CHAPTER-1

INTRODUCTION
1.1. Introduction

1.1.1. Glaucoma

Glaucoma is one of the leading causes of blindness. Glaucoma is actually a term used to describe several types of eye conditions that affect the optic nerve. In most cases, damage to the optic nerve is caused by increased pressure in the eye, also known as intraocular pressure (IOP). In glaucoma, the nerve cells in the front of the optic nerve (the ganglion cells) die and the process is irreversible. Previously, it was believed that glaucoma was almost always due to increased intraocular pressure. However, glaucoma has occurred in many patients with normal and even low eye pressure, so damage to the optic nerve is now key for diagnosis. In most views, the IOP increases with age especially for people over 40 years old. But this may not always be true, the IOP of Japanese, unlike Europeans and Americans decreases with age over 40. Some clinicians reported that (Ledhecker, 1976) the average IOP in a total of 10,000 individuals was 15.5 ± 2.57 mmHg. They suggested that those with 20.5 mmHg or higher IOP could be suspected of having glaucoma and those of above 24 mmHg are definite cases of glaucoma. The major types of glaucoma are classified as primary, congenital, and secondary glaucoma.

− Primary Open-Angle Glaucoma

Most people with glaucoma have the form called primary-open-angle glaucoma (also called chronic open-angle glaucoma). In primary open-angle glaucoma (POAG), poorly functioning drainage channels prevent fluid from being released from the eye at a normal rate. This in turn causes a rise in intraocular pressure. The drainage angle remains open, but tiny drainage channels in the trabecular meshwork pathway become clogged. This pathway is responsible for most aqueous humor fluid outflow. An imbalance then occurs as fluid continues to be produced but does not drain out efficiently. In rare instances, the pressure is high because the eye produces too much aqueous humor. The fluid in the eye’s anterior chamber builds up and increases pressure within the eye. The intraocular pressure exerts force on the optic nerve at the back of the eye. Over time, the persistent pressure coupled with other factors irreversibly damages the delicate long fibers of the optic nerve, called axons, which convey images to the brain. Optic nerve damage is the basic glaucoma condition. If it is untreated, eventually the nerve deteriorates until a person loses sight, first in the peripheral vision (the vision in the "corner of the eyes"). If it
becomes severe, the person loses central vision (in the middle of the eyes), and may eventually become blind. Blindness is fortunately nearly always preventable with early treatment. Primary open-angle glaucoma tends to start in one eye but eventually involves both. About half of patients have generalized (spread out) nerve damage. In the other half the disease is localized, causing wedge-shaped abnormalities in the nerve fiber layers of the retina.

- **Normal Tension Glaucoma**

Normal-tension glaucoma (NTG) is a form of open-angle glaucoma characterized by glaucomatous optic neuropathy in patients with IOP measurements consistently lower than 21 mmHg. Other factors are present that cause optic nerve damage but do not affect IOP. NTG has several distinguishing features. As the name implies, NTG patients have a higher propensity for optic nerve damage at relatively low levels of IOP. Visual field defects typically appear deeper, steeper and closer to fixation in patients with NTG than in patients with POAG. Also, the amount of visual field loss in NTG tends to be greater than one would expect on the basis of optic nerve appearance alone. As with other forms of glaucoma, patients with NTG who have asymmetrical IOP readings demonstrate greater visual field damage in the eye with the higher IOP. On ophthalmoscopy, patients with NTG tend to have more localized defects of the retinal nerve fiber layer and an increased propensity for optic disc hemorrhages compared with patients with POAG.

- **Closed-Angle Glaucoma**

Closed-angle glaucoma (also called angle-closure glaucoma) is responsible for 15% of all cases. The iris is pushed against the lens, sometimes sticking to it, closing off the drainage angle. This can occur very suddenly, resulting in an immediate rise in pressure. It often occurs in genetically susceptible people when the pupil shrinks suddenly. Closed-angle glaucoma can also be chronic and gradual, a less common condition.

- **Congenital Glaucoma**

Congenital glaucoma (CG) refers to a specific form of developmental glaucoma characterized by an isolated maldevelopment of the trabecular meshwork (isolated trabeculodysgenesis) not associated with other developmental ocular anomalies or ocular disease that can raise the IOP. Also called primary infantile glaucoma, it is the most common form of developmental glaucoma.
The condition is typically bilateral, but 25–30% of the cases may be unilateral. This may be an inherited condition and often can be corrected with microsurgery.

