### CHAPTER 1

**Chapter 1: Introduction**

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CHAPTER 1

INTRODUCTION

1.1 Introduction:

Inflammation is the indication of cellular and vascular response of the tissue to injury, which may be caused by physical agents, chemical agents, ischemia, invasion of pathogens and hypersensitivity or autoimmunity mechanisms. Inflammations make ease the immune response for the removal of antigenic material and damaged tissue. Topical application of drugs is most preferred route for effective management of inflammation, as it provides high ocular drug concentration and avoids possible systemic adverse affects associated with oral administration. Because of physiologic restrictions of eye, only few anti-inflammatory agents can be successfully formulated in to suitable ophthalmic dosage form for effective management of ocular inflammations [1].

Despite the accessibility of the eye and the easy application of topical dosage forms, the delivery pathways of drugs across ocular tissues is undoubtedly a complicated process in which drugs encounter many barriers. These barriers hinder drugs delivery to the targeted tissue through the most convenient route i.e. the trans-corneal route [2]. To figure out the barriers and how to improve ocular drug delivery, it is crucially important to be familiar with the structure of the eye.

1.2 Human Eye Structure:

The human eye is composed of two different sized anterior and posterior spherical segments that are linked by limbus (a circular tissue) [3]. A diagrammatic representation of the main parts of the human eye is presented in Figure 1.1. In these two, anterior segment is the smaller (one-sixth of the whole eye, 8 mm radius) with an outer focal component that is a multilayer transparent tissue termed as the cornea and posterior segment is the larger (12 mm radius) with an outer white opaque collagen layer called the sclera. The eye weight in a human adult is approximately 7.5 g, has a volume of 6.5 cm³ and a 24 mm vertical diameter which is usually less than the horizontal diameter [4].

Both anterior and posterior chambers are filled with solution called aqueous humour (anterior: zone between cornea and iris, posterior: zone between iris and lens), whereas a clear gel filling the vitreous chamber (zone between retina and lens).
The anterior chamber mainly consists of cornea, iris, ciliary body and the lens while the posterior part consists of sclera, choroid and the retina. The retina (inner component of the posterior segment) consists mainly of light sensitive photocells which are responsible for converting observed light to electrical signals [5, 6].

### 1.2.1 Anterior Eye Segment:

#### 1.2.1.1 The Conjunctiva:

![Figure 1.1 Human eye structure and main components.](image)

The conjunctiva is a clear vascularised mucous membrane covering eyeball’s anterior surface and eyelids inner surface (Figure 1.1). The conjunctiva can split into three morphologically distinct areas: bulbar (covering the anterior side of the eyeball), palpebral (covering the inside of the eyelids) and fomix or cul-de-sac (a transition zone between the bulbar and the palpebral conjunctivas) [7]. The conjunctival tissue consists of 2 to 3 layers of epithelial cells with tight junctions only on the apical surface layer and cuboidal basal cells. The conjunctival tissue also contains mucous goblet cells which are responsible for secreting the mucus required for the stability of
tear film and corneal transparency. This tissue has a rich supply of blood vessels and plays a significant role in systemic clearance of topically applied formulations [8].

1.2.1.2 The Cornea:

It is an avascular translucent tissue covering approximately 15% of the surface area of the eye. It is primarily responsible for focusing light and protecting internal ocular tissues from xenobiotics (foreign materials). As it is devoid of vasculature the corneal tissue receives nourishment from the tear film, aqueous humour and limbal vessels [9]. The cornea in an adult human has an average dimension of 11.5 mm horizontally, 10.5 mm vertically [10] and an average central corneal thickness of 0.53 mm.

