2015

Development and preliminary in vitro evaluation of nanomicelles laden in situ gel of dexamethasone for ophthalmic delivery

Pallabita Chowdhury
University of Toledo

A Thesis

entitled

Development and Preliminary *In vitro* Evaluation of Nanomicelles Laden *In situ* Gel of Dexamethasone for Ophthalmic Delivery

By Pallabita Chowdhury

Submitted to the Graduate Faculty as partial fulfillment of the requirements for

The Master of Science Degree in Pharmaceutical Sciences,

Industrial Pharmacy Option

_____

Sai HS. Boddu, Ph.D., Committee Chair

_____

Kenneth S. Alexander, Ph.D., Committee Member

_____

Caren L. Steinmiller, Ph.D., Committee Member

_____

Patricia R. Komuniecki., PhD., Dean
College of Graduate Studies

The University of Toledo
June 2015

An Abstract of

Development and Preliminary *In vitro* Evaluation of Nanomicelles Laden *In situ* Gel of Dexamethasone for Ophthalmic Delivery

By Pallabita Chowdhury

Submitted to the Graduate Faculty as partial fulfillment of the requirements for

The Master of Science Degree in Pharmaceutical Sciences,
Industrial Pharmacy Option

The University of Toledo
June 2015

In our previous work we developed and characterized 0.1% dexamethasone mixed nanomicelles (DMN) prepared using polyoxyl 40 stearate (P40S) and polysorbate 80 (P80) for topical drug application in treating posterior segment eye diseases. The present study builds on the previous work by developing and evaluating nanomicelles laden *in situ* gel of 0.1% dexamethasone (DMN-ISG) with potential for treating anterior segment eye inflammations. *In situ* gels are known to reduce pre-corneal drug elimination and thus may result in better ocular availability. DMN-ISG was prepared by mixing the basic 2X formulation of DMN with appropriate concentrations of gellan gum, mannitol, benzododecinium bromide and tromethamine. DMN-ISG was characterized for gelation, viscosity, transparency, morphology using Transmission Electron Microscopy (TEM), thermal analysis using Differential Scanning Calorimetry (DSC), *in vitro* drug release and sterility. DMN prepared with an optimized composition of P40S/P80=7/3 by weight was used in the preparation of DMN-ISG. The pH of DMN-ISG was within the range of 6.5-7.4, which is close to the pH of tear fluids 7.31-7.62.

The dispersion of DMN-ISG appeared clear at room temperature on visual examination and transformed into a gel when diluted with simulated tear fluid (STF) in the ratio of 25:7 at 34°C. DMN-ISG exhibited a non-Newtonian behavior which was asserted by pseduoplastic flow and shear thinning behavior. TEM images of DMN-ISG showed the presence of dexamethasone nanomicelles a size ranging between 20-40 nm entrapped in the gel structure. The DMN-ISG exhibited an endothermic peak with an onset at 94°C and a peak temperature at 107°C, which mostly corresponds to water. The light transmittance of DMN was around 90-100%. However, DMN-ISG showed reduced light transmittance of 56-78% and it is mainly attributed to the presence of gellan gum in the formulation. *In vitro* release data showed that more than 50% of the drug was released from DMN-ISG in the first few hours and the remaining drug was released in a sustained manner for up to 30 h. Aseptically prepared DMN-ISG formulation remained sterile for up to 14 days. The preliminary findings of our investigation suggest that DMN-ISG could potentially be used to treat anterior segment eye inflammations. Further *in vivo* evaluation of DMN-ISG is warranted.

I dedicate this thesis to my family and friends. A special gratitude to my loving parents whose faith in me kept me motivated and focused, my sister and brother–in-law for their constant encouragement and Saptarshi who had never left my side. Lastly my beloved nephew whose cheerfulness was much needed in my life. Thank you all.

Acknowledgements

I wish to thank all of my committee members who were more than generous with their expertise and precious time. I express my deepest gratitude to Dr. Sai Boddu for providing me an opportunity to be a part of his research group. My achievement would not have been possible without his advice, support and the provided resources all throughout my research. I would like to take this opportunity to thank Dr. Caren Steinmiller and Dr. Kenneth S. Alexander for being a part of my defense committee. I would like to specially thank Dr. Jerry Nesamony for allowing me to use the HPLC and UV spectrophotometer. I would also like to thank Dr. Zahoor Shah for serving as a graduate faculty representative. I thank Dr. Joseph Lawrence, for helping me with the TEM study and Dr. Lidia Rodriguez for helping me with the LC-MS (Center for Materials and Sensor Characterization, Bioengineering, University of Toledo). I would like to thank all my friends and fellow lab mates for their love and support throughout this two years. I hugely extend my thanks to Yangjie Wei for sharing his knowledge and extending his help whenever I needed. A special thanks to Rami for his help and patience with the cell culture study. Also I would take the opportunity to thank Prajakta and Andrea for their cheerfulness at home always. Finally, I would like to thank my wonderful parents (Dipak and Alpana Chowdhury), my cute nephew (Aharshi), my sister and brother-in-law and Sapatarshi, for their love and emotional support throughout this journey.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

The human eye is a complex organ and measures about 24 mm in a fully grown adult. Drug delivery to the eye is an arduous task due to its intricate anatomical and physiological barriers. The structure of the eye can be classified into anterior and posterior segments. The anterior segment makes up the visible one-third portion of the eye which mainly consists of cornea, conjunctiva, aqueous humor, iris, ciliary body, and lens. The remaining two-thirds of the eye is known as the posterior segment or the back of the eye. The posterior segment mainly consists of vitreous humor, retina, choroid, and optic nerve [1, 2]. The anterior and posterior segments of the eye are affected by various vision threatening diseases. A few diseases affecting the anterior segment could be, but not limited to, glaucoma, allergic conjunctivitis, anterior uveitis, and cataract. Some of the vision threatening diseases affecting the posterior segment of the eye include age-related macular degeneration (AMD) and diabetic retinopathy macular edema [DME], proliferative vitreoretinopathy [PVR], posterior uveitis, and cytomegalovirus [CMV]) [1]. It has been estimated that there are 161 million visually impaired individuals in the world and among them 37 million are blind [3]. This raises the dire need for drug delivery to the eye [4].

The anterior ocular tissues are subjected to external environmental threats such as dust, microbes, diseases, fungi, parasites, and bacteria, causing inflammations. Also the penetration of proteins and cells from the peripheral circulation may also result in inflammations. The immune cells are capable of suppressing immune responses, on the other hand the blood aqueous barrier successfully resists it. However, changes occur in the blood aqueous barrier due to ocular inflammation and surgical trauma. So all of them results in entry and accumulation of immune cells and inflammatory mediators in ocular tissues which further causes redness, pain, swelling, and itching [4].

Drug delivery to the anterior segment of the eye is typically obtained through topical administration of drugs which includes conventional dosage forms such as solutions, suspensions, and ointments. It is estimated that 90% of marketed ophthalmic formulations are conventional dosage forms [5] which include 62.4% solutions, 17.4% ointments, and 8.7% suspensions [6]. It is widely believed that the topical route is able to deliver drugs efficiently to the anterior ocular tissues including cornea, conjunctiva, iris, and ciliary body. However, topical administration results in low ocular bioavailability (< 5%) due to various barriers such as non-productive absorption, tear production, transient residence time, and impermeable corneal epithelium. A normal eye has a tear volume of ~7-9 µl with a turnover rate of 0.5-2.2 µl/min [7], whereas a typical eye dropper could deliver ~35-56 µl, which causes an increase in tear volume and thus blinking reflex rate. The fate of the excess volume of eye drops is drainage through the nasolacrimal duct and entry into the systemic circulation [8]. The systemic absorption of topically administered drug is also affected by the conjunctival blood. All the above mentioned factors result in the elimination of approximately 95% of the administered dose. The remaining

dose, of about 5% or less, remains in the pre-corneal area and its entry into the eye is limited by the impermeable corneal epithelium (to be discussed later under the cornea as a barrier).

1.1. Barriers to anterior segment delivery

1.1.1. *Epithelial Tight Junction (ZO)* – The corneal epithelium is a part of the cornea and is the prime barrier for drug absorption into the eye. The stratified corneal epithelium consists of a basal layer of columnar cells, two to three layers of wing cells, and one or two outermost layers of squamous polygonal structured superficial cells [9]. The superficial cells are mostly surrounded by the intercellular tight junctions (zonula occludens). These tight junctions (as shown in Fig. 1.1) serve as a selective barrier for small molecules and complete barrier to macromolecules via the paracellular route. A tight junctions are composed of a belt-like structure called anastomotic strands, which determine the resistance for the paracellular route [10]. There are four tight junction associated proteins, namely- ZO-1 [11], cingulin [12], ZO-2 [13], and occludin [14]. It is assumed that occludin is the most important protein for the tight junctions. Permeability through these tight junctions are influenced by high extracellular and low intracellular calcium levels for permeability [15]. If the cytoskeletal structure is disrupted or the extracellular calcium ions are removed by EDTA, the permeability increases through the tight junctions [16, 17]. The pores of the corneal epithelium are negatively charged at physiological pH, hence negatively charged molecules permeate slowly when compared to positively charged molecules [18]. Also the cellular calcium levels and actin filaments present on the cytoskeleton, exhibit an important role in the integrity of tight junctions [10, 15, 16]. The use of hypertonic solutions increases the leakiness through the tight junctions [19].

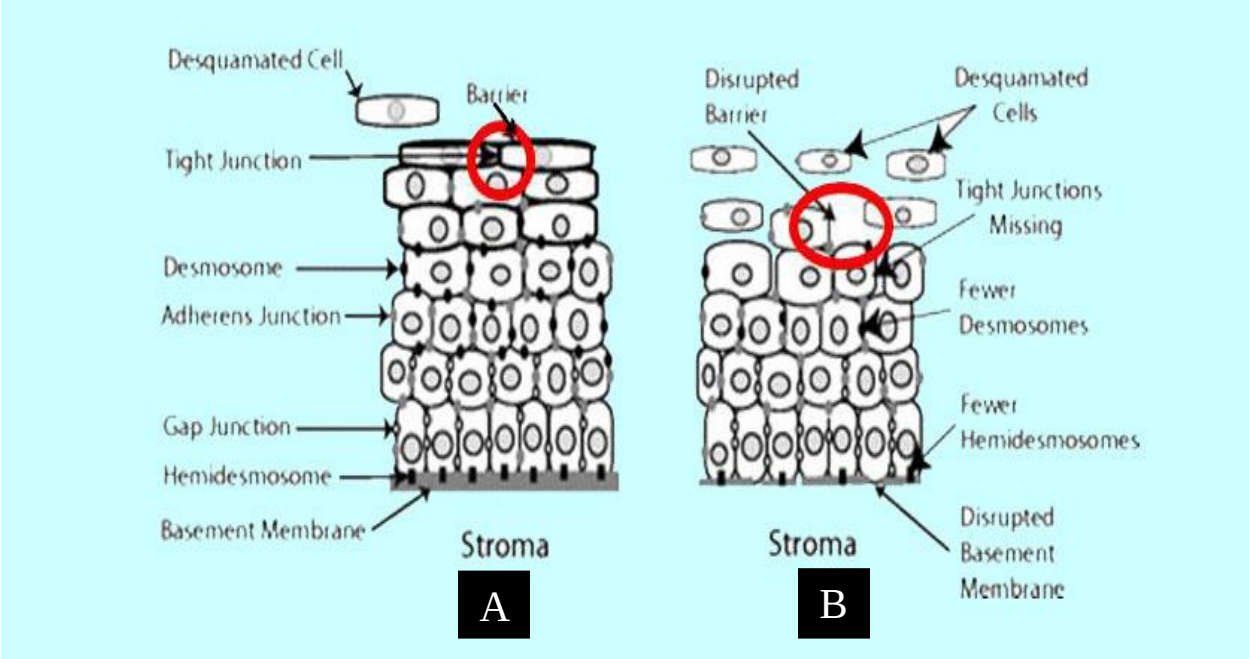


Figure 1.1: Epithelial tight junctions: tight junction is intact (A), tight junction is broken (B)

1.1.2. *Reflex Blinking* – A normal eye dropper can deliver 25-56 µl of formulation into the eye. However, an eye can hold upto 7 µl and the rest is lost either by nasolacrimal drainage or by reflex blinking. The reflex blinking can increase the drainage of solution from the eye and thus decreases the overall drug available for therapeutic action [20]. The normal blink frequency in humans is about 5-7 min^{-1} [21, 22]. According to Ahmed *et al.* drainage of lacrimal fluids due to blinking every 12 seconds causes rapid elimination of eye drops [23].

1.1.3. *Metabolism in ocular tissues* – The human eye has a number of drug metabolizing enzymes such as mercapturate synthesis. Drugs containing aromatic hydrocarbons are metabolized in the pigmented epithelium and ciliary body to their corresponding

epoxides and phenols or further metabolized by other enzymes present in the eye [24]. According to Hayakawa *et al.* poor penetration of peptide drugs and insulin is due to extensive metabolism before and during conjunctival penetration across the conjunctiva of albino rabbits [25]. Schoenwald *et al.* suggested that ocular clearance was higher than clearance via aqueous humor turnover in rabbit eyes, proving that most drugs are eliminated via metabolic pathways [22, 26].

1.1.4. *Tear Turnover* – One of the significant impediments in drug delivery to the eye is tear turnover. A commercial eye dropper can deliver 25-56 µl of solution with an average volume of around 39 µl [27]. However, the human eye can momentarily accommodate upto 30 µl of the instilled solution [28]. The sudden increase in the volume of solution in the cul-de-sac causes reflex blinking and increased tear secretion. Due to increased lacrimation on administration of irritating drugs and vehicles, drug loss is rapid from the precorneal area [29]. This rapid loss of instilled solution occurs either due to tear turnover or nasolacrimal drainage until the tear volume in the conjunctival cul-de-sac returns to its normal value of 7-9 µl in humans [30]. The initial first order drainage rate of eye-drops from the ocular surface is 1.2 µl/min in humans [22, 31] and 0.5-0.7 µl/min in rabbits [32]. This rate is altered with a larger volume of eye drops with a decrease in the viscosity of the solution. A considerable amount of drug is lost from the precorneal pocket due to excessive tear production/tear reflux, thus reducing availability [33].

1.1.5. *Nasolacrimal Drainage* – As mentioned above, most of the instilled drug is lost due to tear turnover or nasolacrimal drainage. About 95% of the dose administered is absorbed systemically via the conjunctiva and nasolacrimal duct [34]. The lacrimal drainage system in human adults serves as a conduit for tear flow from the eye to the nasal cavity and it consists of the puncta, canaliculi, lacrimal sac, and nasolacrimal duct. Histologically, the walls of the lacrimal sac and the nasolacrimal duct are vascularized and hence are potential sites for systemic drug absorption. After topical application, the eye drop solution first mixes with lacrimal fluid. The contact time of the drug with ocular tissues is approximately 1-2 min due to the constant production of lacrimal fluid. About half of the drug flows into the upper canaliculus and the rest into the lower canaliculus into the lacrimal sac. This further opens into the nasolacrimal duct and from there is drained into the nose [5], as shown in Fig. 1.2. A few factors that determine the topically applied drug concentration are volume of the installed drug solution, reflex blinking by the patient and the patient's age. Larger instilled volumes easily get into the nose as they are able to easily distend the nasolacrimal sac [35], whereas smaller volumes are easily absorbed from the lacrimal sac [8]. The loss of active ingredients by the nasolacrimal duct via transconjunctival absorption or transnasal absorption is unwanted, because of loss of drug into the systemic circulation and the encountered systemic side effects on major organs [36].

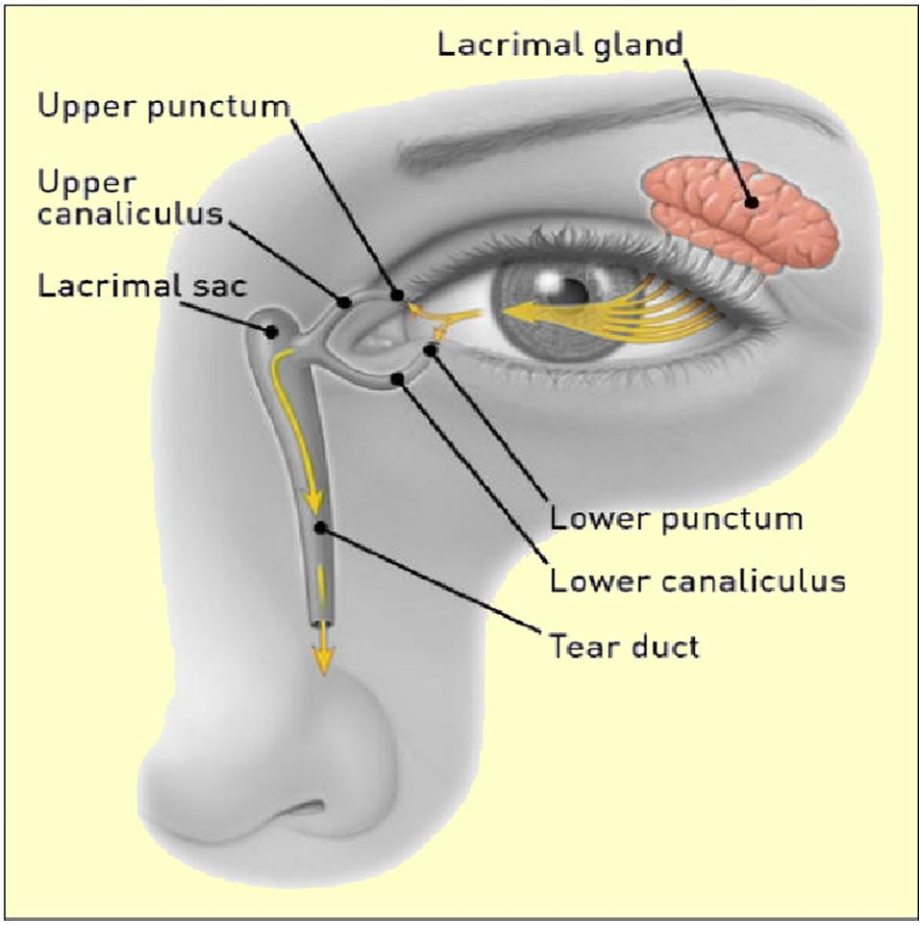


Figure 1.2: Nasolacrimal drainage. Modified from

http://www.corkeyeclinic.ie/dry-eyes-treatment

1.1.6. *Efflux pumps* – Efflux pumps are transporters which are responsible for moving toxic substances and drug molecules out of the cell. The efflux protein may be located either on the apical or basolateral membranes. This protein either restricts or enhances drug absorption depending on its location [37]. ATP-binding cassette, more commonly known ABC proteins, is a superfamily of proteins that is encoded by the MDR1 gene and is responsible for the efflux of various substrates across the plasma membrane and cytoplasm into the extracellular fluid [38]. These ABC proteins help in the detoxification of cells by regulating the transport of various noxious agents such as sterols, lipids, endogenous metabolic products and xenobiotics. ABC proteins are also responsible for drug resistance in cancer cells [37, 39]. There are primarily two multidrug efflux pumps that are responsible for chemoresistance: (a) P-glycoprotein (ABCB1), which restricts entry of amphipathic compounds both in normal and cancer tissue causing resistance to wide variety of drugs, and (b) multidrug resistant protein (MRP) (ABCC1), which is known to efflux organic anions and conjugated compounds [37, 40].

P-glycoprotein 1 (P-gp) also known as MDR1 or ABCB1 is a 170 kDa ATP dependent efflux pump. It is located in the apical surface of polarized cells [41] and is responsible for decreasing drug accumulation in multidrug resistant cells and also mediates the development of resistance to anticancer drugs. P-gp is present in the eye on the conjunctival epithelial cells [42], ciliary non-pigmented epithelium [43], human and rabbit cornea [44], iris and ciliary muscle cells and retinal capillary endothelial cells [45]. P-gp has been detected at the mRNA level in the human cornea, rabbit corneal epithelium, and primary cultures of rabbit corneal epithelial cells [44]. According to

Constable *et al.*, the presence of P-gp on three human RPE cells (ARPE19, D407, and h1RPE) have been studied. It was seen that only D407 cells express P-gp and hence can be used for *in vitro* drug transport studies without any modification [46].

MRP is a 190 kDa membrane bound efflux protein encoded by the ABCC1 gene. It is generally found in the basolateral surface of the intestine, hepatocytes and kidney cells [47, 48]. It acts as a multi-specific organic anionic transporter with glutathione, cysteinyl leukotrienes, glucoronides, sulfate conjugates and bile salts [49]. By quantitative analysis using RT-PCR in human corneal epithelium MRP expression has been detected at the RNA level [50]. MRP5 was expressed at a higher level than MDR1, MRP1–MRP4, MRP6 and BCRP [37]. Zhang *et al.* studied drug transporter and cytochrome P450 mRNA expression in ocular drug disposition. They concluded that both BCRP and MRP2 have very low expression levels in the human cornea, while MRP1 was moderate and MRP3 had low expression levels in the human cornea. Thus designing drugs that could efficiently evade MRP1 efflux play an important role in enhancing the ocular penetration [51].