**The Aqueous Humor.**

To understand glaucoma, it is important to first consider aqueous humor, the clear, watery fluid that circulates continuously through the front (anterior) chamber of the healthy eye and is a primary focus of glaucoma research. This fluid is not related to tears, nor is it the dense jelly-like substance called vitreous humor that is contained in the rear chamber.

**Draining the Fluid and Intraocular Pressure.**

The aqueous fluid is continuously produced within the front of the eye, causing pressure known as intraocular pressure (IOP). To offset the in-flowing fluid and to maintain normal IOP, the fluid drains out between the iris and cornea (an area known as the drainage angle). It does so through two channels within this angle. The trabecular meshwork, a sponge-like, porous network, and its connecting passageways are referred to as the "conventional" outflow pathway. Most of the eye fluid outflow occurs in this region and flows from the trabecular meshwork to a group of vessels encircling the anterior chamber, called Schlemm's canal. From here, the fluid enters collection chambers and then flows out into the general blood circulatory system of the body. The uveoscleral pathway is located behind the trabecular meshwork and is called the "unconventional" pathway. Up to 30% of the fluid flows out through this channel.

Increased IOP is, indeed, present in most cases of glaucoma, but some patients have normal IOP, which is usually maintained at measurements of 10 - 20 mmHg. Measurements above this, however, do not necessarily predict glaucoma. For example, only about 10% of people with IOP levels of 21 - 30 mmHg will actually develop glaucoma. This still puts such patient at considerable risk for glaucoma.

**Treatment:**

There is no cure for glaucoma, but treatment can help reduce intraocular pressure thus preventing optic nerve damage and blindness. Glaucoma is usually treated with medications, although surgery may also be recommended for some patients.
1.2. Physiological constraints of the eye:

The cornea is a transparent membrane in the front part of the eyeball and is considered to be the primary pathway for the drug entry into anterior chamber. After topical administration of eye drops, drug has to be absorbed and reached the target site to certain level in order to achieve the therapeutic effect such as the absorption of carbonic anhydrase inhibitor in ciliary body to reduce production of aqueous humor (Maren 1987). In addition to the barrier properties of the cornea, physiological factors such as lachrymal drainage and blinking can further influence the rate and extent of ocular drug absorption. A lachrymal gland is a compound tubuloacinair gland located at the superior latter portion of each orbit. The lachrymal fluid is secreted and enters into the two small openings (puncta lacrimalia), then passes into the lachrymal canal and is next conveyed into lachrymal sac. The lachrymal sac, superior expanded portion of the nasolacrymal duct which transports the lachrymal secretions into the inferior meatus of the nose (Tortora 1980). Lachrymal drainage of an instilled drug solution competes for drug along with the corneal penetration and can account for a considerable loss of drug. When an eye drop is administered, two processes occur simultaneously: the solution is diluted by reflux tearing and the added volume in excess of the normal lachrymal volume is drained from the eye. This partly facilitated by reflux blinking. Tears flow over the cornea finally down the nasolachrymal duct, potentially giving scope for systemic absorption of drug. The normal lachrymal volume in human is approximately 7µl and if blinking does not occur, the human eye can hold about 30µl without spillage onto the cheek. If blinking occurs, then the human eye can hold approximately 10µl. For a commercial ophthalmic drop of 50-70µl, therefore, significant reduction in the bioavailability of applied drug results due to the drug spillage out of the eye (Aker and Schoenwald, 1977).

1.3. Antiglaucoma drugs:

A. Beta-blockers:

1. Non-selective agents: Timolol (Timoptic, Betimol), Levobunolol (Betagan, Akbeta), Carteolol, Metipronolol.

2. β1 selective agents Betaxolol (Kerlone,Betoptic)
B. Cholinergic agonists: Pilocarpine

C. Prostaglandins: Latanoprost (Xalatan), Travoprost, Bimatoprost.

D. Carbonic anhydrase inhibitors: Acetzolamide, Dorzolamide (Trusopt) and Brinzolamide (Azopt)

E. Adrenergic agonists: \( \alpha-2 \) agonist-Apraclonidine (Iopidine) & Brimonidine (Alphagen) \( \alpha-1 \) agonist- Dipevefrin.