It is composed of five distinct layers (Figure 1.2) namely, corneal epithelium (the outer layer), corneal endothelium, Bowman's layer, Descemet's membrane, corneal and stroma [11]. The epithelium layer consists of 5-7 layers of superficial, wing and basal epithelial cells that form a main barrier to ophthalmic topical formulation because of tight juncture between the cells; especially in the superficial cells [2]. The thickness of the epithelial layer is approximately 50 μm [11]. The stroma mainly resides of an extracellular matrix, stromal cells and approximately 4% glycosaminoglycans [12] with an approximate thickness of 500 μm which accounts for >90% corneal thickness. The endothelium layer consists of a monolayer of cuboidal cells with an approximate thickness of 5 μm [9]. The prime function of this cuboidal cell monolayer is to regulate fluid and solute transport between aqueous humour and corneal stroma. In comparison to the corneal epithelium the endothelium cells do not regenerate and upon cell death in the endothelium layer the cells flatten and enlarge in volume, covering the area occupied by the dead cell.

1.2.1.3 Aqueous Humour:

It is a clear fluid filling the anterior inner space of the eyeball (approximately 250 μL); it exists in the anterior and posterior chambers. The aqueous humour has a pH of 7.2 and it contains small amount of proteins, glucose, various ions, high levels of ascorbic acid and lactic acid in comparison to the blood plasma [13]. Both cornea and lens were nourished by this fluid and give the cornea (front of the eye) its shape. The aqueous humour culvert out of the anterior segment by the trabecular meshwork.
(spongy tissue near the ciliary body) through a set of channels called Schlemm’s canal to the episcleral venous system. The aqueous humour turnover and drainage (2.75 μL. min⁻¹) plays a prime role in the clearance of instilled topical formulations [14].

Figure 1.2 Cross sectional view of the corneal tissue depicting the five layers of the cornea.

1.2.1.4 The Iris and Ciliary Body:

The posterior and anterior segments were divided by iris, which is circular tissue and regulates the extent of light entering the eye and divides the anterior and posterior chambers (Figure 1.1). The ciliary body is mainly responsible for aqueous humour production and for changing the structure of the lens through the zonule of Zinn (connective tissue)[14].

1.2.2 Posterior Segment:

1.2.2.1 The Sclera:

The sclera is a vascular, tough and opaque white-yellow protective layer consisting of collagen, elastic fibers and proteoglycan [15]. Its importance is to maintain the shape of eyeball and to provide resistance to internal and external force; and it is composed of four layers; 1) Tenon’s capsule, 2) episclera, 3) scleral stroma and 4) lamina fusca layers. The Tenon’s capsule layer is a hypocellular layer which contains arranged compact collagen bundles. The episcleral layer is a well-
vascularised layer which contains elastic tissue and macrophages. The scleral stroma has interwoven dense collagen fiber bundles and lamellae with scattered elastic fibers present within. The deepest layer of the sclera (lamina fusca) has collagen bundles smaller than those that exist in the scleral stroma and branch extensively to blend with the underlying choroidal tissue. This layer has a noticeable increase in cell numbers (sclerocytes and melanocytes) in comparison to the scleral stroma [15].

The sclera covers approximately 80% of the eye surface and varies in thickness. The thickness of sclera in an adult human is thickest at the posterior pole (1-1.18 mm), decreasing progressively to the equator (0.4-0.6 mm) and progressively increasing towards the limbs (0.8 mm). The thinnest sclera part is immediately behind the recti muscles (0.3 mm). Besides not having epithelium and endothelium cell layers, the scleral tissue differs primarily from the corneal tissue in the uniformity of the arrangement of the collagen fibers and the degree of hydration [15]. The sclera has irregular collagen fibers when correlated to the cornea (scleral collagen fibrils diameter 25-230 nm, corneal collagen fibrils diameter approximately 25 nm) and a fourfold lower concentration of proteoglycans, which results in a lower water content (68% in comparison to 78% in the corneal stroma). Additionally the sclera is perforated by blood vessels and has an extensive nerve supply.

1.2.2.2 The Choroid:

The choroid along with ciliary body and iris form the vascular middle layer of the eyeball. The choroid lies in between the sclera and retina and is dull reddish brown in colour. The choroid thickness in human eyes is approximately 220 μm at the posterior side and 100 μm at the anterior. Within this vascular layer, large vessels are located near the sclera (vascular lamina) and extended smaller vessels (choriocapillaris) are pressed against the light sensitive layer of the retina. These capillaries provide nourishment and oxygen to the outermost layers of the retina [16].