1.2. Ocular Drug Absorption by the Topical Route

1.2.1. *Role of the cornea* - The eye is broadly divided into two segments - anterior and posterior segments. The cornea is the outermost transparent tissue surrounding the anterior segment and represents a highly impermeable barrier for drug penetration. The cornea has an average central thickness of 0.52 mm and a central radius of 7.8 mm [52] and is composed of five layers-*viz.* the outer lipophilic epithelium, the

Bowman's layer, the hydrophilic stroma, the Descemet's membrane, and the endothelium (Fig. 1.3). The corneal epithelium is the outermost layer and is composed of 5-6 layers of stratified, squamous, non-keratinized cells that possess tight gap junctions [53] called ZO. In terms of drug absorption, the ZO serves as a diffusion barrier similarly as the blood aqueous barrier, which can hinder drug delivery available through the systemic blood flow. The paracellular delivery of hydrophilic drugs is impeded by ZO, hence hydrophilic drugs are less permeable than lipophilic drugs in the cornea as well as the conjunctiva [54]. Below the corneal epithelium are the corneal stroma and endothelium layers. The corneal epithelium and endothelium are impermeable to hydrophilic molecules, whereas the stroma is pervious to hydrophobic molecules. Therefore, drugs should have an optimum oil to water partition coefficient (log P) for desired corneal permeation [55]. However, the drug permeability is altered in cases of distortions to these structures due to injury or disease [56]. A very common example would be to use benzalkonium chloride as the preservative in most ophthalmic preparations, which temporarily disrupts cell membranes and tight junctions and increases the corneal permeability [57]. Patients with dry eye syndrome are facilitated by topical medications due to enhanced permeability of corneal epithelium because of disrupted epithelial cellular barriers [58]. Most of the clinically approved drugs for topical administration are slightly lipophilic with low molecular weights, hence favored by corneal absorption over conjunctival absorption [54]. However, the cornea is found to be 15-20 times less permeable to Polyethylene glycol (PEG) molecules compared to conjunctiva and sclera, thus indicating that drugs of molecular weights around 200-1000 Da are better

delivered via trans-scleral route [59]. Drug absorption through the cornea is also hindered due to the presence of transmembrane efflux pumps on the corneal surface. Drug efflux pumps which have been reported so far are P-gp, MRP and BCRP that restrict drug penetration into the ocular tissues [44, 60-63].

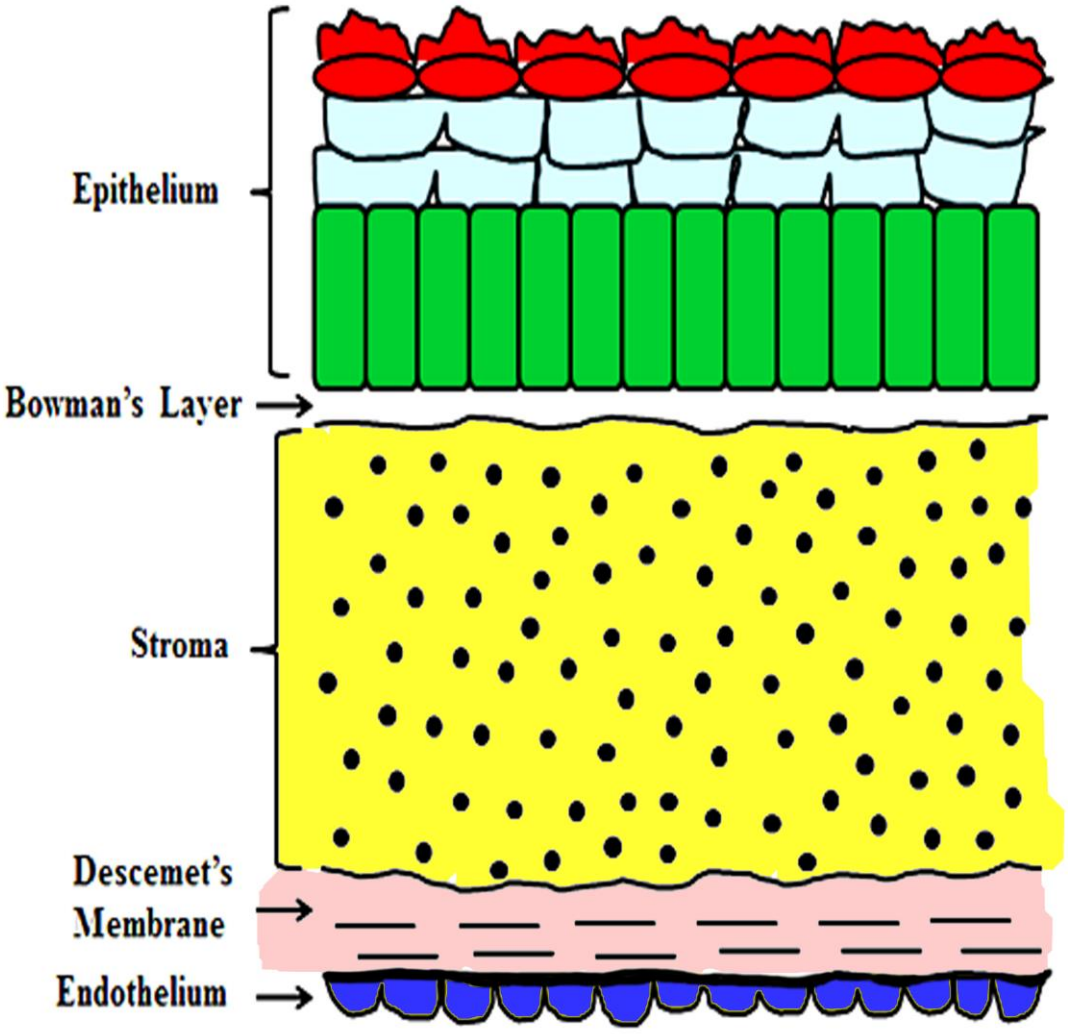


Figure 1.3: Layers of cornea. As modified from [64].

1.2.2. *Role of the conjunctiva*- The conjunctiva is a thin, transparent membrane that covers almost 80% of the anterior ocular surface. It is mainly involved in the formation and maintenance of tear film, and is primarily composed of 2 layers, *viz.* the 2-10 layer outer epithelial layers made of stratified epithelial cells and the inner stroma made of substantia propia [63]. Several secretory cells and glands are found embedded within the conjunctiva which helps in tear formation [65] and mucin [66]. This secreted mucin further helps in adhering and maintaining tear film, which helps in providing protection and nourishment to the cornea [67]. The outer apical epithelial cells form tight junctions (zonal adherens) that inhibit drug permeability by paracellular transport [68]. The stroma is richly supplied with nerves, blood and lymph vessels thus inhibiting hydrophobic drugs. However, administered drugs are cleared away by the blood and conjunctival lymph nodes, posing a major threat to drug absorption [4]. Further the conjunctival blood vessels do not have tight junctions [69] thus enabling drug loss into blood circulation by pinocytosis and/or convective transport through paracellular pores in the endothelial layer [70]. Drug permeation across the conjunctiva is altered by physiochemical properties of drug molecules such as molecular weight and hydrophilicity. Hydrophilic molecules less than 20 kDa in size can permeate through the conjunctiva [68, 71].

The presence of efflux proteins on the cell membrane impedes drug transport by effluxing drugs from the cell cytoplasm and reducing drug concentration in conjunctival cells. It was reported by Lee *et al*. that 10% of small molecular weight hydrophilic compounds (sodium fluorescein) administered through sub-conjunctival

space is eliminated by lymphatics within the first hour in rat eyes [72]. Drug absorption through non-corneal pathways involve the conjunctiva, mucous membrane that lines the anterior globe of eye called bulbar conjunctiva and the underlying surface of inferior and superior lids [58]. Non-corneal absorption plays a significant role in absorbing hydrophilic and large molecules such as pilocarpine and inulin [29, 73]. The conjunctiva (palpebral and bulbar) is permeable to PEG molecules which facilitates sustained delivery of proteins and polymers. This is due to the fact that PEGylation retards degradation thus providing water solubility to hydrophobic drugs and proteins, hence making way for enhanced delivery [59, 74].

1.3. Nanocarriers for anterior segment drug delivery

Despite intense research efforts by pharmaceutical scientists; drug delivery to the eye in an effective manner remains a challenge. The anatomical position of the eye allows local delivery of drugs along with non-invasive clinical assessment of disease, while the physiological barriers prevent the entry of foreign substances. For efficient treatment of diseases, drug molecules should circumvent the protective physiological barriers without causing permanent tissue damage. Both anterior and posterior segments provide unique barriers to the entry of drugs [75, 76]. Drug delivery for anterior-segment diseases vary from topically applied conventional dosage forms such as solutions, suspensions, and ointments to novel dosage forms such as liposomes [77], nanoparticles [78] and implants [75, 79]. However, more than 90% of marketed formulations are conventional dosage forms [80]. Among them, 62.4% are solutions, 17.4% are ointments and 8.7% are suspensions [81]. Much of the research is directed

toward the development of delivery systems with high patient compliance and ocular bioavailability [82]. Over the last decade, nanocarrier systems received significant attention in the delivery of drugs to specific eye tissues. Nanocarriers such as microemulsions, nanosuspensions, liposomes, dendrimers, niosomes, cubosomes, nanoparticles, polymeric micelles, and solid lipid nanoparticles are tested for ocular delivery to overcome the typical problems of topical therapy, such as low corneal penetration and poor drug availability [76]. These systems are easily administered as eye drops for topical delivery or injections for intraocular delivery [83]. The nanocarriers most widely used in treating anterior segment diseases will be highlighted in the subsequent section.

1.3.1. *Microemulsions*

Definition and description- A microemulsion is a dispersion of water and oil that is stabilized by surfactants or co-surfactants to reduce the interfacial tension. They are clear in appearance, thermodynamically stable with a small droplet size (~100 nm) [84].

Use in ocular drug delivery with examples- Microemulsions because of their intrinsic structure and properties have a high drug dissolving capacity. An oil-in-water type of microemulsion with the presence of surfactant and co-surfactant is able to increase membrane permeability in corneal drug delivery [85]. They are generally low in viscosity and able to deliver drug in a sustained manner, thus increasing the overall absorption capacity. The increased permeability and sustained release of drugs makes this system an attractive vehicle for ophthalmic drugs [86]. Apart from having a low

surface tension, they have good spreading ability thus allowing the drug to spread on the cornea and mix well with the precorneal fluid. This improves the contact time of drugs with the corneal epithelium [87]. Insoluble drugs such as indomethacin and chloramphenicol have shown improved solubility due to the presence of surfactant and co-surfactant in the system [86]. They can be sterilized by filtration for ocular delivery as eye drops [88, 89]. Several drugs such as indomethacin [90], delta-8-tetrahydrocannabinol [91], pilocarpine [92], and timolol [93] have been incorporated into microemulsions. *In vivo* studies in rabbits showed a delayed effect and improved bioavailability [94]. Pilocarpine based microemulsion has shown to reduce dosing frequency from four instillations a day (conventional eye drops) to two times a day [88]. This is due to the enhanced permeation across the cornea by the use of combination of lecithin, propylene glycol and PEG 200 as the surfactant and co-surfactant. Microemulsion systems containing pilocarpine hydrochloride were able to convert to different forms such as crystalline liquid and emulsion with a change in water content in the system. This causes a change in the rheological behavior and results in a higher viscosity. This results in a longer retention of the formulation in cornea and so better effect [95]. In spite of these several advantages, availability of a narrow range of surfactants and oils that are physiologically acceptable and devoid of toxicity diminish the success of microemulsions in ocular drug delivery.

1.3.2. *Nanosuspensions*

Definition and description- Nanosuspensions are sub-micron colloidal dispersions, which consist of a poorly water-soluble drug dispersed in an appropriate dispersion

medium stabilized by surfactants or polymers. They usually consist of a colloidal carrier such as a polymeric resin which is inert in nature for enhancing drug solubility and improving bioavailability. Unlike microemulsions they are non-irritant in nature and are a desirable system for ocular drug delivery [96].

Use of nanosuspensions in ocular drug delivery with examples- The inert carriers used in nanosuspensions are non-irritant to the cornea, iris, and conjunctiva [84]. Nanosuspensions increase the precorneal residence time and enhance solubility and ocular bioavailability of drugs that are insoluble in the tear fluid. Glucocorticoids such as dexamethasone, prednisolone and hydrocortisone are widely used in treating anterior segment inflammatory diseases [96]. Glucocorticoid should be repeatedly administered for controlling the inflammation. However, repeated administration of high glucocorticoid doses induces cataract formation and may damage optic nerves. Formulating glucocorticoids as nanosuspension have resulted in increased ocular bioavailability due to sustained drug release in the anterior chamber relative to microsuspensions of similar drugs [97]. Flurbiprofen loaded in polymeric nanosuspension has been shown to prevent myosis during extracapsular cataract surgery. Flurbiprofen helps in decreasing post-surgical edema after intra-ocular surgery. Flurbiprofen loaded in polymeric nanosuspension uses a solvent evaporation technique during preparation, and hence is favored for ophthalmic preparations [98, 99]. The positive charge on the nanoparticles causes them to adhere to the negatively charged corneal surface with ease [100]. According to Adibkia *et al*., animal studies revealed that nanosuspension systems consisting methylprednisolone had a better anti-inflammatory effect than microsuspension when used on rabbits with endotoxin-

induced-uveitis [101, 102]. These studies concluded that nanosuspension is an attractive alternative to conventional eye drops in ocular drug delivery.

1.3.3. *Liposomes*

Definition and description- Liposomes are lipid vesicles composed of one or more phospholipids bilayers with a central aqueous compartment and are 25 nm -10 µm in diameter [103]. They are capable of incorporating both hydrophilic and lipophilic drugs due to the presence of a central aqueous compartment and lipid layer. Liposomes have a high degree of biocompatibility than a polymer-based system [104].

Uses in ocular drug delivery with examples- Liposomes can form intimate contact with the cornea and are favorable for drugs with low solubility and partition coefficient values, higher molecular weight, and poor absorption [105]. The positive charge on the liposomes allows them to bind to the negatively charged mucin coated on the corneal epithelium. A positively charged liposome is shown to enhance the transcorneal flux of penicillin G about four-fold suggesting enhanced corneal permeability [105]. However, the association between drug molecules and lipid vesicles is a major factor that determines the drug disposition in liposomal drug delivery. A review of the literature shows that liposomal drug delivery is more favorable for lipophilic rather than hydrophilic drugs. As stated by Milan *et al.* [106] and Pleyer *et al.* [107] liposomes could be an attractive alternative over an oily

vehicle for the lipophilic drug cyclosporin. Ganciclovir in liposomes is found to be 2-10 times higher in sclera, cornea, iris, lens and vitreous humor when compared to the plain ganciclovir solution, thus suggesting improved delivery to both anterior and posterior ocular tissues [108]. C6-ceramide loaded in liposomes were capable of treating inflammations of the anterior segment of the eye, but there was no effect associated with corneal wound healing [109]. Arakawa *et al.* suggested dexamethasone loaded sugar-chain surface-modified liposomes could efficiently deliver the drug to the anterior segment of the eye for treating ocular inflammation unlike dexamethasone suspension, which showed non-specific drug delivery to other body tissues [110]. Natarajan *et al.* prepared a liposomal formulation with latanoprost that was able to lower the intra-ocular-pressure in rabbits relative to topical formulation of the same drug. A single administration of the liposomal formulation was proficient in lowering intra-ocular pressure. A similar effect was achieved after topical administration for 50 days [111].

Although liposomes provide advantages such as ease of application similar to eye drops and reducing frequency of administration by sustaining drug release there are some disadvantages associated with liposomes. The drawbacks of liposomes for the anterior segment delivery include loss of the topically applied dose through tear turnover, short shelf-life, limited drug-loading capacity, and problems in sterilization [84].

1.3.4. *Dendrimers*

Definition and description- Dendrimers are made up of polymeric macromolecules with a highly branched star-shaped structure. Dendrimers are nanoconstructs with unique physical and chemical properties such as high water solubility, encapsulation ability, monodispersity, and a large number of surface functionalizable groups. The ability to functionalize surface groups make them suitable candidates for delivery of both hydrophilic and lipophilic drugs [112, 113].

Uses in ocular drug delivery with examples- Earlier bioadhesive polymers such as poly (acrylic) acid were used to improve ocular drug delivery by prolonging contact time and better absorption. However, the use of this polymer was limited due to the blurring of vision and formation of a veil in the precorneal area leading to the loss of eyesight [114]. In order to overcome this limitation, dendrimers consisting of polyamidoamine (PAMAM) with carboxylic and hydroxyl surface groups were introduced. PAMAM were able to increase the number of branches in the dendrimers which would lead to the development of higher generation dendrimer (G0, G1, G2 and so on) where G stands for "generation". PAMAM not only improves drug solubility but also allows surface conjugation of targeting ligand and/or drugs. Dendrimers are suitable for ophthalmic drug delivery since they can solubilize lipophilic and hydrophilic drugs in their core and have an exterior region of terminal moieties thus providing sustained drug release [115-117]. Vandamme *et al.* studied the effect of pilocarpine nitrate and tropicamide using PAMAM dendrimers and found that the bioavailability was improved due to better bioadhesion capability and sustained drug release [118]. Drug delivery using dendrimers could be further improved by PEGylation of dendrimer surfaces. Drug delivery by dendrimers can be

altered by selecting the right surface groups such as amine, carboxylic and hydroxyl or size or molecular weight of the dendrimers.

1.3.5. Niosomes and Discomes

Definition and description- Niosomes are bilayered nanosized vesicles made up of amphiphilic nonionic surfactants that are biodegradable, biocompatible, and non-immunogenic. They are chemically stable with 10 to 1000 nm in size and capable of incorporating both hydrophilic and lipophilic drugs [84]. Another novel carrier system are the discomes which are large structures (12 - 16 mm) derived from niosomes by the addition of non-ionic surfactants such as Solulan C24 [119].

Uses in ocular drug delivery with examples- Niosomes are a preferred carrier systems for ocular drug delivery due to their low toxicity associated with the use of nonionic surfactants. Niosomes do not need any special handling during preparation and are able to deliver good bioavailability in a sustained manner at specific ocular sites. Niosomal formulation of cyclopentolate could deliver the drug independent of pH and significantly improved bioavailability, thus they could be used as an efficient vehicle for ocular drug delivery [105]. According to Aggarwal *et al*., timolol maleate niosomes coated with chitosan and carbopol lowered the intra ocular pressure in albino rabbits in a controlled manner for 8 h when compared to timolol maleate solution [120]. Discomes, when prepared, cause the surfactant to partition into the lipid bilayer which forms a large disc like structure. Discomes have a longer residence time in the cul-de-sac and less systemic drainage due to their large size [105]. Entrapment efficiency of drugs is also higher in discomes than niosomes. *In*

vivo bioavailability of drugs in discomes was found to be better than nisomes, as reported by Vyas *et al.* [119]. It is found that entrapment efficiency of naltrexone hydrochloride is 5 times greater in discomes than niosomes. Although *ex vivo* study of transcorneal permeability reveals that niosomes are competent enough to prolong drug release, thus improving corneal permeation [121]. Therefore, niosomes can be considered as a safe option for sustaining transcorneal drug delivery.

1.3.6. *Cubosomes*

Definition and description- Cubsomes are self-assembled liquid crystalline particles or nanoparticles [122].

Uses in ocular drug delivery with examples- Cubosomes loaded with dexamethasone were developed with monoolein and polymer 407, using an emulsification technique. Ethyl rhodamine B (Rh B) was used to label the nanoparticles, whereas Rh B solutions and Rh B carbopol gel served as the control. It was found that dexamethasone, in cubosomes increases the AUC by 2.5 - 3.5 times relative than Rh B solutions and Rh B carbopol gel, whereas no significant difference in clearance rate was observed for the control groups. This indicated that cubsomes were able to improve the ocular residence time and also bioavailability of the drug in ocular tissues. An *in vivo* microdialysis study reveals that dexamethasone in cubosomes increased the concentration in aqueous humor by 1.8-fold when compared to dexamethasone eye drops and by 8-fold when compared to dexamethasone

suspension. Hence it can be concluded that cubosomes can be a good alternative since it also has good biocompatibility with the corneal surface [122].

1.3.7. *Nanomicelles*

Definition and description- A nanomicelles are colloidal structured carrier systems that range from 5 to 200 nm in size. They are made up of amphiphilic surfactant molecules that may be anionic, cationic or zwitterionic in nature [104] or diblock polymers. Micelles could be spherical or cylindrical or star-shaped depending on the molecular weight of the core and corona forming blocks [123]. These amphiphilic molecules orient themselves to form normal or reverse micelles. When the hydrophobic portion forms a cluster in the core while the hydrophilic part aligns towards the outer surface allowing maximal contact with water, normal micelles are formed. Similarly, when an opposite alignment occurs those clustered aggregates are called reverse micelles. Normal micelles are used to encapsulate, solubilize and deliver hydrophobic drugs, whereas reverse nanomicelles are used to encapsulate and deliver hydrophilic drugs [124]. Nanomicelles are suitable and safe alternatives for ocular drug delivery because of their ability to solubilize less water soluble drugs in the hydrophobic core and form a clear aqueous formulation which is otherwise difficult to achieve for a hydrophobic drug. Nanomicelles offer myriad advantages such as ability to formulate hydrophobic drugs into a clear aqueous solution, high water solubility, monodispersity, form nanosize constructs, ability to minimize drug degradation, reduced toxicity, enhanced permeation through tissues and higher bioavailability [124-126].

1.3.7.1. Surfactant nanomicelles- Amphiphilic molecules usually possess a hydrophilic head and a hydrophobic tail. The hydrophilic head group carries an anionic or cationic charge (ionic surfactant) or both positive and negative charges (zwitterion surfactant) or no charge (nonionic surfactant). Sodium dodecyl sulfate (SDS) is an example of anionic surfactant, while dodecyltrimethyl ammonium bromide (DTAB) is an example of cationic surfactant, and dioctanoyl phosphatidylcholine (C8-lecithin) is an example of a zwitterionic surfactant. Non-ionic surfactants are considered to be least toxic and some examples include dodecyl tetra (ethylene oxide) (C12E4); vitamin E TPGS; and octoxynol-40 [127, 128]. The surfactants that are used at lower concentrations are likely to be absorbed at the surface or the interface, thus lowering the surface or interfacial free energy. Surfactants tend to form clustered aggregates as micelles at or above the critical micelle concentration (CMC). Micellization occurs when a balance exists between the intermolecular forces such as hydrophobic, steric, electrostatic, hydrogen bonding and Vander Waals forces. Other parameters that dictate micellization include the shape and size of surfactant monomers, ionic strength, pH, temperature, total surfactant concentration, and number of surfactants used [124, 125, 129]. Several reports published in the literature supported the use of surfactant micelles for improved penetration of topically applied drugs through the cornea and enhanced ocular bioavailability.

