, this is the commonly used term to describe the materials used for contact lenses. Therefore, the materials studied in the current work will be referred as silicone hydrogels as their final potential application is contact lens drug delivery.

5. Results & Discussion

In the current work, pHEMA/TRIS and DMA/TRIS hydrogel materials were investigated as potential delivery devices for hydrophilic ocular therapeutic agents. The goal was to obtain materials which showed extended release of at least two to three weeks and which therefore had the potential to be worn as drug delivering contact lenses. The hydrophilic monomers HEMA and DMA were used to achieve water and ion permeability while the TRIS monomer was used to provide oxygen permeability to enable extended wear as well as to enhance the modulus of elasticity. Addition of EGDMA as crosslinker provided improved and adequate structural integrity, mechanical strength and transparency to silicone hydrogel materials. Although these properties were not actually measured, it is believed that the materials formed have pathways that enable the transport of both oxygen and water/ions as has been shown to be necessary for successful lens materials.

5.1 Model pHEMA/TRIS and DMA/TRIS Silicone Hydrogel Synthesis

pHEMA/TRIS and DMA/TRIS materials were synthesized for evaluation as drug delivery materials. The crosslinker (EGDMA) as well as the drugs used, timolol maleate (TM) and ketotifen fumarate (KF), were soluble in the polymerization solution of both DMA/TRIS and pHEMA/TRIS materials. Timolol maleate (TM) which is known to have relatively high chemical stability, showed no apparent changes in structure when assessed by UV spectrophotometry (Wulff, 1995) since the characteristic peak of TM at 295 nm was the only peak present in the UV spectrum (200-350 nm) used for drug release analysis. The pHEMA/TRIS and DMA/TRIS silicone hydrogels did not exhibit any

visual change upon polymerization with the drug, suggesting that there was good compatibility between the drug and the polymer. In the case of ketotifen fumarate, the DMA/TRIS materials turned slightly yellowish after polymerization when loaded at concentrations higher than 2 wt%, thus 1 wt% was the highest concentration used for drug release studies with these materials. The photoinitiator Irgacure® OXE01 previously used to generate similar materials (van Beek et al., 2008; Weeks et al., 2012) resulted in slightly yellow materials. Hence it was replaced with Irgacure® 184, which showed improved solubility and compatibility with the polymer system, leading to clear and colourless silicone hydrogel materials.

The wetting agent, HA, was poorly miscible and precipitated in the polymerization mixture. Therefore the addition of HA was accomplished under vigorous stirring in order to achieve improved dispersion. As an alternative wetting agent, widely used in silicone hydrogel contact lenses as well as in artificial tear solutions, PVP (10 kDa) was used for comparative studies. PVP (10 kDa) was completely soluble in the hydrophilic monomers, however upon addition of the TRIS monomer, a slight increase in the turbidity of the materials was observed. DMA/TRIS materials exhibited a threshold of PVP addition of 2 wt%. Therefore in the current work, DMA/TRIS materials containing PVP (10 kDa) at a loading of 1 wt% were used in the current study, as there was no visible alteration in the materials upon water immersion. In materials containing greater than 2 wt% PVP, after being soaked into milliQ water, were found to form a white layer on both surfaces. After further water absorption, it was observed that this layer would start peeling off (Figure 5.1). This was assumed to be the result of incompatibility between PVP and the DMA monomers due to their physical properties. We attribute this incompatibility to the

hydrophilic monomer because in these materials, the DMA was at a higher molecular concentration (81 mol%) than the silicone monomer TRIS.

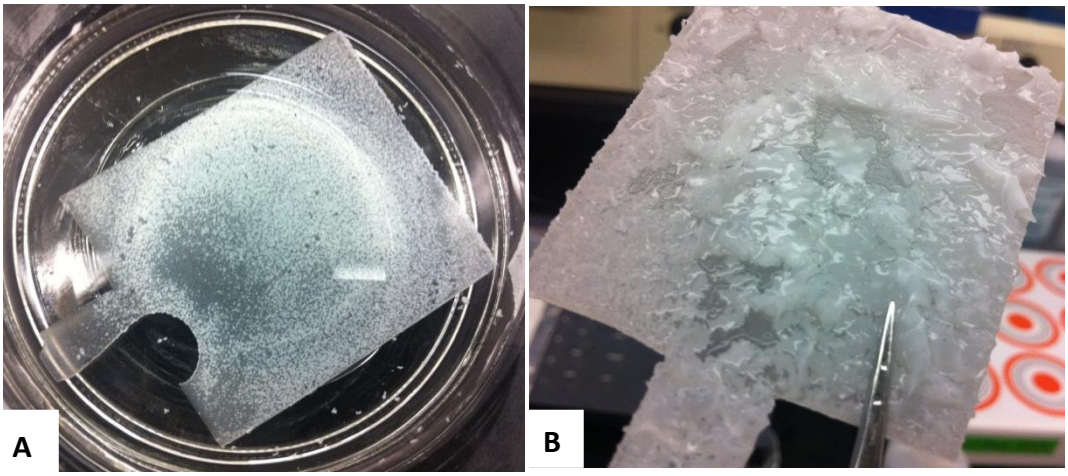

Figure 5.1: PVP-containing DMA/TRIS hydrogel discs immersed in milliQ water A. 2wt% PVP (10 kDa) and B. 5 wt% PVP (10 kDa) was incorporated during synthesis. Incompatibility between the hydrophilic phase DMA and the wetting agent PVP created a layer on the materials surface that peeled off upon hydration.

Incompatibility between these two polymers has been previously reported by Wang et al. (Wang et al., 2002) who mixed high concentrations of DMA monomer in a PVP buffer solution. Furthermore, Liska et al. (Liska et al., 2005) mentioned that in a polymerization process between DMA and low-molecular PVP (MW 10kDa), the latter seemed to suppress polymer formation. However, further experimental research should be done in order to understand better the incompatibility between DMA and PVP. Furthermore, the pHEMA/TRIS materials did not exhibit any similar behaviour with the presence of the PVP (10 kDa). Therefore, in this case, materials loaded with as much as 10 wt% PVP were examined in the current work in order to fully evaluate the impact of the addition of a wetting agent on such material properties as equilibrium water content, transparency and surface wettability as well as on the release of drugs. Direct

entrapment of HA or PVP led to materials with uniformly dispersed and releasable in swollen state wetting agent within the silicone network.

Upon polymerization, the silicone hydrogels, particularly the pHEMA/TRIS materials, were stiff and brittle in dry state. The materials remained flat (i.e. did not roll) in the swollen state confirming uniform polymerization and similar surface characteristics on both sides. In dry state, the DMA/TRIS materials were transparent while pHEMA/TRIS materials were translucent. A decrease in optical transparency occurs due to phase separation between the hydrophilic (HEMA) and hydrophobic (TRIS) monomers. This phase separation is the result of the immiscibility of the silane-based monomer TRIS and the hydrophilic HEMA, when cohesive interactions between chemically identical or similar molecules are stronger than the adhesive interactions between chemically different molecules (Nicolson et al., 2001). The silicone hydrogel might also have domains where there is a transition phase in which the material composition and properties are a combination/blend of these two polymers (amphiphatic phase mixture or blend of pHEMA or DMA and TRIS). This could be the reason why, in swollen state, the pHEMA/TRIS materials were significantly more transparent than in dry state. In general, it is preferable to have separate co-continuous phases within the material in order to allow separate diffusion of oxygen and ions. Nicolson et al. (Nicolson et al., 2001) mentioned that optically clear microphase separated systems can be formed when the two separate phases are less than 100 nm long, since the size of each phase is significantly less than the wavelength of light. This is what we speculate in the case of our DMA/TRIS materials, but TEM images of section materials need to be further evaluated in order to support this idea.

5.2 Bulk Characterization

5.2.1 Swelling Study – Equilibrium Water Content (EWC) Measurement

The ability of the silicone hydrogels to absorb and retain relatively high levels of water within their matrix is an important property, since hydrophobic silicone domains tend to adhere on the ocular surface causing undesired side effects. As a result, the presence of water improves the on-eye movement of the lens. The ability of the silicone hydrogels to reabsorb adequate water during wear is highly correlated to high water swellability. An equilibrium water content (EWC) above 40 wt% is typically desirable as this contributes to the softness and comfort of these lenses during wear without compromising oxygen permeability. The EWC of hydrogels is the result of a balanced state between the osmotic driving forces that lead water molecules to enter the hydrogel matrix and the resistant forces caused by the polymer chains opposing to expansion (Peppas & Yang, 1981).

The EWC of silicone hydrogel contact lenses is altered by many different parameters during wear, affecting the physiological performance of the polymer, ocular integrity and wearer tolerance. In crosslinked polymeric matrices, the water penetrates the network forming a gel phase in the wetted regions that eventually reaches equilibrium when the retractive forces of the network equalize the swelling forces.

Generally, parameters that can affect the EWC include polymer-solvent interactions, crosslinking density of polymeric matrix, polymerization conditions and the presence of wetting and therapeutic agents within the network. In the current study, the only significant variable in the materials studied was the presence of the wetting agent and the drug. Moreover, the EWC of the materials will play a significant role in drug release because the overall rate of drug release has been shown in numerous studies to

be controlled by the rate of the water influx (Peppas & Yang, 1981). During water uptake, as the polymers swell, diffusion of loosely entrapped drug through the aqueous phase may be significant, leading to a burst release. The release is based on water diffusion into the silicone hydrogel, the chain formation and relative mobility. When diffusion is the main factor of drug release, the more the polymeric matrix swells, the faster the drug is released. As a result, kinetic swelling studies can contribute to a better understanding of the release profile for the drugs studied.

5.2.1.1 The impact of HA and PVP on the EWC of pHEMA/TRIS and DMA/TRIS hydrogel discs

Swelling studies of the materials studied were performed in order to assess the effect of wetting agent and drug entrapment into pHEMA/TRIS and DMA/TRIS materials. Completely dry disc samples were weighed and placed in milliQ water at room temperature for 48 hours, at which time they were weighed again. The discs were assumed to have reached equilibrium when no further change in the weight was observed.

The presence of HA can affect the formation of polymeric chains as well as associated crosslinking leading to an increase in the mobility of the hydrogel chains and thus altering water uptake. The result of the swelling study, shown in Table 5.1, demonstrated that the non-covalent incorporation of HA into the silicone hydrogels can increase the EWC of both pHEMA/TRIS and DMA/TRIS discs ($p<0.05$). However, despite being statistically significant, the changes were relatively small. As expected, increasing the concentration of the wetting agent led to a slight increase in the swelling of the materials ($p<0.05$).

Table 5.1: Mean Equilibrium Water Content (EWC) (±SD) in wetting agent-containing silicone hydrogel materials and statistically significant increase (%) in the EWC (n=4).

Material	pHEMA/TRIS EWC (%)	DMA/TRIS EWC (%)
Control (no WA*)	24.35 ± 0.67	31.1 ± 0.95
HA (7.5 kDa) 0.1 wt%	24.97 ± 0.71	31.06 ± 0.44
HA (7.5 kDa) 0.5 wt%	26.04 ± 1.19*****	33.06 ± 0.77*****
HA (7.5 kDa) 2 wt%	27.99 ± 0.25*****	32.78 ± 1.02*****
HA (132 kDa) 0.1 wt%	25.34 ± 0.31*****	30.04 ± 1.32
HA (132 kDa) 0.5 wt%	25.42 ± 0.14*****	32.42 ± 0.9
HA (910 kDa) 0.5 wt%	25.13 ± 0.49	30.14 ± 0.66
PVP (10 kDa) 0.1 wt%	25.73 ± 0.90	30.06 ± 1.00
PVP (10 kDa) 0.5 wt%	25.71 ± 1.15	29.96 ± 0.83
PVP (10 kDa) 1 wt%	-	30.7 ± 0.40
PVP (10 kDa) 10 wt%	28.88 ± 1.25*****	-

*WA: wetting agent, i.e. HA or PVP
*****statistically significant compared to control ($p<0.05$)

For pHEMA/TRIS discs, a statistically significant increase in the EWC was observed when HA (7.5 kDa) and HA (132 kDa) was added to the silicone hydrogel materials ($p<0.05$) but not when 910 kDa HA was used. However, for the PVP-containing materials, only the incorporation of 10 wt% of the wetting agent led to a significant increase of 18.6% relative to the control ($p<7^{x}10^{-4}$). The swellability of the materials was also improved by increasing the concentration of HA, as expected, with discs loaded with HA (7.5 kDa) at 2 wt% exhibiting a 15% increase in the EWC ($p<5.2^{x}10^{-5}$). This result suggests that the size and thus the mobility of the HA chains contribute to a small increase in the nature of the hydrophilic domains in the silicone hydrogels. Interestingly, pHEMA/TRIS materials with HA (7.5 kDa) or PVP (10 kDa) of similar concentration and comparable MW were characterized by similar EWC. However this does not necessarily mean that the interactions between the pHEMA/TRIS and the two wetting agents are the same.

The presence of the wetting agent within the matrix of DMA/TRIS discs did not have the same impact as it did in the pHEMA/TRIS materials. Direct entrapment of high MW of HA did not affect significantly the swellability of the DMA/TRIS materials, although a small but statistically significant increase (~ 6%) ($p<0.05$) in the EWC of the discs was observed when 0.5 wt% of low MW HA (7.5 kDa) was added. Increase in the MW of HA incorporated did not alter the EWC of the DMA/TRIS hydrogel discs, suggesting that the lower molecular weights of HA, led to better dispersion and mobility of the polymer chains.

Based on the above results, the addition of low amounts of wetting agent into the pHEMA/TRIS and DMA/TRIS hydrogels did not significantly alter their EWC. Therefore, it is reasonable to conclude that the presence of the releasable wetting agent did not significantly alter the hydrophilic domains of the silicone hydrogel matrix as no significant change in bulk properties of the materials occurred.

It is of interest to note that in all cases of PVP-loaded DMA/TRIS materials, non-covalent incorporation of this wetting agent led to slight but not statistically significant decrease in EWC of the materials (1-3%, $p>0.1$). The latter in combination with the problems occurred post polymerization of PVP-containing DMA/TRIS hydrogel discs mentioned above, suggest that incompatibility between PVP and DMA may have led to microphase separation. Wang et al. (Wang et al., 2002) found that the pore size of the interfaces in a PVP-DMA polymer blend was smaller than in the polymer formed with DMA alone leading to poor material properties. These interfaces are probably characterized by really small pores which negatively affect the swellability of the materials.

Typically, 24-38% is the acceptable range of EWC for overnight used of silicone hydrogel materials, depending on the constituent materials (Austin & Hills, 2009). For example, the water content of NIGHT & DAY® lens (Ciba Vision) is listed on the package as 24%.

5.2.1.2 The impact of HA and PVP on the EWC of TM loaded-pHEMA/TRIS and DMA/TRIS hydrogel discs

In order to determine the impact of the wetting agent on the matrix of the drug-loaded pHEMA/TRIS and DMA/TRIS materials, the water uptake was determined. Tables 5.2 and 5.3 show the EWC (%) of timolol maleate (TM) loaded-pHEMA/TRIS and DMA/TRIS hydrogel discs.

Table 5.2: Mean Equilibrium Water Content (EWC) (±SD) of 0.5 wt% timolol maleate (TM)-containing pHEMA/TRIS and DMA/TRIS hydrogel materials (n=4).

Material	pHEMA/TRIS EWC (%)	DMA/TRIS EWC (%)
Control (no TM/WA)	24.35 ± 0.67	31.1 ± 0.95
TM 0.5 wt%	25.75 ± 0.97	30.36 ± 0.71
TM 0.5wt% / HA(7.5 kDa) 0.1wt%	25.67 ± 0.52	29.6 ± 0.89
TM 0.5wt% / HA(7.5 kDa) 0.5wt%	25.65 ± 0.43	32.72 ± 1.11*
TM 0.5wt% / HA(132 kDa) 0.1wt%	26.16 ± 1.17	30.42 ± 0.89
TM 0.5wt% / HA(132 kDa) 0.5wt%	24.64 ± 1.11	30.7 ± 0.55
TM 0.5wt% / PVP(10 kDa) 0.1wt%	24.40 ± 1.17	30.15 ± 0.91
TM 0.5wt% / PVP(10 kDa) 0.5wt%	25.1 ± 1.01	30.39 ± 0.43

*****statistically significant compared to control ($p<0.002$)

Swelling studies on pHEMA/TRIS materials that contain TM as therapeutic agent and HA or PVP as wetting agent, all incorporated into the matrix through the method of direct entrapment, showed that there was no significant change in the water uptake of materials when low concentrations of the wetting agent were loaded. However, a further increase in the concentration of PVP (10 kDa) from 0.5 wt% to 10 wt% led to an increase

in EWC (Figure 5.3) (12.3%, $p<0.008$) compared to the non PVP-containing discs assuming that the presence of such amount of PVP stays longer in the polymer matrix and thus increases the hydrophilic domains of the pHEMA/TRIS hydrogel. Since TM is a hydrophilic drug, its presence in the hydrogel matrix was also studied as another parameter that can affect the swellability of the materials. When pHEMA/TRIS discs that contained 2 wt% of TM and no wetting agent were compared to those that did not contain any drug (control), a 10% increase ($p<0.009$) was observed. Therefore, increase in the EWC of the silicone hydrogel could lead to alteration of the release profile of the respective TM-loaded pHEMA/TRIS hydrogel. Indeed, drug release results of TM-loaded pHEMA/TRIS hydrogels verified this assumption (Figure 5.9). Furthermore, a higher MW of HA did not lead to an increase in the EWC of the pHEMA/TRIS hydrogel discs loaded with timolol maleate. Comparing the two wetting agents, when loaded with the same concentration, PVP-containing materials were characterized by a slightly higher EWC (Figure 5.3) (5% for 0.1 wt% and 10% for 0.5wt%, $p<0.005$). The latter observation can likely be attributed to better dispersion of PVP within the hydrogel network, as PVP is soluble in the HEMA monomer compared to HA which is relatively insoluble.

Table 5.3: Mean Equilibrium Water Content (EWC) (±SD) of 2 wt% timolol maleate (TM)-containing pHEMA/TRIS and DMA/TRIS hydrogel materials (n=4).

Material	pHEMA/TRIS EWC (%)	DMA/TRIS EWC (%)
Control (no TM/WA)	24.35 ± 0.67	31.1 ± 0.95
TM 2 wt%	26.78 ± 0.46	31.04 ± 0.71
TM 2wt% / HA(7.5 kDa) 0.1wt%	25.40 ± 0.74	32.07 ± 1.18
TM 2wt% / HA(7.5 kDa) 0.5wt%	26.06 ± 1.19	31.88 ± 1.01
TM 2wt% / HA(132 kDa) 0.1wt%	25.15 ± 0.78	30.69 ± 1.33
TM 2wt% / HA(132 kDa) 0.5wt%	26.80 ± 0.84	30.61 ± 1.06
TM 2wt% / HA(910 kDa) 0.5wt%	26.80 ± 0.74	29.68 ± 0.44
TM 2wt% / PVP(10 kDa) 0.1wt%	26.65 ± 0.34	30.89 ± 0.65
TM 2wt% / PVP(10 kDa) 0.5wt%	27.97 ± 0.39*	32.46 ± 0.27^{+}
TM 2wt% / PVP (10 kDa) 10wt%	29.42 ± 0.77*	-

*****statistically significant compared to control ($p<0.008$)

$^{+}$statistically significant compared to control ($p<0.02$)

The incorporation of releasable wetting agent in the DMA/TRIS materials led to small alterations of the water uptake. Low MW of HA resulted in a 7% ($p<0.02$) increase in the EWC (Figure 5.2); PVP of similar molecular weight (10 kDa) at 0.5 wt% in higher concentrations of TM showed a similar effect (Figure 5.3). While it may be assumed, that using a higher MW of the wetting agent would lead to greater uptake of water but this was not the case. Apparently the degree of dispersion within the hydrogel matrix also plays a significant role in case of direct entrapment since lower molecular weights of HA exhibited a better dispersion in the silicone hydrogels during synthesis. Hence, better distribution of the HA chains throughout the matrix in combination of higher chain mobility (due to lower MW) is thought to lead to higher impact on the hydrogel structure. It is of interest to mention that an increase in the water uptake was observed in PVP-containing DMA/TRIS hydrogels when the materials were loaded with 2 wt% TM (Figure 5.3) ($p<0.02$). However, this increase was attributed to the hydrophilicity of TM and not to

PVP since no change of the EWC was observed in the respective 0.5 wt% TM-loaded DMA/TRIS discs (Figure 5.3).