1.2.2.3 The Retina:

The deepest layer of the posterior chamber of the eyeball consists of the retina (approximately 32 mm diameter) and it is a complex part of the eye. The retina consists of ten layers which are depicted in Figure 1.3. The retinal tissue is bifurcated into two parts namely, retinal pigmented epithelium (RPE) and neural retina (the rest
of the cell layers). The RPE is a tight barrier separating the neural retina from choroidal circulation. It nourishes the retina and removes metabolic end products from the retina [5].

The deepest layer (inner limiting membrane) of the retina is having connection with the vitreous body, while the outermost layer (RPE) is having connection with the choroid. Thickness of the retina across mammalian species is around 217 μm [17]. In humans the retina is very lean at fovea centre (approximately 178 μm) and thickest at the foveal rim (approximately 320 μm), after the fovea the retina progressively thins until the equator (approximately 120 μm). The retina is packed with photosensitive cells called rods and cones (photoreceptor outer segment in Figure 1.3). There are three kinds of cone cells each responding to green, red and blue. Rod cells function at dark light and are sensitive to light but insensitive to colour.

An immensely pigmented yellow spot called macula is present in the vicinity the centre of retina. Close to the macula centre is the fovea, the fovea is a small pit (5-6 mm in diameter) [4] dominated by tightly packed cone cells responsible for central vision. The peripheral part of retina consists of rod cells responsible for nocturnal vision. The retina is the one of the most metabolically active tissue in the human body. Therefore its functional integrity relies upon on nutrients supply and the expulsion of waste. This is achieved through the inner retinal blood and choroidal circulations.

1.2.2.4 Vitreous Humour:

The vitreous humour is an acellular (apart from a few phagocyte cells) transparent gel (approximately 4 mL) that fills a gap between the lens and the retina (vitreous chamber). The constituents of the vitreous body are approximately 98-99% water (the water percentage increases with age), collagen and natural macromolecules like hyaluronic acid (HA). The collagen confers the gel-like property of the vitreous, whereas the HA stabilizes the collagen network. Unlike the aqueous humour which is continuously secreted and drained, the vitreous humour is undynamic. Therefore, any haemorrhage and inflammation in the vitreous cavity will cause vision obstruction and surgical intervention might be required to remove the debris (blood and cells). The vitreous humour is targeted as a drug depot for retinal drug delivery.
1.3 Common eye disorders:

There are many diseases concerned with the eye varying in severity, prevalence and in duration, some of them are discussed briefly in the following sections [18].

1.3.1 Inflammatory conditions:

- **Hordeolum and Chalazion**: Chalazia and hordeola (styes) are ocular inflammatory diseases which causes swellings of the eyelids. A non-infectious meibomian gland blockage causes chalazion and a hordeolum is generally due to infection. Both these conditions primarily cause hyperemia, edema, swelling and pain in eyelids. With time chalazion becomes a little non painful bud in centre of eyelid where as hordeolum have pain and localizes to an eyelid margin.

- **Endophthalmitis**: Is an inflammatory condition of the internal ocular structure caused by bacteria or fungal microorganisms. It often occurs due to complications
in intraocular surgery, particularly cataract surgery or intravitreal injections. Endophthalmitis is often having severe pain and in severe cases it can result in vision loss [19]. Endophthalmitis can be treated using antibiotic (intravitreal or topical) and corticosteroids (intravitreal or topical) reducing the inflammation and swelling to the damaged tissues [20].

- **Blepharitis**: Is a chronic eyelids and/or eyelashes inflammation, which may be due to, poor eyelid hygiene, excess oil production by the meibomian gland, a bacterial infection or an allergic reaction.

- **Dacryocystitis**: It is a lacrimal sac infection, secondary to nasolacrimal duct blockage at the lacrimal sac junction. It causes pain, redness, and swelling over the inner aspect of the lower eyelid and epiphora. When obstruction of nasolacrimal duct is secondary to a congenital barrier, it is known as dacrocystocele and commonly caused by *Staphylococcus aureus* and *Streptococcus pneumoniae*.

