1.1 DEVELOPMENT OF OCULAR CARRIER

Drug carrier can be defined as the administration of drug from natural or synthetic origin, in order to control in vivo availability of drug molecules for pharmacological effects. After the application of dosage, usually a small amount remains available to the sites of interest and major fraction to the unwanted sites giving side effects. Development of drug carrier intended to reach tissues to utilize maximally giving minimum side effects. Drug carrier systems are become more difficult because of the, arrival of low-molecular-weight molecules. Also emergence of bio-macromolecules with poor, aq. solubility and tissue permeation and increased use of bio-materials with less understanding of physical properties, all limits research in drug carrier systems. Controlled delivery to the eye of drugs is limited by the effective protective mechanisms offering by corneal tissues. Penetration into cornea is prerequisite for absorption to take place into the eye and also more contact time. One of the way of optimization of drug carrier system to eye is by improving precorneal contact time by development of gels, use of soluble polymers or erodible and non-erodible inserts etc. [1,2,3]. To optimize drug carrier systems, various characteristics are required such as, good precorneal passage, improved contact time, ease of instillation, non-irritative and comfortable form and optimum viscosity [4].

Although eye medicine in drop form covers about 90% of entire ophthalmic carrier systems, there is a significant research is being carried to override the existing failure and limitations. Various drug carrier systems such as hydrogels, liposomes, microparticles, nanoemulsions, nanogels, collagen shields, nanoparticles etc. have been investigated and studied so far. An ultimate ocular carrier system would be designed as in the form of drops with no blurred vision/irritation. This would need one to two instillations a day. The usefulness to the patient is simplicity, a reduced
frequency of instillation, minimum toxicity and untoward effects. Still existing most carrier systems are, “superficially primitive and less effective” [5]. However, one of the most facilitating and difficult target facing by the researchers is drug delivery to eye. The structural composition, its function and biochemistry make this organ unreachable to foreign bodies. The target of the formulator is to overcome the pre corneal barriers without causing any severe tissue damage [6]. Eye drops with their limitations, are still given preferences/priority since they are relatively simple to prepare, filter and sterilize for a formulator [7].

1.2 ANATOMY OF THE EYE

1.2.1 The eye ball

The human eye is spherical in shape and about one inch across. The eye is comprised of different layers and internal structures, which performs distinctive functions. The detailed description of eye is given below in Fig.1