F. Osmotic agents: Mannitol 20% IV solution, Glycerol 50% syrup.

Pilocarpine is a traditional antiglaucoma drug in the treatment of open-angle glaucoma. Its side effects in miosis and three time dosing requirement may decrease the compliance of the patient. Long acting dosage forms such as ocusert and pilocarpine gel may improve IOP control and patient compliance (March et al 1982). Dipivalyl epinephrine (dipivefrin) is a prodrug of epinephrine. This prodrug has more lipophilicity than parent compound and penetrates the cornea 17 times more readily. Thus 0.1% solution of dipivefrin has equivalent ocular hypotensive effect as dose 1% to 2% solution of epinephrine with less systemic absorption (Kohn et al., 1979). Carbachol is a synthetic derivative of choline with a direct action like that of pilocarpine on parasympathetic receptors. The drug solution is available in strengths of 0.75% to 3.0%. tin the treatment of open-angle glaucoma, one drop is instilled usually 2 or 3 times daily. For beta blockers, their therapeutic ocular hypotensive effect was suggested more than four decades ago. Timolol became available for topical use nearly worldwide in the late 1970’s and early 1980’s. After that, there are dozens more becoming available as eye drops in the treatment of glaucoma. Their pharmacological effect of decreasing IOP is primarily through the reduction of the production of aqueous humor. Most famous of these these drugs, betaxolol, befunolol, bupranolol, carteolol, levobunolol, metipranolol, pindolol and Timolol have been distributed as eye drops in some countries of the world. Since 1954, carbonic anhydrase inhibitors have been used systematically in the treatment of glaucoma. Acetzolamide, methazolamide and dichlorphenamide became the effective agents for treating glaucoma because of their ability to reduce aqueous humor formation in the ciliary body and lower IOP. However systemic administration of these drugs frequently cause undesirable side effects.
1.4. Formulation approaches to improve ocular therapy

Novel drug delivery systems are claimed and proven to have a great potential to overcome the problems of patient compliance and together with local, sustained delivery of drugs with minimum side effects. As topical administration of drugs can be difficult for elderly patients, designing a delivery system to circumvent the aspect of patient compliance and minimize the side effects will be of great benefit. For treatment of ocular conditions effective drugs are present but lack in effective delivery systems to improve patient care and clinical outcomes. Currently available drugs for ocular conditions need to be administered two or more i.e. multiple times a day, and the associated poor patient adherence makes the treatment less clinically effective (Sleath et al., 2006; Schwartz and Quigley, 2008). Studies suggest that <1% of topically administered drug reaches aqueous humor (Lee and Robinson, 1979). Up to 80% of systemic absorption of drug occurs followed by topical administration of eye drop causing systemic side effects. To reduce the number of instillations per day, several gel formulations, liposome composed for phospholipids, niosome based on non-ionic surfactants, PMMA dendrites, PLGA based nanocapsules, nanoparticles, nanoparticles laden hydro gels based on water soluble polymers were developed that increase the viscosity of the solution thereby improves the residence time of drug in cul de sac. However they were not much popular commercially as they tend to blur the vision (Uusitalo et al., 2006) and with associated discomfort.

Currently, the challenge faced by scientific community in ophthalmic pharmaceutical research is to improve ocular bioavailability from less than 1-5 % to at least 15-20 % (Saettone et al., 1996). Numerous strategies have been developed to increase the bioavailability of ophthalmic drugs. The emphasis is given to maximize precorneal drug absorption by minimizing precorneal drug loss, increasing the drug residence time by developing novel drug delivery systems and enhance the corneal permeability by virtue of permeation enhancers. The benefits to the patient should include ease in administration, prolonged contact time with ocular tissues, reduce frequency of administration, reduced spillage, be acceptable to the patients, non-toxic, reduce side-effects, non-irritant and comfortable. The additives used should not affect the corneal permeability of the drug and vision of the patient (Van- Ootegehem, 1987). The use of a water-soluble polymer to enhance the contact time and possibly also the penetration of the drug was first proposed by Swan (Swan, 1945). Although eye-drops represent 90 % of all ophthalmic dosage forms, there is
a significant effort directed towards development of new drug delivery systems to overcome the disadvantages of eye-drops. All novel ophthalmic drug delivery systems are intended to increase the bioavailability of the drug, to sustain/control the drug delivery to the pre and intraocular tissues and to reduce systemic side effects. The primary determinant of efficacy for a controlled release system applied to the eye is the time during which the drug remains in contact with the cornea.