5.2.1.3 The impact of HA and PVP on the EWC of KF loaded-pHEMA/TRIS and DMA/TRIS hydrogel discs.

The results of the EWC (%) study of KF-loaded pHEMA/TRIS and DMA/TRIS materials are presented in the Tables 5.4 and 5.5 respectively. In the pHEMA/TRIS hydrogel discs, low concentrations (0.5 wt%) of releasable wetting agent did not significantly enhance the swellability of these materials, whereas KF-loaded discs containing HA (7.5 kDa, 2 wt%) or PVP (10 kDa, 10 wt%) exhibited an 11% ($p<0.0005$) and 22% ($p<4^{x}10^{-5}$) increase in the EWC respectively. A 2-fold increase in the concentration of the therapeutic agent led to a 5% increase ($p<0.04$) in the EWC of the pHEMA/TRIS materials. The MW of the wetting agent did not seem to affect the swelling properties of the materials. Both HA and PVP drug-containing silicone hydrogels were characterized by the same EWC, when loaded with the same drug and wetting agent concentrations.

Table 5.4: Mean Equilibrium Water Content (EWC) (±SD) in ketotifen fumarate (KF) and wetting agent-containing pHEMA/TRIS silicone hydrogel materials (n=4).

Material (pHEMA/TRIS)	EWC (%)
Control (no KF/WA)	24.35 ± 0.67
KF 1wt%	24.36 ± 0.74
KF 1wt% / HA(7.5 kDa) 0.1wt%	25.04 ± 0.68
KF 1wt% / HA(7.5 kDa) 0.5wt%	25.13 ± 1.25
KF 1wt% / HA(7.5 kDa) 2wt%	27.05 ± 0.27*
KF 1wt% / HA(132 kDa) 0.5wt%	25.1 ± 0.80
KF 1wt% / HA(910 kDa) 0.5wt%	25.23 ± 1.24
KF 1wt% / PVP(10 kDa) 0.5wt%	25.13 ± 0.79
KF 1wt% / PVP(10 kDa) 10wt%	29.78 ± 0.68*****
KF 2wt%	25.66±0.55
KF 2wt% / HA(7.5 kDa) 0.5wt%	24.76±1.03
KF 2wt% / HA(910 kDa) 0.5wt%	25.5±0.82
KF 2wt% / PVP(10 kDa) 0.5wt%	25.28 ± 0.57

*****statistically significant compared to control ($p<0.0005$)

In the DMA/TRIS samples, direct entrapment of either HA or PVP did not lead to significant increase in the EWC of the materials. Therefore, the releasable wetting agent did not affect the hydrophilicity of the silicone hydrogel matrix, probably due to the small amount of the wetting agent loaded that might have already been released by the time that swelling study took place (48 hours). Moreover, drug concentration did not affect the swell ability of the DMA/TRIS discs since an increase in the KF concentration loaded during synthesis did not result in any alteration of their EWC. As a result, any change observed in the release profile of the KF-containing DMA/TRIS materials should be attributed to interactions developed between the wetting agent and the drug. Consistent with the previous results of the swelling study, KF-loaded DMA/TRIS hydrogels containing PVP (10 kDa) as wetting agent were characterized by lower EWC (3%, $p<0.04$) as well.

Table 5.5: Mean Equilibrium Water Content (EWC) (±SD) in ketotifen fumarate and wetting agent-containing DMA/TRIS silicone hydrogel materials (n=4).

DMA/TRIS discs	EWC (%)
Control (no KF/WA)	31.1 ± 0.95
KF 0.5wt%	31.9 ± 0.94
KF 0.5wt% / HA(7.5 kDa) 0.1wt%	31.6 ± 0.8
KF 0.5wt% / HA(7.5 kDa) 0.5wt%	31.65 ± 0.6
KF 0.5wt% / HA(7.5 kDa) 2wt%	32.7 ± 0.91
KF 0.5wt% / HA(132 kDa) 0.5wt%	32.7 ± 0.3
KF 0.5wt% / HA(910 kDa) 0.5wt%	32.13 ± 0.90
KF 0.5wt% / PVP(10 kDa) 0.5wt%	30.73 ± 0.67
KF 0.5wt% / PVP(10 kDa) 10wt%	30.54 ± 0.17
KF 1wt%	31.1 ± 0.70
KF 1wt% / HA(7.5 kDa) 0.5wt%	32.2 ± 0.75
KF 1wt% / HA(910 kDa) 0.5wt%	30.7 ± 0.56
KF 1wt% / PVP(10 kDa) 0.5wt%	30.6 ± 0.3

Therefore, it is clear that the non-covalent incorporation of a wetting agent generally did not lead to increase in EWC as was the case when HA was crosslinked into the polymer matrix of the silicone hydrogel materials (van Beek et al., 2008; Weeks et al., 2001). The releasable wetting agent, due to the concentration difference between the hydrogel matrix and PBS, is probably diffusing faster than actually aggregating in the polymer matrix. As a result, it is not able to contribute significantly to water retention in the network, and thus no change in the EWC was observed. The latter can be also correlated to the increase in the water content of those materials containing 2 wt% HA and 10 wt% PVP, assuming the release duration in this case lasts longer than that of the less concentrated silicone hydrogels.

5.2.2 Optical Transparency Study

One requirement for contact lens use is optical transparency which can be correlated to the morphology of the material. Phase separation between the hydrophilic and the hydrophobic domains reduces visible light transmission, causing substantial image distortion. Therefore, the lens must have a morphology that allows at least 80%, and more preferably greater than 90% visible light transmission. In case of transparent materials, the silicone and hydrogel domains are distributed in phase separated nano-scale regions such that the material is optically clear while achieving the required properties to maintain corneal health and lens movement.

The silicone hydrogel discs used in the current research are 1 mm thick, or approximately 10 times thicker than the average commercially available contact lenses. The transparency is directly correlated with the thickness of relatively transparent materials. Therefore, we would expect that the optical clarity of the studied materials would be significantly improved with thinner dimensions.

5.2.2.1 Optical Transparency Study of control pHEMA/TRIS and DMA/TRIS hydrogels

The results of the transparency study are presented in the Tables 5.6 and 5.7. Data are summarized by presenting transmittance (%) at 600 nm along with average transmittance over the entire range in order to examine the transparency of the materials.

Table 5.6: Transmittance (%) (±SD) in control pHEMA/TRIS silicone hydrogel materials (n=4).

Materials	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
Control (no drug, no WA)	79.3 ± 5.85	85.2
HA (7.5 kDa) 0.1 wt%	76.4 ± 3.4	74.7
HA (7.5 kDa) 0.5 wt%	11.5 ± 0.8	9.5
HA (7.5 kDa) 2 wt%	3.2 ± 0.5	3.0
HA (132 kDa) 0.1 wt%	69.2 ± 7.9	65.3
HA (132 kDa) 0.5 wt%	36 ± 7.7	36
HA (910 kDa) 0.5 wt%	40.1 ± 5.1	38.7
PVP (10 kDa) 0.5 wt%	85.8 ± 0.8	78.7
PVP (10 kDa) 10 wt%	86.6 ± 3.6	84.1

Table 5.7: Transmittance (%) (±SD) in control DMA/TRIS silicone hydrogel materials (n=4).

Materials	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
Control (no drug, no WA)	97.1 ± 0.5	96.4
HA (7.5 kDa) 0.1 wt%	79.2 ± 3.2	77.5
HA (7.5 kDa) 0.5 wt%	33.9 ± 2.4	31.8
HA (7.5 kDa) 2 wt%	6.1 ± 1.3	5.5
HA (132 kDa) 0.1 wt%	69.7 ± 5.65	65.3
HA (132 kDa) 0.5 wt%	57.7 ± 14.9	59.7
HA (910 kDa) 0.5 wt%	56.7 ± 11.7	58.6
PVP (10 kDa) 0.1 wt%	66.6 ± 11.2	67.8
PVP (10 kDa) 0.5 wt%	52.8 ± 4.7	49.8
PVP (10 kDa) 1 wt%	21 ± 6.14	19.7

The transparency study showed that both pHEMA/TRIS and DMA/TRIS discs in swollen state can have adequate optical properties to be used as a drug delivery system in ophthalmic applications. The presence of the wetting agent and especially HA, led to materials with reduced optical clarity. Comparing control pHEMA/TRIS to DMA/TRIS discs, the former exhibited lower transparency, as expected, suggesting that the size of the hydrophilic/hydrophobic domains in the pHEMA/TRIS materials is larger than in DMA/TRIS.

5.2.2.2 Optical Transparency Study of drug-loaded pHEMA/TRIS and DMA/TRIS hydrogels

For non-wetting agent containing samples, the addition of the therapeutic agent during synthesis did not affect the optical acuity (Table 5.8 and 5.9). The data of the transparency study are presented in the Tables A5.1 - A5.4 of the appendix. Based on these results, a further increase in drug concentration from 0.5wt% to 2wt% affected the transparency of TM-loaded DMA/TRIS materials only (Table A5.1), but the transmittance of these discs was still greater than (85%).

Table 5.8: Transmittance (%) (±SD) in drug-loaded pHEMA/TRIS silicone hydrogel materials (n=4).

Material	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
TM 0.5 wt%	85. 2 ± 6. 1	78.8
TM 2 wt%	85.1 ± 7.4	81
KF 1 wt%	85.2 ± 12.05	78.28
KF 2 wt%	83.4 ± 6.0	83.5

Table 5.9: Transmittance (%) (±SD) in drug-loaded DMA/TRIS silicone hydrogel materials (n=4).

Material	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
TM 0.5 wt%	89.5 ± 4.9	91.4
TM 2 wt%	85.9 ± 1.2	85
KF 0.5 wt%	85.2 ± 12.05	78.28
KF 1 wt%	83.4 ± 6.0	83.5

Moreover, direct entrapment of HA decreased the transparency of both pHEMA/TRIS and DMA/TRIS materials, as expected ($p<0.005$). Since HA is insoluble in the prepolymerization mixture, upon polymerization, releasable HA is trapped within the polymer matrix. Despite the release of HA, even though the materials were more transparent at the end of the drug release duration, the domains of HA within the disc were still visible. This could suggest that when a non-soluble wetting agent of relatively high MW is non-covalently incorporated in a polymer mixture before polymerization, some of the dispersed polymer will be released eventually, but a large amount of the wetting agent will remain trapped and not able to get released from the hydrophobic/silicone domains. Increasing the concentration of the HA during synthesis significantly reduced ($p<5^{x}10^{-5}$) the transparency of the model silicone hydrogels. On the other hand, incorporation of a higher MW weight of HA led to more transparent drug-loaded pHEMA/TRIS materials ($p<0.05$). DMA/TRIS discs exhibited similar optical characteristics among each other with the exception of TM 2 wt%, where the addition of HA (132 kDa) led to materials with higher transparency ($p<0.002$) compared to HA (7.5 kDa) (Table A5.1). Inconsistencies between the materials made it impossible to make general conclusions, although the pHEMA/TRIS materials were characterized by either similar or slightly lower transmittance than the DMA/TRIS materials.

Of note, the presence of PVP within pHEMA/TRIS materials did not affect the optical properties to the same extent as HA, even with the addition of high amounts (10 wt% PVP (10 kDa)) ($p>0.08$). This can be probably attributed to the fact that PVP is miscible in HEMA monomer and also showed a good compatibility and no significant precipitation within the polymer mixture prior to polymerization. Since high amounts of PVP within the silicone hydrogel matrix significantly increased the swellability of the materials as well, it is reasonable to suggest that a high degree of swelling may lead to smaller size interphase domains (between HEMA and TRIS) that do not interact with light and cause significant alteration in the optical properties. Contrary to pHEMA/TRIS, DMA/TRIS materials were significantly less clear when loaded with PVP even at low concentrations (0.1 wt%) ($p<0.0001$). This was not unexpected given the reported incompatibility between DMA and PVP (Wang et al., 2002) demonstrating the importance of marrying the properties of the matrix and the wetting agent. Further analysis of these materials is required to better understand the impact of wetting agent on their bulk structure.

5.3 Surface Characterization

5.3.1 Surface wettability and Contact Angle Measurement in non-drug-loaded model silicone hydrogels

The captive bubble technique was used for analysis of the surface wettability of the materials since this gives more representative results. Lower contact angles between the air bubble and the solid surface are indicative of more wettable surfaces (Petrucci; 2002). In case of really wettable surfaces, we expect that the air bubble is not able to stay on the surface of the studied material due to the extremely low interfacial tension

between the bubble and the hydrophilic surface as well as the buoyancy of the bubble in the chamber. The contact angles reported are the average of the contact angles measured at both edges of the air bubble on both sides of the materials to provide more representative results. No significant differences were found between the two edges of the air bubble or between different sides of the discs.

The contact angle study showed that the incorporation of wetting agent led to decreased contact angles in both pHEMA/TRIS ($p<0.005$) and DMA/TRIS ($p<0.001$) materials (Figure 5.2). The releaseable wetting agent migrates to the surface and by attracting the water molecules due to its high hydrophilicity, generates a surface of improved wettability. For pHEMA/TRIS materials, increasing the concetration of both HA (7.5 kDa) and PVP (10 kDa) resulted in significantly reduced contact angles. More specifically, for HA (7.5 kDa) 2wt% and PVP (10 kDa) 10wt% materials, a 55% ($p<6\cdot10^{-5}$) and 61% ($p<1.5\cdot10^{-5}$) decrease in contact angles was observed respectively. In this case, it is reasonable to assume that the "layer" of wetting agent at the surface was reinforced by increasing the concetration. Thus, the surface wettability was ameliorated. These results are in agreement with previous studies (van Beek, Weeks et al., 2008; van Beek, Jones et al., 2008) where the presence of dendrimer crosslinked and photocrosslinked HA, immobilized within the silicone hydrogel matrix, led to reduced contact angles, measured using the sessile drop technique and therefore improved surface wettability. The MW of HA did not play a significant role in the alteration of the contact angles since HA-containg materials that had the same concentration but different MW of HA, exhibited similar contact angles. Based on the assumption that increasing the MW of HA polymer would lead to decreased polymer mobility, materials with low MW of HA should accumulate more evenly and in higher amounts on the surface, hence improving more

effectively the surface wettability. However, the lack of MW effect was previously observed (Weeks et al., 2012) for DMA/TRIS materials where HA was covalently incorporated within the polymer network. Finally, somewhat surprisingly, both HA and PVP-loaded materials were characterized by similar contact angles, therefore both contributed to the surface wettability to the same extent ($p>0.3$).

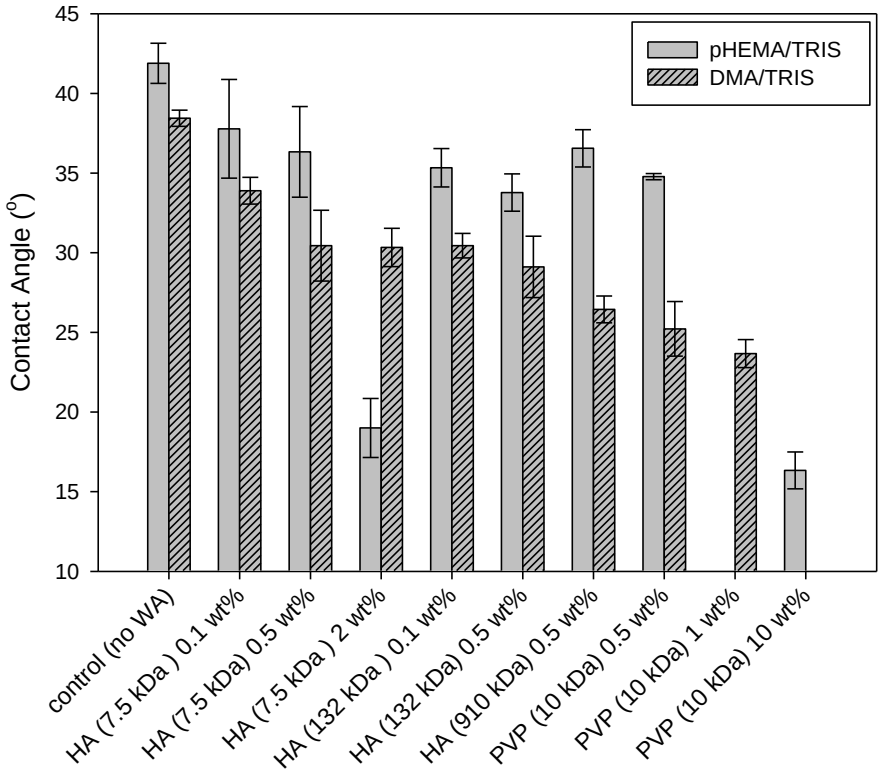

Figure 5.2: Mean contact angles (±SD) on swollen wetting agent-loaded pHEMA/TRIS and DMA/TRIS hydrogel materials, using captive bubble technique.

Despite the fact that the presence of either HA or PVP decreased the contact angles ($p<0.002$) in DMA/TRIS discs, when compared with pHEMA/TRIS materials, the trends were quite different (Figure 5.2). Specifically, an increase in the concetration of the HA loaded during synthesis led to statistically significanlty lower contact angles (20%

decrease) only in the case of HA (7.5 kDa) 2 wt% ($p<0.02$) and not to the same extent as was observed in the pHEMA/TRIS materials. Furthermore, in DMA/TRIS hydrogel discs, contrary to pHEMA/TRIS discs, a higher MW of HA contributed to improvement of surface wettability. Increase in the MW from 7.5 kDa to 910 kDa led to reduced contact angles ($p<0.05$), a somewhat unexpected observation based on the assumed mobility of the lower molecular weight HA chains, leading to greater migration to the surface. This suggests that the distribution of HA in DMA/TRIS materials is perhaps different compared with that of pHEMA/TRIS; as well as that longer chains of HA seem to migrate more easily to the surface for DMA/TRIS materials. When the same concentration of wetting agent was used, PVP was observed to have a stronger impact on the improvement of surface wettability for DMA/TRIS materials ($p<0.04$) with PVP-containing samples exhibiting a 35% decrease in contact angles, compared with HA-containing samples where only a 20% decrease was observed.