![Fig. 1.1 Structure of Eye-Ball](image-url)
The sclera covers major surface of eyeball having mean surface area of 16.30 square centimeters. The cornea is controlling factor for the refraction of light which is extension of sclera of eye ball. The conjunctiva extends the posterior layer of the eyelids, the anterior sclera and the superior and inferior conjunctival fornices. The sclera is composed of episclera, stroma and lamina fusca. Episclera is made of loosely packed collagen fibers that are connected to the sheath of eyeball. At the limbus it connects to the cornea and composed of collagen fibers with different sizes and orientation. The mean thickness of sclera is 0.53 mm near the limbus, near the optic nerve is about 0.9 to 1.0 mm and near the equator is 0.39 mm. Stroma composed of larger collagen fibers and elastic tissue. Lamina fusca is the innermost layer of sclera forms the uveal tract with the choroids [8, 9]. The cornea is supplied with oxygen and nutrients via the lacrimal fluid, aqueous humor and the blood vessels at the cornea-scleral junction. The cornea is very thin at its center (0.5–0.6 mm) and thick in the periphery (1.2 mm). The cornea is composed of five layers as, the epithelium: a stratified squamous epithelium. It is made of five layers of cells that have total thickness of around 50 to 100μm. Bowman's membrane lies between the most underlying membrane of the epithelium and the stroma and is composed of acellular interwoven collagen fibers. The stroma is mainly composed of water and collagenous lamellae that gives the strength and structure for this layer. It allows the penetration of light. The Descemet's membrane is composed of collagen fibers and the endothelium is composed of a single layer of flattened cells that are connected through tight junctions. It controls the hydration of the cornea by limiting access of water from the aqueous humor and by energy active transport [10]. The conjunctiva helps in the tear layer formation by releasing electrolytes and mucin. Conjunctiva is comprised of two layers as outer epithelial layer which is continuous with the corneal
epithelium and an underlying stroma layer. The conjunctival epithelium is made of 5 to 15 layers. The stroma layer of the conjunctiva contains the nerves, lymphatics and blood vessels and it attaches loosely to the sclera [11, 12]. Pupil is dark centre of the eye, but can be described as the circular hole in the centre of the iris through which light enters into the eye. The size of the pupil and therefore the amount of light is regulated by the pupillary reflex. The iris is located in front of the lens and behind the cornea. The iris is a diaphragm ie functions as a curtain of variable size. Its function is to adjust the size of the pupil to control the amount of light admitted into the eye. It is the coloured part of the eye. The lens is structured behind the pupil and encircled by the ciliary muscles. It helps to refract light travelling through the eye. The lens focuses light into an image on the retina as the shape of the lens is changed according to the distance from the eye of the object(s). The screen of retina is a light-sensitive structure lining the interior of the eye. Striated smooth muscles in the middle layer of eye are called as ciliary muscle. Ciliary muscle controls accommodation for viewing objects and regulates the flow of aqueous humour into schlemm’s canal. The muscle has parasympathetic and sympathetic innervations. The curvature of the lens is altered by contraction-relaxation of the muscle of cilia. The retina is located at the deep in the human eye and its function is to collect the information contained in image. It contains rods and cones photosensitive cells and their associated nerve fibers that transfer the light impulses that are then received by brain along the optic nerve. The choroid is between the retina and the sclera, a highly vascularized tissue. Macula is the center of the retina. Fovea is the site of our vision at the very center of the macula. Macula consists of the vessel layer and Bruch's membrane. There is the suprachoroidal thin space between the sclera and the choroid. The choriocapillaris
provides nutrition to the RPE. Between the pigment epithelium of retina and the choriocapillaris is the Bruch's membrane composed of five layers, the basement membrane of the RPE, an interior collagenous layer, the elastic layer, the outer collagenous zone and the basement membrane of capillary endothelial cell. The optic nerve (a bundle of over one million nerve fibers) transmits nerve signals from the eye to the brain. The front surface of the optic nerve is called the optic disk [13, 14].

1.2.2 Fluid composition of the eye

Tears are continuously secreted by lacrimal glands and the goblet cells. The normal tear fluid volume in eye is about 7 µl, secretes at a rate of about 1.2 µl/min and under reflex tearing the secretion rate may increase. The meibomian glands secrete lipids of the tear film. The lipid layer of tear film reduces loss of moisture due to evaporation from the ocular surface, lubricates the ocular surface and controls the surface energy of the tear film. The thickness of the oil film on the tear fluid is about 32 to 80 nm. Tear fluid contains proteins in more amount. The mucus layer is secreted on the eye surface by the goblet cells. Mucus consists of glycoproteins, proteins, lipids, electrolytes, enzymes, mucopolysaccharides and water. The primary component of mucus is mucin which is a high-molecular-weight glycoprotein, negatively charged at physiological pH. The pH of normal tear is 7.4±0.2. The viscosity of tears falls in between 1.3 to 5.9cps with a average of 2.9 cps. The mean surface tension value of tear fluid is about 44 mN/min. The bicarbonate and proteins are present in the tear fluid. The tear fluid has more than twice the buffer capacity to resist drops in pH. As a result of this, solutions in the pH range of 4.0 to 8.5 will cause minimal shift on the surface of the eye and will be easily neutralized by the tear fluid. Solutions with higher buffer capacities may be uncomfortable to the eye if they result in a significant shift of the tear fluid pH. Osmosity values for tear usually falls in
between about 300 to 320 mOsm. The salt content of the lacrimal fluid includes NaCl, sodium bicarbonate, KCl, calcium chloride and magnesium chloride. Aq. humor is a clear fluid secreted by the cilia body via filtration of blood passing through the ciliary body capillaries. It maintains shape of the eye by controlling its pressure. It provides essential components to the cornea and lens and carries out transport of drain materials far away from surrounding tissues.