1.4.1. Liquid dosage forms

(a) Viscous drug solutions

Conventional aqueous solutions applied topically to the eye have the disadvantage that most of the instilled drug is lost within the first 15-30 sec after instillation. A simple and most popular approach to prolong the precorneal residence time thereby increasing the ocular bioavailability is to increase vehicle viscosity by incorporating soluble polymers into an aqueous solution. The increased vehicle viscosity reduces the drainage of the formulation, thus the drug, into the nasolachrymal duct and slower elimination from the preocular area and hence a greater transcorneal penetration of the drug into the anterior chamber (Saettone et al., 1982). Viscous vehicles increase the contact time of the preparations to varying degrees, but so far no marked sustaining effect has been attained (Saettone et al., 1989). Acetazolamide formulated in carboxymethyl cellulose (CMC), compared with the saline solution of the drug in patients with unilateral open-angle glaucoma, was found to have a longer duration of action. However, the results were not spectacular and were significant only when using high drug concentrations (Kaur and Kanwar, 2002). Viscous solutions of ofloxacin prepared using chitosan hydrochloride (CH-HCl) and N-carboxymethyl chitosan have maintained drug levels for 2.4 folds more time than reference vehicle (Di Colo et al., 2004). Nevertheless, viscosity alone cannot significantly prolong the residence time. This can be considered, in part, as the premise of using bioadhesive polymers to enhance drug absorption. The capacity of some polymers to adhere to the mucin coat covering the conjunctiva and the corneal surfaces of the eye forms the basis for ocular mucoadhesion. Due to interactions with the mucus layer or the eye tissues, an increase in the precorneal residence time of the preparation, therefore prolongs the residence time of a drug in the conjunctival sac. In these systems clearance of instilled dose is controlled by the much slower
rate of mucus turnover than the tear turnover rate. Bioadhesive polymers are usually macromolecular hydrocolloids with numerous hydrophilic functional groups and possess the correct charge density (Robinson and Mlynek, 1995). These bioadhesive polymers can be natural, synthetic, or semi-synthetic in nature. Ocular bioavailability of rufloxacin has been enhanced with tamarind seed polysaccharide (TSP) (Burgalassi et al., 2006). Viscosity inducing and bioadhesive polymer like sodium hyaluronate (Aragona et al., 2002; Vico et al., 2005), trehalose (Matsuo et al., 2002), hydroxypropyl-guar (Ubels et al., 2004; Hartstein et al., 2005) were used to prepare artificial tears for the treatment of conditions like preservative induced damage and dry eye syndrome.

(b) Aqueous suspensions and oily preparations:

Insoluble drugs can be delivered effectively by formulating an aqueous or oily dispersion. Suspensions are dispersions of finely divided relatively insoluble drug substances in an aqueous vehicle containing suitable suspending and dispersing agents. Because of a tendency for the particles to be retained in the cul-de-sac, the contact time and duration of action of a suspension exceed those of a solution (Le Bourlais et al., 1998). While the retention increases with an increase in the particle size, as does the irritation of the eye, the rate of dissolution of the suspended drugs increases with decreasing particle size. Thus an optimum particle size has to be selected for each type of drug, and it is recommended that the particles in an ophthalmic suspension should be lower than 10 µm in size (Sieg and Robinson, 1975). The spillover and drainage of a suspension leading to the loss of both solution and suspended solid can also affect the drug availability and absorption. Moreover, a change in crystal structure, i.e., polymorphism, may occur during storage, resulting in an alteration in the suspension characteristics causing solubility changes reflected in an increased or decreased bioavailability. Ocular bioavailability of ketorolac was enhanced by two folds when sesame oil and soybean oil based formulations were instilled into rabbit eyes as compared to aqueous ketorolac (Malhotra and Majumdar, 2005).