Finally, as expected, DMA/TRIS materials exhibited lower contact angles, thus better surface wettability than pHEMA/TRIS samples due to the presence of the more hydrophilic DMA ($logP_{DMA}=0.1$ versus $logP_{HEMA=}0.6$). We would expect a bigger difference between the contact angles of pHEMA/TRIS and DMA/TRIS surfaces. However, due to higher phase separation in the pHEMA/TRIS materials, we would assume that HEMA domains contribute to surface hydrophilicity more than DMA domains. In the latter case, transparency results suggest that the TRIS domains are better dispersed within the polymer matrix. In addition, different parameters affect the surface wettability of pHEMA/TRIS and DMA/TRIS hydrogels when either HA or PVP is non-covalently entrapped into the polymer matrix, presumably due to differences in the

structure. Clearly further experimentation is necessary to understand the relationship between surface structure and bulk composition.

5.3.2 Surface wettability and contact angle measurement in drug-loaded model silicone hydrogels.

5.3.2.1 Timolol maleate-loaded pHEMA/TRIS and DMA/TRIS discs

The impact of TM in combination with the wetting agents on surface wettability was also examined. In pHEMA/TRIS materials, low concentration (0.5 wt%) drug loaded discs did not exhibit significant changes in the surface wettability (Figure 5.3). However an increase in the concentration of TM in combination with the entrapment of HA or PVP within the hydrogel network, led to lower contact angles, thus ameliorating the surface wetting state (Figure 5.4).

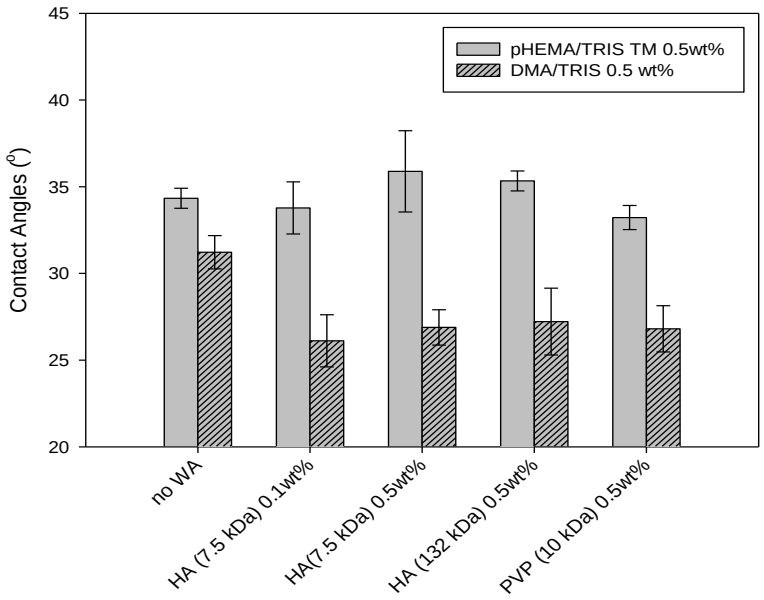

Figure 5.3: Mean contact angles (±SD) on swollen 0.5 wt% TM-loaded pHEMA/TRIS and DMA/TRIS hydrogel materials, using captive bubble technique.

The most noticeable change occurred in pHEMA/TRIS discs containing TM 2wt% and PVP (10 kDa) 10wt% where a 30% decrease in contact angles was detected. It is interesting to mention that the presence of TM in the 10wt% PVP-containing materials caused a significant increase in the contact angles compared to those materials loaded with PVP 10 wt% only (1.5 times). This result could possibly be attributed to the low octanol-water partition coefficient of TM affecting the degree of hydrophilicity between the therapeutic and the wetting agent. Under the same drug concentrations, neither the MW nor the nature of the wetting agent led to an impact on surface wettability. In case of DMA/TRIS materials, the presence of the TM and particularly that of the wetting agent, led to lower surface contact angles.

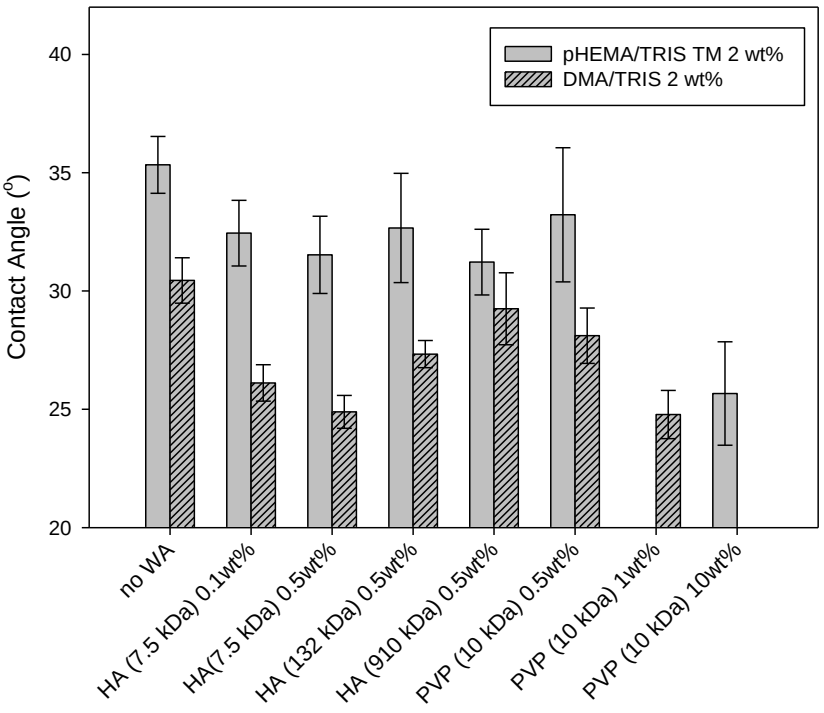

Figure 5.4: Mean contact angles (±SD) on swollen 2 wt% TM-loaded pHEMA/TRIS and DMA/TRIS hydrogel materials, using captive bubble technique.

5.3.2.2 Ketotifen fumarate-loaded pHEMA/TRIS and DMA/TRIS discs.

In case of KF-loaded silicone hydrogel materials, direct entrapment of wetting agent improved the surface wettability of both DMA/TRIS and pHEMA/TRIS materials containing low concentrations of drug ($p<0.01$ and $p<0.005$). Increasing the KF concentration in both model silicone hydrogels led to materials with similar surface wettability. In pHEMA/TRIS materials, a significant decrease (25%, $p<0.001$) in contact angle was observed with low MW of HA (2 wt%) and PVP (10 wt%) compared to the control (Figure 5.5).

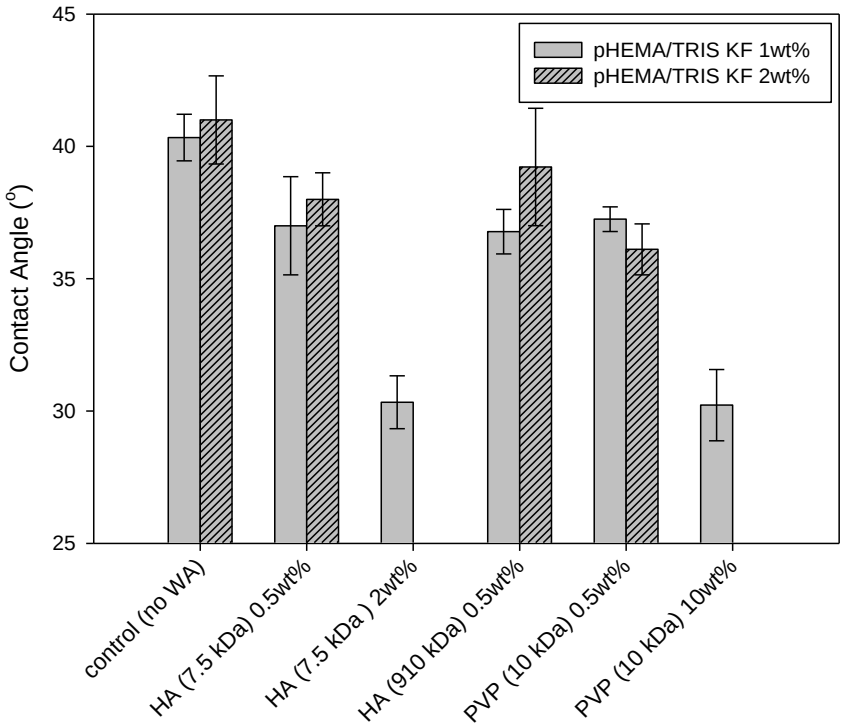

Figure 5.5: Mean contact angles (±SD) on swollen KF-loaded pHEMA/TRIS hydrogel materials, using captive bubble technique. The presence of wetting agent improved the surface wettability ($p<0.01$).

In general, these results are in agreement with the results for the non-drug loaded control materials (Figure 5.2). Similarly to TM-containing materials, the presence and concentration of the therapeutic agent KF affected the surface wettability in both wetting agent-containing pHEMA/TRIS and DMA/TRIS materials. The most significant change due to KF was observed in the case of pHEMA/TRIS HA (7.5 kDa) 2 wt% and PVP 10wt% where a 60% and 85% ($p<4^{x}10^{-4}$) increase in contact angles was observed respectively (Figure 5.5), suggesting a KF migration closer to surface prior release that by its turn interacts with the surface "shield" caused by the presence wetting agent.

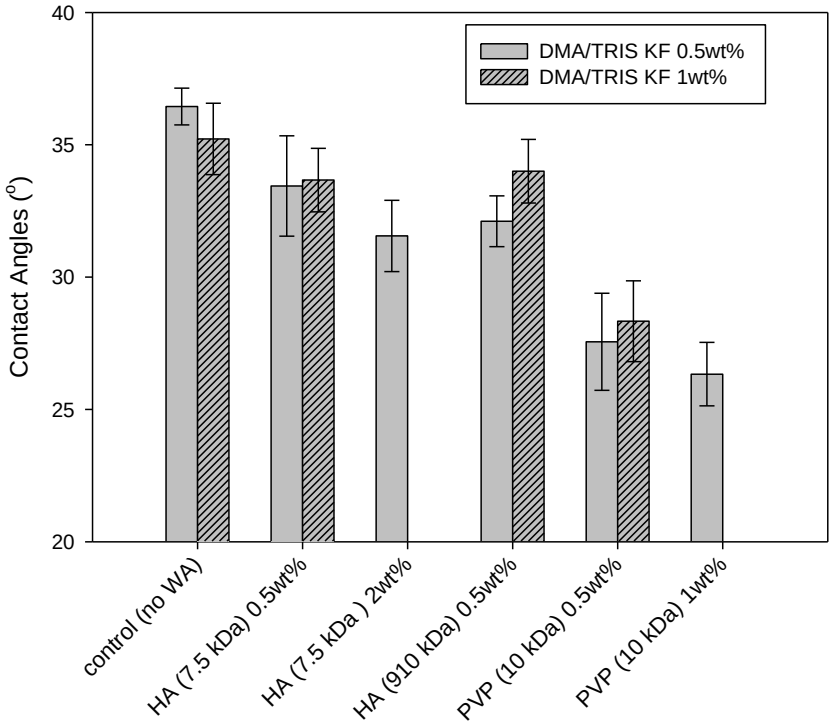

Figure 5.6: Mean contact angles (±SD) on swollen KF-loaded DMA/TRIS hydrogel materials, using captive bubble technique.

5.3.2.3 TM vs KF loaded pHEMA/TRIS and DMA/TRIS model hydrogels

Comparing the contact angles of TM with those of KF-loaded materials, it is noticeable that TM-loaded model silicone hydrogels were characterized by lower contact angles when HA was used as a wetting agent, possibly due to the different degree of hydrophilicity between the two therapeutic agents. Further characterization of the drug-loaded surface should be made in order to determine the impact that the drug has on the surface of wetting agent-containing materials.

Overall, it can be stated that low MW HA and PVP can improve the surface wettability because the small polymer chains are more mobile and thus more capable of migrating to the surface than the larger polymer chains. On the other hand, in some cases, high MW HA improved the surface wettability. In this case, it is likely that the extremely slow release expected with these high molecular weights will lead to significant shielding of the surface.

Commercially available contact lens materials with surface treatment in comparison with internal wetting agent were characterized by similar surface wettability (Santos et al., 2007). Generally, contact angles are often quoted by manufacturers in their marketing literature, but it is impossible to correlate these angles as they vary widely depending on the method used to obtain the measurement, the liquid probe that was used, and if the mentioned contact angles are referring to static or dynamic measurements (Read et al., 2011).

5.4 *In vitro* Drug Release Study

Fickian release kinetics are followed when the rate of release of a drug from a delivery device is proportional to the concentration gradient between the drug within the device and the release medium. In practical terms, this leads to a decreasing driving force with drug release. Zero-order (i.e., time independent) release is often the target in drug delivery, providing constant release of the therapeutic agent for an extended time period. The hypothesis of the current research is that altering the hydrophilicity of the polymer domains within a silicone hydrogel network, the release kinetics can be tailored providing sustained and/or extended release of at least two weeks duration.

The pHEMA/TRIS and DMA/TRIS discs used in the current research were initially in a dry state prior being immersed in PBS release medium. Therefore, we have a monolithic device that is initially a “swelling-controlled” system. After reaching the EWC, the system becomes a “diffusion-controlled” system. Drug release occurs via diffusion of the therapeutic agent through the macromolecular mesh of the aqueous phase or the water-filled pores. Consequently, the changes in the dimensions of the polymer matrix may lead to time dependent diffusion coefficients resulting in a complex kinetic process (Korsmeyer & Peppas, 1981; Korsmeyer & Peppas, 1984; Bae & Okano, 1992). Silicone hydrogels used as ophthalmic drug delivery devices would be in an equilibrium swollen state when inserted in ocular environment. Therefore, release kinetics would be controlled only by diffusion of the drug through the polymer or through the aqueous channels in the polymer under these conditions. For this reason, assuming that both pHEMA/TRIS and DMA/TRIS materials reached their EWC within the first day, the first 24 hours should not be considered for the calculation of the release rate of TM and KF in

order to assess the efficacy of model pHEMA/TRIS and DMA/TRIS silicone hydrogels as drug delivery systems.

The release mechanism of the TM and KF is mainly determined by the hydration of the silicone hydrogels combined with the interactions between the matrix and the therapeutic and the wetting agent. Therefore, the release kinetics shown were determined based on the composition of the materials, as well as the nature and the concentration of both therapeutic and wetting agent.

Finally, since experimental conditions such as temperature, pH and ionic strength play an important role on the behavior of the materials and may affect interactions with the therapeutic agent, drug release studies were carried out at physiological temperature (37^{o}C) and in phosphate buffer solution (PBS, pH=7.4), a media that imitates the lens preserving liquid and lachrymal fluid.

Assessment of kinetics was based on the following assumptions:

- Diffusion was the main mechanism of release
- In pHEMA/TRIS hydrogels the TRIS domains are in really small amounts. Therefore the diffusivity of TM in HEMA was assumed to determine the release profile of the drug.

5.4.1 Timolol Maleate Release

Timolol maleate (TM), is a β-blocker used for glaucoma treatment, with a relatively small molecular weight (MW=432.5 Da) and multiple sites for interaction that can be used to control its release (Figure 5.7). TM has a pKa value of 9.2 (Jouyban et al., 2003), meaning that TM is almost entirely protonated during the polymer synthesis as well as during drug release duration in PBS of pH=7.4. As a result, TM typically shows high

water solubility, and consequently was thought to be released through the aqueous domains not through the silicone phase of the polymer matrix (Kim et al., 2010).

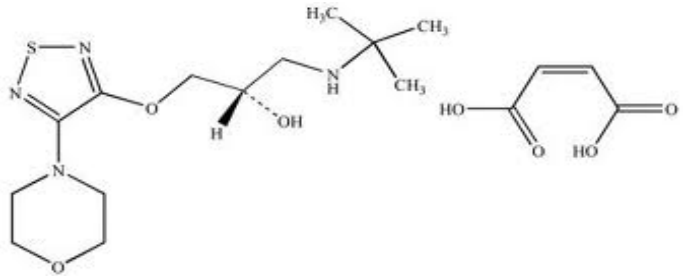

Figure 5.7: Chemical structure of timolol maleate

Interactions between the silicone domains, TM and HA may occur but not on a significant level. On the other hand, HA has a pKa value of about 3.0 (Brown & Jones, 2005b) and is in salt form (sodium hyaluronate). Therefore under the prepolymer mixture and release conditions HA is negatively charged. A shift in ionization of HA alters the intermolecular interactions between the HA leading to the assumption of decreased entanglement within the polymer matrix.

Hiratani et al. (Hiratani & Alvarez-lorenzo, 2002) took advantage of the electrostatic interactions between positively charged TM and methacrylic acid (MAA), which was used as a functional monomer, in order to control the drug/matrix affinity through molecular imprinting. As a result, the loading amount of TM was improved leading to the design of conventional contact lenses capable of providing sustained delivery.

On the other hand, based on the chemistry of TM and the wetting agent PVP, the only interaction that may possibly affect the TM release is that of hydrogen bonding between the carbonyl groups (C=O) groups in PVP and the hydroxyl groups (-OH) of TM (Ng & Swami, 2008; Auzély-Velty et al., 2002) and only when hydrogels are in hydrated state.

5.4.1.1 pHEMA/TRIS model silicone hydrogels

5.4.1.1.1 Timolol maleate release from non-wetting agent containing pHEMA/TRIS hydrogels

The release of TM loaded at 0.5 wt% lasted for 14 days, with 90% of the drug being released within 9 days (Figure 5.8). Diffusion of the TM is thought to occur through the hydrophilic pHEMA domains with more than 75% of the TM initially loaded getting released within the release duration.

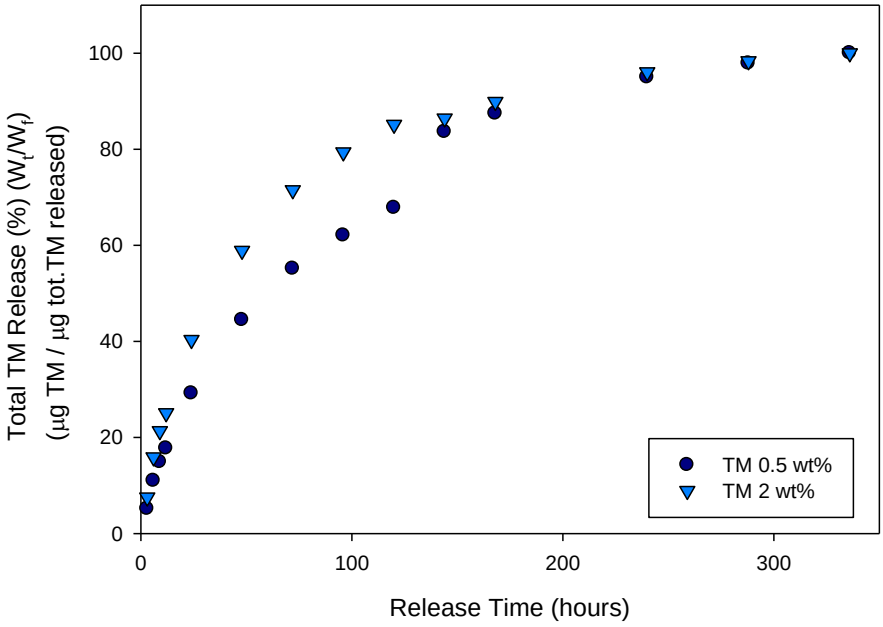

Figure 5.8: Comparison of the release kinetics of TM 0.5 wt% and 2 wt% loaded pHEMA/TRIS discs in PBS. TM and the wetting agent were loaded during synthesis by direct entrapment. Data are shown as mean (±SD) with n=4.