The aqueous humor is mainly composed of water, high concentrations of ascorbic acid, glucose, amino acids and limited levels of proteins. The entire volume of the aqueous humor is around 0.2ml and is replaced every one to two hour. Vitreous humor is a gel-like material occupies the space between the lens and the retina. The vitreous humor is mainly composed of water (98–99.7%), collagen fibrils and hyaluronic acid. It supports the posterior surface of the lens and helps to keep the neural part of the retina in place [15, 16, 17].

1.3 OCULAR ROUTES OF ADMINISTRATION

There are different routes of drug carrier to the different tissues of eyes (Fig.1.2). A) transcorneal permeation into the anterior segments, B) non-corneal drug permeation, C) the blood stream drug distribution into anterior segments, D) the anterior segment elimination of drug, E) the aqueous humor drug elimination, F) drug distribution from circulation into the posterior segments, G) intravitreal drug administration, H) vitreous via posterior route drug elimination and I) drug elimination from the vitreous passing by anterior.
Fig. 1.2  Routes of drug kinetics

1.3.1 Topical route of drug administration

Use of eye drops, provide very short contact upon instillation onto the eye surface. This leads to the overall less drug activity; can be made prolonged by altering formulation criteria.
Fig. 1.3  Schematic representations of topical administration

During the minimum time duration of drug on the cornea it penetrates to the epithelium. In case of hydrophobic molecule it remains in the upper layer and is slowly passes over to the stroma and next to the inner segment. After instillation of eye formulation, the maximum concentration is generally reached after half an hour in the anterior segment. From the aq. humor drug enters easily to the iris and ciliary body. Melanin bound depot of drug diffuses drug very slowly to the other cells, causes prolonged drug activity.
1.3.2 Subconjunctival route

Drug penetrates across more permeable sclera than cornea. The sclera is highly permeable to the molecules of protein size. However, carrier to the retina is rather complex, because the drug should cross through the choroid and RPE. For the permeation of hydrophilic compounds RPE is dense barrier than sclera.

1.3.3 Intravitreal route

A small molecule diffuses rapidly into the vitreous while passage of larger cationic molecules is limited [18]. Carrier from the vitreous fluid to the choroid is difficult and tedious because of the barrier of the RPE.

1.4 BARRIERS FOR OCULAR DRUG CARRIER

1.4.1 Eye surface drug loss

Commercially available eye drop volume is normally 25-56 µl. When an eye drop is instilled, cul-de sac may approximately contain a 30µl volume. The excess is rapidly drain out from the conjunctiva to the lacrimal drainage until the tears volume returns to normal volume of 7 µl. The lacrimal turnover is about 1µl/60 sec., excess amount instilled usually flows to the nasolacrimal duct. Systemic absorption of drugs can take place through the lacrimal drainage, nasopharynx, mouth and GIT. The mucosa of nose is more active than conjunctiva in contributing to systemic absorption which is greater than 70-90% of used dose. Additionally short ocular contact time as long as of 5-10 minutes is necessary for minimizing systemic absorption. Also systemic absorption can be lower by instilling lower volume.
1.4.2 Lacrimal fluid barriers

Epithelia of cornea regulate access of hydrophilic drugs from the lacrimal fluid into the eye. Lipophilic drugs have greater permeability in cornea while the water soluble drugs are less. Transcorneal perfusion is the major way of drug issue to the aqueous humor. The conjunctiva has porous epithelium with its surface area twenty times much than cornea.

1.4.3 Blood-eye barriers

This is present in anterior region; consists of the endothelial cells that are present in the uvea. The accessibility of plasma protein and hydrophilic drugs into the aq. humor is prevented here. Blood ocular barrier has two parts as, blood-aq. and blood retina. The integrity of this may be disrupted by inflammation. This causes major drug to reach to the anterior chamber. The existence of posterior hindrances among blood flow and eye is composed of epithelium of retinal pigment and the compact sides of capillaries of retina. Drugs radially get access to the choroide. Retinal distribution is limited by RPE and endothelia. There is high blood perfusion to the choroid but constitutes less fraction of the total circulation in the body. Specific targeting units are required to give access to the retina and choroid of the intravenous or oral drug dose [18, 19].