(c) Vesicular or colloidal systems

These systems were exploited for both intraocular and topical administration. The rationale for the development of various particulate systems for the delivery of ophthalmic drugs was based on possible entrapment of the particles in the ocular mucus layer and the interaction of
bioadhesive polymer chains with mucins inducing a prolonged residence, and slow release. Furthermore, controlled drug release and enhanced absorption or even endocytosis in the case of nanoparticles have been found to improve bioavailability (Kreuter, 1993; Le Bourlais et al., 1995; Zimmer and Kreuter, 1995; Le Bourlais et al., 1998; Saettone et al., 1999; Alonso and Sanchez, 2003). Various vesicular or colloidal systems used for ocular therapy includes liposomes, microemulsions, niosomes, pharmacosomes, microparticles, nanoparticles and dendrimers. When appropriately formulated for ophthalmic delivery, the particles are retained in the ocular cul-de-sac and the drug released at a rate that is neither too fast nor too slow to allow adequate drug penetration into ocular tissues. They can be used to target the drug molecule to a specific tissue. The particle size of ophthalmic controlled-release formulations has proved to be very important in balancing between the drug release rate, bioavailability improvement, patient comfort and ease of use (Shekunov et al., 2007). Nanoparticles (typically about 300 nm) without bioadhesion can be eliminated from the precorneal site almost as quickly as aqueous solutions. Microparticles (mean diameter 1-3 µm) may be better suited for controlled release, but the presence of coarse particle fraction above 25 µm makes them less tolerable and can cause irritation to the eye. One of the main challenges in developing such particulate systems is the manufacturing complexity and particle size control during large-scale manufacturing (Ding, 1998).

1.4.2. Semi-solid dosage forms

(a) Ointments

Ointments are useful as drug carriers for improving bioavailability, sustaining drug release and improving drug stability. There is more flexibility in the choice of drug to be incorporated into an ointment base, as even drugs with low water solubility can be suitably delivered to the eye. The ointments are reported to remain on the surface of the eye for up to 2-4 or even 8 hr after application (Gebhardt and Kaufman, 1995). Ophthalmic ointments containing different sorption promoters have also been formulated and reported to show significantly higher release rates, relative to ointments without these promoters. However, dosage variability with ointments is greater than with solutions and the ointments interfere with vision unless their use is limited to bedtime instillation. Drug molecules entrapped within the ointment base may or may not be
release at the site of action due to partitioning towards the base (Kaur and Kanwar, 2002). Flavin adenine dinucleotide sodium ointment applied topically in humans extended the drug levels upto 1 hr (Takaoka et al., 2004).

(b) Aqueous gels/hydrogels

The use of highly viscous aqueous solutions leads to an improvement in the precorneal retention of drugs and a better miscibility with the lachrymal fluid. It also leads to a reduction in the dosage frequency due to enhanced bioavailability. As a consequence, there is a decrease in the drug concentration required, with a concomitant reduction in the potential for side effects. A number of water-soluble or insoluble natural, synthetic, and semi-synthetic viscous vehicles have been developed during the last 50 years (Kaur and Kanwar, 2002). Bioadhesive polymers enhance the possibility of interactions with mucus thereby increase residence time of drugs. An optimal concentration of mucoadhesive polymer will produce maximum adhesion (Lee et al., 2000). The drug should be slowly released during its stay in the cul-de-sac. Water soluble drugs will release fast, so alternative approaches were developed by incorporating slightly soluble drug complexes, micelles, and liposomes into the gel. Another possibility is the non-covalent or covalent binding of the drug molecule or a polyethelene glycol (PEG)–drug conjugate to the polymer chains (Ludwig, 2005). Tethering of long PEG chains on acrylate based hydrogels improves mucoadhesion properties due to enhanced anchoring of the chains with the mucus layer (Bures et al., 2001). Pilocarpine HCl or chloramphenicol loaded co-polymeric hydrogels constituting of vinylpyrrolidone and methacrylic or acrylic acid repeat units have extended the drug release (Barbu et al., 2005). Important disadvantages are irritation, blurred vision, sticky eyelids, poor control of drug release, prone to bacterial contamination and difficulty in administration (Kaur and Kanwar, 2002; Ludwig, 2005).