Mitra *et al.* reported a mixed nanomicellar system of vitamin E TPGS and octoxynol-40 for delivery of drugs such as voclosporin, dexamethasone, and rapamycin. An *in vivo* study conducted in rabbit eyes and canines revealed enhanced drug bioavailability to anterior segment ocular tissues with no symptoms of ocular irritation or toxicity [130, 131]. Vadlapudi *et al.*, solubilized biotin-12hydroxystearic acid-acyclovir (B-12HS-ACV) in nanomicelles of vitamin E TPGS and octoxynol-40 and evaluated their biocompatibility in human corneal epithelial cells (HCECs). B-12HS-ACV released in a sustained manner from the nanomicellar formulation for a period of 4 days when compared to 100% release of B-12HS-ACV in about 6 h from an ethanol solution [132]. Kuwano *et al.* compared the pharmacokinetics and distribution of cyclosporine A resulting from topical application as an oil-based medium (polyoxyl 60 hydrogenated castor oil (HCO-60)), o/w emulsion, and cyclosporine A aqueous clear solution containing a surfactant (polyoxyl 40 stearate (MYS-40)) in rabbit ocular tissues. Higher solubility was reported for the insoluble cyclosporine A with surfactants when compared to the other oil based mediums and emulsions. *In vivo* studies revealed MYS-40 as a solubilizer of cyclosporine A which showed improved ocular drug accumulation with a single topical administration compared to the other oil based mediums and emulsions. A significant increase in AUC is observed in corneal stroma–endothelium, bulbar conjunctiva, and lacrimal gland as compared to oil- and emulsion-based formulations [133].

1.3.7.2. Polymeric nanomicelles- Polymeric nanomicelles are synthesized from block copolymer. They form amphiphilic monomeric units which have distinct hydrophilic

and hydrophobic monomeric units; generally the hydrophobic core is surrounded by the hydrophilic shell. They contain polymer chains which are self-assembled due to hydrophobic or ion pair interactions between polymer segments [130]. The polymer blocks are arranged differently as diblock (A-B type), triblock (A-B-A type) or even grafted or branched type copolymers, where A and B are different polymers used. Ideally, the polymers utilized to prepare nanomicelles should be biodegradable and/or biocompatible [123]. The polymers commonly used in the preparation of nanomicelles include polyethylene glycol, polyethylene oxide, poly (D, L-lactic acid), polypropylene oxide, polyamino acids like polyaspartic acid, polyglutamine acid, poly-L-lysine, and poly-histidine [104]. As the length of the hydrophilic segment increases, copolymers tend to exist in aqueous solvents as unimers, whereas copolymers tend to form non-nanomicellar structures known as rods and lamellae with increase in the length of hydrophobic segment [134]. If the core-forming block structures are efficiently monitored the nanomicelles may have good thermodynamic and kinetic stability, thus can enable a variety of drugs to be incorporated for drug loading, release, activation, and effective therapy. It is found that polymeric micelles are more stable than nanomicelles made from conventional surfactants [134]. Literature suggests that these polymeric nanomicelles are able to retain drug molecules for a long time even in a diluted environment in systemic fluids due to their low CMC values [135]. Polymeric nanomicelles have also been used in active drug targeting [136]. Lastly, polymeric micelles offer advantages like extended circulation time, sustained release, favorable biodistribution, reduced side effects and

lower toxicity [125, 137, 138]. Thus, polymeric nanomicelles are an attractive option for ocular drug delivery.

Polymeric micelles containing ketorolac and copolymers such as N-isopropyl acrylamide (NIPAAM), vinyl pyrrolidone (VP), and acrylic acid (AA) cross-linked with N, N'-methylene bis-acrylamide (MBA) were able to improve the ocular bioavailability by two fold with no corneal damage when compared to an aqueous suspension containing the same amount of ketorolac [139]. Dexamethasone was entrapped in nanomicelles prepared from PEG and/or carbon chain conjugated with a polyhydroxyethyl-aspartamide system (PHEA-C and PHEA-PEG-C). Higher drug permeation was observed in primary cultured rabbit conjunctival and corneal epithelial cells than dexamethasone solution or suspension. The ocular bioavailability of dexamethasone was also improved with PHEA-PEG-C16 polymer suggesting that micelles have the potential to be a colloidal drug carrier for ocular drug delivery [140]. Cyclosporin A was formulated in polymeric micelles of methoxy poly (ethylene glycol)-hexylsubstituted poly (lactides) (MPEG-hexPLA). Results indicated excellent *in vitro* and *in vivo* ocular biocompatibility, transparency, and stability of the formulation. This provides the potential of MPEG-hexPLA micelles as carriers for cyclosporine A in treating dry eye syndrome and autoimmune uveitis, or for the prevention of corneal graft rejection [141]. Pilocarpine formulated with triblock copolymer Pluronic F127 poly (oxyethylene)/ poly (oxypropylene)/poly (oxyethylene) showed improved pharmacokinetics and prolongation of miotic response when compared with standard pilocarpine solutions. Such augmentation in

mitotic response was attributed to the productive absorption of drug-loaded nanomicelles [142]. Pepic *et al.* developed and characterized dexamethasone loaded nanomicellar formulation with polyoxyethylated nonionic surfactant Pluronic F127 and chitosan. The *in vitro* release of dexamethasone and transport across Caco-2 cell monolayers was found to be higher in the presence of chitosan as compared to chitosan free Pluronic F127 micelles. Polymeric micelles Pluronic F127 and chitosan showed excellent ocular bioavailability with 2-4 fold increases when compared to standard dexamethasone suspension. The improved intraocular absorption of dexamethasone from the micellar systems was attributed to the higher permeability and mucoadhesive nature of chitosan [143]. Metipranolol was delivered by using a chitosan-pluronic nanomicellar formulation. Despite the immediate release of the drug with 0.5% chitosan/pluronic micelles an increase in the AUC of metipranolol occurred by 1.67-fold relative to commercial metipranolol eye drops. The increment in the AUC of chitosan/pluronic micelles could be because of bioadhesive property of chitosan. However, no significant difference in AUC was observed against commercial eye drops of metipranolol when F127 was only used. It was attributed to the lack of bioadhesion leading to the elimination of micelles from the precorneal area [144].

1.3.7.3. Polyion complex nanomicelles– These nanomicelles are formed by electrostatic interactions between polyion copolymers (comprised mainly of the neutral segment and ionic segments) and oppositely charged ionic drugs [145]. Polyion complex (PIC) nanomicelles are mainly used for gene and antisense

oligonucleotide delivery [146, 147]. In general the block copolymer is hydrophilic in nature, the neutral block is polyethylene glycol (PEG) and the ionic block is neutralized by oppositely charged species that forms the hydrophobic core [148]. For ocular drug delivery, PEG stabilizes the hydrophobic polyion-drug complex thus forming the PIC micelles. PIC micelles offer reduced side effects as it is target specific and thus is a promising carrier system for ocular delivery of ionic macromolecules [123].

Uses in ocular drug delivery with examples- Ideta *et al.* studied the distribution of PIC micelles of PEG-P (Asp) for the treatment of choroidal neovascularization (CNV). FITC-P (Lys) was used as a model drug. Accumulation of PIC micelles in rats with CNV lesion was observed from the first hour and resided for up to 168 h, which could be due to the enhanced permeation and retention of PIC micelles. Also PIC micelles were also found to be very stable and their distribution in the body mainly depended on size and surface properties rather than the properties of loaded drugs [149]. Another novel approach for using photodynamic therapy (PDT) with dendrimerporphyrin (DP) loaded PIC micelles was for the selective accumulation in the pathologic corneal neovascularization area without affecting normal ocular vessels. Dendrimer zinc porphyrin (DP, photosensitizer) was prepared with PEG-P (Lys) block copolymer resulting in PIC micelles. PIC micelles accumulated in the corneal neovascular tissue following intravenous delivery with no accumulation of DP-micelles in normal limbal vessels thus suggesting improved targetability with enhanced permeability and retention effects [150].

Nanomicelles have been used for gene delivery to the anterior segment ocular tissues by means of eye drops. This strategy has been used for treating corneal diseases such as corneal neovascularization, dry eye syndrome, corneal scarring, corneal angiogenesis and inflammation because of enhanced bioavailability [130]. Liaw *et al.* have used a nonionic copolymeric system poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) for ocular gene delivery. Stable and efficient delivery of plasmid DNA encapsulated with the *LacZ* gene was observed in rabbit and mice eyes. Results indicated a promising potential for copolymers in DNA transfer [151]. Another polymeric system composed of PEO-PPO-PEO was developed by the same group to deliver genes for cornea-specific promoters (keratin 12 and keratocan). Significant elevation of β-Gal activity occurred (which was considered as a measurement of transgene expression) after administration of 6 doses of the DNA-encapsulated micellar system. This was attributed to endocytosis and particle size dependent paracellular transport of polymeric micelles [152]. The polymeric micellar formulation improved m-RNA levels by 2.2-fold suggesting the increased permeability of nanomicelles in the anterior ocular tissues [153].

1.3.8. ***Nanoparticles*–**Nanoparticles are colloidal drug carriers with a size ranging from 10 to 1000 nm. Depending on the mode of preparation they are further classified into :-

- Nanocapsules- The drug in nanospheres remain at the center which is surrounded by a polymeric membrane. The central core is either oily or aqueous in nature and the shell is solid.

- Nanospheres- The drug in nanospheres is entrapped inside the polymeric matrix in a uniform manner. They are composed of both biodegradable and non-biodegradable polymers depending on the disease condition being treated [76, 104].

In general, nanoparticles used in ophthalmic preparations are made up of lipids, proteins and natural or synthetic polymers such as albumin, sodium alginate, chitosan, poly (lactide-co-glycolide) (PLGA), polylactic acid (PLA) and polycaprolactone [154]. Nanoparticles have been used to deliver drugs to both the anterior and posterior eye segments. Nanoparticles have several advantages: 1) provides less irritation due to small size, 2) provides sustained drug release thus avoiding frequent administration, 3) does not allow premature degradation or non-specific uptake, 4) improves intracellular penetration and provides better absorption, and 5) provides target specific delivery to the desired tissue [76, 155]. However, they have the tendency to drain from the precorneal pockets similar to aqueous solutions. Ocular diseases which involves angiogenesis, such as central retinal vein occlusion (CNVO), choroidal neovascularisation (CNV), and diabetic retinopathy (DR) have drugs delivered using nanoparticle drug targeting [84]. Both lipophilic [156] and hydrophilic drugs [157] can be loaded into nanoparticles. Hydrophilic drugs are entrapped in PLGA nanoparticles using a water-in-oil-in-water (W/O/W) double emulsion technique, whereas hydrophobic drugs are entrapped in PLGA nanoparticles using an oil-in-water (O/W) emulsion technique [76].

1.3.8.1. ***Polymeric Nanoparticles*****-** The unique structure of polymers such as shape, size, physical state and surface render polymeric nanoparticles as the most compliant material used for ocular drug delivery [158]. Polymeric nanoparticles use biodegradable and non-

biodegradable polymers for sustained drug delivery for treatment of anterior segment inflammations. To overcome the shortcoming of precorneal elimination of nanoparticles they are often coated with polyethylene glycol, chitosan, and hyaluronic acid or even thermosensitive gels to impart mucoadhesive property and thus improving the drug bioavailability [154].

Uses in ocular drug delivery with examples- Gan *et al.* prepared self-assembled liquid crystalline nanoparticles of ethyl rhodamine B (Rh B) using monoolein and poloxamer 407. The bioavailability of Rh B from nanoparticles increased 3.5 and 2.5-fold against Rh B solution and Rh B carbopol gel, respectively, no significant difference in clearance rate was observed between Rh B solution and Rh B carbopol gel [122]. Other drugs such as ibuprofen, flurbiprofen, and indomethacin were incorporated into nanoparticles for treating anterior segment inflammations. Ibuprofen loaded nanoparticles were able to improve bioavailability of the drug in aqueous humor of rabbit eyes when compared with ibuprofen aqueous eye drops [159]. Similarly, flurbiprofen loaded in nanoparticles showed improved interactions with corneal surface, probably because the cationic nature of nanoparticles interacts better with the anionic corneal surface and thus improves bioavailability [160, 161]. Chitosan is the most widely used polymer for increasing the precorneal residence time of nanoparticles. Cyclosporin-A loaded nanoparticles has a positive zeta potential with smaller particle size. It improved the precorneal retention by 2-fold in cornea and 4-fold in conjunctiva when compared to Cs-A eye drop solutions or suspensions [162]. Improved ocular bioavailability was observed when gatifloxacin was incorporated into mucoadhesive polymer (HA) coated with Eudragit nanoparticles (RS 100 and RL 100) [163]. Mitra *et al.* developed a pentablock copolymer system which is

capable of forming nanoparticles with thermosensitive gelling capacity. These polymeric systems have been used to deliver drugs for treating chronic anterior ocular diseases [164]. Human cornea and conjunctival cells have CD44 HA receptors located on them. They were monitored to check the uptake of hyaluronic acid-chitosan oligomer based nanoparticles (HA-CSO NPs) and found that HA-CSO NPs undergoes active transport which is mediated by CD44 HA receptors via caveolin-dependent endocytosis pathways [165]. Active drug targeting using surface modified nanoparticles has also been pursued by ophthalmic researchers. Nanoparticles functionalized with antibodies, vitamins, peptides, and aptamers show enhanced uptake in specific ocular tissues. According to Kompella *et al.* surface functionalized nanoparticles with deslorelin and transferrin showed 64% and 74% higher transport respectively compared to un-functionalized nanoparticles. This indicates that surface modification provides a rapid and efficient delivery of nanoparticles into and/or across cornea and conjunctiva [166]. Nevertheless, nanoparticles have some disadvantages such as low drug loading and burst release of drugs. Nanoparticles generally exhibit a biphasic release pattern, with an initial burst release followed by sustained release. Jwala *et al.* demonstrated that the burst release of drugs from nanoparticles could be eliminated by dispersing nanoparticles in thermosensitive gels such as PLGA-PEG-PLGA [55].

1.3.8.2. ***Nanoparticle loaded contact lenses-*** Conventionally used topical eye drops of ophthalmic drugs result in limited bioavailability mainly due to drainage of the drug into the systemic circulation by the nasolacrimal duct and less residence time in the precorneal area. Nanoparticles loaded into contact lens are considered as an alternative

because they can maximize the residence time of the drug and thus improve the drug permeation through the cornea. They also provide a continuous drug release due to the slow diffusion of drug molecules through nanoparticles and the lens matrix. Drugs can be loaded into the nanoparticles either by soaking the contact lens in a drug solution or by entrapping drugs in nanovesicles and then dispersing these vesicles throughout the contact lens. However, there are a few limitations associated with both methods: soaked contact lenses deliver drugs only for a limited time period and the amount of drug to be loaded depends on the equilibrium solubility of the drug in the lens matrix, which is generally small for most drugs. Thus soaked contact lenses are not a convenient alternative for long term drug delivery, whereas the entrapment of drugs in nanoparticles results in an additional barrier that prevents the immediate release of drugs [84].

Uses in ocular drug delivery with examples- Gulsen *et al*. dispersed drug in an o/w microemulsion of poly-2-hydroxyethyl methacrylate (p-HEMA) hydrogels. Results confirmed that there is an initial burst release of 50% drug due to the drug absorbed on the surface of nanoparticles and the hydrogel. Later the drug entrapped in the o/w microemulsion droplets was released in a slow fashion [167]. However, a part of the drug is also lost due to tear drainage, absorption via conjunctiva and the majority by post lens tear film. The drug stays in the post lens tear film for about until 30 min till it is absorbed by the cornea. A small portion of the drug is lost by pre-tear film, however it is not a significant loss [168]. Kim *et al.* were able to demonstrate that silicon hydrogel material releases timolol and dexamethasone in a zero order fashion for up to 120 days with a negligible burst release of <5% on the first day [169]. Nakada *et al.* developed a compound contact lens with a hollow cavity that binds two contact lenses. This when

soaked in drug solution resulted in drug loading and allowed the drug to be released following insertion into the eye. However, low oxygen and carbon-dioxide permeability limits its use [170].

1.3.8.3. Solid-Lipid-Nanoparticles (SLN)- SLN can be defined as a solid lipid matrix in the nanometer range accommodating a drug that is stabilized by one or more surfactants [171]. They offer advantages such as controlling drug release, drug targeting, long-term stability, and endless biotoxicity due to the use of physiological lipids [172]. However, SLN has a limited drug loading capacity (around 25% of lipid matrix) and leads to burst release of hydrophilic drugs during the initial period [173]. SLN stops or retards the degradation process for lipophilic drugs because the mobility of the reactive agents is hindered in solid state than the liquid state [174]. Impediments associated with SLN led to the modification of SLN to a nanostructured lipid carrier (NLC). NLC is capable of accommodating a larger quantity of drugs thus improving their release profile. This is because the space between the fatty acid chains of glycerides is increased allowing more drug to be accommodated, and the formation of lipid crystals which avoids drug expulsion during storage [175]. NLC contains around 30% liquid lipids but the final product is in solid state with no crystalline structure [171].

Uses in ocular drug delivery with examples- SLN are considered a favorable alternative for ocular drug delivery due to their nano-size range, enhanced corneal absorption, improved bioavailability, and prolonged ocular retention in the conjunctival sac. They also are easily dispersible in aqueous media, hence they can be formulated into eye drops

[176]. Drugs loaded in SLN are capable of crossing the corneal epithelium and the anionic nature of the corneal epithelium enables absorption of cationic SLN [177]. Cavalli *et al.* demonstrated that SLN enhance the ocular bioavailability of tobramycin by increasing the residence time on the corneal surface and conjunctiva when compared to an equal dose of tobramycin aqueous solution [178]. SLN of diclofenac sodium were developed with a combination of homo-lipid from goat (goat fat) and phospholipid (phospholipon 90G®), whereas the control formulation contained diclofenac sodium without phospholipid. Drug loading capacity was approximately 90% and SLN showed a sustained release of the drug. On the other hand, the control formulation showed low drug loading with a burst release. Permeation across the cornea was improved probably due to high loading capacity and a sustained release profile of diclofenac sodium [179]. Gokce *et al.* prepared an SLN formulation of cyclosporine A using Compritol 888 ATO as the base lipid. The entrapment efficiency of cyclosporine A was found to be 95%. SLN of cyclosporine A were further tested in rabbit eyes. Results showed that the therapeutic level of cyclosporine A was achieved in aqueous humor after 3 h, which was attributed to internalization/uptake of SLN by the corneal epithelium. SLN of cyclosporine A retained in the precorenal area for up to 40 min, while the retention time of cyclosporine A solution was only 10 min [180].

NLC due to increased liquid lipid concentration results in enhanced ocular retention and improved corneal penetration [178, 181]. Shen *et al.* incorporated cyclosproin A in NLC with 2% Tween 80 as an emulsifier and 2% polyethylene glycol stearate (PEG-SA) as a surface modifier. It resulted in smaller particles with an enhanced drug loading capacity,

although there was no significant difference in entrapment efficiency. The release profile was improved with NLC with a faster drug release during the first 12 h followed by a sustained release thus prolonging ocular retention and better corneal permeability [181].

1.4. Dexamethasone in the treatment of anterior segment eye inflammations

1.4.1 Dexamethasone delivery to the anterior segment eye diseases

Dexamethasone is a corticosteroid which is widely used as an anti-inflammatory agent and immunosuppressant. Dexamethasone is a glucocorticoid agonist and its anti-inflammatory action is due to the interference in function of phospholipase A2 inhibitory protein, lipocortin, which helps in the biosynthesis of prostaglandins and leukotrienes (which induce inflammation). Glucocorticoids such as rapamycin or dexamethasone are used in the form of eye drops for cataract surgery, corneal procedures, uveitis, macular edema, fibrin deposition, and retinal vein occlusion [182, 183]. Corticosteroids generally have a log octanol–water partition coefficient between 2 and 3, and the logP value of dexamethasone is 1.83, which can be considered moderately lipophilic. In general, the concentration of corticosteroids used in an ophthalmic treatments is 0.1 to 1.0%. Dexamethasone is generally administered in the form of suspensions and ointments for treating anterior segment inflammations, due to its low aqueous solubility [184]. Some commercially available dosage forms of dexamethasone for treating ocular inflammations are shown in Table 1.1. In a suspension dosage form, finely divided drugs are dispersed in an aqueous solvent and stabilized by a suspending or dispersing agent. The suspended drug particles tend to be retained longer in the precorneal area and thus improve residence time and duration of action relative to simple eye drop solutions. Particle size

plays a prime role in ocular bioavailability, where a smaller size is assumed to increase drug bioavailability [98, 159], whereas larger particles are retained longer thus decreasing drug dissolution [185]. Ophthalmic suspensions of corticosteroids such as dexamethasone are considered to be acceptable for delivery since it is presumed that drug particles persist in the conjunctival sac giving rise to a sustained-release effect [186].

Table 1.1: Commercially available dosage forms of dexamethasone for treating ocular inflammations.