1.4.4 Drainage by nasolachryma

The nasolachrymal drainage is made up of 3 parts; consists of very secreting units. These are activated by blinking action and variation in temperature. Reflex secretors posses distinct parasympathetic supply of nerve and acts in response to physical as well emotional changes. The distributive part covers the eyelids and round the edges of lid which causes tears to spread onto eye by blinking. This prevents
drying of areas. The secreting units of the nasolachrymal excretory system consist of: the lachrymal puncta, superior, inferior and canaliculi; lachrymal sac and duct. Tears are majorly absorbed by the mucus coverings of the ducts and lachrymal pouch and very less to the nasal passage [20, 21, 22, 23].

Fig. 1.4 Nasolachrymal drainage systems

1.5 OCULAR DRUG ABSORPTION

1.5.1 Corneal absorption

Usually, the tight nodes of corneal epithelium worked as eliminating barrier for low molecular weight molecules. The tidy junction is made up of a belt band. Stroma is hydrophilic, contain mostly water. Due to the relatively open structure, molecules of size up to 500,000 can diffuse in stroma. Stroma is a rate controlling barrier for less hydrophilic drugs.
1.5.2 **Absorption via other than cornea**

Drug absorption from other than corneal route involves passage through bulbar conjunctiva and beneath sclera into posterior segments.

1.5.2.1 **Conjunctiva**

Superficial conjunctival epithelium prevents drug penetration through conjunctiva. Intercellular voids in the epithelium of conjunctiva are broader than the corneal epithelium. The cut off molecular size for this penetration is about 20000 to 40000 [24, 25]. The bulbar conjunctiva is initial hindrance for permeation of local medicine via this route [26].

1.5.2.2 **Sclera**

A topically instilled drug penetrates and crosses the sclera through peri-vascular voids, through the aq. media of muco-polysaccharides or through empty voids within the collagen. Sclera is more permeable comparatively.

1.5.3 **Physicochemical drug properties**

Hydrophobic drugs prefer the transcellular route. Paracellular pathway is suitable for hydrophilic drugs; involves passive and/or altered diffusion. Hydrophobicity, water solubilizing capacity, size of molecule, its shape, present charge and ionization decides the route and extent of permeation. Permeation of an ionizable drug depends on the balance of equilibria between the ionized and nonionized drug in formulation. Charge of the molecule limits corneal penetration. Above isoelectric point, the corneal epithelium is negatively charged (pI 3.2). Below the isoelectric point the cornea is selective to anions; however, these conditions are too acidic and irritating [19, 27, 28].
1.6 OCULAR DRUG DISTRIBUTION AND ELIMINATION

If drug is instill topically, passes into the eye and crosses the cornea and enters into the aq. humor and later over to the different tissues, i.e., iris-ciliary body, lens, vitreous and choroid-retina. The aq. humor volume is app. 0.3 ml, but ocular Vd is larger due to more than one compartment kinetics. Reported values for volume of distribution are among 0.24 ml and 0.62 ml. Small ranges suggests less tissue distribution because of protein binding in aqueous humor. Ocular clearance ranges from 4.7µl/min to 15µl/min. Aq. humor half-lives of most drugs ranges from 0.8-3.0 hr, which is longer than aq. humor turnover rate (t_{1/2}0.77 hr). Aq. humor half-lives may be sustained due to binding of drug to specific tissues. Efflux of drug takes place from these tissue depots [19, 23 27].