A further increase in the concentration of the therapeutic agent in the pHEMA/TRIS discs, led to systems with different release characteristics. The total release of TM 2 wt% lasted for 14 days, with 90% of the drug being released within 7 days (Figure 5.8). The remaining TM was released gradually reaching a plateau, exhibiting a concentration

dependent release profile. More than 70% of the TM initially loaded in the silicone hydrogel was released within the studied release duration.

Comparing the release kinetics of the low (0.5 wt%) and high (2 wt%) concentration of TM in pHEMA/TRIS, suggests that more controlled release was observed in low concentration TM silicone hydrogels (Figure 5.8). Increasing the concentration of the hydrophilic therapeutic agent resulted in faster release but similar release duration. It is clear that, as expected, a greater difference in TM concentration leads to a higher release rate from these materials. Moreover, the water content of pHEMA/TRIS materials loaded with 2wt% TM was higher (Table 5.3), hence higher degree of swelling led to faster release of TM throughout the silicone matrix. Somewhat surprisingly however, the total percentage of TM released by 2 wt% TM-loaded materials was lower than the 0.5 wt% TM-containing materials ($p<0.009$) (Figure 5.13). Presumably this unreleased drug is trapped in the crosslinked silicone domains. This suggests that there was a threshold in the loading amount beyond which no further drug is released.

5.4.1.1.2 Timolol maleate release from HA-containing pHEMA/TRIS hydrogels

The presence of releasable HA within the silicone hydrogel polymer matrix led to different release kinetics based on the drug concentration and the HA molecular weight (Figure 5.9 and 5.10) compared to the non-HA materials, while the percentage of the total amount of TM released from the former materials was decreased (up to 20%, $p<2^{x}10^{-4}$) (Figure 5.13). Initially, for the first 5 days HA and non-HA pHEMA/TRIS materials released the same amount of TM with a similar release rate, but after this time

interval the presence of HA within the matrix decreased the release rate (Figure 5.9) and thus the total amount of TM released was decreased.

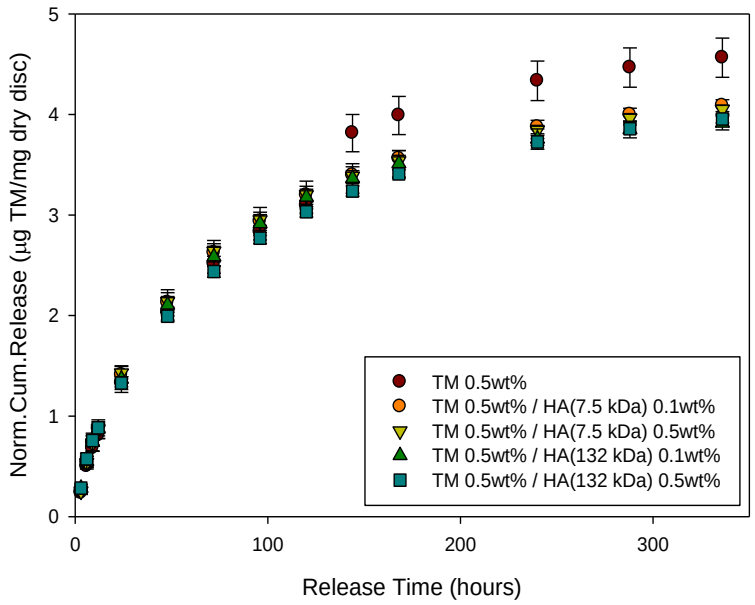

Figure 5.9: Timolol maleate cumulative release in PBS from pHEMA/TRIS loaded with different concentrations and MW of HA. TM and wetting agent were loaded during synthesis by direct entrapment. Data are shown as mean (±SD) with n=4.

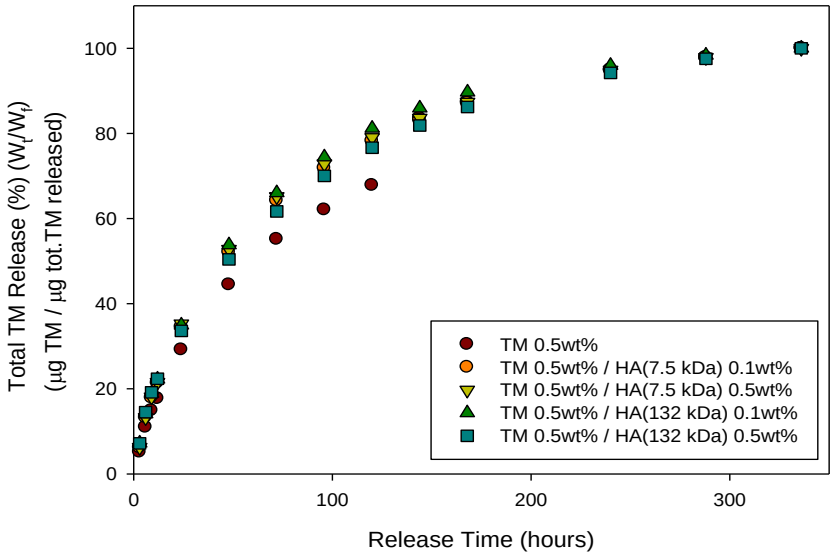

Figure 5.10: Timolol maleate release kinetics from pHEMA/TRIS loaded with different concentrations and MW of HA. Data shown as mean (±SD) with n=4.

This suggests that sterically hindered HA which has a higher MW than the therapeutic agent slowly diffuses throughout the silicone hydrogel matrix controlling the release rate of TM. Based on Figures 5.9 and 5.13 less cumulative mass of TM was released ($p<0.009$) within the same release duration when compared to control (no wetting agent incorporated) or PVP-containing pHEMA/TRIS discs, suggesting that HA not only determines the release rate but also the amount of TM released. The release of HA should be also examined in order to confirm that. The tortuosity as well as the size of the pores should be studied using imaging techniques like Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The porosity of the material determines the mesh size of the matrix and the water content, and as a result also the release of the therapeutic and wetting agents (Yañez et al., 2008). In addition, in the prepolymer mixture TM and HA are electrostatically bound and since TM is a lot smaller than HA, the drug is likely attached to the HA chains. However, due to the low miscibility of the HA in the prepolymer mixture, it is only dispersed in the silicone hydrogel matrix. As a result, during synthesis an amount of HA/TM complex is getting trapped in the polymer matrix and cannot be released. This explains why the transparency of the HA-loaded materials is not improved at the end of the release.

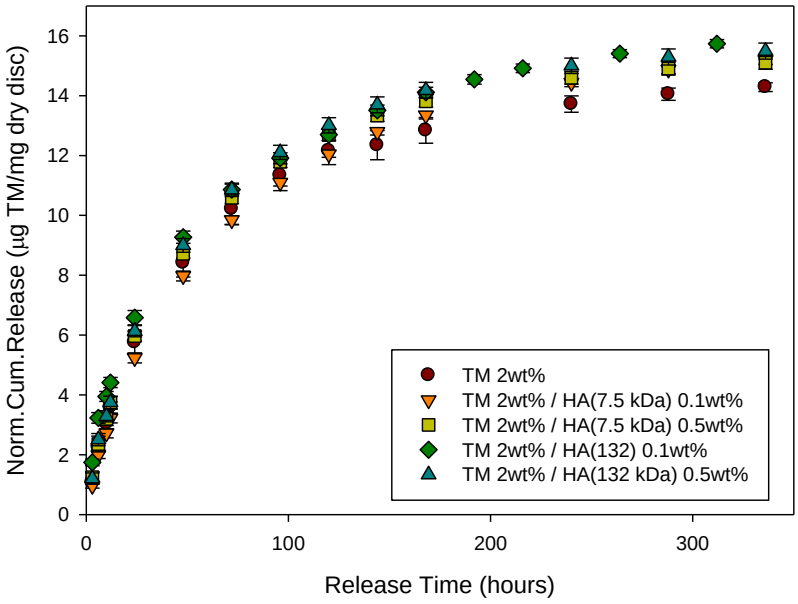

Figure 5.11: Timolol maleate cumulative release profile in PBS by pHEMA/TRIS loaded with different concentrations and MW of HA. TM and wetting agent were loaded during synthesis by direct entrapment. Data shown as mean (±SD) with n=4.

However, when the concentration of the therapeutic agent was increased while maintaining the concentration of HA, the system exhibited different release behavior (Figure 5.11). A 4-fold increase in the concentration of TM (from 0.5 wt% to 2 wt%) led to materials with higher release rate, releasing more TM (average 20%, $p<0.007$) without though altering the release kinetics of the system (Figures 5.12). Neither the MW nor the concentration of HA affected the amount of TM released ($p>0.8$). In this case we can assume that, since the concentration of TM is 4 times higher than that of HA, the TM is controlling the release not the wetting agent. The latter can be verified only through the release kinetics of HA.

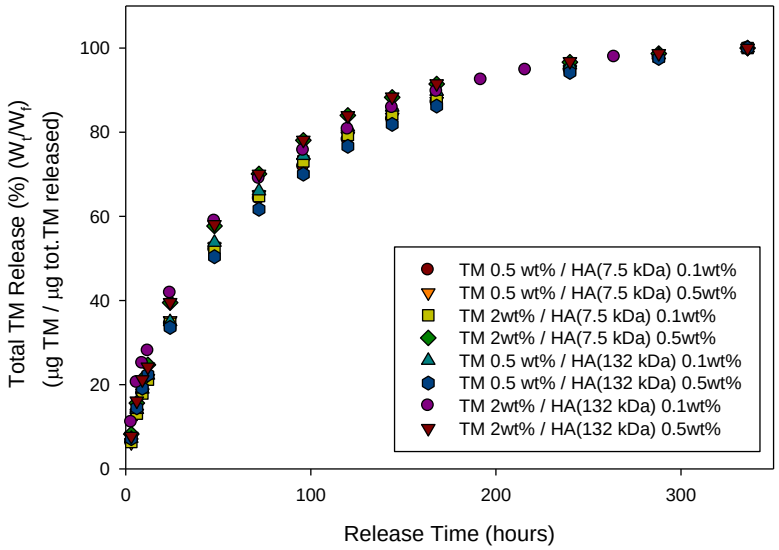

Figure 5.12: Timolol maleate release from pHEMA/TRIS loaded with different concentrations and MW of HA. Data are shown as mean (±SD) with n=4.

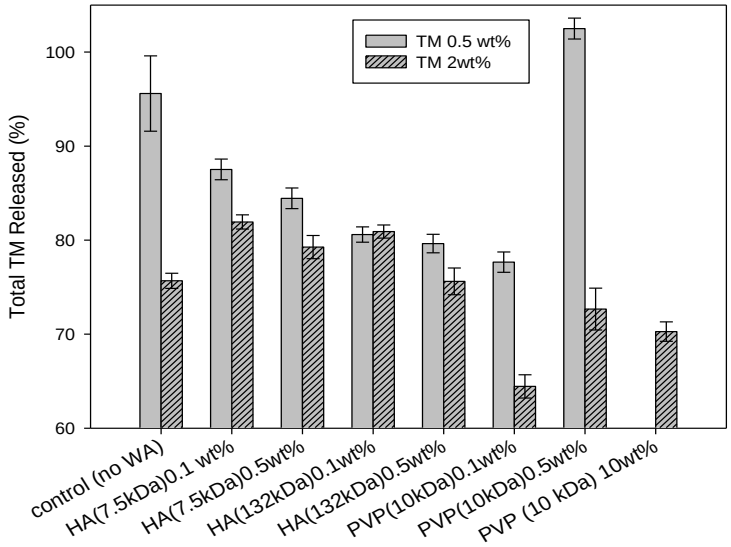

Figure 5.13: Total amount of timolol maleate released from pHEMA/TRIS materials, based on the initial concentration loaded, when different amounts and MW of HA and PVP were loaded during silicone hydrogel synthesis. Data are shown as mean (±SD) with n=4.

5.4.1.1.3 Timolol maleate release from PVP-containing pHEMA/TRIS hydrogels

The presence of PVP as an internal releasable wetting agent led to different release profiles and kinetics (Figure 5.14). A further increase in the amount of PVP (0.5 wt%) further controlled the release kinetics of TM as shown in Figure 5.14. The wetting agent which has a high affinity for the release medium was probably released faster than the drug without affecting the diffusion of the hydrophilic drug through the hydrogel domains.

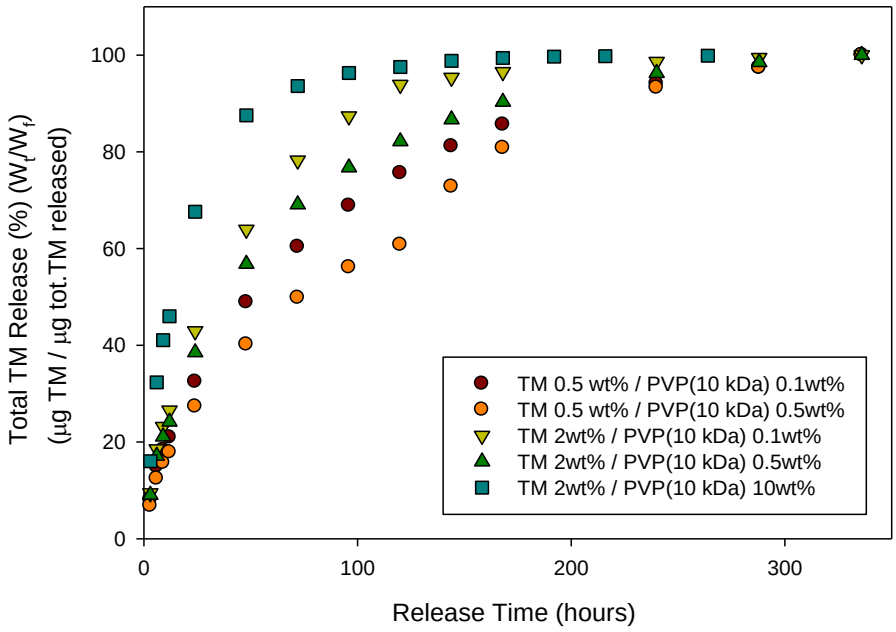

Figure 5.14: Timolol maleate release from PVP-containing pHEMA/TRIS loaded with different concentrations and MW of HA and PVP. Data are shown as mean (±SD) with n=4.

A completely different release profile was observed when a significantly higher amount of releasable PVP (10 wt%) was loaded. Following a burst release, 90% of the drug was released within the first three days with the remaining TM released over the next 4 days by diffusion through the silicone hydrogel matrix (Figure 5.14). As expected, the presence of a wetting agent at high concentration accelerated the release of the

hydrophilic drug, TM, shortening the release duration. The burst release is attributed to the improved hydrophilicity of the HEMA domains due to the presence of PVP, as evidenced by the increase in the EWC. However, even though the release rate of TM in the silicone hydrogel's domains was increased by the presence of the PVP, the total amount of TM released from the 10 wt% PVP-loaded pHEMA/TRIS discs was lower than from the control ($p<3^{x}10^{-4}$) (Figure 5.13).

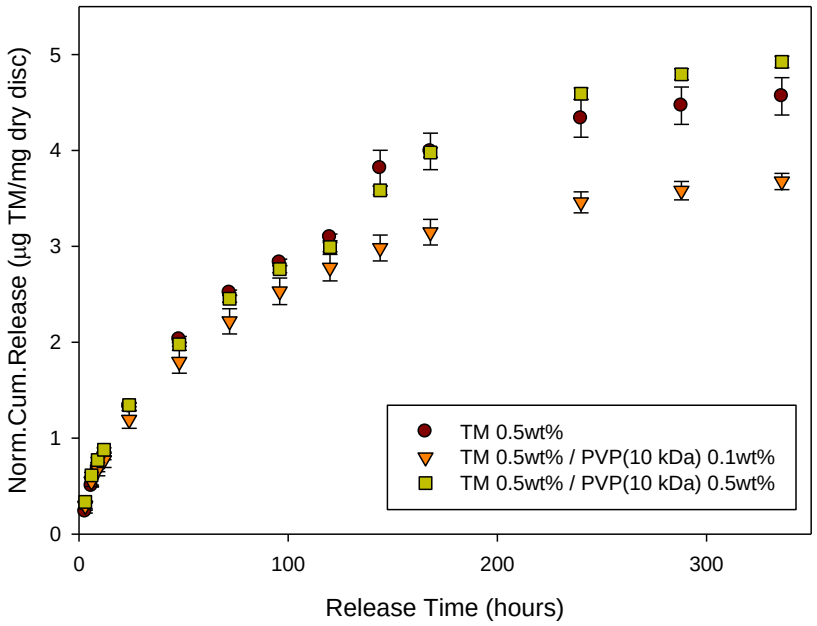

Figure 5.15: Timolol maleate release from PVP-containing pHEMA/TRIS materials. Data are shown as mean (±SD) with n=4.

In 0.1 wt% PVP-loaded pHEMA/TRIS discs, the release profile of TM exhibited an initial burst release phase, which was more controlled in low concentration TM (Figure 5.15). As a result, the total amount of the TM released from 0.1 wt% PVP-containing materials was significantly lower than from the control ($p<0.0001$) (Figure 5.13). This observation was not expected and further research should be conducted in order to

further evaluate why TM release was restricted by the presence of small amounts of releasable PVP. The release profile of PVP under these conditions would possibly help us further understand the mechanism of TM release and the complications observed. In addition, a small increase in the total amount of the TM released ($p<0.02$) was found in the case of low concentration TM materials containing 0.5wt% PVP, which suggests that diffusion of TM from the hydrophilic domains was improved (Figure 5.13 and 5.15).

A comparison between HA and PVP of similar molecular weights was performed demonstrating that while the release profiles were similar for the first 9 days, thereafter the presence of HA increased the amount of TM released while PVP obstructed its release. This could be due to the affinity of the anionic HA with the HEMA chains of the silicone hydrogels, leading to slower or longer release than with PVP.

5.4.1.2 DMA/TRIS model silicone hydrogels

5.4.1.2.1 Timolol maleate release from non-wetting agent containing DMA/TRIS hydrogels

In DMA/TRIS materials, the release of TM at a loading of 0.5 wt% lasted for 4 days, with 90% of the drug being released within 60 hours (2.5 days), followed by a slower release rate where the limiting step is probably the diffusion of TM through the aqueous domains (Figure 5.16). More than 90% of the TM initially loaded within the DMA/TRIS discs that did not contain a wetting agent, was released from the silicone hydrogel matrix (Figure 5.17).