(c) *In situ* gelling systems

*In situ* gelling systems are viscous hydrogel systems, which undergo a sol-gel phase transition after exposure to the physiological conditions in the cul-de-sac, forming a viscoelastic gel. These systems have advantages like easy, accurate and reproducible administration of a dose compared to the application of preformed gels and thus resulted in increased patient compliance due to ease of administration and low frequency of administration (Ludwig, 2005). Different polymers
exhibiting reversible phase transitions were used in these systems. The phase transition is triggered by the pH of the tears (e.g. carbomer, cellulose acetate phthalate (Gurny et al., 1985; Ke et al., 2001; Srividya et al., 2001; Xu et al., 2002; Sultana et al., 2006; Wu et al., 2007), or the temperature at the eye surface (e.g. methyl cellulose, xylöglucan, poloxamer 407) (Miyazaki et al., 2001; Wei et al., 2006) or the monovalent and divalent cations present in the tear film (e.g. alginic acid, sodium alginate, gellan gum) (Demailly et al., 2001; Balasubramaniam et al., 2003; Trinquand et al., 2003; El-Kamel et al., 2006; Takiyama et al., 2006). These polymers were used alone and in combination with other in situ gel forming polymers, viscosity enhancing agents and bioadhesive polymers. Once gelled, the formulation resists the natural drainage process from the precorneal area. Residence at the site of drug absorption is prolonged and subsequently, the bioavailability of the drug is increased (Rozier et al., 1997). The rate of in situ gel formation is important because between instillation in the eye, and before a strong gel is formed, the solution or weak gel is prone to elimination by the fluid mechanics of the eye (Carlfors et al., 1998). CAP latex coagulates when its native pH of 4.5 is raised by the tear fluid to pH 7.4. Gelrite® is a low-acetyl gellan gum, which forms a clear gel in the presence of mono- or divalent cations. The electrolytes of the tear fluid and especially Na⁺, Ca²⁺ and Mg²⁺ cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution into the cul-de-sac. Poloxamers or Pluronics®, block copolymers, undergo thermal gelation or sol-gel transition in the 25-35°C temperature range. Below transition temperature, poloxamer solutions allow a comfortable and precise delivery by the patient in the cul-de-sac. Due to inherent surface active properties, poloxamers were employed as solubilizer, and proposed as artificial tears. In order to reduce the total polymer content of a formulation and to improve the rheological behaviour and gelling characters of the delivery system, combination of polymers were employed. Several researchers explored the advantage of using various in situ gelling polymers with different phase transition mechanisms in ophthalmic drug delivery. Desai and Blanchard (1998) showed that the addition of methyl cellulose and hydroxypropylmethylcellulose to Pluronic® F-127 gels slowed down the gel dissolution rate and pilocarpine release. Miotic response was extended in rabbits for the gels compared to the aqueous solution. Poloxamers were tested as a vehicle for various drugs or drug complexes such as liposomes or cyclodextrins (Bochot et al., 1998; Kim et al., 2002). Also various polymers such as water-soluble cellulose derivatives (Desai and Blanchard, 1998), polysaccharides, poly(acrylic acid) (Lin and Sung, 2000) and hyaluronan were added to
poloxamer gels. Copolymerization of the bioadhesive polymer poly(acrylic acid) with Pluronic®, a thermally-induced, phase-separating graft polymer, has been reported to yield a bioadhesive vehicle with a prolonged residence time plus a prolonged drug release period in contact with mucosal surfaces such as the eye.

1.5. Microemulsion

Microemulsion was developed firstly by Hoar and Schulman in 1943. These are clear liquids in which surfactant molecules with or without co surfactant gets incorporated in between the oil and water interface and thereby reducing the interfacial tension to minimal. Microemulsions have been employed widely in petrochemical extraction, as reaction mediators and template for nano-synthesis of various nanomaterials. Apart from this they are also employed in electro kinetic chromatographic separation process. Microemulsions were also explored as vehicles for oral, topical and parenteral routes and was being appreciably beneficial in terms of safety, efficacy and bioavailability. More recently many formulation scientists/clinicians attempted to evaluate its potential for ocular drug delivery applications.

A high surfactant concentration usually tend to form a solubilised system which exhibits small droplet diameter with additional thermal, kinetic stability. The surfactant solubilised system generally forms reverse inverted micelles composed of surfactant molecules and display a slight difference of droplet size with the microemulsion. The microemulsion is said to have a droplet diameter in the range of 100-600Å. Whereas the dimension of anionic micelles in aqueous solution is around 50Å and non-ionic micelles are double in size. It is known that the solubilization process swells the non-ionic micelles and the resulting swollen micelle phase is termed as microemulsion phase. Both the solubilization and microemulsion formation occurs spontaneously without any external energy input and so form thermally and kinetically stable system. The large amount of surfactant present in microemulsion reduces the interfacial tension to a metastable negative value which spontaneously form smaller droplets to prevent phase separation. As the droplets become smaller, the interfacial area increases and the emulsifier molecule gets depleted by adsorption until the interfacial tension goes to less than zero value and further maintains the state of equilibrium.
The inherent stability exhibited by microemulsion mainly due to presence of high concentration of surfactant molecule constantly co-exist with various continuously interchanging geometric structures such as cubic phase, lamellar phase and columnar rods. Modulation in temperature for the most part promotes the event of transition of various micelle structures. Other factors that determine the formation of specific type of structure formed by a surfactant include the alkyl chain length, surfactant concentration, temperature, salt concentration and the presence of a co-surfactant.