1.7 FORMULATION CONSIDERATION

Development of ophthalmic preparations must satisfy stability, sterility, tolerance and efficacy simultaneously. No sign of physical or chemical alteration should be detected. The stability may be influenced by the pH of the lachrymal film or the preparation. The stability may be also influenced by the sterility of the preparation. Thus, the use of preservatives or special packaging devices maintaining the sterility of multi dose ophthalmic formulation is required. Another important issue is the ocular tolerance. Comfort to eyes is largely affected by the buffer capacity of the formulations. Eye can bear minor alterations in pH. A wide difference may causes damage to the ocular surface, leading to ocular burning and irritation. Ocular tolerance of aqueous formulation is influenced by the osmolarity. Instillation of more osmotic solutions causes lachrymation a burning sensation and cell desquamation. Hypotonic solution may cause corneal edema. Therapy efficacy is most important for
ophthalmic preparation. It depends on the stability, tolerance of the preparation and on the corneal permeability. The use of absorption promoters also can be used to improve the bioavailability and therapeutic response. These absorption promoters are chemicals that modify transiently the integrity of the corneal epithelium, thus promoting the penetration of drugs through the cornea [29, 30].

1.8 POLYMERIC CARRIER SYSTEMS

The systems made for ocular drug carrier can be grouped into three different groups as viscosity imparting polymers which add viscosity to the formulation, resulting reduced lacrimal fall and improved bio-availability. Mucoadhesive interact with mucin, increases the residence. ISG polymers are those undergo liquid-to-gel phase conversion in response to the physiological changes that occurs inside the eye [31].

1.8.1 Viscosity enhancing polymers

Various polymers are invented and used to improve the consistency of eye drops. Thus prolongs precorneal contact and consequently improving ocular bio-availability of the drug. These are high molecular weight cannot go through biological membranes. In the literature by Patton and Robinson et. al. [32] mentioned that the corneal passage of moiety could be highest at a viscosity of about 15 to 150cP. Polymer solutions generally exhibit either Newtonian or non-Newtonian behavior [33]. Solutions having Newtonian mean the viscosity remains constant at constant conditions (temperature and pressures). While non-Newtonian or pseudo-plastic characteristic means viscosity decreases with increase of shearing, thereby giving considerably less resistance to the movement of the lid and thus show much greater acceptance [34].
1.8.2 Mucoadhesive polymers

Attachment of drug molecule to a biological tissue covered by a mucin is called as mucoadhesion. Being an efficient adhesive, polymers should show strong H₂ bonding, strong negative charge, more molecular weight and chain plasticity, surface energy to facilitate covering on mucusa. Ultimate zero contact angles allows maximum contact with the mucin [35]. The process of mucoadhesion includes sequences of steps. The mucoadhesive formulation should form closed contact with the absorbing surface. Further includes passage of the polymer links to the attaching sites of the tissue surface and entanglement with the mucus chains [36]. These polymers improve the residence of a formulation [27, 37, 38].

1.8.3 ISGS

ISGS are viscous polymer based liq. systems that undergoes liquid-to-gel state conversion onto the eye responding to change in ionic contents, temperature or pH. They can easily be instilled in fluid form and are able to prolong the residing time due to gelling [39]. The benefits of ISGS are the ease, accuracy and reproducibility of administration [38]. The concept of forming gels IS was first documented in the early 1980s, [40]. There are total three mechanisms are available to cause liquid to gel conversion: change in temperature, pH and ionic contents.

1.8.3.1 Ion activation systems

Gelrite is a polysaccharide, in the solution of mono or divalent positive ions forms transparent gels [41]. The concentration of sodium in tears, 2.6g/l is optimum to facilitate gelation of the solution when locally applied on the conjunctival sac [42]. Gelrite is very well tolerated and can be developed as tonic neutral solution with less concentration.
Alginates consist of 1 to 4 linked beeta-D-mannuronic acid and alpha-L guluronic acid. Composition and sequence usually varies. Alginate with a high G content shows improved gelling properties. The alginate forms 3-dimensional matrices, due to interaction of ions with the G moieties results in homogeneous gel. The properties are mechanical strength and porosity. G:M ratios, type of crosslinker, concentration and viscosity were important [23].

1.8.3.2 Temperature sensitive systems

Another approach is the application of a polymer those changes from liquid to gel at temperature of eye (33 to 34°C). PXM are thermal setting polymers in ocular sciences. These are block co-polymers of poly (oxyethylene) and poly (oxypropylene) units. They radially undergo gel formation and remains as a liquid at 4°C. PXM combinations were used to obtained synergism [43].