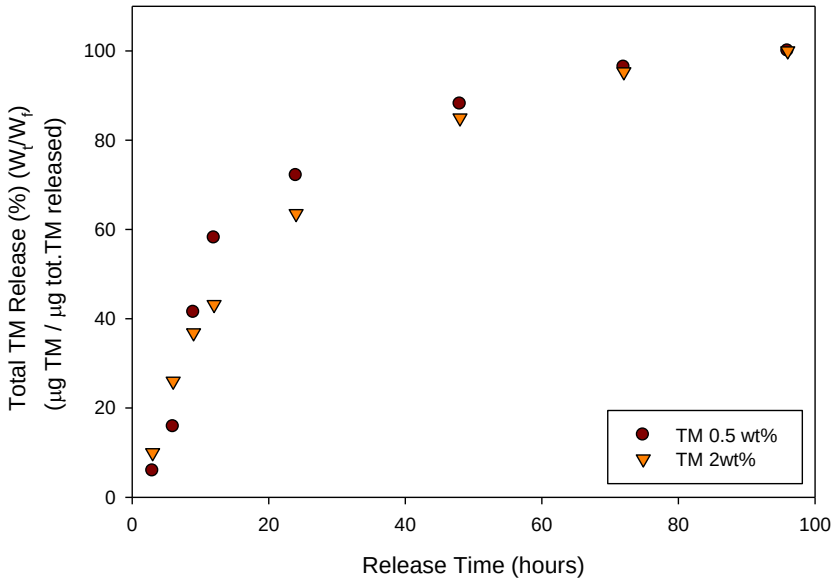

Figure 5.16: Release kinetics from 0.5 wt% and 2 wt% TM-loaded DMA/TRIS discs. Data shown as mean (±SD) with n=4.

A further increase in the concentration of the therapeutic agent in DMA/TRIS discs did not change the release profile, contrary to pHEMA/TRIS materials, or the duration of TM release. Following an initial burst release, the release rate slowly decreased. The release kinetics from both low and high TM materials were similar (Figure 5.16), whereas the total amount (%) of TM released was significantly lower in case of TM 2 wt% (Figure 5.17). This decrease is attributed to the possible physical or chemical entrapment of the TM, during synthesis, in the crosslinked silicone domains of the hydrogel.

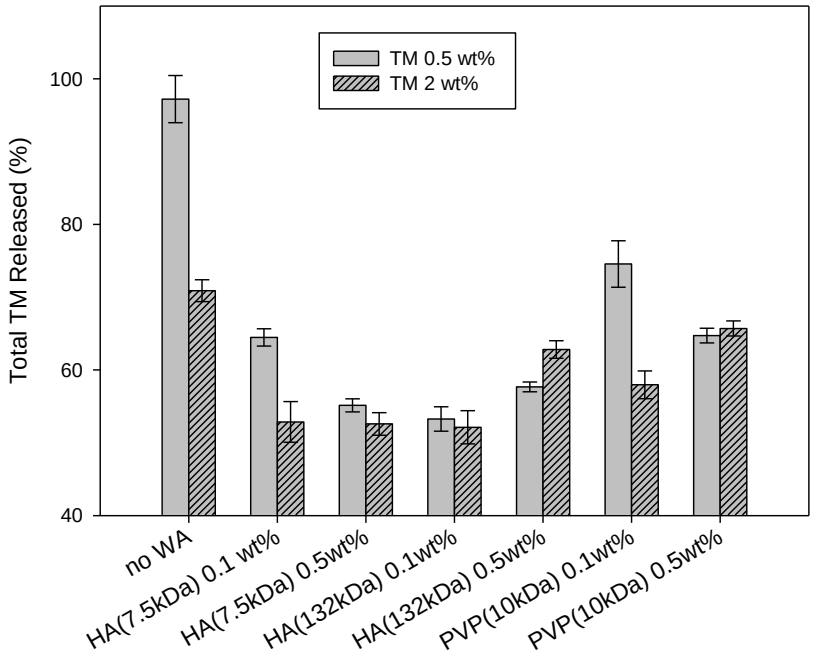

Figure 5.17: Total amount of timolol maleate released from DMA/TRIS materials, based on the initial concentration loaded, with different amounts and MW of HA and PVP loaded during silicone hydrogel synthesis. Data are shown as mean (±SD) with n=4.

5.4.1.2.2 Timolol Release from HA-containing DMA/TRIS hydrogels

In order to verify the electrostatic interactions between TM and HA, the release kinetics of HA (7.5 kDa) 0.5wt% were compared with the TM release kinetics from HA-containing DMA/TRIS materials. As shown in Figure 5.18, the release kinetics were similar verifying that interactions between the TM and the HA led to the HA controlling TM release while a significant reduce in the amount of TM released up to 50% ($p<4^{x}10^{-7}$) was noticed (Figure 5.17).

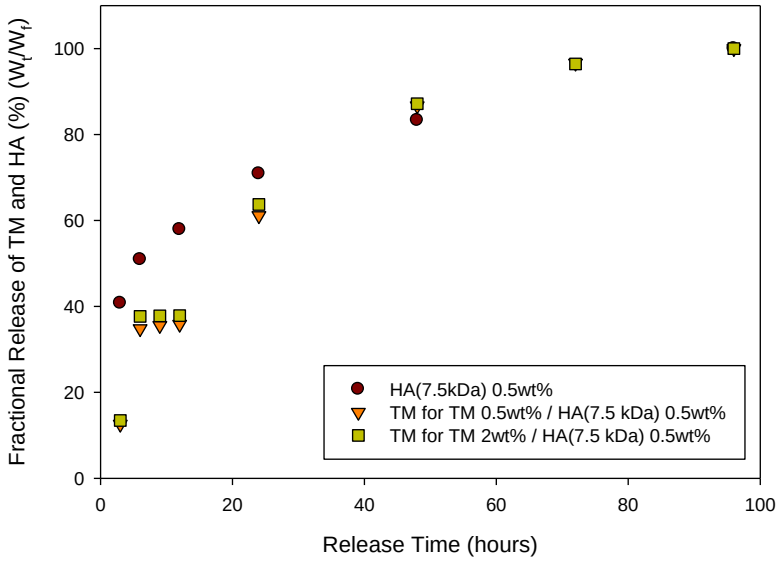

Figure 5.18: Release kinetics of both TM and HA from DMA/TRIS loaded with different concentrations and MW of HA. Data are shown as mean (±SD) with n=4.

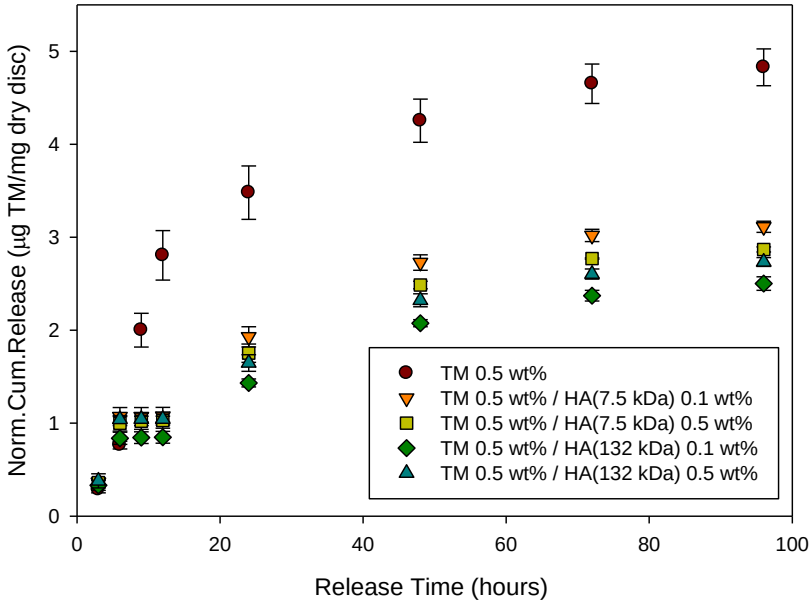

Figure 5.19: Cumulative release of timolol maleate loaded at 0.5 wt% from DMA/TRIS materials with different concentrations and MW of HA. Data are shown as mean (±SD) with n=4.

The presence of releasable HA within the silicone hydrogel matrix decreased the release rate without changing the release kinetics (Figures 5.19 and 5.20). A further increase in the MW of HA caused a further decrease in the release rate of TM, especially in the 0.1 wt% HA-containing samples ($p<4^{x}10^{-5}$), while increasing the concentration of HA (132 kDa) led to higher amounts of TM release ($p<0.003$) (Figures 5.17 and 5.19). Despite any increase in the release rate, the cumulative mass of TM released was still lower than that of the control. As in the HA-loaded pHEMA/TRIS hydrogels, the HA chains, which are significantly longer than TM, slowly diffuse through the water filled pores, controlling the drug release rate. In addition, some of the HA/TM complex is likely trapped in the crosslinked network during synthesis due to either physical entrapment in tightly bound regions or chemical entrapment due to reactions (Kim & Chauhan, 2008).

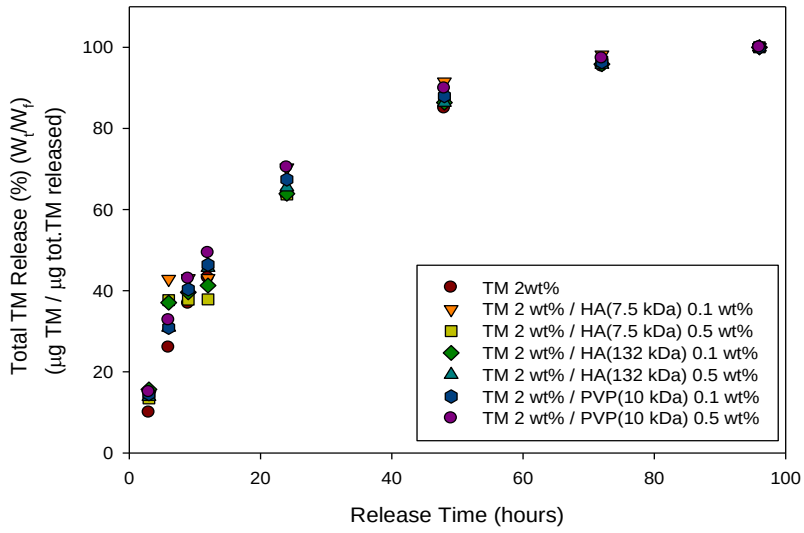

Figure 5.20: Release kinetics of TM (0.5%) loaded DMA/TRIS discs. Data are shown as mean (±SD) with n=4.

Finally, all HA-containing materials were characterized by the same release kinetics without showing any change in the release duration compared with the control DMA/TRIS hydrogels.

5.4.1.2.3 Timolol maleate release from PVP-containing DMA/TRIS hydrogels

In DMA/TRIS hydrogel materials the presence of PVP as an internal releasable wetting agent led to different release profiles, controlled by the concentration of the therapeutic agent TM and that of PVP, without any change in release duration. In all cases, the release kinetics were the same. Similar to HA, non-covalent incorporation of PVP into the DMA/TRIS network caused decrease in the TM release rate suggesting an interaction between the drug and the wetting agent (Figure 5.21) that needs to be further examined. It was expected that a further increase in the concentration of PVP would improve the diffusivity of TM by reinforcing the hydrophilic domains of the polymer matrix. Instead, as shown in Figure 5.21, the release rate was further decreased.

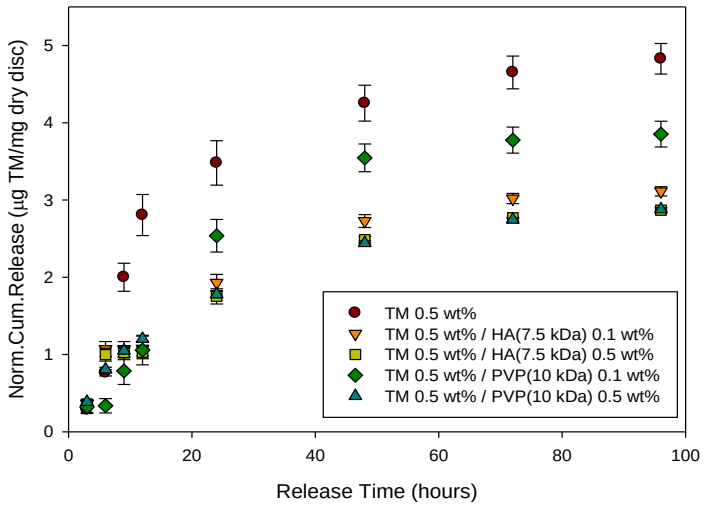

Figure 5.21: Timolol maleate 2 wt% cumulative release from DMA/TRIS loaded with different concentrations and MW of HA. Data are shown as mean (±SD) with n=4.

On the other hand, a 4-fold increase in the concentration of the therapeutic agent overcame the impact of PVP on the release, leading to similar release profiles and duration of TM as that of the materials loaded with TM only (data not shown). As a result,

there was not a general trend that could suggest a specific release mechanism of TM from PVP-containing DMA/TRIS materials.

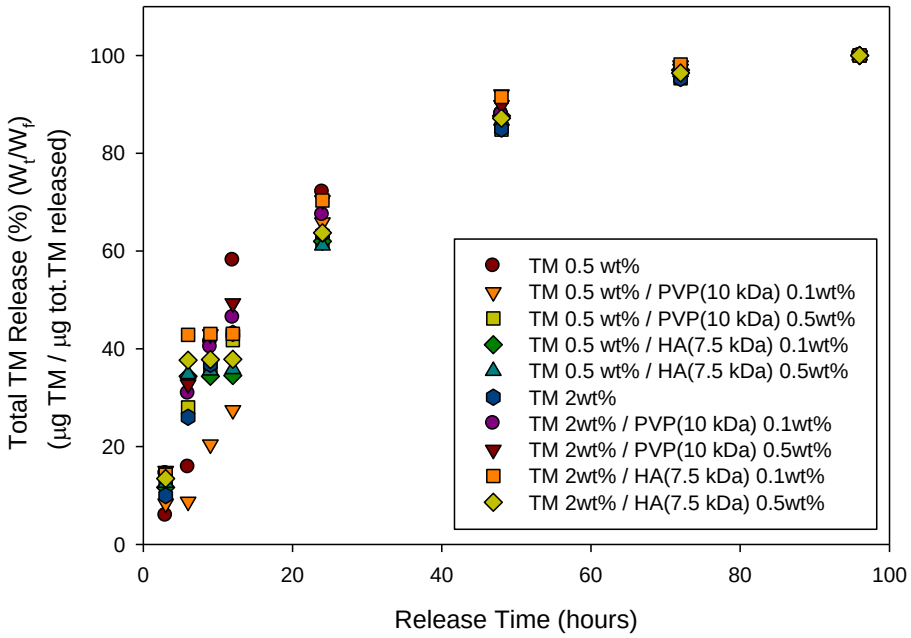

Figure 5.22: Timolol maleate release kinetics from DMA/TRIS materials. Data are shown as mean (±SD) with n=4.

Finally, when the impact of PVP was compared to that of HA (Figure 5.22) all samples exhibited similar release kinetics independent of the TM and wetting agent concentrations. It is of interest to mention that PVP-containing samples did not exhibit that initial release delay that HA-containing samples did (Figure 5.22). This observation should be further examined.

5.4.1.3 pHEMA/TRIS vs DMA/TRIS hydrogels loaded with timolol maleate

The cumulative release from the pHEMA/TRIS materials did not show similar kinetics when compared to the respective DMA/TRIS hydrogels (Figure 5.23). Based on

comparative studies between the cumulative release profiles and kinetics, the composition of the silicone hydrogel, the nature as well as the concentration of both therapeutic and wetting agents can be used in order to tailor the release of a protonated drug like TM.

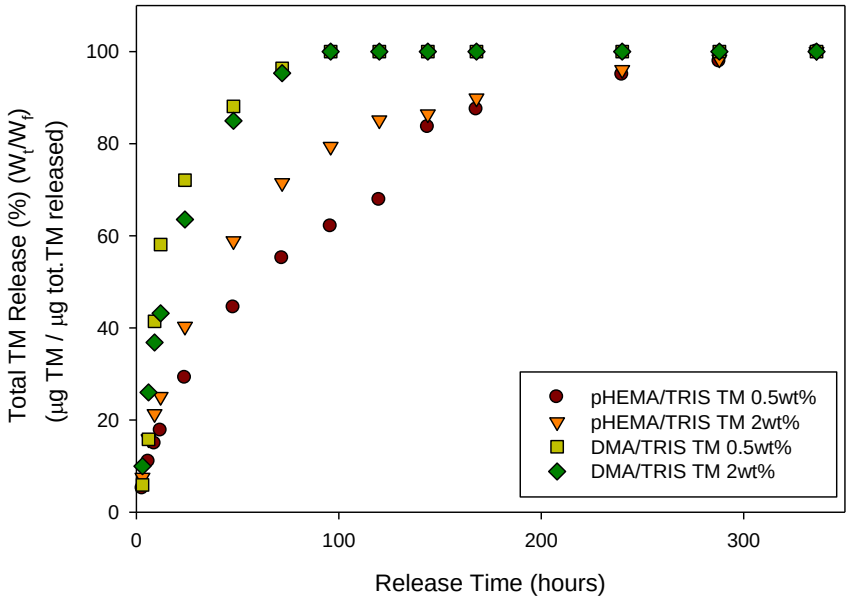

Figure 5.23: Timolol maleate release kinetics of pHEMA/TRIS and DMA/TRIS hydrogels. Data are shown as mean (±SD) with n=4.

The composition of the silicone hydrogel matrix played a significant role in determining the release duration of TM in PBS under physiological conditions. The DMA/TRIS materials would be expected to show more controlled and extended release of hydrophilic TM since the concentration of TRIS is higher in these materials while also the pHEMA/TRIS materials have higher phase separation. The release duration and kinetics are controlled by the interactions developed between the drug and polymer domains, the water content of the silicone hydrogel as well as the interactions between the wetting agent and the drug. Therefore, in pHEMA/TRIS-based materials the

intramolecular binding interactions between the TM and the HEMA domains were the predominant factor affecting release. More specifically, the hydroxyl groups of HEMA monomers interact through hydrogen bonding with the amino, ether and hydroxyl groups of TM (Hiratani & Alvarez-Lorenzo, 2004) allowing slower drug diffusion through these domains. On the other hand, in DMA/TRIS-based materials, the release duration is determined by the increased hydrophilicity of the DMA domains since intramolecular interactions between the drug and the neutral ion-permeable DMA are not that significant. The higher degree of swellability of the latter materials suggests that there are more aqueous domains in the polymer matrix, compared to pHEMA/TRIS hydrogels, with the swelling rate and the pore size being the determining factors in TM release. Therefore, it is not surprising that shorter release durations were observed with DMA/TRIS hydrogels. This is agreement with previous studies were it was found that HEMA-based conventional contact lenses have exhibited greater affinity with timolol maleate compared to other lenses (Hiratani & Alvarez-Lorenzo, 2004).

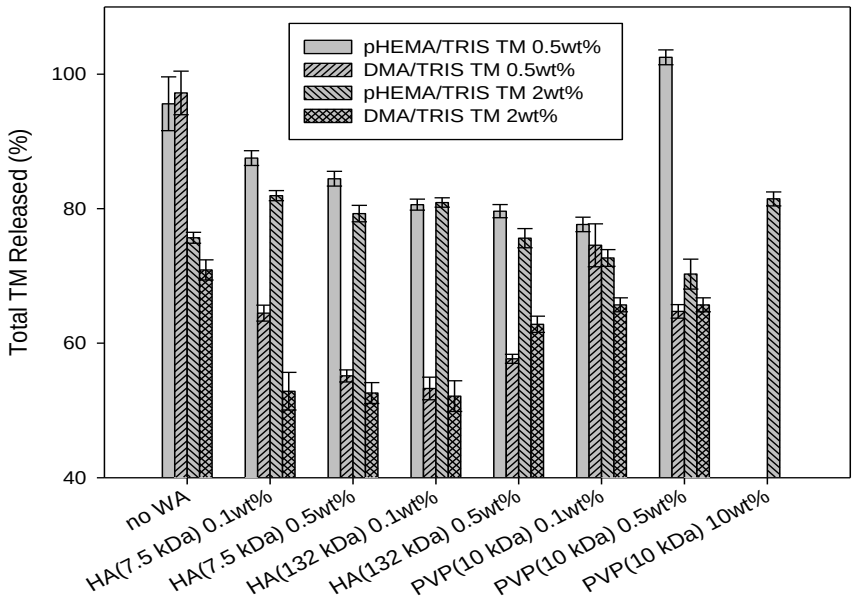

Figure 5.24: Total amount of timolol maleate (TM) released from pHEMA/TRIS and DMA/TRIS materials, based on the initial concentration loaded, when different amounts and MW of HA and PVP were loaded during silicone hydrogel synthesis. Data are shown as mean (±SD) with n=4.