Brand Names	Active Ingredients	Available forms	Use
Maxidex®/ Minims®	0.1% Dexamethasone	Eye drops/ Eye ointments/ Suspension	Inflammations of bulbar conjunctiva, cornea, anterior eye segment, conjunctivitis, corneal injury, including postoperative inflammations
Ozurdex®	0.7 mg Dexamethasone	Intravitreal implant	Macular edema following branch retinal vein occlusion (BRVO) or central retina vein occlusion (CRVO), uveitis or diabetic macular edema
Maxitrol®/ Dexair®/ Dexasporin®	Polymyxin B Sulfate+ neomycin sulfate+ Dexamethasone	Eye drops/ Eye ointment/ Suspension	Bacterial blepharitis, bacterial conjunctivitis, trachoma and inflammations if palpebral, bulbar conjunctiva, cornea and anterior eye segment
Tobradex®	0.3%Tobramycin+ 0.1%dexamethasone	Suspension/Eye drops/ Eye ointments	Bacterial conjunctivitis and infections after surgery or trauma
TobraDex®ST	0.3%Tobramycin+ 0.05%dexamethasone	Suspension/ Eye drops	For ocular infections and inflammations of palpebral or bulbar conjunctiva, cornea, anterior eye segment
Neodecadron®	Dexamethasone sodium phosphate and Neomycin Sulfate	Eye drops/oral tablets	Bacterial infections and inflammations for bulbar conjunctiva, cornea, anterior eye segment, chronic anterior uveitis, corneal injury.

1.4.2. Problems associated with the use of dexamethasone suspension and eye drops

The most commonly marketed dexamethasone suspension is Maxidex®.Though it is efficient in treating ocular inflammations it suffers from lower aqueous humor bioavailability [187]. In general, ophthalmic suspensions are known to have several drawbacks. The particle size of drugs in ophthalmic suspension dosage forms should be less than 10 µm in order to prevent any irritation to the sensitive ocular tissues [186, 188, 189]. Drug absorption from a suspension dosage form is highly unpredictable and identical formulations with the same concentration of active and inactive ingredients tend to exhibit differences due to varying physicochemical properties and lack of dose uniformity [190]. The erratic absorption of drugs from a suspension dosage form is attributed to clearance of a large percentage of drug particles from the precorneal region before dissolution and absorption can occur. Moreover, the intrinsic dissolution rate of the drug varies due to constant inflow and outflow of lachrimal fluids [186, 190]. Eye drop suspensions are also known to cause ocular adverse effects such as irritation, redness of eye and blurred vision. The chronic administration is also likely to increase drug content in the systemic circulation which may lead to severe systemic complications. The use of preservatives in these formulations will likely induce adverse reactions [191].

Dexamethasone prodrug such as dexamethasone sodium phosphate has also been widely used in ophthalmic formulations. However, the hydrophilic nature of dexamethasone sodium phosphate restricts its permeation through the lipophilic corneal epithelium, thus decreasing ocular bioavailability [192]. In an attempt to overcome the above-mentioned

disadvantages associated with eye drop suspensions, alternate strategies have been developed for delivering required amounts of dexamethasone into the ocular tissues.

1.4.3. Alternate strategies for delivering dexamethasone to the eye

1.4.3.1. Cyclodextrins: Cyclodextrins, especially 2-hydroxypropyl beta cyclodextrins (HP-β-CD), have been widely used in the delivery of dexamethasone [193]. Cyclodextrins are inert in nature and HP-β-CD can be used as high as 45% w/v without producing any discomfort [194]. A clinical study on human subjects with 0.32% dexamethasone HP-β-CD solution showed a 2.6-fold increase in aqueous humor relative to dexamethasone suspension [195]. A 0.7% dexamethasone solution with 10% w/v HP-β-CD was compared against the marketed dexamethasone suspension (Maxidex®). It was found that the 0.7% dexamethasone solution administered once daily was able to prolong the effect similar to Maxidex® suspension administered thrice daily [196]. Cyclodextrin eye drops, prepared with dexamethasone for topical use, also showed promising results for posterior segment eye diseases such as diabetic macular edema [197]. In spite of having potential to prolong the residence time, cyclodextrins for lipophilic molecules must be used in limited quantity otherwise it may result in decreased drug availability [198, 199]. Moreover, there is not much evidence available on the toxicological data for cyclodextrins which may circumscribe their use in ophthalmic formulations.

1.4.3.2. Bioadhesive polymers: Durasite® core technology, which involves the use of a bioadhesive polymer, was developed by InSite Vision Inc. to prolong the duration of action of topically applied drugs to the eye in order to improve bioavailability. AzaSite

Plus™ uses Durasite® technology containing 0.1% dexamethasone and 1.0% azithromycin, for treating blepharitis, infections and inflammations and is in Phase III development clinical trials. DexaSite™ consists of 0.1% dexamethasone and uses Durasite® technology developed by the same company and is also in Phase III development [200].

1.4.3.3. Use of xanthan gum: TobraDex® is an ophthalmic suspension commercially manufactured by Alcon Laboratories Inc, (Fort Worth, TX) containing a mixture of tobramycin 0.3% and dexamethasone 0.1%, used as an anti-inflammatory and anti-infective for blepharitis. Tobramycin is effective in treating ocular inflammation and infection. However being viscous and exhibiting low concentration of both tobramycin and dexamethasone in human and rabbit eye limits its use. To improve this, Scoper *et al.* developed TobraDex ST® with 0.3% tobramycin and 0.05% dexamethasone. Results indicated a significant improvement in suspension characteristics and quality, tear film kinetics, and tissue penetration of drugs by using xanthan gum, an anionic polysaccharide with a repeating unit of two D-glucose, two D-mannose and one D-glucuronic acid residues. The viscosity of the new formulation was improved following interaction with tear fluids in the eye and thus improved overall bioavailability. Clinical studies also showed higher concentrations of dexamethasone in the aqueous humor, better pharmacokinetics and anti-bacterial properties and higher patient compliance compared to TobraDex® eye drops [201].

1.4.3.4. Microemulsions: Microemulsions have been used in ophthalmic delivery to solubilize lipophilic drugs and to reduce the dosing frequency by prolonging the release of drugs. A 0.1% w/v dexamethasone loaded in microemulsion eye drops prepared using Cremophor EL and propylene gylcol as surfactants and co-surfactants respectively, provided a prolonged release of the drug for up to 8h relative to the marketed formulation. Microemulsions also enhance the drug permeation through the corneal membrane. This microemulsion system of dexamethasone (0.1%) was able to penetrate faster into the aqueous humor (approximately 10 minutes after instillation) relative to dexamethasone solution [87]. This system also has an optimum viscosity to enable dispensing of the formulation in the form of eye drops, but greater viscosity than solutions, thus improving residence time without any discomfort or blurred vision. The same dexamethasone microemulsion system was compared against other formulations, *viz.* - dexamethasone loaded in cyclodextrins, 0.1% dexamethasone suspension, 1% dexamethasone suspension, and 0.1% dexamethasone disodium phosphate solution. Results revealed that 0.1% dexamethasone in a microemulsion system showed the highest concentration and was well tolerated in rabbits [87]. Though microemulsions offer excellent advantages, use of higher surfactant and cosurfactant concentrations in the manufacture of microemulsions tends to irritate sensitive ocular tissues and thus limits their use.

1.4.3.5. Use of thermosensitive polymers: A dexamethasone hydrogel solution was prepared by Gao *et al.* using thermosensitive polymer, poly-lactide-co-glycolide-polyethylene glycol-poly-lactide-coglycolide (PLGA-PEG-PLGA). This system was able

to prolong the precorneal residence time and improve the drug concentrations in the aqueous humor by 7-fold compared to dexamethasone eye drops [202].

Chapter 2

Significance of Research

To date, anterior segment diseases are mainly treated using topical eye drops. Eye drops, which account for approximately 90% of ophthalmic market formulations [80, 188], are widely used in the delivery of anesthetics, antihistamines, β-receptor blockers, non-steroidal anti-inflammatory drugs (NSAIDs), parasympatholytics, parasympathomimetics, prostaglandins, steroids, and sympathomimetics [33]. Ocular bioavailability of drugs following topical administration is less than 5% due to nasolacrimal drainage and high tear fluid turnover [79]. Regardless of the low ocular bioavailability, eye drops are preferred because of their affordability, ease in scale-up, and manufacturing processes.

Dexamethasone eye drops are widely used in treating anterior segment inflammations. Dexamethasone is a glucocorticoid with an optimum log P value that allows its permeation into the lipophilic corneal epithelium. However, it is marketed in the form of an eye drop suspension due its limited water solubility. Maxidex® is a marketed suspension of dexamethasone. Drug absorption from a suspension dosage form is highly unpredictable and identical formulations with the same concentrations of active and inactive ingredients tend to exhibit differences due to varying physicochemical properties. The inherent disadvantages of suspension dosage forms have encouraged researchers to look for alternate strategies to deliver dexamethasone. Nanocarrier systems such as nanomicelles are capable of delivering drugs to the anterior segment of the eye in the form of eye drops. Nanomicelles are considered a suitable and safe alternative for ocular drug delivery because of their ability to solubilize less water soluble drugs in the hydrophobic core and form a clear aqueous formulation, which is otherwise difficult to achieve for a hydrophobic drug. Nanomicelles can facilitate enhanced permeation through ocular tissues and provide higher bioavailability [124-126]. A preliminary study done in our lab revealed that mixed nanomicelles of polyoxyl 40 stearate (P40S) and polysorbate 80 (P80) could efficiently solubilize 0.1% dexamethasone in their core. The results also indicated that mixed nanomicelles could be utilized as a potential delivery system for delivering dexamethasone to treat "back of the eye" diseases such as posterior uveitis after topical application [64]. The present study builds on the previous work by developing and evaluating nanomicelles laden *in situ* gel of 0.1% dexamethasone (DMN-ISG) with potential for treating anterior segment eye inflammations. *In situ* gels are known to reduce pre-corneal drug elimination and thus may result in better ocular availability. We hypothesize that mixed nanomicelles laden *in situ* gels could be a potential

strategy in reducing the precorneal drainage and sustaining the drug release for a longer period of time following topical application.

The aim of this study is to prepare 0.1% dexamethasone nanomicelles laden *in situ* gel with potential for treating anterior segment eye inflammations. Dexamethasone nanomicelles were dispersed in gellan gum which has the ability to undergo sol-gel phase transition in response to mono- and di-valent cations in the eye. We hypothesize that *in situ* gelation would increase drug bioavailability by lowering drainage from the cul-de-sac. Drug loaded nanomicelles were dispersed in gellan gum and evaluated for pH, osmolality, viscosity, morphology, thermoanalysis, light transmittance and *in vitro* drug release.

Chapter 3

Development and Preliminary *In vitro* Evaluation of Nanomicelles laden in *In situ* Gel of Dexamethasone for Ophthalmic Delivery

3.1. Abstract

In our previous work we developed and characterized 0.1% dexamethasone mixed nanomicelles (DMN). DMN were prepared using surfactants polyoxyl 40 stearate (P40S) and polysorbate 80 (P80), which are approved by the FDA for ocular use. The present study builds on the previous work by developing and evaluating nanomicelles laden *in situ* gel of 0.1% dexamethasone (DMN-ISG) with potential for treating anterior segment eye inflammations. DMN-ISG was prepared by mixing the basic 2X formulation of DMN with appropriate concentrations of gellan gum, mannitol, benzododecinium bromide and tromethamine. DMN-ISG was characterized for gelation, viscosity, transparency, morphology using Transmission Electron Microscopy (TEM), thermoanalysis using Differential Scanning Calorimetry (DSC), *in vitro* drug release and sterility. DMN prepared with an optimized composition of P40S/P80=7/3 by weight were used in the preparation of DMN-ISG. TEM image of DMN-ISG showed the presence of dexamethasone nanomicelles in the size range between 20-40 nm entrapped in the gel structure. More than 50% of the drug was released from DMN-ISG in the first few hours and the remaining drug was released in a sustained manner for up to 30 h. Aseptically prepared DMN-ISG formulation remained sterile for up to 14 days. The preliminary findings of our investigation suggest that DMN-ISG has the potential for use in treating anterior segment eye inflammations. Further *in vivo* evaluation is warranted.

3.2. Introduction

The anatomical position of the eye allows local delivery of drugs via topical route for treating anterior segment inflammations. However, the physiological barriers prevent the entry of drug substances into the eye. For efficient treatment of diseases, drug molecules should circumvent the protective physiological barriers without causing permanent tissue damage [75]. The corneal epithelium acts as a barrier to the absorption of hydrophilic drugs while the stroma acts as a barrier for the passage of hydrophobic drugs [203, 204]. Therefore, compounds with an optimum oil to water partition coefficient will exhibit maximum corneal penetration [203]. In a comparative study of steroids, the corneal permeability was found to be highest for compounds having a log octanol–water partition coefficient value between 2 and 3 [205]. In addition to physiological barriers and physicochemical properties of drug molecules, the type of dosage form also plays a key role in ophthalmic delivery. Drug delivery for anterior segment diseases vary from topically applied conventional dosage forms such as solutions, suspensions, and ointments to novel dosage forms such as liposomes [77], nanoparticles [78] and implants [75, 79]. However, more than 90% of marketed formulations are conventional dosage forms [80]. Among them 62.4% are solutions, 17.4% are ointments and 8.7% are suspensions [81]. Water-soluble drugs are generally administered topically as aqueous eye drop solutions and water-insoluble drugs (such as glucocorticoids) are administered as suspensions.

Dexamethasone is widely used as a topical ocular corticosteroid for both anterior and posterior segment eye diseases. Dexamethasone with a log P value of 1.83 is considered moderately lipophilic like most glucocorticoids. Due to the limited water solubility of dexamethasone in an aqueous eye drop formulation, it is marketed as a suspension product (Maxidex®, Alcon Laboratories, Fort Worth, TX) or as a hydrophilic water-soluble salt, such as 0.1%

dexamethasone sodium phosphate. Formulation of a pharmaceutically elegant, sterile and effective suspension such as Maxidex® is more complex and challenging than conventional ophthalmic solutions. A formulator should take into consideration the homogeneity of the dosage form, aggregation of the suspended particles, settling of suspended particles, cake formation, and resuspendability during the development of a suspension formulation [206]. Particle size of the drug plays a key role in maintaining the physical stability and ocular bioavailability of drug products. The particle size of drugs in ophthalmic suspension dosage forms should be less than 10 μm in order to prevent any irritation to the sensitive ocular tissues [188].

Drug absorption from a suspension dosage form is highly unpredictable and identical formulations with same the concentration of active and inactive ingredients tend to exhibit differences due to varying physicochemical properties. The erratic absorption of the drug from a suspension dosage form is attributed to clearance of a large percentage of drug particles from the precorneal region before dissolution and absorption can occur. Moreover, the intrinsic dissolution rate of the drug varies due to constant inflow and outflow of lacrimal fluids [207]. To increase the ocular bioavailability of dexamethasone in a predictable fashion, it is necessary to increase the concentration of the drug in aqueous eye drops and enhance the precorneal retention of the drug. We hypothesize that mixed nanomicelles laden *in situ* gels could be a potential strategy in accomplishing these goals.

Mixed nanomicelles are vesicular systems used for the delivery of lipophilic substances [208]. Very recently their potential as vehicles for topical ocular drug delivery has been investigated [209, 210]. Mixed nanomicelles are spherical in structure with a size in the nanometer range.

They are formed spontaneously when amphiphilic molecules consisting of hydrophobic and hydrophilic segments are dissolved in water [211, 212]. Velagaleti *et al.* [210] developed a nanomicellar formulation of vitamin E TPGS and octoxynol-40 for voclosporin, and ocular distribution studies following topical application in rabbits resulted in therapeutic concentrations of voclosporin in the retina and choroid. In a recent study, we demonstrated that topical application of 0.1% dexamethasone mixed nanomicelles prepared using polyoxyl 40 stearate (P40S) and polysorbate 80 (P80) resulted an increased therapeutic retinal drug concentration in rabbits [64]. P40S and P80 were used in the preparation of mixed nanomicelles since they have been previously used in commercial ophthalmic products and are generally regarded as non-toxic and non-irritant materials [213].

As an extension of our previous work, the present study involves the development and preliminary evaluation of nanomicelles laden *in situ* gel of 0.1% dexamethasone (DMN-ISG) with potential for treating anterior segment eye inflammations. Gellan gum, a water-soluble anionic polysaccharide, was used an *in situ* gelling polymer as it forms a gel in the presence of monovalent and divalent cations. We hypothesize that nanomicelles dispersed in gellan gum would increase the precorneal retention of the drug and can deliver drugs at a slow rate for a longer period compared to nanomicelles alone.

3.3. Materials and Methods

3.3.1. Materials

Dexamethasone (Lot C137572) was procured from PCCA (Houston, TX). Polysorbate 80 (Lot 20589) was procured from Fisher Scientific (Pittsburgh, PA). Polyoxyl–40–stearate (Lot 109K0160V) was procured from Sigma Aldrich (St. Louis, MO). Ethanol (Lot B0522876) was supplied by ACROS (Fair Lawn, NJ). Gelzan™ (Gelrite) (Lot V12030603) was purchased from Plantmedia (Dublin, OH). D-Mannitol (Lot 086810) was procured from Fisher Scientific (Pittsburgh, PA). Benzyldodecyldimethyl ammonium bromide (Lot H3012) was procured from Santa Cruz Biotechnology (Dallas, TX). Tris (Lot R28030) was procured from MP Biochemicals (Santa Ana, CA). DMEM/F-12 (Lot 0000414827) was procured from Lonza (Walkersville, MD). Trypsin EDTA, 1X (Lot 25052393) was procured from Cellgro (Manassas, VA). TACS® MTT reagent (Lot 31205J14) was supplied from Trevigen. DMSO (Lot 104549) was procured from Fisher Scientific (Pittsburgh, PA). Tryptic soy broth (Soyabean Casein Digest medium-Bacto™, Lot 2030828) and Mueller-Hinton broth (Lot 3240477) were purchased from Fisher Scientific (Pittsburgh, PA). High Performance Liquid Chromatography (HPLC) solvents, including acetonitrile (Lot 121151) and methanol (Lot 113904) were supplied by Fisher Scientific (Pittsburgh, PA). Distilled deionized water was used throughout the study.

3.3.2. Preparation of 0.1% dexamethasone mixed nanomicelles (DMN)

Mixed nanomicelles of dexamethasone were prepared and used as a control in characterization studies. Nanomicelles were prepared by the rotary evaporation method with an optimized composition of P40S/P80 = 7/3 by weight. In brief, dexamethasone, in required amount, and

P40S (4.2 g) were dissolved in a sufficient amount of ethanol. Ethanol was then evaporated under vacuum at 50°C, leaving behind a thin film of dexamethasone and P40S. The film was rehydrated using 100 ml of water containing P80. The final solution was passed through a 0.22 µm sterile Nylon membrane filter (Millex® Syringe Filter, Sterile, 0.22 µm).

3.3.3. Preparation of nanomicelles laden in situ gel of 0.1% dexamethasone (DMN-ISG)

Mixed nanomicelles laden *in situ* gel was prepared by mixing the basic 2X formulations of dexamethasone with other inactive ingredients as shown in Table 3.1. A basic (2X) formulation was prepared by dissolving 4.2 g of P40S and dexamethasone in a required amount of ethyl alcohol in a round bottomed flask. The ethyl alcohol was then evaporated under vacuum at 50°C, leaving behind a thin film of P40S and dexamethasone. The thin film was rehydrated using 50 ml of water containing the required amount of P80. This 2X nanomicellar solution was passed through a 0.22 µm sterile Nylon membrane filter (Millex® Syringe Filter, Sterile, 0.22 µm). Gellan gum was slowly dispersed in 25 ml of water and hydrated in the presence of heat and sterilized by autoclaving at 121°C and 15 Pa for 20 minutes. In a separate beaker, mannitol, benzododecinium bromide and tromethamine were dissolved in 15 ml of water and passed through a 0.2 µm filter (Millex® Syringe Filter, Sterile, 0.22 µm) and collected in a pre-calibrated sterile vial containing the gellan gum solution. Fifty milliliters of sterile, basic 2X nanomicellar formulations was added to the sterile vial and mixed well. The final volume was brought up to 100 ml. The osmolality was measured using an Osmette-Precision Osmometer (Sudbury, MA) and the tonicity adjusted between 285-310 mOsmol/kg with mannitol solution. The pH was adjusted to 6.5 with tromethamine (commonly known as Tris Buffer) using an Accumet® excel XL 25 pH meter (Fisher Scientific, Pittsburgh, PA).

Quantitative composition of nanomicelles laden *in situ* gel of 0.1% dexaemthasome *(DMN-ISG)*	
Basic formulation (2X)	50 ml
Gellan gum	0.5%
Mannitol	2%
Benzododecinium bromide	0.012%
Tromethamine	q.s. pH6.5
Purified Water	q.s. 100 ml

Table 3.1: Quantitative composition of nanomicelles laden *in-situ* gel of 0.1% dexamethasone

3.3.4. Gelation study

Gelation of nanomicelles laden *in situ* gel was carried out in the presence of simulated tear fluid (STF) composed of sodium chloride (0.670 g), sodium bicarbonate (0.200 g), calcium chloride dehydrate (0.800 g) in 100ml of distilled deionized water. The temperature was maintained at 34 ºC to mimic the conditions of the eye [214]. Nanomicelles laden *in situ* gel was diluted with STF in 7.8:2.2 ratio [215]. Gelation of dispersion in the presence of STF was checked by visual examination.

3.3.5 Viscosity Study

A Brookfield RVDV-II with spindle no. 21 (Brookfield engineering Laboratories, Middleboro, MA) was used to determine the viscosities of *in situ* gel samples. Nanomicelles dispersed in gellan gum was diluted with STF in ratios of 8.8:1.2, 7.8:2.2, and 7.2:2.8 [216]. The viscosity of the samples was determined in a temperature controlled Brookfield Programmable Bath (Brookfield engineering Laboratories, Middleboro, MA) at 34ºC. The viscosity measured at an angular velocity from 0.5 to 100 RPM at a controlled ramp speed of 10 seconds. The speed was

reversed (100 to 0.5 rpm) with a wait time of 10 seconds. The viscosity values were calculated by taking the average of the two readings.