The simplest straightforward representation of a microemulsion can be made by using a droplet model. Droplet model suggests formation of reverse micelles which with increasing water concentration forms hydrated swollen micelles, where the surfactant molecules develop into micelles incorporating within the internal phase, and projecting the tail towards the external phase. The required curvature for the surfactant film to attain the minimal interfacial tension is assisted by the presence of a co-surfactant. The micelle attains a definite shape depending on the concentration of surfactant and temperature. The change in micelle shape is governed by change in surfactant orientation in the formed micelle with a marked change in the viscosity and polydispersity.

1.6. Factors affecting microemulsion formation

a) Polarity and type of oil:

The type of oil intended to be used in microemulsion must interpenetrate and associate with the interfacial film. The above requirement demands that the oils to be used to prepare microemulsion with a given emulsifier combination must be structurally similar to the emulsifiers and should be of equal or smaller alkyl chain length. Oils with long carbon chains such as triglycerides are too large to penetrate the hydrophobic chain of surfactant and thus tend to promote formation of lamellar phases as opposed to clear microemulsion region (Aboofazeli, 1995). It is seen that the size rather than the polarity shows significant difference in the phase behaviour of the microemulsion system. This suggests use of smaller oils favouring microemulsion formation. However some considerations must be given while using oils with short hydrocarbon chains, as these may significantly penetrate the surfactant molecules of the interfacial region thereby acting more as co-surfactant and possibly altering the locus of drug
solubilisation. Along with the size, nature of oils is also important in determining the drug loading capacity which makes it essential to examine the effect of oil in microemulsion formation.

b) Surfactant related parameters:

i) Surfactant packing parameter:

Systematic trends in the micelle morphology can be rationalized using surfactant packing parameter (SPP). It can be stated by:

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\text{SPP} = \frac{v}{a_o} \times l_c 
\]

the ratio of the alkyl chain volume \((v)\) to the area occupied at the interface by the polar head group \((a_o)\) and the critical alkyl chain length \((l_c)\). This gives an estimation of balance between polar head group repulsion and alkyl chain penetration. The volume of molecular chains in the surfactant molecules can be estimated from the volume contributions of the chemical group it contains (Chevalier and Zemb, 1990). Surfactants with a SPP of less than or equal to one-third form spherical micelles, between one-third and half forms rod like micelles, between one-half and one forms bilayers and above one forms inverted structures like reverse micelles. Based on the literature available, the non-ionic polyoxyethelene ether surfactants were found to show SPP values ranging from 0.43-0.72 (Simmons et al 2002). Phosphatidylcholine (lecithin) has a significantly larger head group and a smaller packing parameter of 0.6 by forming structures of lower curvature. Indeed, in non-polar solvents, lecithin forms wormlike cylindrical reverse micelles that can incorporate water up to a water/lecithin molar ratio of 20.

ii) Curvature \((H_o)\):

Microemulsion microstructure is set by the curvature of the surfactants at the water-oil interface. This curvature acts as a main balance between oil penetration into surfactant tails, and head group repulsion. The alkyl chain length in the oil penetrates in the surfactant chain, if alkyl chain length is much less than surfactant chain length \((l)\), the oil penetrates strongly swelling up the hydrophobic area. By contrast when the chain length exceeds that of the surfactant, surfactant does not penetrates the alkyl chains of oil which fails to form microemulsion (Chen et al 1986).
In addition to the oils used, the alcohols which usually used as co-surfactants also have a marked effect on the curvature. They show a little effect on surfactant head group and mostly act to swell the effective chain volume at the interface to allow substantial oil uptake.