The presence of wetting agent altered the cumulative release profile. Attractive electrostatic interactions between the protonated drug TM and anionic HA polymer were the limiting step in determining the release rate of TM in HA-containing materials, without altering the release kinetics. The impact of HA and its MW was more obvious in the DMA/TRIS materials. Interactions between the carbonyl groups (C=O) in PVP and the hydroxyl groups of TM molecules (Ng & Swami, 2008; Auzély-Velty et al., 2002) also affected the release profile of TM, while the release kinetics were altered in the pHEMA/TRIS hydrogels but not in DMATRIS. However additional studies are necessary to better understand interactions in order to draw more defined conclusions.

In addition, the release duration of TM was not affected by the presence of the wetting agent. Based on Figure 5.24 the total amount of TM released was consistently

lower from the DMA/TRIS hydrogels. As mentioned above, it is believed that some of the drug and wetting agent are immobilized in the crosslinked silicone domains during synthesis and are not being released. When the TRIS concentration was lower (pHEMA/TRIS samples), less drug was immobilized in these domains, suggesting irreversible interactions between the drug and the polymer backbone. This can be attributed possibly to either physical entrapment of the drug and/or the wetting agent in the tightly bound regions, and especially the silicone ones, or chemical entrapment due to reactions (Kim & Chauhan, 2008).

The released HA/TM compounds will presumably dissociate in the presence of tear film due to the presence of salt (NaCl). As well, this combination may allow the mucoadhesive properties of HA to increase the residence time of TM on the corneal surface, diminishing the undesired side effects. PVP was also found to enhance ocular TM adsorption in case of topical administration, reducing the instilled volumes thus reducing systemic adsorption (Podder et al., 1992). Therefore, the studied wetting agent-containing materials have potential applications as drug delivery systems/devices for sustained TM release used for the treatment of chronic pathologies such as glaucoma.

5.4.2 Ketotifen Fumarate Release

Ketotifen Fumarate (KF) was used as an alternative therapeutic agent in order to examine the release profile of wetting agent-containing pHEMA/TRIS and DMA/TRIS hydrogels. KF is an amphiphilic antihistamine (neutral or positively charged), used to treat ocular allergies (allergic conjunctivitis) (Alkhamis et al., 2008) with a MW of 425.5 Da (Figure 5.25). In the literature, two different values of pKa have been mentioned pKa=6.7 (AA, 2011) and pKa= 8.43 (Ketotifen Fumarate pKa).

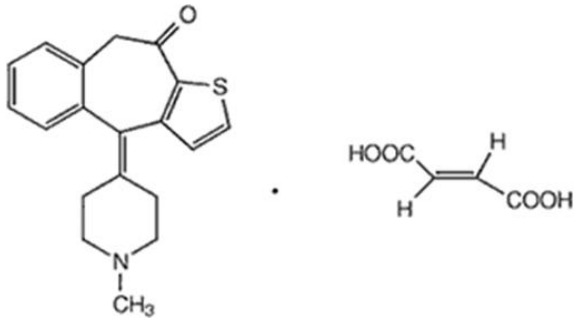

Figure 5.25: Chemical structure of ketotifen fumarate.

On the other hand, HA has a pKa value of about 3.0 (Brown & Jones, 2005b) and therefore under the prepolymer mixture and release conditions is negatively charged. Moreover, based on EWC study results the incorporation of neither HA nor PVP in the pHEMA/TRIS and DMA/TRIS hydrogels during synthesis by direct entrapment altered the swellability of the studied materials with the exception of the pHEMA/TRIS discs containing 2 wt% HA (7.5 kDa) and 10 wt% PVP (10 kDa). Therefore, any change in the release kinetics occurs due to interactions between the drug, the wetting agent and the matrix of the silicone hydrogel during KF diffusion.

5.4.2.1 pHEMA/TRIS model silicone hydrogels

5.4.2.1.1 Ketotifen fumarate release from non-wetting agent containing pHEMA/TRIS hydrogels.

The total release of KF 1 wt% lasted for 36 days, with 90% of the drug being released within 25 days (Figure 5.26). Based on the extended release profile we could speculate that KF might associate with and diffuse through the silicone and the hydrogel domains, due to its amphiphilic nature. Within the first 24 hours, approximately 20% of KF was released probably due to the dissolution of drug located at/or near the surface of

the silicone hydrogel discs during transition from the glassy to the rubbery state (Figure 5.26). The drug release rate was determined by the diffusion of KF through the mesh and the PBS-filled pores of the hydrogel (Xu, Li et al, 2011b). More than 75% percent of the KF initially loaded was released within the release duration (Figure 5.27).

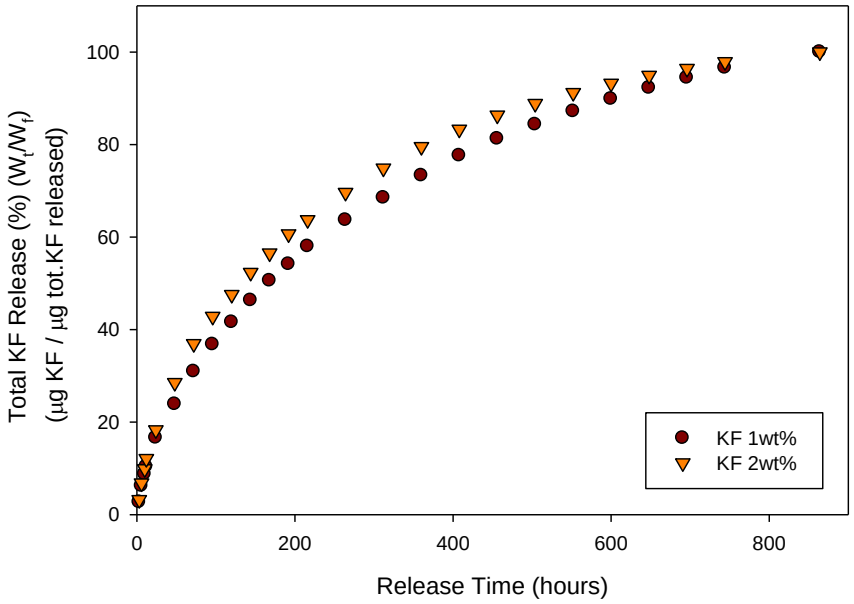

Figure 5.26: Cumulative release from Ketotifen Fumarate (KF)-loaded pHEMA/TRIS. Data are shown as mean (±SD) with n=4.

A further increase in the concentration of the therapeutic agent in pHEMA/TRIS discs, led to a system with slightly faster cumulative release rate (Figure 5.26). While the release duration of KF 2 wt% was the same as KF 1wt%, 90% of the drug got released within 23 days, exhibiting a concentration dependent cumulative release profile. The same explanation given for high concentration TM-loaded pHEMA/TRIS materials, could be given here too. Increasing the concentration of the KF dispersed within the matrix, the release rate and kinetics were increased because the driving force for mass transfer is the concentration differences between the two release media. A greater difference led to

faster release rate and, most of the time, a higher amount of KF released. However, in the case of control samples (no wetting agent), a 2-fold increase in the initial amount of drug loaded during synthesis did not lead to higher cumulative mass (%) ($p>0.08$) (Figure 5.27).

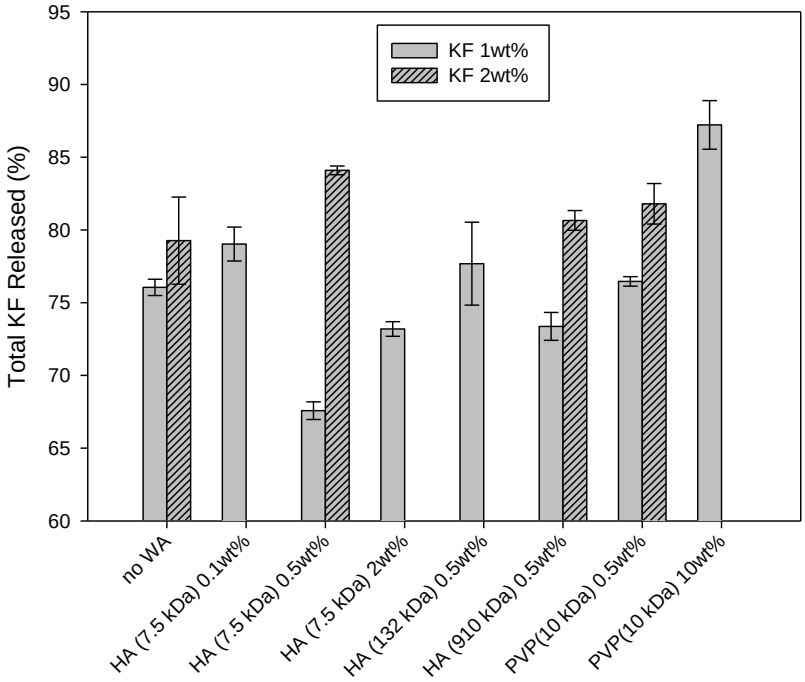

Figure 5.27: Total amount of KF released from pHEMA/TRIS materials, based on the initial concentration loaded, when different amounts and MW of HA and PVP were loaded during silicone hydrogel synthesis. Data are shown as mean (±SD) with n=4.

5.4.2.1.2 Ketotifen fumarate release from HA-containing pHEMA/TRIS hydrogels

The presence of releasable HA slightly affected the release of KF (Figure 5.28). Normally, assuming an interaction between KF and HA in case of choosing as pKa=8.43 hence drug in protonated form, similar to that of HA and TM but in a lower degree due to the chemical structure of KF, we would expect a change in either the release rate or the

release kinetics of KF. Therefore, further research on release duration of HA as well as on the interactions developed between HA, the drug and the polymer matrix should be conducted.

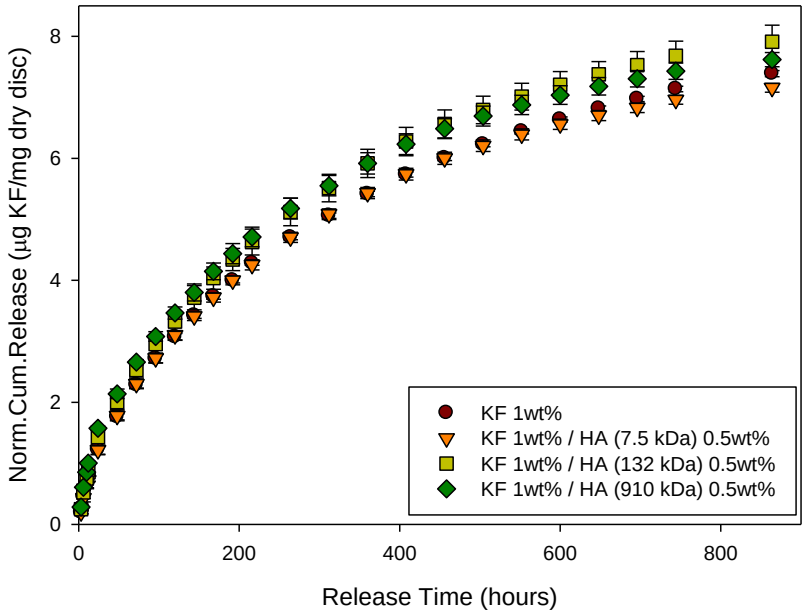

Figure 5.28: Cumulative release of KF-loaded pHEMA/TRIS hydrogels containing HA of different MW. Data are shown as mean (±SD) with n=4.

As shown in Figure 5.28, increasing the MW of HA led to a slight increase in release rate without however affecting the release kinetics or release duration. Moreover, when the MW of HA was kept constant (7.5 kDa) and the cumulative release was examined, increasing the HA concentration resulted in different cumulative mass amounts although no specific trend was observed. The samples containing HA (7.5 kDa) 0.1wt% exhibited increased cumulative mass ($p<0.004$) (Figure 5.27) while samples containing HA(7.5 kDa) 2wt% demonstrated a reduced amount of KF released, even though the EWC was increased. Higher EWC should lead to higher KF release from these materials due to change in the mesh and pores size, although this was not

observed. Therefore, the direct entrapment of HA may have the potential to improve the bulk properties of the model pHEMA/TRIS hydrogels during its release, but not to control the simultaneous release of an amphiphilic therapeutic agent like KF.

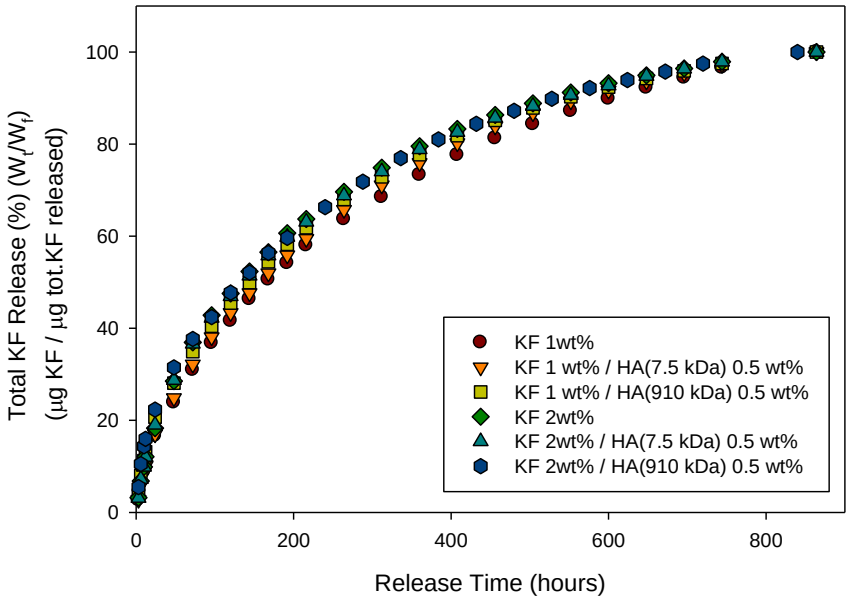

Figure 5.29: KF release kinetics from pHEMA/TRIS loaded with different concentrations and MW of HA. Data shown as mean (±SD) with n=4.

Furthermore, all HA-containing materials exhibited the same release kinetics independent of the KF concentration (Figure 5.29). All of the afore mentioned observations suggest that there were no specific interactions between the KF and the negatively charged HA in agreement with previous studies where the molecular binding between KF and HA was examined (Uccello-Barretta et al., 2008). Surprisingly, these compounds are used together in eye drops - Hyalcrom NF from Bausch and Lomb. It would therefore be useful to track the release of HA during the simultaneous release of KF in order to see if the presence and release of a bigger anionic molecule like HA, affects the release profile of a unionized drug like KF.

5.4.2.1.3 Ketotifen fumarate release from PVP-containing pHEMA/TRIS hydrogels

As shown in Figures 5.30 and 5.31, the incorporation of 0.5 wt% releasable PVP (10kDa) as an internal wetting agent did not show significant impact on the cumulative release profile and release kinetics compared to the control (only KF) samples, resulting in the same release duration independent of the KF concentration. This can be attributed to the fact the low amount of the hydrophilic agent PVP diffuses through the matrix more quickly than the therapeutic agent and as a result does not impact on its release profile. The release profile for PVP would help to understand the mechanism of the simultaneous release of the therapeutic and wetting agent.

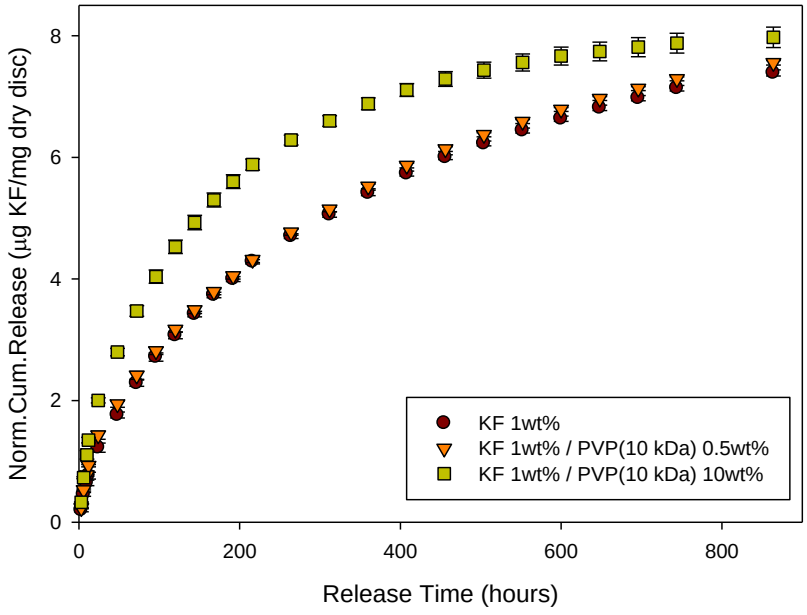

Figure 5.30: Cumulative release of KF from PVP-containing pHEMA/TRIS loaded. Data are shown as mean (±SD) with n=4.

The cumulative release and release kinetics changed when a significantly higher concentration (10 wt%) of PVP was incorporated during synthesis (Figures 5.30 and

5.31), leading to a faster release rate without altering the release duration. Based on the fractional cumulative release profile (Figure 5.31) 90% of the drug was released within 17 days while the same percentage of KF was released within 25 days in 0.5wt% PVP-containing materials. The remaining drug within the silicone hydrogel network slowly diffused within the next 21 days, exhibiting a concentration dependent diffusion profile. The high PVP concentration materials were characterized by higher EWC (22%) that resulted in a more swollen hydrogel matrix where the bigger pore size also contributed to faster diffusion of KF though the aqueous channels. Moreover, this significant increase in the PVP content led to pHEMA/TRIS hydrogels with reinforced hydrophilic domains that decreased the affinity between the matrix and the amphiphilic KF therapeutic agent. As a result, 14% more KF was released from these materials compared to that of control ($p<1.5 \cdot 10^{-4}$) (Figure 5.27).