3.3.5. Transparency of DMN and DMN-ISG

Light transmission of DMN and DMN-ISG were studied with a UV-Visible spectrometer (Agilent 8453, UV-Vis Spectroscopy System) in the visible range of 400–700 nm. Light transmission of blank formulations was also measured as a control. Distilled deionized water was used as a blank for the study.

3.3.6. HPLC analysis of dexamethasone

HPLC (Waters Alliance e2695 separation module, Milford, MA), equipped with a 2998 PDA detector and reverse-phase C8 column (5 µm, 100A, Luna, Torrance, CA, USA), was used to quantify dexamethasone in the formulation. The solution was analyzed by an isocratic method with a mobile phase containing water and acetonitrile (50:50) pumped at a flow rate of 1.0 ml/min. The absorbance of dexamethasone was measured at a wavelength of 242 nm. The drug content was determined quantitatively by plotting a calibration curve. A stock solution with a strength of 1 mg/ml of dexamethasone in methanol was prepared and calibration standards ranging from 0.39 - 50 µg/ml were prepared in the mobile phase. Each standard concentration was analyzed in triplicate and the average peak area was plotted against concentration to obtain the calibration curve for dexamethasone quantification. The standard curve obtained was linear with an r^2 value of 1.00. The percentage recovery of dexamethasone ranged from 99.04%-

101.97%. The intra-assay precision of dexamethasone was found to be in the range of 0.51% to 0.79% and is considered satisfactory; the relative standard deviation did not exceed 2%.

3.3.7. Transmission Electron Microscopy (TEM)

TEM (HITACHI HD-2300 A, Ultra-thin Film Evaluation System, Hitachi High Technologies America, Pleasanton, CA) was used to study the morphology of dexamethasone nanomicelles in gellan gum. Negative staining of dexamethasone nanomicelles was made with 2% phosphotungstic acid solution (Fisher Scientific, Pittsburgh, PA) before the addition of gellan gum. A small drop was then placed on a holey carbon 400 mesh copper grid (Ted Pella, Redding, CA) and dried overnight undisturbed. The samples were then observed using the TEM.

3.3.9. Differential Scanning Calorimetry (DSC)

Physical state and thermal properties of dexamethasone, gellan gum and *DMN-ISG* were determined using a Differential Scanning Calorimeter (DSC) (822e Mettler Toledo) equipped with a TS0800GCI gas flow system attached to a nitrogen gas cylinder. Samples were placed (5-10 mg) in an aluminum crucible using the Mettler MT 5 microbalance and sealed. DSC studies were performed at a heating rate of 10°C/min over a range of 20 - 300°C. Nitrogen gas was purged at a rate of 20 ml/min. Star-e software V8.10 was used to obtain the scans.

3.3.10. In vitro drug release study

The *in vitro* release of dexamethasone from *DMN-ISG* was performed using a dialysis bag (Fisherbrand® Dialysis tubing, MWCO: 12,000-14,000 Da) and the release profile was compared to the drug release from DMN. One milliliter of DMN or DMN-ISG was placed in the

dialysis bag and sealed. These dialysis bags were immersed into tubes containing 50 ml of phosphate buffer (pH 7.4) containing 0.025% (w/v) Tween 80 in order to maintain sink conditions. The vials were then placed into a shaker bath maintained at 37±0.5°C and 60 oscillations/min. At predetermined time intervals one milliliter sample was withdrawn from each tube and replaced with an equal volume of fresh buffer. The study was conducted in triplicate and samples withdrawn at each time point were analyzed using HPLC.

3.3.11. Sterility Test

The final DMN-ISG formulation was checked for sterility in order to verify that the formulation was microorganism free. The sterility testing was performed using plate as well as direct/tube inoculation methods as per our published protocols [217]. For direct/tube inoculation method Trypsin soy broth (TSB) was used. In TSB, liquid culture of *Staphylococcus aureus* Rosenbach ATCC BAA 1692 was grown at 37°C for 24 h in a shaker water bath. After diluting, the final concentration of liquid culture was 10^2 CFU/ml. Samples for direct inoculation included a negative control vial, positive control vial, positive sample control vial, and aseptically prepared DMN-ISG vial. The negative control vial contained 1 ml of sterile water and 9 ml of the un-inoculated medium. The positive control vial contained 1 ml of water containing 10^2 CFU/ml and 9 ml of un-inoculated medium. The positive sample control vial contained 1 ml of DMN-ISG containing 10^2 CFU/ml and 9 ml of un-inoculated medium. The aseptically prepared DMN-ISG vial contained 1 ml of DMN-ISG and 9 ml of the un-inoculated medium. All of four vials were incubated at 37°C to speed up the growth of the bacteria. For the plate inoculation, samples of 100 µl were withdrawn by the direct inoculation method from each of the vials on 0, 7 and 14 days. These samples were transferred and uniformly spread on Mueller Hinton (MH) agar plates.

The plates were then incubated at 37°C for 12 h and observed for bacterial growth visually at 25°C. The entire study was performed in aseptic conditions in a laminar air flow hood. Glassware was autoclaved prior to use and then placed under the hood. All non-autoclavable materials were thoroughly wiped with isopropyl alcohol to make them free from microorganisms.

3.4. RESULTS AND DISCUSSION

Mixed nanomicelles laden *in situ* gel of dexamethasone (DMN-ISG) was successfully prepared and characterized for pH, osmolality, gelation, viscosity, morphology, drug content, *in vitro* release, light transmittance, and sterility. Mannitol was used adjust the osmolality, benzododecinium bromide was used as a preservative and tromethamine was used to adjust the pH of DMN-ISG. The pH of DMN-ISG was within the range of 6.5-7.4, which is close to the pH of tear fluids 7.31-7.62 [220]. Maintanence of pH is important in order to avoid irritation to the eye from the eye drops. Our intial findings showed that the osmolality of DMN-ISG was 258 mOsms/kg. The tonicity of DMN-ISG was adjusted between 285-310 mOsmol/kg with mannitol solution.

3.4.1. Gelation study

The dispersion of DMN-ISG appeared clear at room temperature on visual examination. Figs. 3.1 (a) amd 3.1 (b) show the sol-gel transition of the dispersion when diluted with STF in the ratio of 25:7 at 34°C. This indicates that DMN-ISG forms a viscoelastic gel in the presence of tear fluids upon topical administration.

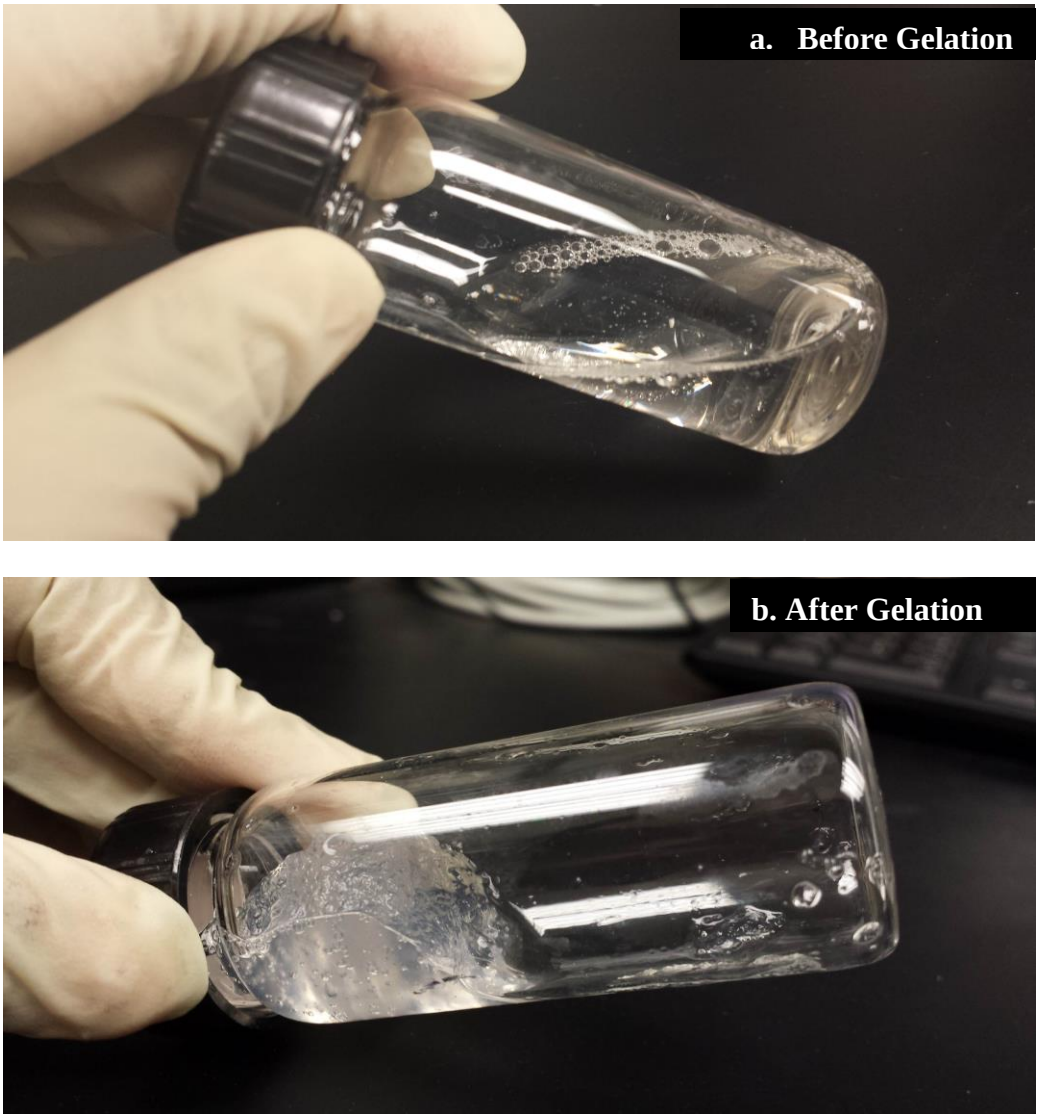


Figure 3.1: Sol-gel transition of dexamethasone mixed nanomicelles dispersed in gellan gum (a), formulation upon dilution with simulated tear fluids in eye (b)

3.4.2. Rheology

The viscosity of the DMN-ISG was measured at varying stress conditions. Simulated tear fluid (STF) was used to study the viscosity of DMN-ISG in various formulations: STF ratios of 8.8:1.2, 7.8:2.2, and 7.2:2.8. The dilution ratio of formulation: STF- 8.8:1.2 corresponds to 50 µl of the eye drop: 7 µl of tear fluid; dilution ratio of formulation: STF - 7.8:2.2 corresponds to 25

µl of the eye drop: 7µl of tear fluids; dilution ratio of formulation: STF - 7.2:2.8 corresponds to 25 µl of the eye drop: 10µl of tear fluids. DMN-ISG without any dilution was used as a control. At all dilutions, DMN-ISG exhibited a non-Newtonian behavior which was demonstrated as pseudoplastic flow and shear thinning behavior, i.e. the viscosity of the gel decreased with an increase in shear force, followed by a tendency to thicken when the force was withdrawn, which was indicated by the downward sloping curves (Fig. 3.2). Viscosities were recorded at 34 ºC in order to mimic the temperature of the eye.

This study indicates that dexamethasone nanomicelles dispersed in gellan gum forms a gel-like structure in the presence of lacrimal fluids, which in turn will enhance the residence time of the formulation in the precorneal area. The shape of the downward sloping curve confirms that a significant loss of viscosity was observed at the end of the test for DMN-ISG with no dilution, whereas a minor loss of viscosity occurred for two formulations: STF ratios of 7.8:2.2 and 7.2:2.8. The administration of an ophthalmic preparation should influence as little as possible the pseudo-plastic character of the pre-corneal film [221]. Since the ocular shear rate is very high, ranging from 0.03 s^{-1} during inter-blinking period to 4250 - 28500 s^{-1} during blinking [222], viscoelastic fluids with a viscosity that are high under low shear rate conditions and low under high shear rate conditions are often favored. Thus, DMN-ISG on dilution with the tear fluids in the eye is capable of forming a gel in the presence of tear fluids.

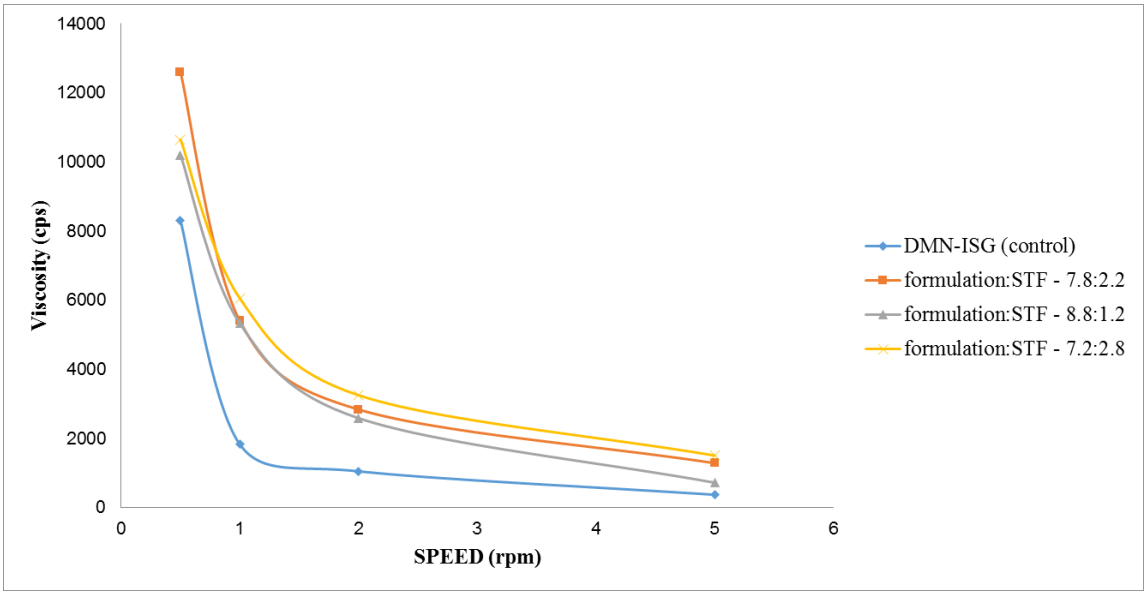


Figure 3.2: Viscosity data of dexamethasone mixed nanomicelles dispersed in gellan gum in the presence and absence of simulated tear fluids (STF).

3.4.3. HPLC Analysis

A HPLC method for analyzing dexamethasone concentration was successfully developed and validated. The retention time for dexamethasone was found to be 2.9 minutes (λ_{max}= 242 nm). A stock solution of 1 mg/ml of dexamethasone was prepared in methanol and different calibration standards were prepared in the mobile phase ranging from 0.39-50 µg/ml. Each standard was analyzed in triplicate and the average peak area was plotted against the concentration for the calibration curve. A straight line (y = 1991x + 767) was obtained with a correlation coefficient value (r^2) of 1.00 (Fig. 3.3). A sample chromatogram of dexamethasone is shown in Fig. 3.4.

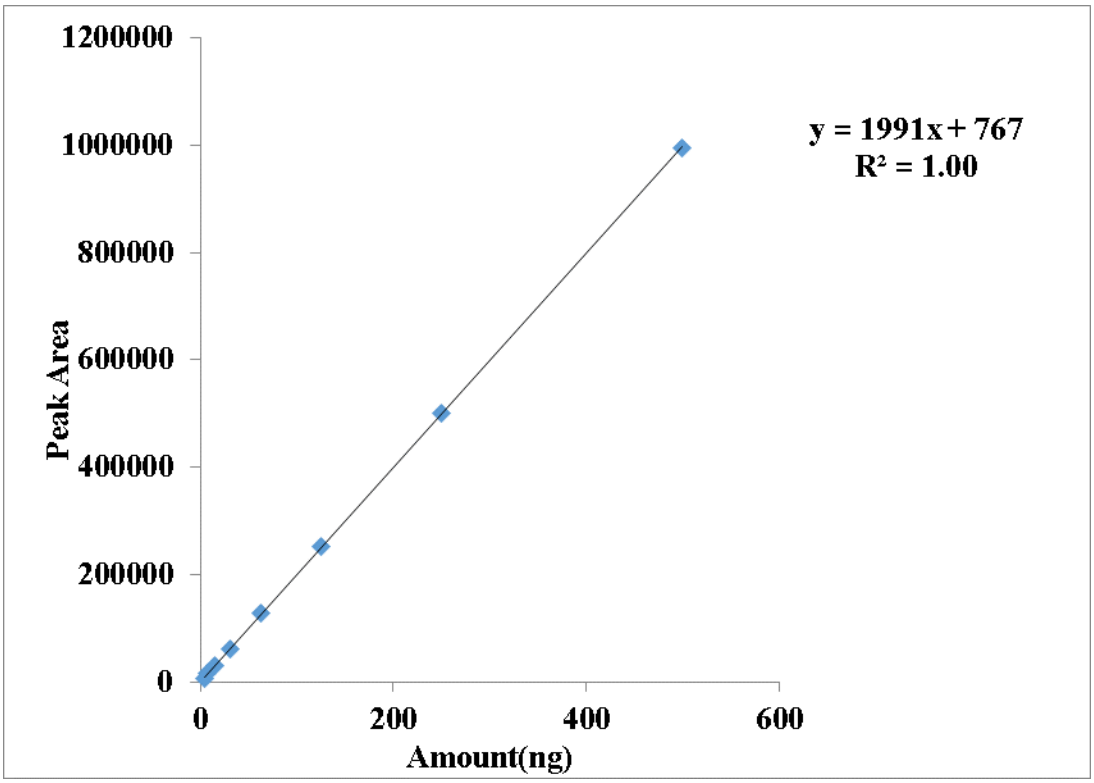


Figure 3.3: Calibration curve of dexamethasone

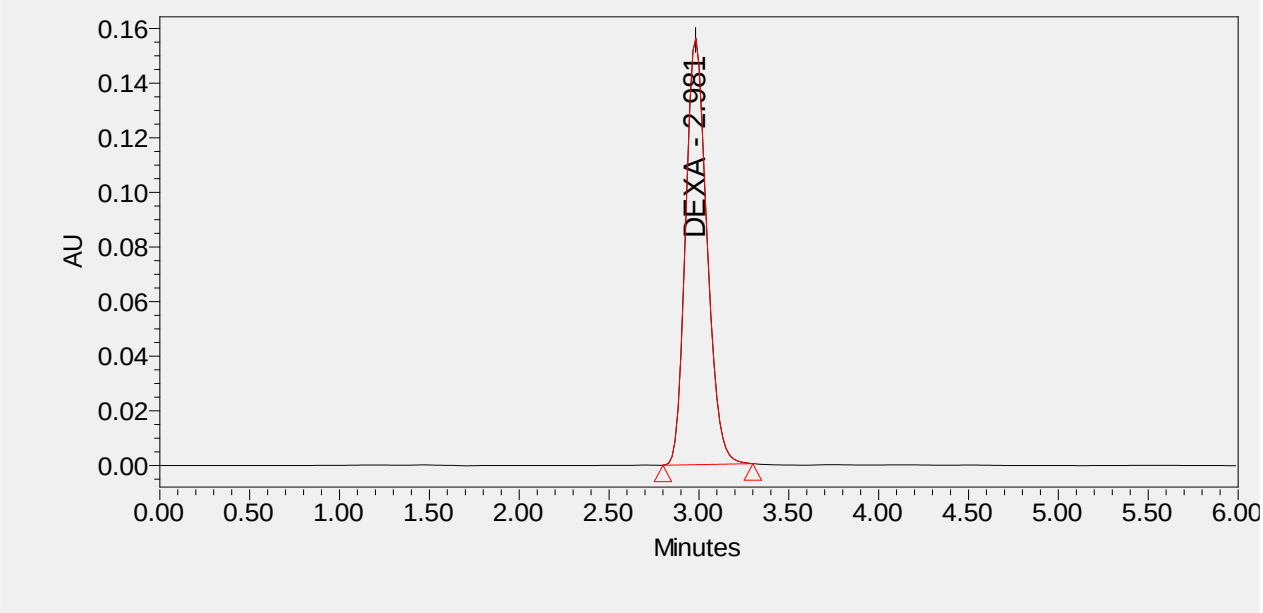


Figure 3.4: A sample HPLC chromatogram of dexamethasone giving the area-under-the-curve (AUC) versus retention time in minutes.