1.8.3.3 pH triggered systems

These are the polymers that undergo liquid-to-gel conversion due to change in pH such as CAP and branched polyacrylic acid derivates; CP, methacrylates and polycarbophils. Latex remains as a liquid at pH 4.4 and undergoes liquid to gel conversion at pH 7.4 to that of the eye tear. CP has pKa values of 4 to 5, resulting in gel formation due to a rise in pH after ocular administration [44].
1.9 TOLERANCE OF EYE

Usually highly viscous solutions are associated with blurred vision, restrict eyelid motion thereby causes discomfort. Amongst various ISG polymers, gellan gums are known to be very well tolerated by the eye [42, 45]. The PXM are safe, tolerated good and non-toxic. Requires in large amounts (20-30%) obtaining a suitable gel. As the concentration of CP increases, it causes rise in pH of formulations. This rise causes burning sensation. Pseudo-latex of CAP has a low buffering capacity. When instilled as a drop, it will gel into the cul-de-sac. CP has apparent pKa in the pH of 4-5 range such that upon instillation, the immediate rise in pH causes a rapid gelation. However, blurred vision due to more viscous gel and the formation of an opaque matrix by gellan was observed. Also concern data of superficial corneal haze/opacity have been reported in previous study. A similar corneal change was observed with CP [46].
1.10 EYE INFECTIONS

Eyes get infected from bacteria, fungi or viruses in different parts. More general eye infections are conjunctivitis, corneal ulcers and endophthalmitis.

1.10.1 Conjunctivitis

Conjunctivitis is swelling (inflammation) or infection of the membrane lining the eyelids (conjunctiva). The main features are infiltration at cell level and exudation. *Staphy A* is the most common cause of bacterial-conjunctivitis and blepharo-conjunctivitis. Many other organisms like *Haemo. I, Strepto P* also causes conjunctivitis. Conjunctivitis can be classified as (1) infective – acute, subacute and chronic (2) allergic conjunctivitis.

1.10.2 Corneal ulcers/ Keratitis

Inflammation of cornea is characterized by swelling of cornea, infiltration at cell level and ciliary congestion. Cornea is the most anterior lobe of eye; it is focused to atmosphere and hence available to get infected radially. Bacterial corneal ulcers are the most commonly caused by viruses and common bacteria.

1.10.3 Endophthalmitis

It is severe form of intraocular inflammation (purulent uveitis) involving ocular cavities and inner coats of eyeball. Responsible organisms include *Streptococci, E.coli, Pseudomonas* etc. Treatment lines include antivirals, antifungals and antibacterial. The general routein antibacterials used topically in the treatment of ocular infectious diseases include sulfonamides, aminoglycosides, polymyxin-based and FQ.
1.11 THE FLUOROQUINOLONES

The fluoroquinolones represents broad-spectrum antibacterial used against ocular infections caused by a gram -ve and anaerobics. The fluoroquinolones are as effective as or more than other antibiotics used in combinations. FQ are also effective against gram +ve organisms, including *Strepto.* and *Staphy.*

1.11.1 Mechanism of action of fluoroquinolones

FQ act by inhibiting 2 enzymes involved in bacterial DNA synthesis, both of which are di-nucleotide adenosine topoisomerase, are essential for bacterial DNA replication [47]. Topoisomerase IV is responsible for removing the bridging of chromosomes (daughter) thus, permitting separation into two daughter cells at the end of a circle of replication. FQ interact with the enzyme-bound di-nucleotide adenosine complex to create structural modifications that ends in the inhibition of routine enzyme functions [48].

1.12 OCULAR PHARMACOKINETICS

Human ocular pharmacokinetics experimentation is limited to the nonoperative procedures of using fluorescent or gamma-scintigraphic probes. Also involves determination of drug concentration in aqueous humor specimens from patients under cataract treatment. In these cases the anterior chamber is open. This latter method is not practiced very frequently and has serious ethical issues/obligations. Precorneal drainage can be studied in mammals doing tear collection and subsequent measurement of drug levels in the tear. The albino rabbit is by far the most precisely used animal model in ocular studies. This animal model has various advantages including its accessibility, docility, ease of handling, economy and comparatively big ocular surface area. Also availability of supporting literature on ocular effects of chemicals and lack of pigmentation enable better observation of possible hyperemia.
especially of the iris. Although the rabbit and human eye have quiet similarities such as nearly same cornea and aqueous humor composition, the rabbit eye differs from an anatomical and physiological point of view from the mammalian eyes [49].
Table 1.1: Comparison of pharmacokinetic factors between rabbit and human eye