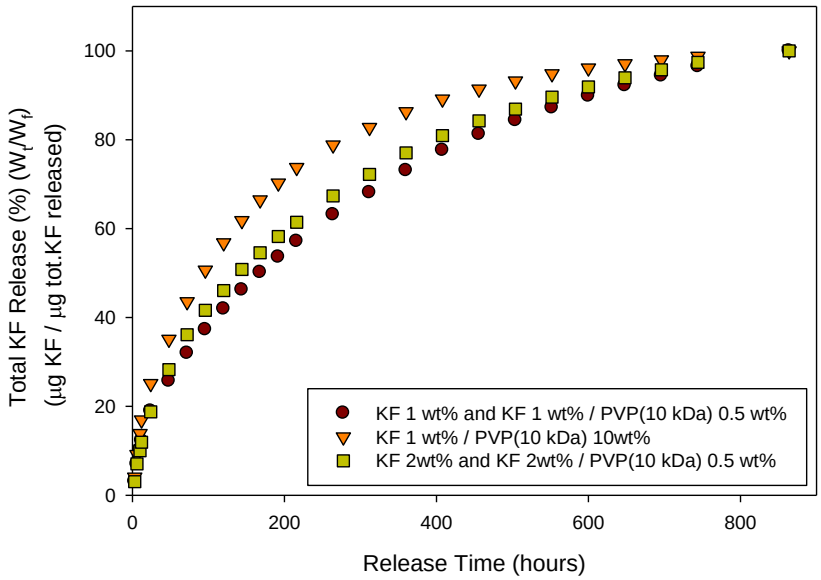

Figure 5.31: Ketotifen fumarate release from PVP-containing pHEMA/TRIS. Data are shown as mean (±SD) with n=4.

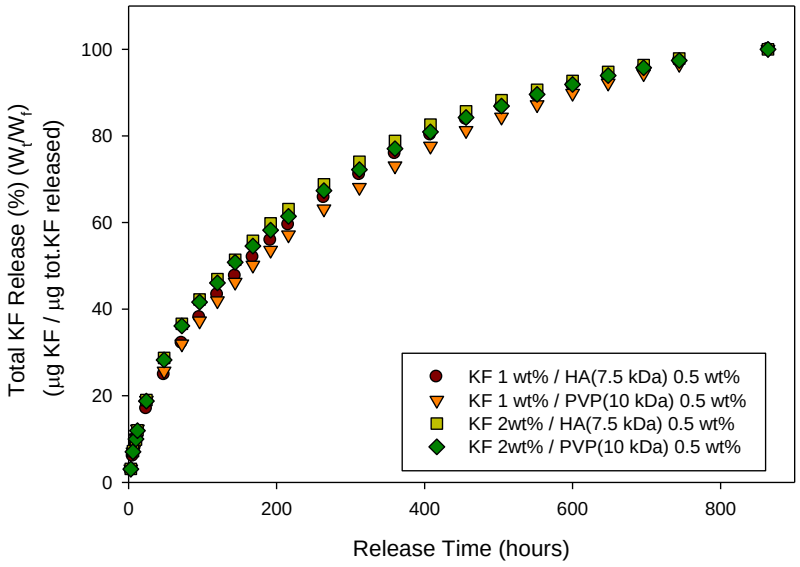

Figure 5.32: Ketotifen fumarate release kinetics from HA or PVP-containing pHEMA/TRIS. Data are shown as mean (±SD) with n=4.

To conclude, a comparative study between similar MW HA and PVP-containing pHEMA/TRIS discs showed a speculated absence of any interaction between the drug and the wetting agent or the wetting agent and the matrix led to almost the same diffusion kinetics (Figure 5.32). Since PVP-containing materials released more KF than those containing HA, further research should be conducted in order to understand the effect of the nature of the drug on the release profile.

5.4.2.2 DMA/TRIS model silicone hydrogels

No change in the EWC was observed in KF-loaded DMA/TRIS with or without the presence of wetting agent, therefore alteration of release profile or kinetics is attributed to interactions between the therapeutic and/or wetting agent and the silicone hydrogel.

5.4.2.2.1 Ketotifen fumarate release from non-wetting agent containing DMA/TRIS hydrogels.

The total release of KF 0.5 wt% lasted for 24 days, with 90% of the drug being released within 14 days (Figure 5.33). Similarly to pHEMA/TRIS materials, the extended release profile of KF can be also attributed to possible interactions with the hydrophobic silicone domains. Approximately 60% of the KF initially loaded in the control DMA/TRIS discs was released from the silicone hydrogel matrix (Figure 5.34). The cumulative release profile of KF in DMA/TRIS materials suggests concentration dependent diffusion of KF through the aqueous domains of DMA being the main mechanism of release.

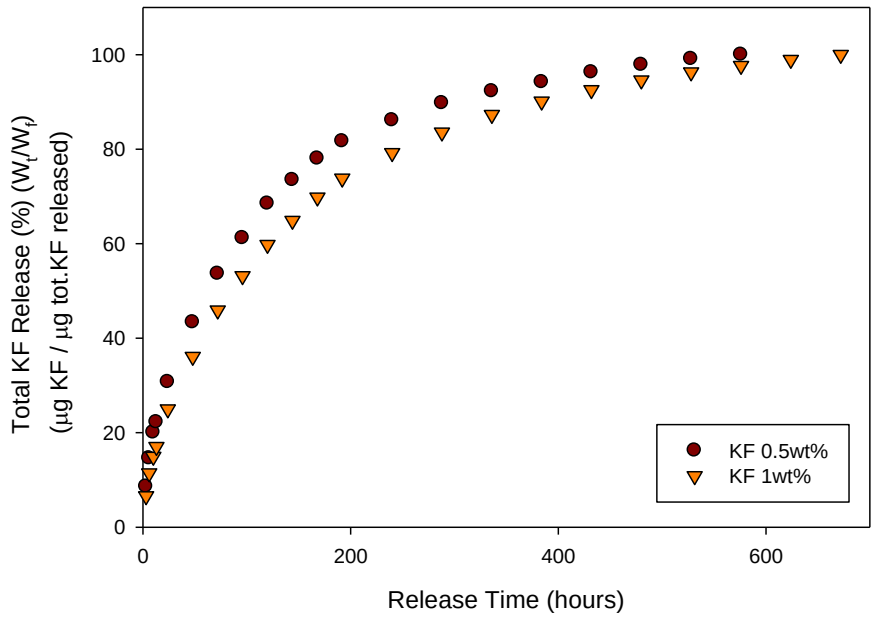

Figure 5.33: Release kinetics of 0.5 wt% and 1 wt% KF-loaded DMA/TRIS discs. Data are shown as mean (±SD) with n=4.

A further increase in the concentration of the KF loaded during synthesis slightly decreased the release rate of the drug resulting in a more sustained release profile (Figure 5.33). Both 1 wt% and 0.5 wt% KF-loaded DMA/TRIS materials released similar

percentage of KF (Figure 5.34), based on the amount of drug initially loaded. Therefore contrary to the pHEMA/TRIS materials, a greater difference between the concentration of the silicone hydrogel matrix and the release medium, leads to a stronger affinity between the therapeutic agent and the material. This suggested that there might be an interaction between the KF and the hydrogel domains that needs to be further examined in order to understand the release mechanism of KF in DMA/TRIS hydrogels.

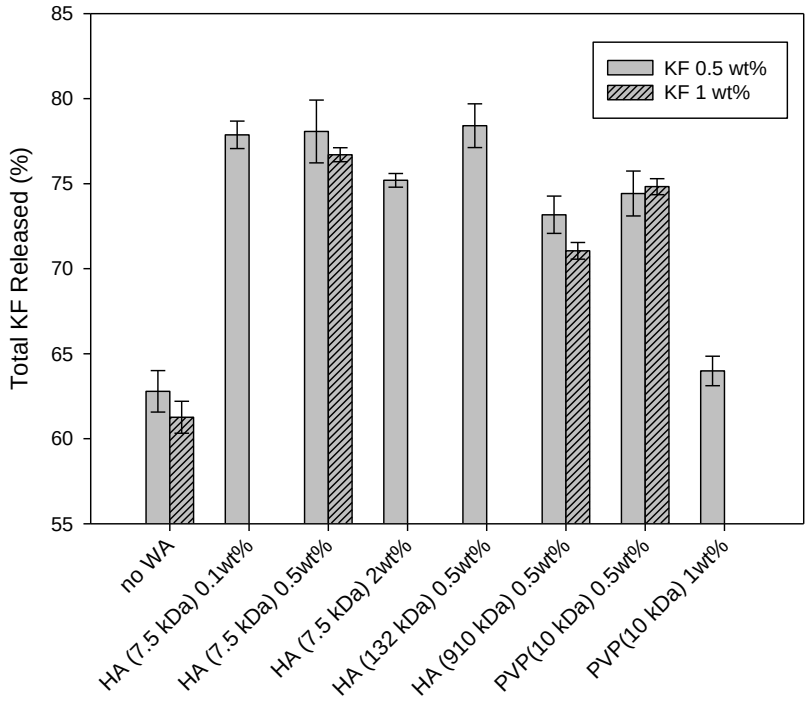

Figure 5.34: Total amount of KF released from DMA/TRIS materials, based on the initial concentration loaded, when different amounts and MW of HA and PVP were loaded during silicone hydrogel synthesis. Data are shown as mean (±SD) with n=4.

5.4.2.2.2 Ketotifen fumarate release from HA-containing DMA/TRIS hydrogels

In DMA/TRIS materials, the non-covalent incorporation of an anionic glycosaminoglycan, like HA, during the synthesis of KF-loaded hydrogels led to materials

with the same release kinetics but higher release rates (Figures 5.35 and 5.36) when compared to the control (no wetting agent). As a result, even though the release duration was not prolonged, greater amounts of KF were released with the presence of HA in the silicone hydrogel matrix ($p<10^{-4}$) (Figures 5.34 and 5.35).

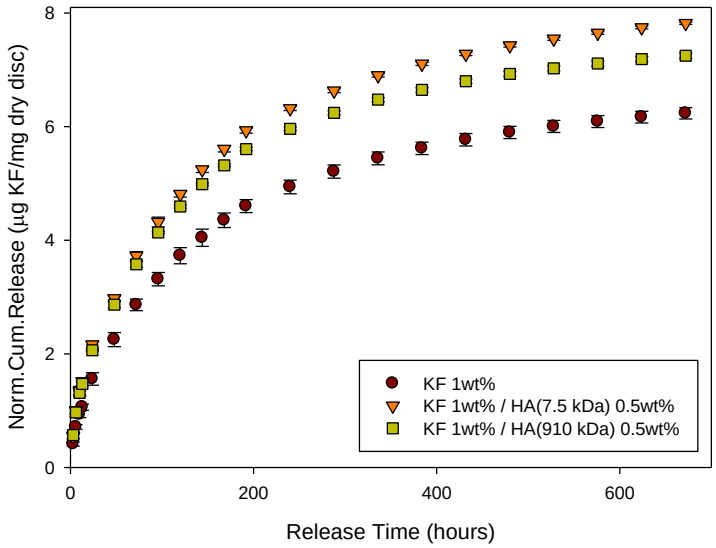

Figure 5.35: Cumulative release profile of KF-loaded DMA/TRIS hydrogels containing HA of different MW. Data are shown as mean (±SD) with n=4.

The release rate was inversely proportional of the MW of HA, with this observation being more obvious in the case of KF 1 wt% (Figure 5.35). Increasing the MW of the HA reduced the amount of KF released which was still though greater than that of the control. Since there is no specific binding affinity between HA and KF (Uccello-Barretta et al., 2008), there might be interactions developed between the polymer matrix and HA, that affect the release of KF. It can be speculated that direct entrapment of the wetting agent within the silicone hydrogel matrix during synthesis does not lead to the formation of big hydrophilic aggregates within the network that can control the drug release kinetics or alter the water content. However assuming uniform dispersion, HA and/or PVP might

coat the polymer channels, facilitating the diffusion of the drug without altering the release (kinetics). For this reason, it would be suggested to track also the release profile of HA as well as to examine the structure of these materials in order to better support this idea.

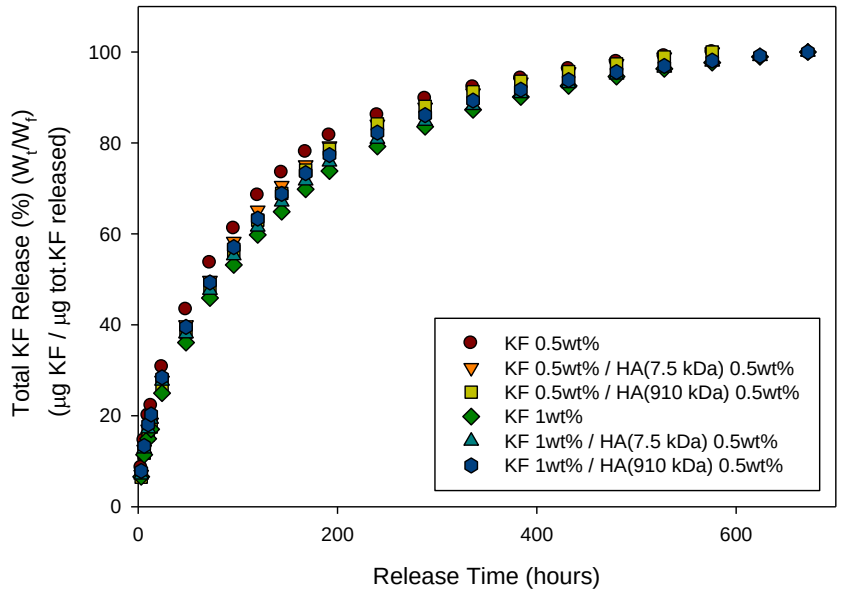

Figure 5.36: KF release kinetics from DMA/TRIS loaded with different concentrations and MW of HA. Data are shown as mean (±SD) with n=4.

Based on Figure 5.36, the presence of HA led to materials with release kinetics bounded between the release kinetics of the control materials of different KF concentration, without significant differences being observed.

5.4.2.2.3 Ketotifen fumarate release from PVP-containing DMA/TRIS hydrogels

The simultaneous release of PVP and KF from DMA/TRIS discs increased the release rates of KF through the silicone hydrogel matrix independently of the initial concentration of KF, suggesting that the wetting agent facilitated the diffusion of KF. As a

result, a higher cumulative mass of KF was released during the same release duration compared to that of control samples (Figure 5.37).

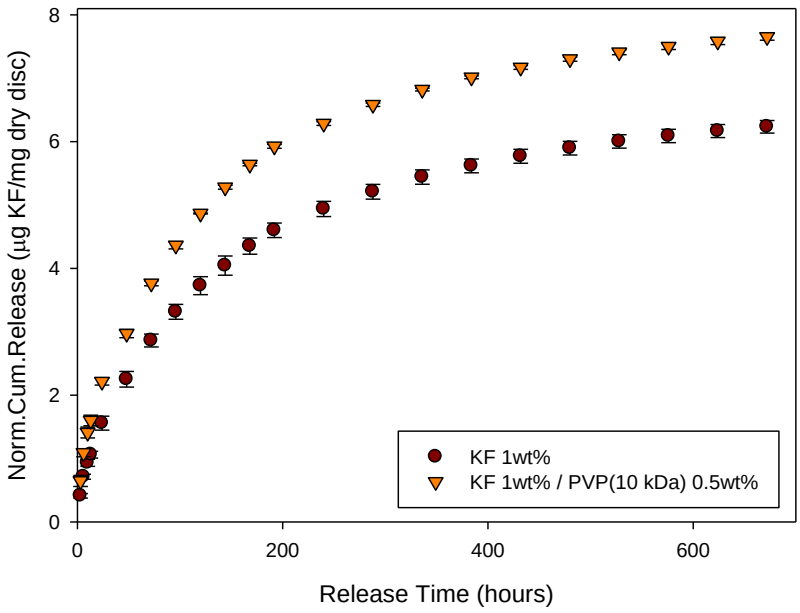

Figure 5.37: Cumulative release from PVP-containing pHEMA/TRIS loaded. Data are shown as mean (±SD) with n=4.

A further increase in the concentration of PVP, however, decreased the release rate of KF leading to a release profile similar to that of the control (data not shown). This behavior even though was not expected, can be directly correlated to the decrease in the EWC that was observed as well as the aforementioned threshold concerning the incompatibility between the PVP and the DMA in DMA/TRIS hydrogels.

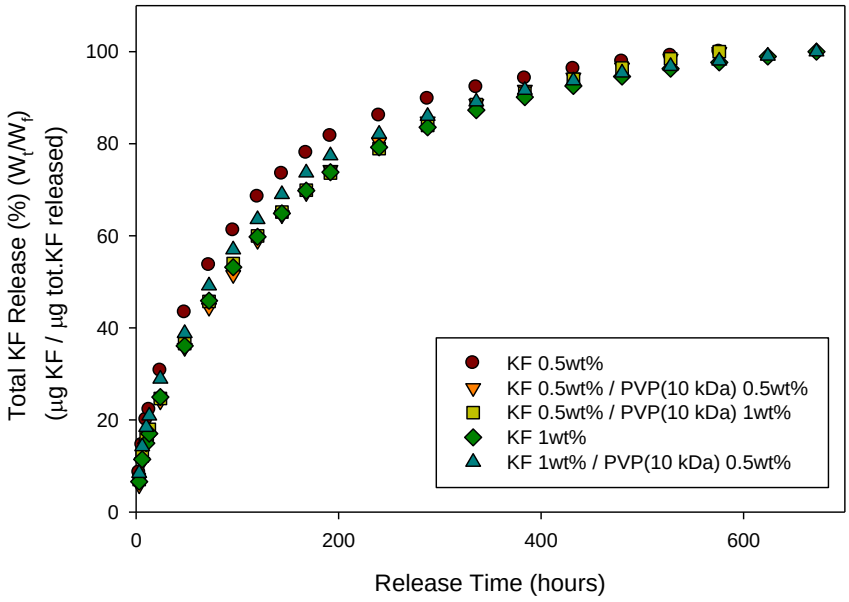

Figure 5.38: Ketotifen fumarate release from PVP-containing pHEMA/TRIS. Data are shown as mean (±SD) with n=4.

The release kinetics were similar suggesting a concentration dependent release profile with diffusion as the mechanism of mass transfer between the matrix and the release medium. Determination of the release duration and kinetics of PVP would help us understand better the contribution of the wetting agent to the higher rate of KF diffusion through DMA/TRIS hydrogels.

Finally, comparing the impact of HA to that of PVP, both wetting agent-containing materials were characterized by the same release profile and kinetics during the same release period (data not shown). HA-containing DMA/TRIS hydrogels released slightly higher amounts of KF ($p<0.05$) compared to PVP-containing hydrogels (Figure 5.34).

5.4.2.3 pHEMA/TRIS vs DMA/TRIS hydrogels loaded with ketotifen fumarate

The pHEMA/TRIS hydrogels exhibited a sustained release profile for KF with a release period of 36 days. On the other hand, KF was released faster from the matrix of DMA/TRIS materials within 24 days. Based on comparative studies between the cumulative release profiles and kinetics (Figure 5.39) the composition of the silicone hydrogel, as well as the nature and the MW of a wetting agent loaded in the polymer matrix along with the nature of the drug can affect the diffusion of an amphiphilic/hydrophilic drug like KF.