3.4.4. Morphology

Transmission electron microscopy (TEM) was used to study the morphology of dexamethasone nanomicelles dispersed in gellan gum (Fig. 3.5). In order to identify a clear contrast, dexamethasone nanomicelles were negatively stained with 2% phosphotungstic acid solution, for contrasting them from the gellan gum. TEM image of dexamethasone nanomicelles is shown as an inset in Fig. 3.5. From the figure it is clearly evident that the size of dexamethasone nanomicelles ranges between 20-40 nm. Dexamethasone nanomicelles dispersed in gellan gum are found to be intact with a similar size. As observed in the Fig. 3.5, gellan gum forms a matrix with double helices which are formed due to the coil rearrangement in the solution, and is widely present in a homogeneous fashion [223]

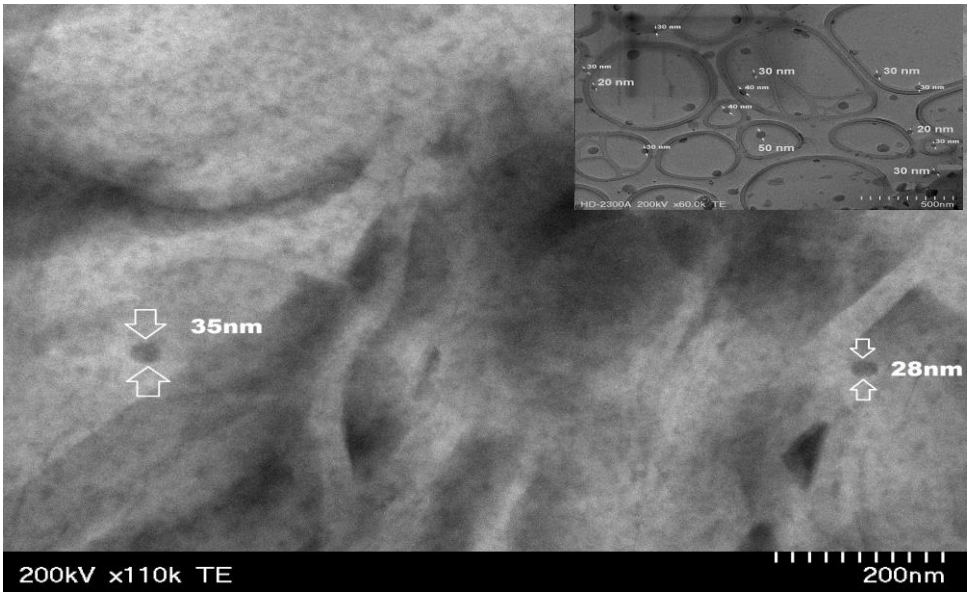
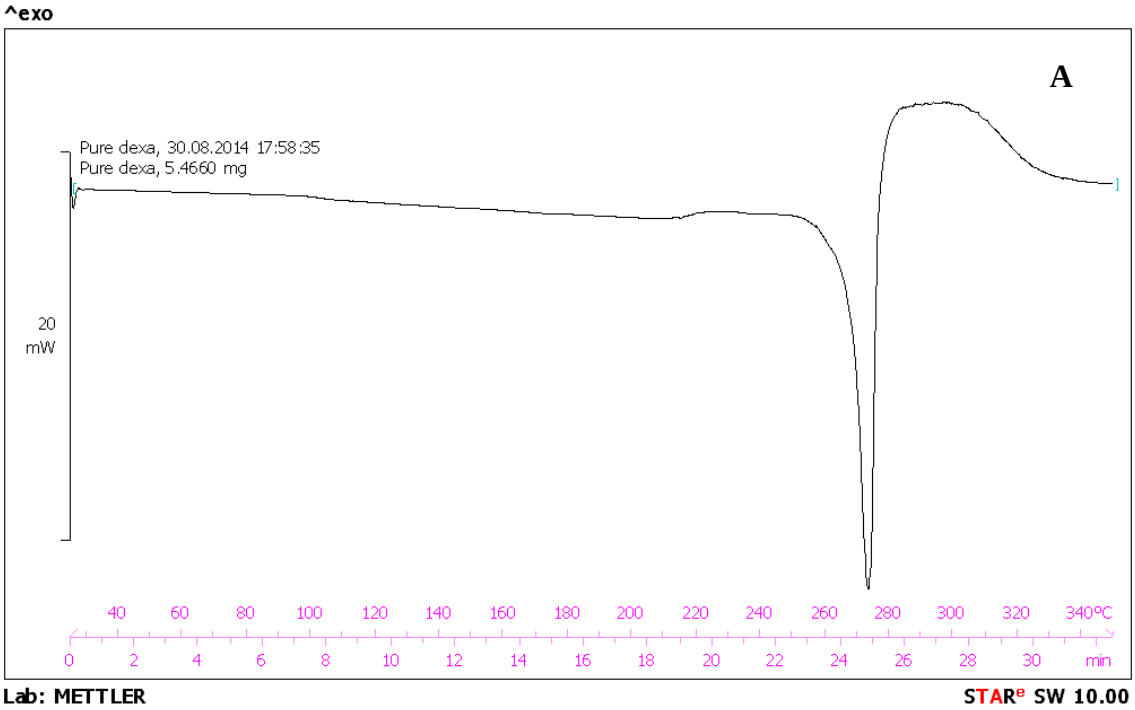
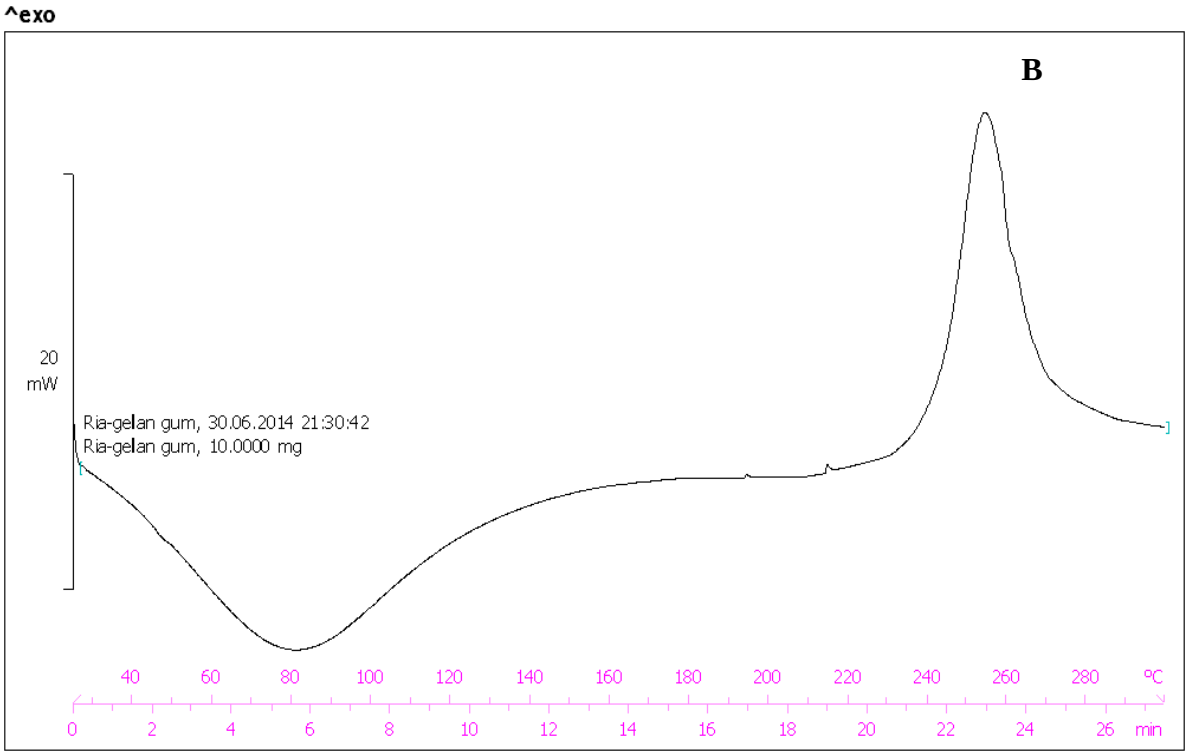


Figure 3.5: TEM image of dexamethasone nanomicelles dispersed in gellan gum, Inset: Basic dexamethasone nanomicelles in water without excipients

3.4.5. Differential Scanning Calorimetry (DSC)

DSC was carried out to confirm the absence of un-dissolved dexamethasone in DMN-ISG. DSC thermograms of pure dexamethasone, gellan gum and DMN-ISG are shown in Fig. 3.6 (a), 3.6 (b) and 3.6 (c). Dexamethasone exhibited a sharp endothermic peak with onset at 267°C and a peak temperature at 270°C,which corresponds to the melting point of dexamethasone [224]. Gellan gum showed an endothermic peak with a onset at 38°C and a peak temperature at 81°C, which corroborates to the melting point of low acyl gellan gum [225]. The DMN-ISG exhibited an endothermic peak with onset 94°C and a peak temperature at 107°C, which mostly corresponds to water. The characteristic sharp endothermic peak of dexamethasone was completely absent in DMN-ISG. This indicated the absence of any un-dissolved dexamethasone in nanomicelles.




^exo
B
20
mW
Ria-gelan gum, 30.06.2014 21:30:42
Ria-gelan gum, 10.0000 mg
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
Lab: METTLER
STARe SW 10.00

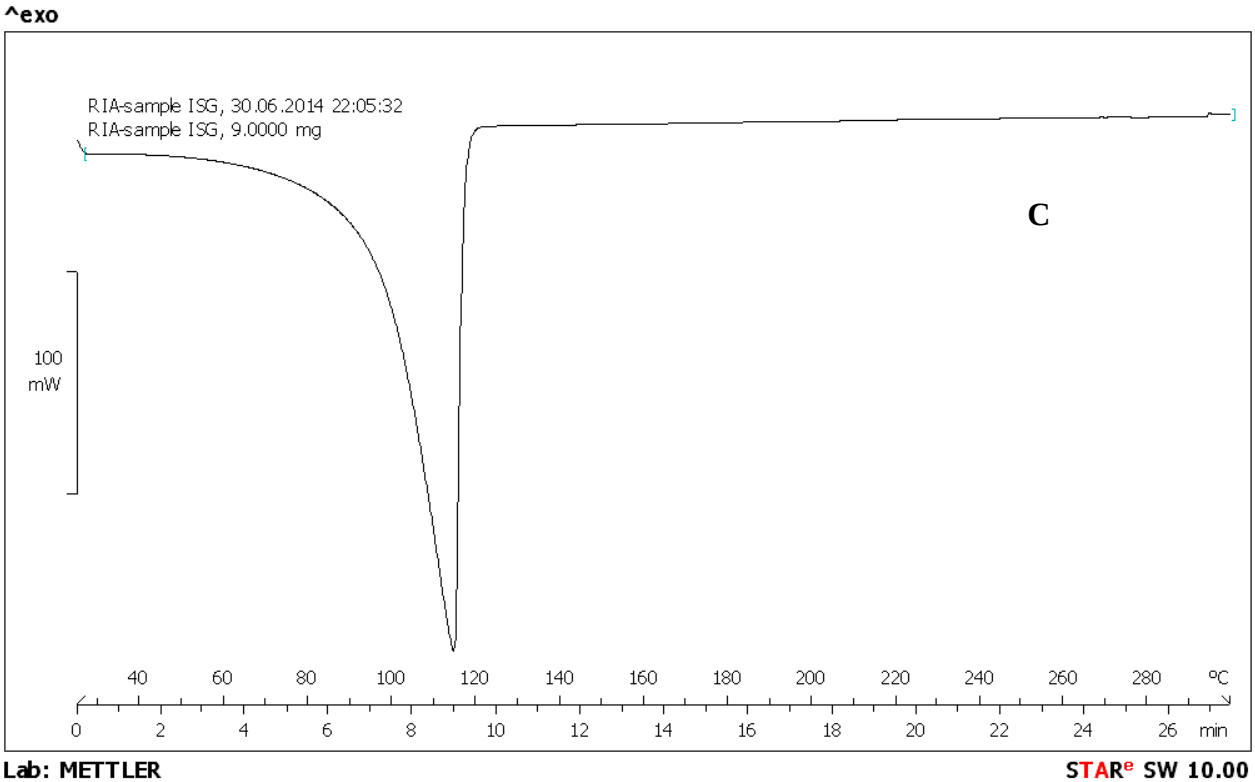

^exo
RIA-sample ISG, 30.06.2014 22:05:32
RIA-sample ISG, 9.0000 mg
C
100
mW
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
Lab: METTLER
STARe SW 10.00

Figure 3.6: DSC thermograms of dexamethasone (A), gellan gum (B), dexamethasone nanomicelles dispersed in gellan gum (C)

3.4.6. Light transmittance

Light transmittance is crucial for any ophthalmic formulation. Light transmittance was studied to establish the ability of light transparency through the ophthalmic formulation. The vision of patients should not be obscured by the ophthalmic formulation. The light transmittance was determined in the visible range (400-700 nm) of the electromagnetic spectrum. As shown in Fig. 3.7, light transmittance was around 90-100% for DMN which indicate that the required light transmittance into the eye occured without blurring the vision. However, DMN-ISG showed reduced light transmittance of 56-78% which is mainly attributed to the presence of gellan gum in the formulation. Literature showed the evidence of gellan gum causing blurred vision due to its physical characteristics [226, 227] but it was recorded only in a limited number of individuals - around 29% of the patients administered [226]. The hindrance to clear vision of gellan gum did not last more than 30 seconds to 5 minutes [228]. Moreover, ophthalmic gels have higher incidence of blurred vision as seen by the use of carbomer 974P in Zirgan (0.15% ganciclovir ophthalmic gels) causing blurring of vision in 58-60% of the patients [229]. We assume that the blurring of vision caused by DMN-ISG will be temporary and will eventually clear upon dilution with STF in the eye. Gellan gum will result in an optimum viscosity that allows the formulation to spread uniformly on the cornea and prevents the formulation from draining out of the eye.

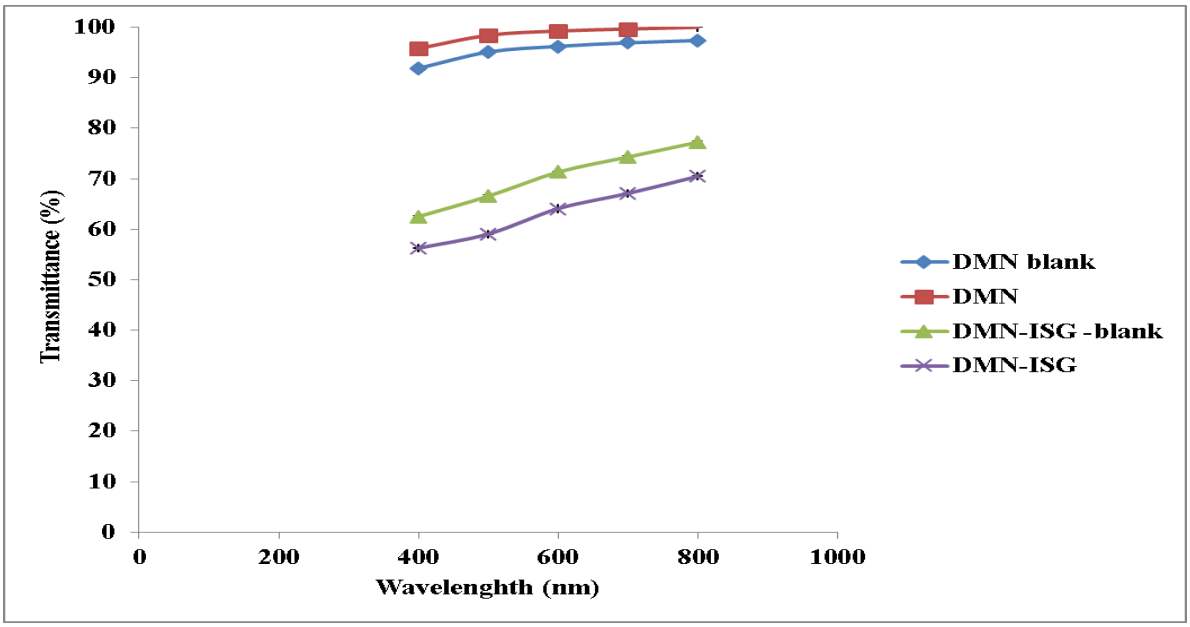


Figure 3.7: Light transmittance of dexamethasone nanomicelles (DMN) and dexamethasone nanomicelles dispersed in gellan gum (DMN-ISG) with and without the drug

3..9.11. In vitro drug release study

The *in vitro* release of dexamethasone from DMN-ISG was investigated and compared with DMN at 34°C. The release profiles of dexamethasone from DMN and DMN-ISG are seen in Fig. 3.8. More than 50% of the drug was released from DMN and DMN-ISG in the first few hours. However, DMN-ISG was able to sustain the release of the remaining dexamethasone for up to 30 h. The rapid release of dexamethasone from DMN is due to the short relaxation time (typically in the range of microseconds to seconds) of nanomicelles and rapid partitioning of dexamethasone in and out of micelles [213]. The sustained release of dexamethasone from DMN-ISG, especially

the last 30% of the drug, was attributed to the presence of gellan gum from which the drug has to diffuse in order to reach the external medium.

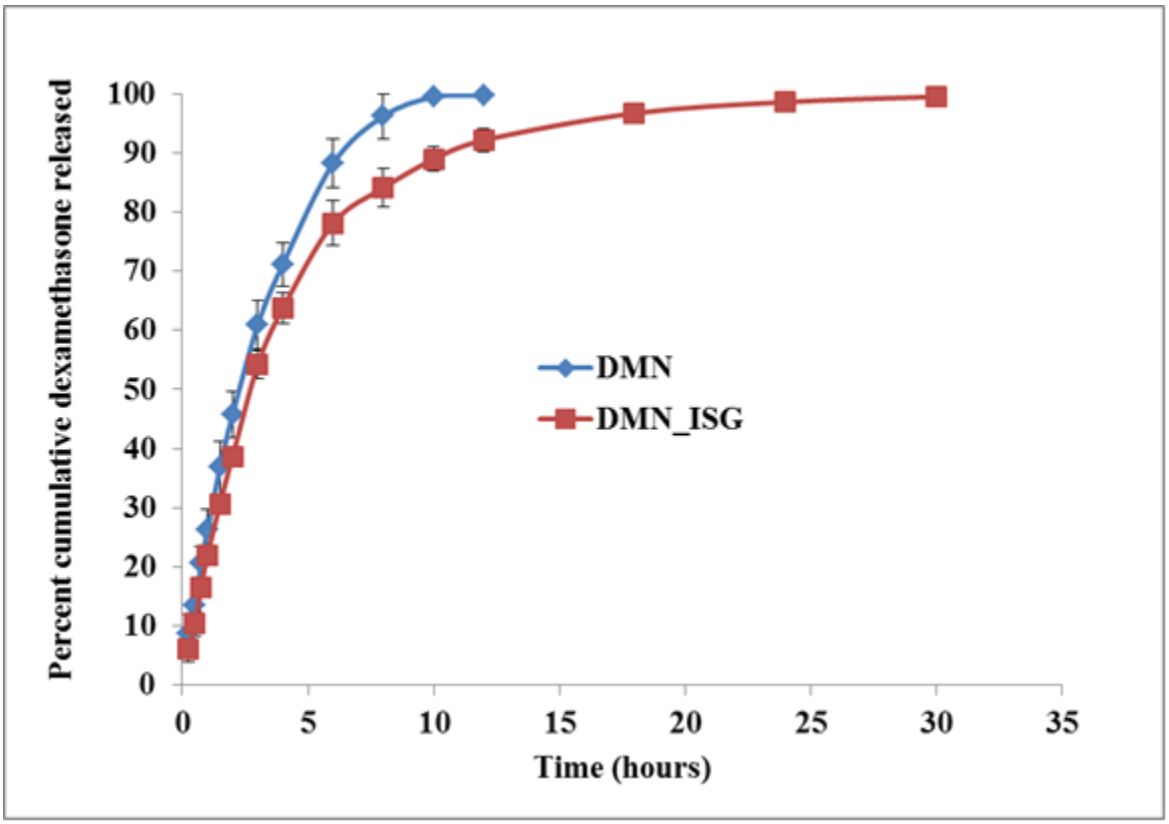


Figure 3.8: *In vitro* release of dexamethasone from dexamethasone nanomicelles (DMN) and dexamethasone nanomicelles dispersed in gellan gum (DMN-ISG). Values are expressed as mean (n=3)

3.4.8. Sterility Testing

According to the USP guidelines, ophthalmic preparations should be sterile at least during the first use of the product. Sterility of DMN-ISG was ensured during the preparation process.

Gellan gum dispersion was sterilized by autoclaving at 121°C and 15 Pa for 20 minutes and the solution of dexamethasone nanomicelles, mannitol, benzododecinium bromide and tromethamine was passed through a sterile 0.2 µm syringe filter for sterilization. Formulations are tested for sterility using direct inoculation and plate inoculation techniques in a TSB broth as per USP guidelines. Samples of 100 µl were withdrawn by the direct inoculation method from each of the vials on days 0, 7 and 14 and then transferred to MH plates for the plate inoculation method. After allowing the plates to incubate for 24 h at 35°C, to mimic the body temperature and promote any microbial growth if it is to occur, they were examined for the presence/absence of microbial growth. As seen in Table 3.2, the negative control and aseptically prepared DMN-ISG did not show any microbial growth during the 14 days of the study period. Positive control and positive sample control showed turbidity and microbial growth due to the presence of bacteria. This study indicates that the aseptically prepared DMN-ISG formulation remains sterile for at least 14 days (Fig. 3.9).

No. of Days	**Aseptically Filtered Formulation**	**Negative Control**	**Positive Control**	**Positive Sample Control**
Day 0	-	-	+	+
Day 7	-	-	+	+
Day 14	-	-	+	+

Tale 3.2: Validation of sterility on 0,7 and 14 days, where (+) is presence and (-) is absence of microbial growth

Figure 3.9: Images of plates after 14days showing presence/absence of microbial growth

3.5. Conclusion

In conclusion, DMN-ISG was successfully prepared by mixing a basic 2X formulation of DMN with appropriate concentrations of gellan gum, mannitol, benzododecinium bromide and tromethamine. The dispersion of DMN-ISG appeared clear at room temperature on visual examination and transformed into a gel when diluted with simulated tear fluid (STF). Dexamethasone nanomicelles appeared intact when dispersed in gellan gum. The preliminary results obtained in our investigation suggest that DMN-ISG could be utilized as a potential delivery system for delivering dexamethasone to treat anterior segment eye inflammations. The *in vitro* characteristics of DMN-ISG suggest further *in vivo* evaluation in a rabbit model.

Future studies

The final aspectically prepared formulation DMN-ISG should be further evaluated by stability studies conducted at room temperature and 4^{o}C for changes in pH and drug content. The cell cytotoxicity study should be conducted by studies such as lactate dehydrogenase (LDH) assay. Ocular irritation study should be carried out in rabbits. Potential of DMN-ISG to treat both anterior and posterior segment eye inflammations following multiple administration should be evaluated.