1.7. Physiological barriers of eye and ocular drug absorption:

The human cornea measures approximately 12 mm in diameter and 520 μm in thickness, and consists of five layers, including the epithelium, basement membrane (Bowman's layer), stroma, descemnet membrane and endothelium. The human corneal epithelium is a stratified, squamous, non-keratinized 50 μm in thickness. It is composed of two to three layers of flattened superficial cells, wing cells, and a single layer of columnar basal cells which are separated by a 10–20 nm intercellular spaces and have regular intercommunications. These desmosome-attached cells can communicate via gap junctions through which small molecules traverse. Tight junctions (zonulae occludens) seal the superficial cells, building a diffusion barrier on the surface of the epithelium. Compared to the stroma and endothelium, the corneal epithelium represents a rate-limiting barrier which hinders permeation of hydrophilic drugs and macromolecules. The stroma displays hydrophilic nature due to an abundant content of hydrated collagen, which prevents diffusion of highly lipophilic agents. The corneal endothelial monolayer maintains an effective barrier between the stroma and aqueous humor (Schoenwald and Huang 1983). Active ion and fluid transport mechanisms in the endothelium are responsible for maintaining corneal transparency. In case of topically applied drugs, small lipophilic molecules are normally absorbed through the cornea, while large hydrophilic molecules such as protein/gene based medicines are absorbed via the conjunctiva and sclera. It has been reported that certain drug properties such as lipophilicity, molecular weight, charge, and degree of ionization can significantly influence its passive permeability across the cornea (Huang et al 1983). Of these factors, lipophilicity has played a key role since transcellular permeation of lipophilic drugs through the cornea is faster and greater as compared to hydrophilic drugs. This route appears to be the main path for absorption of topical drugs. The greater molecular size decreases the rate of paracellular permeation of drugs (Duvvuri et al. 2003). Once the drug diffuses the cornea, the drug can diffuse into the aqueous humor and the anterior segment. However, local administration of conventional drugs via the corneal route fails to provide adequate concentrations within the vitreous and retina. As the drug fails to permeate the differential solubility barriers imposed by corneal membrane due to lack of
differential solubility. For a drug molecule to permeate through corneal barriers it should possess ability to cross the hurdles imposed and further reach the anterior segment. According to Sieg & Robinson (1976): The corneal epithelium serves as both a barrier and a depot, and the corneal stroma, endothelium are kinetically indistinguishable from the aqueous humor. These findings can be correlated to the drug absorption from microemulsion through the lipid-aqueous-lipoidal corneal segment to reach the anterior segment by formation of the nanodroplet reservoir in the corneal segment of eye. The conjunctiva is a mucous membrane consisting of vascularised epithelium (2-3 cell layers thick) and plays an important role as a protective barrier on the ocular surface since tight junctions are present on the apical surface of its cells. In fact, the bulbar conjunctiva represents the first barrier against permeation of topically applied drugs via the non-corneal route, which is the main intraocular route for entry of macromolecules and hydrophilic substances. Due to significant loss of drug through systemic circulation, the conjunctiva/sclera pathway appears to be a non-efficient path. The sclera is about 10 times more permeable than the cornea and half permeable as the conjunctiva. It is poorly vascularised and consists mainly of collagen and mucopolysaccharides, through which drugs can diffuse and enter the posterior segment (uveal tract, retina, choroid, vitreous humor). The topically administered aqueous medications are absorbed mainly by scleral tissues and conjunctival tissues as leading to over 95% loss of drugs into systemic circulation. The vascularised conjunctiva is responsible for the non productive absorption. During the contact of the drug on the corneal surface it partitions into the epithelium and in the case of lipophilic compounds it remains in the epithelium and is slowly released to the corneal stroma and further into the anterior chamber (Janoria et al. 2007). It has been seen that lipophillic character of microemulsion makes it undergo much more absorbed by corneal membrane rather than scleral and conjunctival non productive absorption by this means increasing the local action of topically applied medication.

1.8. Ideal ophthalmic drug delivery

Conventional eye drops suffer from several drawbacks due to physiological conditions in the eye and other barriers. Blinking, baseline and reflex lachrymation, and nasolachrymal drainage remove foreign substances rapidly, including drugs, from the surface of the eye. So, eye drops suffer quick elimination and low bioavailability (1-5 %) and required to be administered
frequently to maintain the drug levels for prolonged periods, often as high as 6-8 times a day. The frequent use of highly concentrated solutions may induce toxic systemic side effects and cellular damage at the ocular surface. Novel ophthalmic formulation would enhance bioavailability by reducing the drug precorneal wastage, prolonging the residence time and sustaining the drug release, with less patient inconvenience. Consequently, delivery systems that prolong the residence time of the applied dose in the conjunctival sac would be expected to reduce systemic drug absorption. This project has targeted to design novel ocular delivery systems for model antiglaucoma drug Timolol maleate, a non specific beta blocking agent.
[BIBLIOGRAPHY]