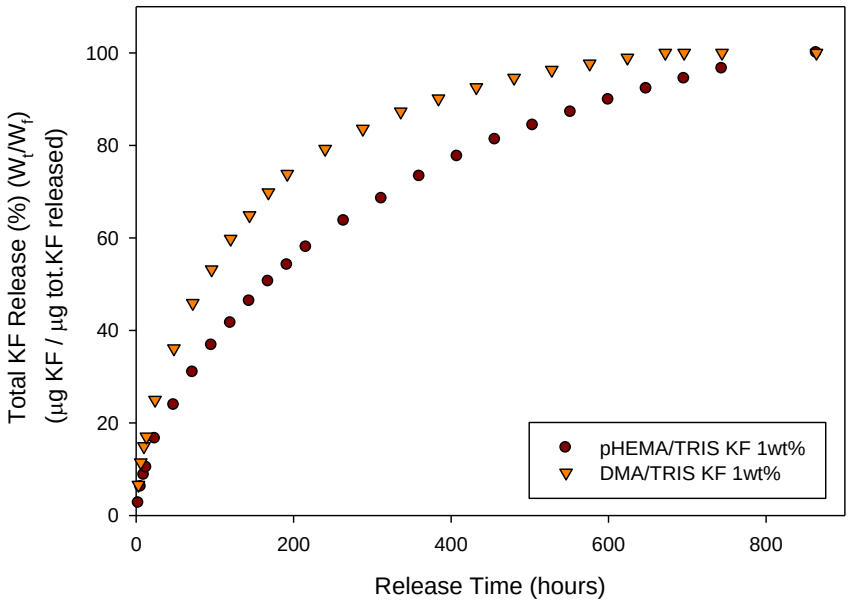

Figure 5.39: Ketotifen fumarate release kinetics of pHEMA/TRIS and DMA/TRIS hydrogels. Data are shown as mean (±SD) with n=4.

The two main factors that can alter the release profile of KF from the studied model silicone hydrogels are the affinity of the drug for the polymer matrix and the water content of the silicone hydrogel. Based on the results of the release study, the hydrophilic/hydrophobic ratio of the HEMA or DMA and TRIS monomers did not play a

significant role since DMA/TRIS materials have higher concentration of TRIS in its matrix compared to pHEMA/TRIS hydrogels but exhibited shorter release duration.

In DMA/TRIS-based materials the release duration and kinetics are determined mainly by the increased swelling degree of the silicone hydrogel caused by the hydrophilicity of the DMA domains. The chemistry of the system does not suggest any interactions between the drug and the neutral ion-permeable DMA. Thus, diffusion of KF through the aqueous domains and the water-filled pores is thought to be the main release mechanism. Contrary, the release duration and kinetics of KF-loaded pHEMA/TRIS hydrogels are controlled predominately by the hydrogen bonds developed between the carbonyl group of KF and the hydroxyl group of HEMA. Interactions between the drug and the silicone domains may also occur due to the amphiphilic nature of KF. As a result, the affinity of the therapeutic agent with pHEMA/TRIS matrix in combination with the high degree of swelling in DMA/TRIS hydrogels leads to the difference between the release duration of DMA/TRIS and pHEMA/TRIS materials. The properties of the therapeutic agent are speculated to affect the release mechanism as the release profile does not appear to be correlated solely with the hydrophilicity of the therapeutic agent. Further investigation of KF properties would therefore help to understand better the aforementioned interactions.

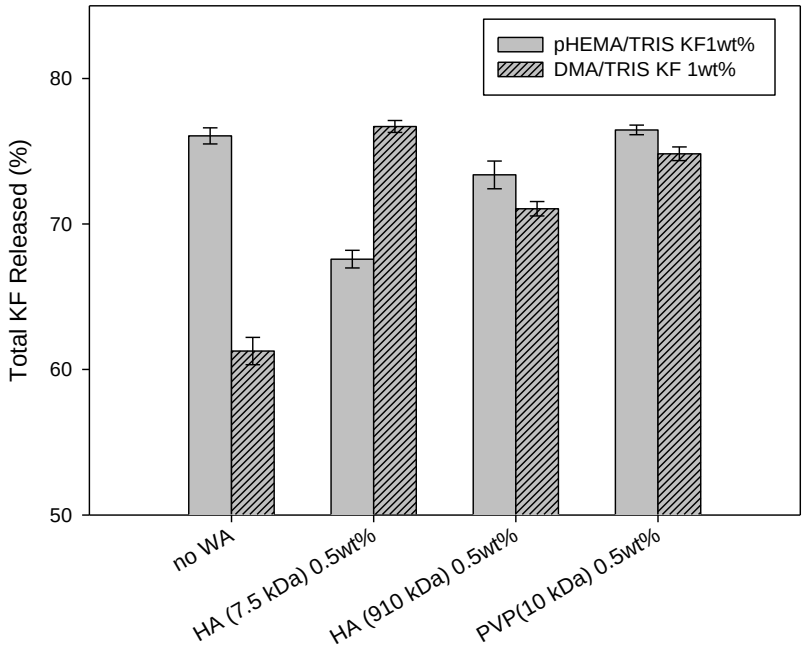

Figure 5.40: Total amount of ketotifen fumarate (KF) released from pHEMA/TRIS and DMA/TRIS materials, based on the initial concentration loaded. Different amounts and MW of HA and PVP were loaded during silicone hydrogel synthesis. Data are shown as mean (±SD) with n=4.

The impact of the wetting agent (0.1 wt% and 0.5 wt%) on the release profile of the studied materials was relatively surprising. The pHEMA/TRIS hydrogels did not exhibit any major change in their cumulative release profile or release kinetics, suggesting a simultaneous release of therapeutic and wetting agent without any significant interactions between KF and HA or PVP. On the other hand, non-covalent incorporation of either HA or PVP in the DMA/TRIS hydrogels, increased the release rate as well as the total amount of KF released (Figure 5.40), without though altering the release kinetics and duration of the system. The co-existence of the wetting agent and KF is thought to exhibit a synergistic effect for KF release that needs further examination.

Consistently to what was observed in TM-loaded pHEMA/TRIS and DMA/TRIS hydrogels, neither the KF-loaded pHEMA/TRIS and DMA/TRIS materials exhibited a 100% release of the KF initially loaded presumably due to immobilization in the crosslinked silicone domains and cannot be released. This irreversibly incorporated KF, has been seen previously (Karlgard et al., 2003). When the TRIS concentration was lower (pHEMA/TRIS samples), less drug was immobilized in these domains. The latter in combination with the sustained release profile of pHEMA/TRIS samples could justify the decreased amount of KF released in DMA/TRIS materials.

6. Conclusions

In this work, model pHEMA/TRIS and DMA/TRIS hydrogels were used as drug delivery systems to study the release profile of two model ocular drugs, timolol maleate (TM) and ketotifen fumarate (KF). In order to improve the water content, the surface characteristics and control the release kinetics a releasable wetting agent, such as HA or PVP, was incorporated along with the therapeutic agent during synthesis via direct entrapment.

The addition of low amounts of wetting agent into the pHEMA/TRIS and DMA/TRIS hydrogels did not significantly alter their EWC. Based on the EWC results, the concentration and the degree of dispersion of the releasable wetting agent had a greater impact than its molecular size on the swellability of the studied materials. Increasing the concentration of HA and PVP to 2 wt% and 10 wt % respectively, the water uptake of the silicone hydrogels was increased. As a result, it is reasonable to conclude that the releasable wetting agent, due to the concentration difference between the hydrogel matrix and PBS, is probably diffusing faster than actually aggregating in the polymer matrix. Hence, it is not able to contribute significantly to water retention in the network, and thus no significant change in the hydrophilicity of the silicone hydrogel domains was observed. Comparative study between HA and PVP-containing DMA/TRIS discs suggested that HA is possibly a better wetting agent for these materials than PVP.

Moreover, the optical transparency of both pHEMA/TRIS and DMA/TRIS was not affected by the incorporation of the therapeutic agent independently of its concentration, with the exception of TM-loaded DMA/TRIS materials. The transmittance, however, in the latter case was still greater than 85%, even though they were approximately 10 times thicker than the commercially available lenses. The optical acuity of the studied materials

was reduced by the direct entrapment of the wetting agent, and especially of the insoluble in the pre-polymer mixture HA. In general, pHEMA/TRIS materials were characterized by the same or lower degree of transparency than DMA/TRIS materials, suggesting larger phase separation between the hydrophilic and hydrophobic domains within these silicone hydrogel materials.

Surface analysis by contact angle study showed that DMA/TRIS materials were characterized by better surface wettability as well as that the incorporation of wetting agent led to decreased contact angles in both silicone hydrogel materials. The releaseable wetting agent migrates to the surface and by attracting the water molecules due to its high hydrophilicity, generates a surface of improved wettability. Both wetting agents imporved the surface characteristics to the same degree while increasing the concentration of the wetting agent initially loaded affected positively the surface wettability. The combination of lower contact angles of DMA/TRIS samples and the higher transparency, even though they have more TRIS in their matrix, suggests that the phase DMA/TRIS domains are characterized by betther phase distribution than those of pHEMA/TRIS.

In vitro release studies of TM and KF-loaded silicone hydrogels, in PBS and under physiological conditions, showed a sustained release profile for extended periods with diffusion being the main mechanism of drug and wetting agent release. More specifically, release duration of hydrophilic TM lasted 14 days for pHEMA/TRIS materials and 4 days for DMA/TRIS. The release duration and kinetics of TM were presumably controlled by the interactions developed between the drug and the material's hydrophilic domains and the water content of the silicone hydrogel as well. Therefore, intramolecular binding between TM and HEMA was the predominent factor providing sustained release while

the higer degree of swelling of DMA/TRIS materials led to higher TM release rates, thus shorter release duration. The incorporation of a wetting agent affected the release profile of TM without though altering the release kinetics and duration. In the case of HA, HA diffusion through the hydrophilic domains of the silicone hydrogel was this limiting step that determined the drug release profile due to the electrostatic interactions between the anionic polymer HA and the protonated TM. Intreactions between PVP and TM were also developed leading to a more controlled drug release, especially in the case of pHEMA/TRIS materials, whereas the release duration remained the same. It is of interest to mention that not all the initial amount of drug loaded into the silicone hydrogel during the synthesis was released, speculating that the drug was either chemically entrapped due to reactions or physically entrapped in tightly bound regions of the silicone hydrogel.

The KF release was characterised by a more sustained profile leading to longer release duration compared to TM. In the case of pHEMA/TRIS 90% of KF was released within 25 days, while the same amount of drug was release within 14 days from DMA/TRIS respectively. The diffusion of the drug through the hydrophilic domains and the water-filled pores of DMA and the hydrogen bonds developed between the carbonyl group of KF and the hydroxyl group of HEMA were thought to be the main factors that determined the release kinetics and duration of KF. Based on the extended release profile, a possible interaction of the ampliphilic KF and the silicone domains is also speculated. Significant increase in PVP concentration that increased hydrophilicity in the network of the pHEMA/TRIS disc resulted in faster KF release. Similarly to the TM results, the affinity of the therapeutic agent for pHEMA/TRIS matrix in combination with the high degree of water content of DMA/TRIS hydrogels can possibly explain the

difference between the release duration of DMA/TRIS and pHEMA/TRIS materials. For pHEMA/TRIS hydrogels, the presence of wetting agent did not alter the release kinetics and duration, suggesting a simulateous release of the wetting and the therapeutic agent without any significant interaction developed between them. On the other hand, in DMA/TRIS hydrogels the presence of HA or PVP increased the release rate leading to higher cumulative mass. Finally, immobilization of KF within the silicone hydrogel matrix was also observed via the method of direct entrapment leading to irrevirsible binding between the drug and the silicone hydrogel materials.

Concluding, the method of direct entrapment of both a hydrophilic drug like TM or amphiphilic drug like KF and of a wetting agent like PVP or HA, led to model silicone hydrogels that exhibited controlled and extended release. Hydrophilic drugs exhibit difficulty in penetrating the corneal epithelium, thus the simultaneous release of a hydrophilic drug such as TM and of a mucoadhesive polymer such as hyaluronic acid is really important. The drug bioavailability might potentially get improved by the increased residence time of drug on the corneal surface of the targeted delivery device and the mucoadhesive properties of HA. Moreover, it is thought that HA improves the stability of tear film during contact lens wear, alleviating potentially the symptoms of ocular dryness. As a result, the simultaneous release of a therapeutic and wetting agent from these materials increases the potentials of designing a wettable ocular drug delivery system capable of delivering therapeutic agents and providing comfort during therapy.

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APPENDIX

Table A4.1: Summary of prepared pHEMA/TRIS and DMA/TRIS hydrogels loaded with timolol maleate.

Timolol Maleate (wt%)*	Wetting Agent	Molecular Weight (kDa)	Amount (wt%)*
0.5 or 2	-	-	-
0.5 or 2	HA	7.5	0.1
0.5 or 2	HA	7.5	0.5
0.5 or 2	HA	132	0.1
0.5 or 2	HA	132	0.5
0.5 or 2	PVP	10	0.1
0.5 or 2	PVP	10	0.5

*wt% in respect to the hydrophilic:hydrophobic monomers ratio

Table A4.2: Summary of prepared KF loaded-pHEMA/TRIS hydrogels

Ketotifen Fumarate (wt%)*	Wetting Agent	Molecular Weight (kDa)	Amount (wt%)*
1 or 2	-	-	-
1	HA	7.5	0.1
1 or 2	HA	7.5	0.5
1	HA	7.5	2
1	HA	132	0.5
1 or 2	HA	910	0.5
1 or 2	PVP	10	0.5
1	PVP	10	10

*wt% in respect to the hydrophilic:hydrophobic monomers ratio

Table A4.3: Summary of prepared KF loaded-DMA/TRIS materials

Ketotifen Fumarate (wt%)*	Wetting Agent	Molecular Weight (kDa)	Amount (wt%)*
0.5 or 1	-	-	-
0.5	HA	7.5	0.1
0.5 or 1	HA	7.5	0.5
0.5	HA	7.5	2
0.5	HA	132	0.5
0.5 or 1	HA	910	0.5
0.5 or 1	PVP	10	0.5
0.5	PVP	10	1

*wt% in respect to the hydrophilic:hydrophobic monomers ratio

Table A5.1: Transmittance (%) (±SD) in TM-loaded pHEMA/TRIS silicone hydrogel materials (n=4).

Material	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
TM 0.5 wt%	85. 2 ± 6. 1	78.8
TM 0.5wt% / HA (7.5 kDa) 0.1wt%	52.6 ± 9.7	53.25
TM 0.5wt% / HA(7.5 kDa) 0.5wt%	15 ± 0.80	13.95
TM 0.5wt% / HA (132 kDa) 0.1wt%	71.6 ± 14.85	69.8
TM 0.5wt% / HA (132 kDa) 0.5wt%	51.3 ± 9.1	50.5
TM 0.5wt% / PVP (10 kDa) 0.1wt%	72.2 ± 13.8	72.3
TM 0.5wt% / PVP (10 kDa) 0.5wt%	91.00 ± 3.4	88.6
TM 2wt%	85.1 ± 7.4	81
TM 2wt% / HA (7.5 kDa) 0.1wt%	72.1 ± 3.35	71
TM 2wt% / HA(7.5 kDa) 0.5wt%	23.6 ± 1.9	22.3
TM 2wt% / HA (132 kDa) 0.1wt%	81.9 ± 4.35	80.5
TM 2wt% / HA (132 kDa) 0.5wt%	47.9 ± 1.48	45.2
TM 2wt% / HA (910 kDa) 0.5wt%	60.55 ± 4.9	57.45
TM 2wt% / PVP (10 kDa) 0.1wt%	93.15 ± 1.0	93.4
TM 2wt% / PVP (10 kDa) 0.5wt%	90.8 ± 2.35	89.5
TM 2wt% / PVP (10 kDa) 10wt%	69.2 ± 9.6	64.4

Table A5.2: Transmittance (%) (±SD) in TM-loaded DMA/TRIS silicone hydrogel materials (n=4).

Materials	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
TM 0.5 wt%	89.5 ± 4.9	91.4
TM 0.5wt% / HA (7.5 kDa) 0.1wt%	72 ± 7.0	72.1
TM 0.5wt% / HA(7.5 kDa) 0.5wt%	38 ± 10.5	41.3
TM 0.5wt% / HA (132 kDa) 0.1wt%	76.1 ± 7.2	75.8
TM 0.5wt% / HA (132 kDa) 0.5wt%	52.1 ± 6.9	55.6
TM 0.5wt% / PVP (10 kDa) 0.1wt%	83.3 ± 3.2	82
TM 0.5wt% / PVP (10 kDa) 0.5wt%	37.65 ± 11.4	33.1
TM 2wt%	85.9 ± 1.2	85
TM 2wt% / HA (7.5 kDa) 0.1wt%	76.8 ± 2.3	74.1
TM 2wt% / HA(7.5 kDa) 0.5wt%	34.7 ± 6.9	29.5
TM 2wt% / HA (132 kDa) 0.1wt%	86.5 ± 5.5	85.2
TM 2wt% / HA (132 kDa) 0.5wt%	69.1 ± 5.0	65.8
TM 2wt% / HA (910 kDa) 0.5wt%	61.4 ± 8.7	56
TM 2wt% / PVP (10 kDa) 0.1wt%	58.35 ± 4.8	54.6
TM 2wt% / PVP (10 kDa) 0.5wt%	11.6 ± 1.0	10.4

Table A5.3: Transmittance (%) (±SD) in KF-loaded pHEMA/TRIS silicone hydrogel materials (n=4).

Materials	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
KF 1wt%	85.2 ± 12.05	78.28
KF 1wt% / HA(7.5 kDa) 0.1wt%	55.2 ± 8.5	56.80
KF 1wt% / HA(7.5 kDa) 0.5wt%	19.3 ± 3.2	18.72
KF 1wt% / HA(7.5 kDa) 2wt%	3.25 ± 0.4	2.9
KF 1wt% / HA(132 kDa) 0.5wt%	43.2 ± 3.12	39.15
KF 1wt% / HA(910 kDa) 0.5wt%	51.1 ± 3.8	50.1
KF 1wt% / PVP(10 kDa) 0.5wt%	63.1 ± 6.4	74.2
KF 1wt% / PVP(10 kDa) 10wt%	59.0 ± 13.9	55.9
KF 2wt%	83.4 ± 6.0	83.5
KF 2wt% / HA(7.5 kDa) 0.5wt%	20.95 ± 1.9	18.75
KF 2wt% / HA(910 kDa) 0.5wt%	49.7 ± 4.60	46.8
KF 2wt% / PVP(10 kDa) 0.5wt%	81.8 ± 8.35	80.3

Table A5.4: Transmittance (%) (±SD) in KF-loaded DMA/TRIS silicone hydrogel materials (n=4).

Materials	Transmittance (%) ± SD at 600 nm	Average Transmittance (%) (range 400-750 nm)
KF 0.5wt%	87.2 ± 6.2	88.3
KF 0.5wt% / HA(7.5 kDa) 0.1wt%	63.8 ± 18.6	66.2
KF 0.5wt% / HA(7.5 kDa) 0.5wt%	28.4 ± 4.0	27.2
KF 0.5wt% / HA(7.5 kDa) 2wt%	3.6 ± 0.7	3.5
KF 0.5wt% / HA(132 kDa) 0.5wt%	27 ± 6.5	25.6
KF 0.5wt% / HA(910 kDa) 0.5wt%	29.0 ± 7.8	27.0
KF 0.5wt% / PVP(10 kDa) 0.5wt%	36.1 ± 2.4	32
KF 0.5wt% / PVP(10 kDa) 10wt%	13.6 ± 1.2	13.2
KF 1wt%	90.00 ± 3.9	87.75
KF 1wt% / HA(7.5 kDa) 0.5wt%	37.1 ± 2.45	34.4
KF 1wt% / HA(910 kDa) 0.5wt%	37.8 ± 5.8	36.4
KF 1wt% / PVP(10 kDa) 0.5wt%	37.3 ± 8.3	31.2