References

1. Ashaben Patel, K.C., Vibhuti Agrahari and Ashim K Mitra., *Ocular drug delivery systems: An overview.* World journal of Pharmacology, June 2013: p. 47-64.
2. Boddu, S.H., et al., *A Brief Overview of Ocular Anatomy and Physiology.* Treatise on Ocular Drug Delivery, 2013. **1**: p. 3.
3. Cavallotti, C. and L. Cerulli, *Age-related changes of the human eye*. 2008: Springer.
4. Kishore Cholkar, S.P.P., Aswani Dutt Vadlapudi, and Ashim K. Mitra, *Novel Strategies for Anterior Segment Ocular Drug Delivery.* Journal of ocular Pharmacology, 2013: p. 106-123.
5. Bourlais CL, A.L., Zia H, et al., *Ophthalmic drug delivery systems-recent advances. Prog Retin Eye Res* Prog Retin Eye Re, 1998: p. 17: 33-58.
6. Saettone MF, G.B., Monti D., *Ophthalmic emulsions and suspensions.* Cutaneous Ocul Toxicol 2001: p. 20: 183-201. .
7. Boddu SH, G.S., Earla R, Mitra AK, *Ocular microdialysis: A continuous sampling technique to study pharmacokinetics and pharmacodynamics in the eye.* Bioanalysis 2010: p. 2: 487-507. .
8. Urtti A, S.L., *Minimizing systemic absorption of topically administered ophthalmic drugs.* . Surv Ophthalmol 1993: p. 37: 435- 56. .
9. Klyce, S.D. and C.E. Crosson, *Transport processes across the rabbit corneal epithelium: a review.* Current eye research, 1985. **4**(4): p. 323-331.
10. Gumbiner, B., *Structure, biochemistry, and assembly of epithelial tight junctions.* American Journal of Physiology-Cell Physiology, 1987. **253**(6): p. C749-C758.
11. Stevenson, B.R., et al., *Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia.* The Journal of cell biology, 1986. **103**(3): p. 755-766.
12. Citi, S., et al., *Cingulin, a new peripheral component of tight junctions.* 1988.
13. Gumbiner, B., T. Lowenkopf, and D. Apatira, *Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1.* Proceedings of the National Academy of Sciences, 1991. **88**(8): p. 3460-3464.
14. Furuse, M., et al., *Occludin: a novel integral membrane protein localizing at tight junctions.* The Journal of cell biology, 1993. **123**(6): p. 1777-1788.
15. Bhat, M., et al., *Regulation of tight junction permeability by calcium mediators and cell cytoskeleton in rabbit tracheal epithelium.* Pharmaceutical research, 1993. **10**(7): p. 991-997.
16. Rojanasakul, Y. and J.R. Robinson, *The cytoskeleton of the cornea and its role in tight junction permeability.* International journal of pharmaceutics, 1991. **68**(1): p. 135-149.
17. Grass, G., R.W. Wood, and J.R. Robinson, *Effects of calcium chelating agents on corneal permeability.* Investigative ophthalmology & visual science, 1985. **26**(1): p. 110-113.
18. Rojanasakul, Y. and J.R. Robinson, *Transport mechanisms of the cornea: characterization of barrier permselectivity.* International journal of pharmaceutics, 1989. **55**(2): p. 237-246.
19. Martinez-Palomo, A. and D. Erlij, *Structure of tight junctions in epithelia with different permeability.* Proceedings of the National Academy of Sciences, 1975. **72**(11): p. 4487-4491.
20. Urtti, A. and L. Salminen, *Minimizing systemic absorption of topically administered ophthalmic drugs.* Survey of ophthalmology, 1993. **37**(6): p. 435-456.

21. Saettone, M., et al., *The validity of rabbits for investigations on ophthalmic vehicles: a comparison of four different vehicles containing tropicamide in humans and rabbits.* Pharmaceutica acta Helvetiae, 1982. **57**(2): p. 47.
22. Maurice, D. and S. Mishima, *Ocular pharmacokinetics*, in *Pharmacology of the Eye*. 1984, Springer. p. 19-116.
23. Ahmed, I. and T.F. Patton, *Disposition of timolol and inulin in the rabbit eye following corneal versus non-corneal absorption.* International journal of pharmaceutics, 1987. **38**(1): p. 9-21.
24. Das, N.D. and H. Shichi, *Enzymes of mercapturate synthesis and other drug-metabolizing reactions-specific localization in the eye.* Experimental eye research, 1981. **33**(5): p. 525-533.
25. Hayakawa, E., et al., *Conjunctival penetration of insulin and peptide drugs in the albino rabbit.* Pharmaceutical research, 1992. **9**(6): p. 769-775.
26. Schoenwald, R.D., *Ocular drug delivery.* Clinical pharmacokinetics, 1990. **18**(4): p. 255-269.
27. Lederer, C.M. and R.E. Harold, *Drop size of commercial glaucoma medications.* Am J Ophthalmol, 1986. **101**(6): p. 691-4.
28. Mishima, S., et al., *Determination of tear volume and tear flow.* Investigative Ophthalmology & Visual Science, 1966. **5**(3): p. 264-276.
29. Lee, V.H.L. and J.R. Robinson, *Mechanistic and quantitative evaluation of precorneal pilocarpine disposition in albino rabbits.* Journal of pharmaceutical sciences, 1979. **68**(6): p. 673-684.
30. Järvinen, K., T. Järvinen, and A. Urtti, *Ocular absorption following topical delivery.* Advanced drug delivery reviews, 1995. **16**(1): p. 3-19.
31. Sugrue, M.F., *The pharmacology of antiglaucoma drugs.* Pharmacology & therapeutics, 1989. **43**(1): p. 91-138.
32. Chrai, S.S., et al., *Lacrimal and instilled fluid dynamics in rabbit eyes.* Journal of pharmaceutical sciences, 1973. **62**(7): p. 1112-1121.
33. Sasaki, H., et al., *Delivery of drugs to the eye by topical application.* Progress in Retinal and Eye Research, 1996. **15**(2): p. 583-620.
34. Lang, J.C., *Ocular drug delivery conventional ocular formulations.* Advanced drug delivery reviews, 1995. **16**(1): p. 39-43.
35. Hurwitz, J., M. Maisey, and R. Welham, *Quantitative lacrimal scintillography. I. Method and physiological application.* British Journal of Ophthalmology, 1975. **59**(6): p. 308-312.
36. Meseguer, G., R. Gurny, and P. Buri, *In vivo evaluation of dosage forms: application of gamma scintigraphy to non-enteral routes of administration.* Journal of drug targeting, 1994. **2**(4): p. 269-288.
37. Mannermaa, E., K.-S. Vellonen, and A. Urtti, *Drug transport in corneal epithelium and blood–retina barrier: emerging role of transporters in ocular pharmacokinetics.* Advanced drug delivery reviews, 2006. **58**(11): p. 1136-1163.
38. Eytan, G.D. and P.W. Kuchel, *Mechanism of action of P-glycoprotein in relation to passive membrane permeation.* International review of cytology, 1999. **190**: p. 175-250.
39. Sarkadi, B., et al., *Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system.* Physiological reviews, 2006. **86**(4): p. 1179-1236.
40. Sharom, F.J., *ABC multidrug transporters: structure, function and role in chemoresistance.* 2008.
41. Bellamy, W.T., *P-glycoproteins and multidrug resistance.* Annual review of pharmacology and toxicology, 1996. **36**(1): p. 161-183.
42. Saha, P., J.J. Yang, and V. Lee, *Existence of a p-glycoprotein drug efflux pump in cultured rabbit conjunctival epithelial cells.* Investigative ophthalmology & visual science, 1998. **39**(7): p. 1221-1226.
43. Wu, J., et al., *P-glycoprotein regulates a volume-activated chloride current in bovine non-pigmented ciliary epithelial cells.* The Journal of Physiology, 1996. **491**(Pt 3): p. 743-755.

44. Dey, S., et al., *Molecular evidence and functional expression of P-glycoprotein (MDR1) in human and rabbit cornea and corneal epithelial cell lines.* Investigative ophthalmology & visual science, 2003. **44**(7): p. 2909-2918.
45. Holash, J.A. and P.A. Stewart, *The relationship of astrocyte-like cells to the vessels that contribute to the blood-ocular barriers.* Brain research, 1993. **629**(2): p. 218-224.
46. Constable, P.A., et al., *P-Glycoprotein expression in human retinal pigment epithelium cell lines.* Experimental eye research, 2006. **83**(1): p. 24-30.
47. Yang, J.J., et al., *Multidrug resistance protein 1 (MRP1) in rabbit conjunctival epithelial cells: its effect on drug efflux and its regulation by adenoviral infection.* Pharmaceutical research, 2007. **24**(8): p. 1490-1500.
48. Roelofsen, H., et al., *Glutathione S-conjugate transport in hepatocytes entering the cell cycle is preserved by a switch in expression from the apical MRP2 to the basolateral MRP1 transporting protein.* Journal of cell science, 1999. **112**(9): p. 1395-1404.
49. Aukunuru, J.V., et al., *Expression of multidrug resistance-associated protein (MRP) in human retinal pigment epithelial cells and its interaction with BAPSG, a novel aldose reductase inhibitor.* Pharmaceutical research, 2001. **18**(5): p. 565-572.
50. Vellonen, K., et al., *Gene expression and activity of efflux proteins in human corneal epithelial cells.* Invest Ophthalmol Vis Sci, 2006. **47**: p. 1597.
51. Zhang, T., et al., *Drug transporter and cytochrome P450 mRNA expression in human ocular barriers: implications for ocular drug disposition.* Drug Metabolism and Disposition, 2008. **36**(7): p. 1300-1307.
52. Tombran-Tink, J. and C.J. Barnstable, *Ocular transporters in ophthalmic diseases and drug delivery*. 2008: Springer.
53. Gaudana, R., et al., *Development and characterization of nanoparticulate formulation of a water soluble prodrug of dexamethasone by HIP complexation.* Journal of microencapsulation, 2011. **28**(1): p. 10-20.
54. Urtti, A., *Challenges and obstacles of ocular pharmacokinetics and drug delivery.* Advanced drug delivery reviews, 2006. **58**(11): p. 1131-1135.
55. Jwala, J., et al., *Ocular sustained release nanoparticles containing stereoisomeric dipeptide prodrugs of acyclovir.* Journal of Ocular Pharmacology and Therapeutics, 2011. **27**(2): p. 163-172.
56. Benson, H., *Permeability of the cornea to topically applied drugs.* Archives of ophthalmology, 1974. **91**(4): p. 313-327.
57. Chen, W., et al., *Corneal alternations induced by topical application of benzalkonium chloride in rabbit.* PloS one, 2011. **6**(10): p. e26103.
58. Molokhia, S.A., et al., *Anterior eye segment drug delivery systems: current treatments and future challenges.* Journal of Ocular Pharmacology and Therapeutics, 2013. **29**(2): p. 92-105.
59. Hämäläinen, K., et al., *Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera.* Investigative ophthalmology & visual science, 1997. **38**(3): p. 627-634.
60. Karla, P.K., et al., *Molecular expression and functional evidence of a drug efflux pump (BCRP) in human corneal epithelial cells.* Current eye research, 2009. **34**(1): p. 1-9.
61. Karla, P.K., et al., *Molecular evidence and functional expression of a novel drug efflux pump (ABCC2) in human corneal epithelium and rabbit cornea and its role in ocular drug efflux.* International journal of pharmaceutics, 2007. **336**(1): p. 12-21.
62. Karla, P.K., D. Pal, and A.K. Mitra, *Molecular evidence and functional expression of multidrug resistance associated protein (MRP) in rabbit corneal epithelial cells.* Experimental eye research, 2007. **84**(1): p. 53-60.

63. Cholkar, K., et al., *Novel strategies for anterior segment ocular drug delivery.* Journal of Ocular Pharmacology and Therapeutics, 2013. **29**(2): p. 106-123.
64. Patel, S., et al., *Development and Evaluation of Dexamethasone Nanomicelles with Potential for Treating Posterior Uveitis After Topical Application.* Journal of Ocular Pharmacology and Therapeutics, 2015.
65. Dartt, D.A., *Regulation of mucin and fluid secretion by conjunctival epithelial cells.* Progress in retinal and eye research, 2002. **21**(6): p. 555-576.
66. Lee, V.H.L. and J.R. Robinson, *Preliminary examination of rabbit conjunctival mucins.* Journal of pharmaceutical sciences, 1980. **69**(4): p. 430-438.
67. Iwamoto, T. and F.A. Jakobiec, *A comparative ultrastructural study of the normal lacrimal gland and its epithelial tumors.* Human pathology, 1982. **13**(3): p. 236-262.
68. Huang, A., S. Tseng, and K. Kenyon, *Paracellular permeability of corneal and conjunctival epithelia.* Investigative ophthalmology & visual science, 1989. **30**(4): p. 684-689.
69. Raviola, G., *Conjunctival and episcleral blood vessels are permeable to blood-borne horseradish peroxidase.* Investigative ophthalmology & visual science, 1983. **24**(6): p. 725-736.
70. Kuno, N. and S. Fujii, *Recent advances in ocular drug delivery systems.* Polymers, 2011. **3**(1): p. 193-221.
71. Horibe, Y., et al., *Polar solute transport across the pigmented rabbit conjunctiva: size dependence and the influence of 8-bromo cyclic adenosine monophosphate.* Pharmaceutical research, 1997. **14**(9): p. 1246-1251.
72. Lee, S.J., et al., *Evaluation of clearance mechanisms with transscleral drug delivery.* Investigative ophthalmology & visual science, 2010. **51**(10): p. 5205-5212.
73. Ahmed, I. and T. Patton, *Importance of the noncorneal absorption route in topical ophthalmic drug delivery.* Investigative ophthalmology & visual science, 1985. **26**(4): p. 584-587.
74. Fishburn, C.S., *The pharmacology of PEGylation: balancing PD with PK to generate novel therapeutics.* Journal of pharmaceutical sciences, 2008. **97**(10): p. 4167-4183.
75. Kaur, I.P. and M. Kanwar, *Ocular preparations: the formulation approach.* Drug Dev Ind Pharm, 2002. **28**(5): p. 473-93.
76. Boddu, S.H., *Polymeric Nanoparticles for Ophthalmic Drug Delivery: An Update on Research and Patenting Activity.* Recent Patents on Nanomedicine, 2012. **2**(2): p. 96-112.
77. Smolin, G., et al., *Idoxuridine-liposome therapy for herpes simplex keratitis.* Am J Ophthalmol, 1981. **91**(2): p. 220-5.
78. Gupta, H., et al., *Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery.* Nanomedicine, 2010. **6**(2): p. 324-33.
79. Lee, V.H. and J.R. Robinson, *Topical ocular drug delivery: recent developments and future challenges.* J Ocul Pharmacol, 1986. **2**(1): p. 67-108.
80. Bourlais, C.L., et al., *Ophthalmic drug delivery systems--recent advances.* Prog Retin Eye Res, 1998. **17**(1): p. 33-58.
81. Saettone, M.F., B. Giannaccini, and D. Monti, *Ophthalmic emulsions and suspensions.* Cutaneous and Ocular Toxicology, 2001. **20**: p. 183 – 201.
82. Gaudana, R., et al., *Recent perspectives in ocular drug delivery.* Pharm Res, 2009. **26**(5): p. 1197-216.
83. Vandervoort, J. and A. Ludwig, *Ocular drug delivery: nanomedicine applications.* Nanomedicine (Lond), 2007. **2**(1): p. 11-21.
84. Sahoo, S.K., F. Dilnawaz, and S. Krishnakumar, *Nanotechnology in ocular drug delivery.* Drug discovery today, 2008. **13**(3): p. 144-151.
85. Lawrence, M.J. and G.D. Rees, *Microemulsion-based media as novel drug delivery systems.* Advanced drug delivery reviews, 2000. **45**(1): p. 89-121.

86. Vandamme, T.F., *Microemulsions as ocular drug delivery systems: recent developments and future challenges.* Progress in retinal and eye research, 2002. **21**(1): p. 15-34.
87. Fialho, S.L. and D. Silva-Cunha, *New vehicle based on a microemulsion for topical ocular administration of dexamethasone.* Clinical & experimental ophthalmology, 2004. **32**(6): p. 626-632.
88. Haβe, A. and S. Keipert, *Development and characterization of microemulsions for ocular application.* European Journal of Pharmaceutics and Biopharmaceutics, 1997. **43**(2): p. 179-183.
89. Derle, D.V., S.H.S. Boddu, and R. Pimpale, *Microemulsion as a vehicle for transdermal permeation of nimesulide.* Indian Journal of Pharmaceutical Sciences, 2006. **68**(5): p. 622-625.
90. Muchtar, S., et al., *Ex-vivo permeation study of indomethacin from a submicron emulsion through albino rabbit cornea.* Journal of Controlled Release, 1997. **44**(1): p. 55-64.
91. Muchtar, S., et al., *A submicron emulsion as ocular vehicle for delta-8-tetrahydrocannabinol: effect on intraocular pressure in rabbits.* Ophthalmic research, 1992. **24**(3): p. 142-149.
92. Naveh, N., S. Muchtar, and S. Benita, *Pilocarpine incorporated into a submicron emulsion vehicle causes an unexpectedly prolonged ocular hypotensive effect in rabbits.* Journal of Ocular Pharmacology and Therapeutics, 1994. **10**(3): p. 509-520.
93. Gasco, M., et al., *Microemulsions as topical delivery vehicles: ocular administration of timolol.* Journal of pharmaceutical and biomedical analysis, 1989. **7**(4): p. 433-439.
94. Fialho, S.L. and A. da Silva-Cunha, *New vehicle based on a microemulsion for topical ocular administration of dexamethasone.* Clin Experiment Ophthalmol, 2004. **32**(6): p. 626-32.
95. Chan, J., et al., *Phase transition water-in-oil microemulsions as ocular drug delivery systems: in vitro and in vivo evaluation.* International journal of pharmaceutics, 2007. **328**(1): p. 65-71.
96. Gaudana, R., et al., *Recent perspectives in ocular drug delivery.* Pharmaceutical research, 2009. **26**(5): p. 1197-1216.
97. Kassem, M., et al., *Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs.* International journal of pharmaceutics, 2007. **340**(1): p. 126-133.
98. Pignatello, R., et al., *Flurbiprofen-loaded acrylate polymer nanosuspensions for ophthalmic application.* Biomaterials, 2002. **23**(15): p. 3247-3255.
99. Kawashima, Y., et al., *Preparation of controlled-release microspheres of ibuprofen with acrylic polymers by a novel quasi-emulsion solvent diffusion method.* Journal of pharmaceutical sciences, 1989. **78**(1): p. 68-72.
100. Marchal-Heussler, L., et al., *[Value of the new drug carriers in ophthalmology: liposomes and nanoparticles].* Journal francais d'ophtalmologie, 1989. **13**(11-12): p. 575-582.
101. Adibkia, K., et al., *Inhibition of endotoxin-induced uveitis by methylprednisolone acetate nanosuspension in rabbits.* Journal of Ocular Pharmacology and Therapeutics, 2007. **23**(5): p. 421-432.
102. Adibkia, K., et al., *Piroxicam nanoparticles for ocular delivery: physicochemical characterization and implementation in endotoxin-induced uveitis.* Journal of drug targeting, 2007. **15**(6): p. 407-416.
103. Ebrahim, S., G.A. Peyman, and P.J. Lee, *Applications of liposomes in ophthalmology.* Survey of ophthalmology, 2005. **50**(2): p. 167-182.
104. VADlApuDi, A.D. and K. CholKAr, *Ocular Drug Delivery.*
105. Kaur, I.P., et al., *Vesicular systems in ocular drug delivery: an overview.* International journal of pharmaceutics, 2004. **269**(1): p. 1-14.
106. Milani, J.K., et al., *Prolongation of corneal allograft survival with liposome-encapsulated cyclosporine in the rat eye.* Ophthalmology, 1993. **100**(6): p. 890-896.
107. Pleyer, U., et al., *Ocular absorption of cyclosporine A from liposomes incorporated into collagen shields.* Current eye research, 1994. **13**(3): p. 177-181.

108. Shen, Y. and J. Tu, *Preparation and ocular pharmacokinetics of ganciclovir liposomes.* The AAPS journal, 2007. **9**(3): p. E371-E377.
109. Sun, Y., et al., *Inhibition of corneal inflammation by liposomal delivery of short-chain, C-6 ceramide.* Journal of leukocyte biology, 2008. **83**(6): p. 1512-1521.
110. Arakawa, Y., et al., *Eye-concentrated distribution of dexamethasone carried by sugar-chain modified liposome in experimental autoimmune uveoretinitis mice.* Biomedical research (Tokyo, Japan), 2007. **28**(6): p. 331-334.
111. Natarajan, J.V., et al., *Sustained release of an anti-glaucoma drug: demonstration of efficacy of a liposomal formulation in the rabbit eye.* PLoS One, 2011. **6**(9): p. e24513.
112. Quintana, A., et al., *Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor.* Pharmaceutical research, 2002. **19**(9): p. 1310-1316.
113. Ihre, H.R., et al., *Polyester dendritic systems for drug delivery applications: design, synthesis, and characterization.* Bioconjugate chemistry, 2002. **13**(3): p. 443-452.
114. Patton, T.F. and J.R. Robinson, *Ocular evaluation of polyvinyl alcohol vehicle in rabbits.* Journal of pharmaceutical sciences, 1975. **64**(8): p. 1312-1316.
115. Milhem, O., et al., *Polyamidoamine Starburst® dendrimers as solubility enhancers.* International journal of pharmaceutics, 2000. **197**(1): p. 239-241.
116. Bhadra, D., et al., *A PEGylated dendritic nanoparticulate carrier of fluorouracil.* International journal of pharmaceutics, 2003. **257**(1): p. 111-124.
117. Ooya, T., J. Lee, and K. Park, *Effects of ethylene glycol-based graft, star-shaped, and dendritic polymers on solubilization and controlled release of paclitaxel.* Journal of controlled release, 2003. **93**(2): p. 121-127.
118. Vandamme, T.F. and L. Brobeck, *Poly (amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide.* Journal of controlled release, 2005. **102**(1): p. 23-38.
119. Vyas, S., et al., *Discoidal niosome based controlled ocular delivery of timolol maleate.* Die Pharmazie, 1998. **53**(7): p. 466-469.
120. Aggarwal, D. and I.P. Kaur, *Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system.* International journal of pharmaceutics, 2005. **290**(1): p. 155-159.
121. Abdelkader, H., et al., *Design and evaluation of controlled-release niosomes and discomes for naltrexone hydrochloride ocular delivery.* Journal of pharmaceutical sciences, 2011. **100**(5): p. 1833-1846.
122. Gan, L., et al., *Self-assembled liquid crystalline nanoparticles as a novel ophthalmic delivery system for dexamethasone: improving preocular retention and ocular bioavailability.* International journal of pharmaceutics, 2010. **396**(1): p. 179-187.
123. Vaishya, R.D., et al., *Controlled ocular drug delivery with nanomicelles.* Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2014. **6**(5): p. 422-437.
124. Trivedi, R. and U.B. Kompella, *Nanomicellar formulations for sustained drug delivery: strategies and underlying principles.* Nanomedicine, 2010. **5**(3): p. 485-505.
125. Torchilin, V.P., *Structure and design of polymeric surfactant-based drug delivery systems.* Journal of controlled release, 2001. **73**(2): p. 137-172.
126. Rangel-Yagui, C.O., A. Pessoa Jr, and L.C. Tavares, *Micellar solubilization of drugs.* J. Pharm. Pharm. Sci, 2005. **8**(2): p. 147-163.
127. Sammalkorpi, M., M. Karttunen, and M. Haataja, *Ionic surfactant aggregates in saline solutions: sodium dodecyl sulfate (SDS) in the presence of excess sodium chloride (NaCl) or calcium chloride (CaCl2).* The Journal of Physical Chemistry B, 2009. **113**(17): p. 5863-5870.
128. Rosen, M.J. and J.T. Kunjappu, *Surfactants and interfacial phenomena*. 2012: John Wiley & Sons.