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Pharmacokinetic factor</th>
<th>Human</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bowman’s membrane</td>
<td>Present</td>
<td>Partially absent</td>
</tr>
<tr>
<td>2</td>
<td>Nictitating membrane</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Lacrimal punctum</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Spontaneous blinking rate</td>
<td>6-15 times /hr</td>
<td>4-5 times / hr</td>
</tr>
<tr>
<td>5</td>
<td>Tear volume (μl)</td>
<td>7-30</td>
<td>5-10</td>
</tr>
<tr>
<td>6</td>
<td>Tear turnover rate (μl/min)</td>
<td>0.5-2.2</td>
<td>0.5-0.8</td>
</tr>
<tr>
<td>7</td>
<td>Protein content of tear fluid (%)</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>pH of lacrimal fluids</td>
<td>7.3-7.7</td>
<td>7.3-7.7</td>
</tr>
<tr>
<td>9</td>
<td>Lacrimal volume (μl)</td>
<td>7</td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>Turnover rate of lacrimal fluids (%min)</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td>Buffering capacity of lacrimal fluids</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>12</td>
<td>Osmolarity of tear fluid (mOsm/l)</td>
<td>305</td>
<td>305</td>
</tr>
<tr>
<td>13</td>
<td>Initial drainage rate constant( min⁻¹)</td>
<td>1.6</td>
<td>0.55</td>
</tr>
<tr>
<td>14</td>
<td>pH of aqueous humor</td>
<td>7.1-7.3</td>
<td>8.2</td>
</tr>
<tr>
<td>15</td>
<td>Aq. humor volume (ml)</td>
<td>0.1-0.25</td>
<td>0.25-0.3</td>
</tr>
<tr>
<td>16</td>
<td>Aq. humor turnover rate (μl/min)</td>
<td>2-3</td>
<td>3-4.7</td>
</tr>
<tr>
<td>17</td>
<td>Protein content of aq. humor (mg/ml)</td>
<td>30</td>
<td>0.5</td>
</tr>
<tr>
<td>18</td>
<td>Corneal thickness(mm)</td>
<td>11-12</td>
<td>15</td>
</tr>
<tr>
<td>19</td>
<td>Corneal diameter (mm)</td>
<td>1.04</td>
<td>1.5-2.0</td>
</tr>
<tr>
<td>20</td>
<td>Ratio of conjunctiva and corneal surface</td>
<td>17</td>
<td>9</td>
</tr>
</tbody>
</table>
Preclinical study plays a crucial part in the development of ocular carriers. Clinical studies are limited to non-operative procedures. Rabbits have commonly used as an animal specimen for ophthalmic research, as many pharmacokinetics parameters of the mammal and the rabbit are reasonably similar as given in Table 1.1. Consequently, they have also been chosen as animal model for evaluation of the ISGS. However, before determining the precorneal residence time as well as the in vivo release characteristics of the formulations, it was essential to initially test the developed systems for their ocular irritation potential. Ocular irritation testing is performed according to the procedure described by Draize et al. [50]. This methodology has been widely challenged due to the subjectivity of the grading system and therefore its reproducibility [51]. Following irritation testing, the precorneal retention of the formulations can be evaluated using gamma scintigraphy. Nasolacrimal drainage is one of the major factors causing precorneal drug loss, leading to poor ocular bioavailability. It is also main route for systemic drug absorption with possible systemic side effects and potential toxicity. It is therefore indispensable to test the ISG formulations for their precorneal retention time, which is anticipated to be greater than that of conventional eye drops due to gelation of the formulation on the ocular surface. Various methods are invented to measure the precorneal stability of ophthalmic dosage forms. Amongst one of the procedure involves direct accumulation of the tear fluid, to measure the disappearance of the drug from the precorneal area [52]. However, as the precorneal tear volume less as much as seven to nine μl, sampling of one μl can imbalance the normal tear fluid dynamics. Moreover, the sampling procedure itself is quite difficult and contact of the sampling device (filter paper, capillary tube or cotton swab) with the ocular surface may result in reflex tearing or even injury of the ocular tissues.
Gamma scintigraphy is the most prominent method to measure the precorneal retention of the ISGS, as this technique has been widely and successfully used to monitor the drainage of a diverse range of ocular delivery systems [53]. Literature includes assessment of the precorneal drainage of polyvinyl alcohol (PVA) solutions in rabbits [54]; the retention of various artificial tear products [55]. The residence time of ophthalmic ointments was determined [56]; liposomal formulations were evaluated [57] and w/o microemulsions were studied for their precorneal retention [58]. Various ISGS with different phase transition properties have also been investigated. In an ion-activated ISGS based on alginate/HPMC found that, a solution containing both polymers retained the drug much better than individual solutions of alginate or HPMC [59].
1.13 MANUFACTURING AND PACKAGING

1.13.1 Sterile Manufacturing

In general, ophthalmic formulations are “sterile dosage forms essentially free from particulate matter preferably compounded and packaged for instillation in the eye”. When developing a formulation it is very important to consider general points to establish a manufacturing process more scaleable, reproducible, and cost and time efficient.

✓ The order of addition of the components.

✓ The time (and temperature) required for mixing and type of mixing.

✓ Possible interaction of formulation components with the manufacturing components, including tubing, filters, filter housing, cleaning agents, etc.

✓ Filter choice.

1.14 STABILITY STORAGE AND TESTING

The International Conference on Harmonization (ICH) guidelines the specific conditions include;

✓ Long-term stability testing (25°C/40%RH);

✓ Intermediate accelerated (if 40°C fails) testing (30°C/40% RH) as per FDA or

✓ Intermediate accelerated 30°C/60%RH as per ICH guidelines and;

✓ Accelerated testing at 40°C/15%RH.

For specialized specific products and packaging systems, a well-planned, durable, commercialized stability study procedure should have to be developed. Such that it may properly guides important product properties during usage and storage. To increase the chances of product approval it is important to develop a well-defined stability protocol that aims to address all international, regional and local
requirements that is approved by the regulatory authorities prior to start of stability studies.

1.15 PACKAGING

Packaging of ophthalmic formulations is very important. The shelf-life of a product is solely based on packaging choice in maximum conditions. The vast majority of ophthalmic formulations excluding the injectables and specialized carrier systems are packaged in polyolefin containers preferably:

- High-density PE (HDPE),
- LDPE (low-density polyethylene),
- Polypropylene (PP),
- Polyethylene terephthalate (PET)

Topical eye drops are typically packaged in 5 to 15 mL LDPE or HDPE bottles with tips that can be of linear low-density polyethylene (LLDPE) or HDPE or PP and caps that are usually HDPE or PP. LDPE is generally preferred for eye drop bottles because of their suitability and ease with which a drop can be dispensed. The quality of the product may be affected by additives/excipients in the polymer which may interact with formulation components such as, binding of preservatives and API, formation of insoluble complexes resulting in haze or opacity over time. They may appear later as contaminants in the form of extractable and leachable. Extractable and leachable may also be contributed by labels and secondary packaging components such as cartons and package leaflets. The FDA is strict about the presence of extractable and leachable in ophthalmic products. To assure the highest quality of the product, bottles have some form of tamper evident or proof seal. All primary packaging components must be sterile.
Sterilization of plastics such as LDPE and PP typically may be by ethylene oxide vapors or by γ irradiation (HDPE, LLDPE). The sterilization method for packaging materials must be routinely validated. In BFS or FFS, operations product is filled into the bottle as it is being formed in a sterile environment. Because of the high temperature of the polymer as it is molded, it is supposed to be sterile and no further sterilization of the finished product is generally required. The dropper tips may be molded as part of the operation or separate preformed, presterilized tips may be inserted followed by capping [17].