129. Chevalier, Y. and T. Zemb, *The structure of micelles and microemulsions.* Reports on Progress in Physics, 1990. **53**(3): p. 279.
130. Cholkar, K., et al., *Novel nanomicellar formulation approaches for anterior and posterior segment ocular drug delivery.* Recent patents on nanomedicine, 2012. **2**(2): p. 82.
131. Mitra, A.K., P.R. Velagaleti, and S. Natesan, *Ophthalmic compositions comprising calcineurin inhibitors or mTOR inhibitors*. 2013, Google Patents.
132. Vadlapudi, A.D., et al., *Aqueous nanomicellar formulation for topical delivery of biotinylated lipid prodrug of acyclovir: formulation development and ocular biocompatibility.* Journal of Ocular Pharmacology and Therapeutics, 2014. **30**(1): p. 49-58.
133. Kuwano, M., et al., *Cyclosporine A formulation affects its ocular distribution in rabbits.* Pharmaceutical research, 2002. **19**(1): p. 108-111.
134. Kabanov, A.V., E.V. Batrakova, and V.Y. Alakhov, *Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery.* Journal of controlled release, 2002. **82**(2): p. 189-212.
135. Batrakova, E.V. and A.V. Kabanov, *Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers.* Journal of Controlled Release, 2008. **130**(2): p. 98-106.
136. Müller, R.H., *Colloidal carriers for controlled drug delivery and targeting: Modification, characterization and in vivo distribution*. 1991: Taylor & Francis.
137. Kwon, G.S. and K. Kataoka, *Block copolymer micelles as long-circulating drug vehicles.* Advanced drug delivery reviews, 1995. **16**(2): p. 295-309.
138. Jones, M.-C. and J.-C. Leroux, *Polymeric micelles–a new generation of colloidal drug carriers.* European journal of pharmaceutics and biopharmaceutics, 1999. **48**(2): p. 101-111.
139. Gupta, A.K., et al., *Ketorolac entrapped in polymeric micelles: preparation, characterisation and ocular anti-inflammatory studies.* International journal of pharmaceutics, 2000. **209**(1): p. 1-14.
140. Civiale, C., et al., *Polyhydroxyethylaspartamide-based micelles for ocular drug delivery.* International journal of pharmaceutics, 2009. **378**(1): p. 177-186.
141. Di Tommaso, C., et al., *Ocular biocompatibility of novel Cyclosporin A formulations based on methoxy poly (ethylene glycol)-hexylsubstituted poly (lactide) micelle carriers.* International journal of pharmaceutics, 2011. **416**(2): p. 515-524.
142. Pepić, I., N. Jalšenjak, and I. Jalšenjak, *Micellar solutions of triblock copolymer surfactants with pilocarpine.* International journal of pharmaceutics, 2004. **272**(1): p. 57-64.
143. Pepić, I., et al., *A nonionic surfactant/chitosan micelle system in an innovative eye drop formulation.* Journal of pharmaceutical sciences, 2010. **99**(10): p. 4317-4325.
144. Lin, H.R. and P.C. Chang, *Novel pluronic-chitosan micelle as an ocular delivery system.* Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2013. **101**(5): p. 689-699.
145. Kataoka, K., A. Harada, and Y. Nagasaki, *Block copolymer micelles for drug delivery: design, characterization and biological significance.* Advanced drug delivery reviews, 2001. **47**(1): p. 113-131.
146. Harada, A. and K. Kataoka, *Novel polyion complex micelles entrapping enzyme molecules in the core: preparation of narrowly-distributed micelles from lysozyme and poly (ethylene glycol)-poly (aspartic acid) block copolymer in aqueous medium.* Macromolecules, 1998. **31**(2): p. 288-294.
147. Zhang, G.-D., et al., *Polyion complex micelles entrapping cationic dendrimer porphyrin: effective photosensitizer for photodynamic therapy of cancer.* Journal of controlled release, 2003. **93**(2): p. 141-150.
148. Castro, E., P. Taboada, and V. Mosquera, *Behavior of a styrene oxide-ethylene oxide diblock copolymer/surfactant system: a thermodynamic and spectroscopy study.* The Journal of Physical Chemistry B, 2005. **109**(12): p. 5592-5599.

149. Ideta, R., et al., *Effective accumulation of polyion complex micelle to experimental choroidal neovascularization in rats.* FEBS letters, 2004. **557**(1): p. 21-25.
150. Sugisaki, K., et al., *Photodynamic therapy for corneal neovascularization using polymeric micelles encapsulating dendrimer porphyrins.* Investigative ophthalmology & visual science, 2008. **49**(3): p. 894-899.
151. Liaw, J., S. Chang, and F. Hsiao, *In vivo gene delivery into ocular tissues by eye drops of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide)(PEO-PPO-PEO) polymeric micelles.* Gene therapy, 2001. **8**(13): p. 999-1004.
152. Tong, Y.C., et al., *Eye drop delivery of nano-polymeric micelle formulated genes with cornea-specific promoters.* The journal of gene medicine, 2007. **9**(11): p. 956-966.
153. Tong, Y.-C., et al., *Polymeric micelle gene delivery of bcl-x L via eye drop reduced corneal apoptosis following epithelial debridement.* Journal of Controlled Release, 2010. **147**(1): p. 76-83.
154. Bu, H.-Z., et al., *Ocular disposition, pharmacokinetics, efficacy and safety of nanoparticle-formulated ophthalmic drugs.* Current drug metabolism, 2007. **8**(2): p. 91-107.
155. Peer, D., et al., *Nanocarriers as an emerging platform for cancer therapy.* Nature nanotechnology, 2007. **2**(12): p. 751-760.
156. Losa, C., et al., *Design of new formulations for topical ocular administration: polymeric nanocapsules containing metipranolol.* Pharmaceutical research, 1993. **10**(1): p. 80-87.
157. Losa, C., et al., *Improvement of ocular penetration of amikacin sulphate by association to poly (butylcyanoacrylate) nanoparticles.* Journal of pharmacy and pharmacology, 1991. **43**(8): p. 548-552.
158. Kumar, E.b.A., *Nanomedicine in drug delivery.* 2013.
159. Pignatello, R., et al., *Eudragit RS100® nanosuspensions for the ophthalmic controlled delivery of ibuprofen.* European Journal of Pharmaceutical Sciences, 2002. **16**(1): p. 53-61.
160. Liaw, J., Y. Rojanasakul, and J.R. Robinson, *The effect of drug charge type and charge density on corneal transport.* International journal of pharmaceutics, 1992. **88**(1): p. 111-124.
161. Rojanasakul, Y., et al., *The transport barrier of epithelia: a comparative study on membrane permeability and charge selectivity in the rabbit.* Pharmaceutical research, 1992. **9**(8): p. 1029-1034.
162. De Campos, A.M., A. Sánchez, and M.a.J. Alonso, *Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A.* International Journal of Pharmaceutics, 2001. **224**(1): p. 159-168.
163. Ibrahim, H.K., I.S. El-Leithy, and A.A. Makky, *Mucoadhesive nanoparticles as carrier systems for prolonged ocular delivery of gatifloxacin/prednisolone bitherapy.* Molecular pharmaceutics, 2010. **7**(2): p. 576-585.
164. Mitra, A.K., and Mishra, *Pentablock polymers.* U.S.
2011, 2010(0250283 A1;).
165. Contreras-Ruiz, L., et al., *Intracellular trafficking of hyaluronic acid-chitosan oligomer-based nanoparticles in cultured human ocular surface cells.* Molecular vision, 2011. **17**: p. 279.
166. Kompella, U.B., et al., *Luteinizing hormone-releasing hormone agonist and transferrin functionalizations enhance nanoparticle delivery in a novel bovine ex vivo eye model.* Mol Vis, 2006. **12**(134-135): p. 1185-98.
167. Gulsen, D. and A. Chauhan, *Dispersion of microemulsion drops in HEMA hydrogel: a potential ophthalmic drug delivery vehicle.* International Journal of Pharmaceutics, 2005. **292**(1): p. 95-117.
168. Gulsen, D. and A. Chauhan, *Ophthalmic drug delivery through contact lenses.* Investigative ophthalmology & visual science, 2004. **45**(7): p. 2342-2347.

169. Kim, J., A. Conway, and A. Chauhan, *Extended delivery of ophthalmic drugs by silicone hydrogel contact lenses.* Biomaterials, 2008. **29**(14): p. 2259-2269.
170. Nakada, K. and A. Sugiyama, *Process for producing controlled drug-release contact lens, and controlled drug-release contact lens thereby produced*. 2000, Google Patents.
171. Sawant, K.K. and S.S. Dodiya, *Recent advances and patents on solid lipid nanoparticles.* Recent patents on drug delivery & formulation, 2008. **2**(2): p. 120-135.
172. Mehnert, W. and K. Mäder, *Solid lipid nanoparticles: production, characterization and applications.* Advanced drug delivery reviews, 2001. **47**(2): p. 165-196.
173. Kaur, I.P., C. Rana, and H. Singh, *Development of effective ocular preparations of antifungal agents.* Journal of Ocular Pharmacology and Therapeutics, 2008. **24**(5): p. 481-494.
174. Helgason, T., et al., *Effect of surfactant surface coverage on formation of solid lipid nanoparticles (SLN).* Journal of colloid and interface science, 2009. **334**(1): p. 75-81.
175. Müller, R., M. Radtke, and S. Wissing, *Nanostructured lipid matrices for improved microencapsulation of drugs.* International journal of pharmaceutics, 2002. **242**(1): p. 121-128.
176. Pal Kaur, I. and M. Kanwar, *Ocular preparations: the formulation approach.* Drug development and industrial pharmacy, 2002. **28**(5): p. 473-493.
177. Seyfoddin, A., J. Shaw, and R. Al-Kassas, *Solid lipid nanoparticles for ocular drug delivery.* Drug delivery, 2010. **17**(7): p. 467-489.
178. Cavalli, R., et al., *Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin.* International journal of pharmaceutics, 2002. **238**(1): p. 241-245.
179. Attama, A.A., S. Reichl, and C.C. Müller-Goymann, *Diclofenac sodium delivery to the eye: in vitro evaluation of novel solid lipid nanoparticle formulation using human cornea construct.* International journal of pharmaceutics, 2008. **355**(1): p. 307-313.
180. Gökçe, E.H., et al., *Cyclosporine A-loaded solid lipid nanoparticles: ocular tolerance and in vivo drug release in rabbit eyes.* Current eye research, 2009. **34**(11): p. 996-1003.
181. Shen, J., et al., *Incorporation of liquid lipid in lipid nanoparticles for ocular drug delivery enhancement.* Nanotechnology, 2010. **21**(2): p. 025101.
182. Haller, J.A., et al., *Dexamethasone intravitreal implant in patients with macular edema related to branch or central retinal vein occlusion: twelve-month study results.* Ophthalmology, 2011. **118**(12): p. 2453-2460.
183. Elg, M. and D. Gustafsson, *A combination of a thrombin inhibitor and dexamethasone prevents the development of experimental disseminated intravascular coagulation in rats.* Thrombosis research, 2006. **117**(4): p. 429-437.
184. Davies, N.M., *Biopharmaceutical considerations in topical ocular drug delivery.* Clinical and experimental pharmacology and physiology, 2000. **27**(7): p. 558-562.
185. Remington, J.P., D.B. Troy, and P. Beringer, *Remington: The science and practice of pharmacy*. Vol. 1. 2006: Lippincott Williams & Wilkins.
186. Aldricha, D.S., et al., *Ophthalmic preparations.* 2013.
187. Boddu, S.H., H. Gupta, and S.P. Bonam, *Preclinical evaluation of a ricinoleic acid poloxamer gel system for transdermal eyelid delivery.* International journal of pharmaceutics, 2014. **470**(1): p. 158-161.
188. Le Bourlais, C., et al., *Ophthalmic drug delivery systems—recent advances.* Progress in retinal and eye research, 1998. **17**(1): p. 33-58.
189. Netland, P.A., *Glaucoma medical therapy: principles and management*. 2008: Oxford University Press, USA.
190. Stringer, W. and R. Bryant, *Dose uniformity of topical corticosteroid preparations: difluprednate ophthalmic emulsion 0.05% versus branded and generic prednisolone acetate ophthalmic suspension 1%.* Clinical ophthalmology (Auckland, NZ), 2010. **4**: p. 1119.

191. Ayaki, M., et al., *Cytotoxicity of ophthalmic solutions with and without preservatives to human corneal endothelial cells, epithelial cells and conjunctival epithelial cells.* Clinical & experimental ophthalmology, 2008. **36**(6): p. 553-559.
192. HULL, D.S., et al., *Permeability of the isolated rabbit cornea to corticosteroids.* Investigative Ophthalmology & Visual Science, 1974. **13**(6): p. 457-459.
193. Loftsson, T., et al., *Dexamethasone delivery to posterior segment of the eye.* Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2007. **57**(1-4): p. 585-589.
194. Javitt, J.C., N.B. Javitt, and P. Mcdonnell, *Topical compositions for the eye comprising a beta-cyclodextrin derivative and a carbonic anhydrase inhibitor*. 1994, Google Patents.
195. Kristinsson, J.K., et al., *Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops. Aqueous humor pharmacokinetics in humans.* Investigative ophthalmology & visual science, 1996. **37**(6): p. 1199-1203.
196. K.M. Saari, H.J., H. Seppa, T. Loftsson, E. Stefansson,, *Acute and Chronic Postoperative Inflammatory actions after Cateract Extraction and Intraocular Lens Implantation.* Excerta Medica International Congress, 1998: p. p. 1158.
197. Tanito, M., et al., *Topical dexamethasone-cyclodextrin microparticle eye drops for diabetic macular edema.* Investigative ophthalmology & visual science, 2011. **52**(11): p. 7944-7948.
198. Van Doorne, H., *Interactions between cyclodextrins and ophthalmic drugs.* European journal of pharmaceutics and biopharmaceutics, 1993. **39**(4): p. 133-139.
199. Loftsson, T. and E. Stefansson, *Effect of cyclodextrins on topical drug delivery to the eye.* Drug development and industrial pharmacy, 1997. **23**(5): p. 473-481.
200. Vision, I.-S.
201. Scoper, S.V., et al., *Ocular distribution, bactericidal activity and settling characteristics of TobraDex® ST ophthalmic suspension compared with TobraDex® ophthalmic suspension.* Advances in therapy, 2008. **25**(2): p. 77-88.
202. Gao, Y., et al., *PLGA-PEG-PLGA hydrogel for ocular drug delivery of dexamethasone acetate.* Drug development and industrial pharmacy, 2010. **36**(10): p. 1131-1138.
203. Prausnitz, M.R. and J.S. Noonan, *Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye.* J Pharm Sci, 1998. **87**(12): p. 1479-88.
204. Huang, H.S., R.D. Schoenwald, and J.L. Lach, *Corneal penetration behavior of beta-blocking agents II: Assessment of barrier contributions.* J Pharm Sci, 1983. **72**(11): p. 1272-9.
205. Schoenwald, R.D. and R.L. Ward, *Relationship between steroid permeability across excised rabbit cornea and octanol-water partition coefficients.* Journal of pharmaceutical sciences, 1978. **67**(6): p. 786-788.
206. Ali, Y. and K. Lehmussaari, *Industrial perspective in ocular drug delivery.* Advanced drug delivery reviews, 2006. **58**(11): p. 1258-1268.
207. Tousif, K., et al., *NANOSUSPENSION: NOVEL APPROACH FOR ENHANCEMENT OF SOLUBILITY AND SUBSEQUENT BIOAVAILABILITY.* Inventi Rapid: NDDS, 2012.
208. Trivedi, R. and U.B. Kompella, *Nanomicellar formulations for sustained drug delivery: strategies and underlying principles.* Nanomedicine (Lond). **5**(3): p. 485-505.
209. Pepic, I., et al., *A nonionic surfactant/chitosan micelle system in an innovative eye drop formulation.* J Pharm Sci, 2010. **99**(10): p. 4317-25.
210. Velagaleti, P., et al., *Topical Delivery of Hydrophobic Drugs Using a Novel Mixed Nanomicellar Technology to Treat Diseases of the Anterior & Posterior Segments of the Eye.* Drug Delivery Technology, 2010 **10**(4): p. 42-47.
211. Torchilin, V.P., *Micellar nanocarriers: pharmaceutical perspectives.* Pharm Res, 2007. **24**(1): p. 1-16.

212. Aliabadi, H.M. and A. Lavasanifar, *Polymeric micelles for drug delivery.* Expert Opin Drug Deliv, 2006. **3**(1): p. 139-62.
213. Jiao, J., *Polyoxyethylated nonionic surfactants and their applications in topical ocular drug delivery.* Adv Drug Deliv Rev, 2008. **60**(15): p. 1663-73.
214. Scott, J., *A finite element model of heat transport in the human eye.* Physics in Medicine and Biology, 1988. **33**(2): p. 227.
215. Carlfors, J., et al., *Rheological evaluation of Gelrite® in situ gels for ophthalmic use.* European journal of pharmaceutical sciences, 1998. **6**(2): p. 113-119.
216. Rozier, A., et al., *Gelrite®: A novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol.* International journal of pharmaceutics, 1989. **57**(2): p. 163-168.
217. Nesamony, J., et al., *Development and characterization of nanostructured mists with potential for actively targeting poorly water-soluble compounds into the lungs.* Pharmaceutical research, 2013. **30**(10): p. 2625-2639.
218. MacCallum, D.K., et al., *Bovine corneal endothelium in vitro: elaboration and organization of a basement membrane.* Experimental cell research, 1982. **139**(1): p. 1-13.
219. Boddu, S.H., et al., *Novel nanoparticulate gel formulations of steroids for the treatment of macular edema.* Journal of Ocular Pharmacology and Therapeutics, 2010. **26**(1): p. 37-48.
220. Janszky, I., et al., *Demonstration of increasing standard pH value of lacrimal fluid with increase of flow rate.* Acta Ophthalmologica Scandinavica, 2001. **79**(2): p. 180-183.
221. Bother, H. and T. Waaler, *Rheological characterization of tear substitutes.* Drug Development and Industrial Pharmacy, 1990. **16**(5): p. 755-768.
222. Kumar, S. and K.J. Himmelstein, *Modification of in situ gelling behavior of carbopol solutions by hydroxypropyl methylcellulose.* Journal of pharmaceutical sciences, 1995. **84**(3): p. 344-348.
223. Nickerson, M., A. Paulson, and R. Speers, *Rheological properties of gellan solutions: effect of calcium ions and temperature on pre-gel formation.* Food hydrocolloids, 2003. **17**(5): p. 577-583.
224. Gómez-Gaete, C., et al., *Encapsulation of dexamethasone into biodegradable polymeric nanoparticles.* International journal of pharmaceutics, 2007. **331**(2): p. 153-159.
225. Bajaj, I.B., et al., *Gellan gum: fermentative production, downstream processing and applications.* Food Technology and Biotechnology, 2007. **45**(4): p. 341.
226. Shedden, A., et al., *Efficacy and tolerability of timolol maleate ophthalmic gel-forming solution versus timolol ophthalmic solution in adults with open-angle glaucoma or ocular hypertension: a six-month, double-masked, multicenter study.* Clinical therapeutics, 2001. **23**(3): p. 440-450.
227. Shedden, A.H., et al., *Plasma timolol concentrations of timolol maleate: timolol gel-forming solution (TIMOPTIC-XE®) once daily versus timolol maleate ophthalmic solution twice daily.* Documenta ophthalmologica, 2001. **103**(1): p. 73-79.
228. Laurence, J., et al., *A double-masked, placebo-controlled evaluation of timolol in a gel vehicle.* Journal of glaucoma, 1993. **2**(3): p. 177-182.
229. Chou, T.Y. and B.Y. Hong, *Ganciclovir ophthalmic gel 0.15% for the treatment of acute herpetic keratitis: background, effectiveness, tolerability, safety, and future applications.* Therapeutics and clinical risk management, 2014. **10**: p. 665.