- **Conjunctivitis**: It is swelling of the thin and clear layer covering the surfaces of inner eyelid and front of eyeball. It is commonly because of an infection (usually viral, but sometimes bacterial) or an allergic reaction.

- **Trachoma**: Trachoma or granular conjunctivitis is an ocular infection caused by *Chlamydia trachomatis* and leads to roughening of the deeper surface of the eyelids. This roughening causes eye pain, corneal or eyes outer surface break down leading blindness.

- **Anterior Uveitis or Iritis**: Is swelling of the median layer of eye, which includes ciliary body.

1.3.2 **Corneal ulcer**: a lesion and loss of tissue of the cornea resulting from an infection.

1.3.3 **Glaucoma**: It is a condition where the eye trabecular meshwork becomes slightly blocked resulting in raised intraocular pressure (IOP). Untreated glaucoma leads to optic nerve damage. There are two varieties of glaucoma (chronic and acute glaucoma), both types can be considered as a type of optic neuropathy. The elevated IOP can be decreased by laser treatment, surgery and/or by the use of drugs. The drugs work by promoting the aqueous humour flow (prostaglandin analogues e.g. tafluprost), decreasing the aqueous humour production (beta-blockers and carbonic
anhydrase inhibitors) or opening up the blocked trabecular meshwork (miotics e.g. pilocarpine) [21].

1.3.4 Diabetic Macular degeneration: A change that occurs in the blood vessels which nourishes retina causes vision blurriness and distortion associated with diabetes [22].

1.3.5 Dry eye: Is a condition where there is an inadequate production of tears, which causes eye irritation, burning and discomfort.

1.3.6 Macular degeneration: Is a condition where changes in “macula” resulting in a gradual loss of central vision: objects appear distorted, colour vision weakens and a dark area appears at the centre of vision.

1.3.7 Neovascularization: Tiny blood vessel growth from conjunctiva to cornea; most often caused by inappropriate or over-wear of contact lenses.

1.3.8 Pinguecula: A slightly raised, benign, whitish thickening on either side of the cornea, caused by over exposure to UV radiation.

1.3.9 Ptergium: A conjunctival growth over the cornea which is due to irritation or dryness of eye by wind and sand or excess exposure to UV radiation.

1.3.10 Retinal detachment: Separation of the retina from the deeper layers of the eye, caused by holes or tears in the retina, or fluid pressure in the area, or by a tumour.

1.3.11 Retinitis pigmentosa (RP): A group of inherited diseases developing inside the pigmented area of the retina. Vision changes comprise night blindness, loss of side vision and “tunnel vision.”

1.4 Ocular Drug Delivery Routes:

There are four main drug delivery channels to eye; namely topical, systemic, transscleral and intravitreal which used to treat various eye diseases [23]. A summary of the four drug delivery channels is illustrated in Figure 1.4. The most effective and convenient route of ophthalmic delivery of drugs to the anterior segment is the topical route (section 1.3.3.1) [24]. But this is not the effective channel for delivery of drug to the posterior segment due to the obstructive nature of the eye barriers toward xenobiotics (foreign materials).

1.4.1 Topical Drug Delivery:

This is the most convenient route, due to the accessibility of the eye and ease of instilling the dosage form. However, this route suffers from many disadvantages
such as vision distortion, systemic clearance and frequent application that may cause inconvenience and disruption to the patient’s daily life [24]. In addition, it is an ineffective route for chronic and posterior segment diseases due to the short drug contact time with the cornea (precorneal barriers). In addition to the corneal barrier, the aqueous humour circulation and the long distance between the anterior part and the retina (approximately 23 mm) are some of the main barriers which are limiting the access of topically applied drugs to posterior segments of the eye [25] which causes less bioavailability.

1.4.2 Systemic Drug Delivery:

Ophthalmic drugs delivered through the systemic route are generally delivered via intravenous injection (e.g. Visudyne®, Novartis AG) [26]. This route is the least desirable route in ophthalmology due to the high drug concentrations needed to overcome the blood retinal barriers and due to the toxicity that might occur in other organs in the body [24].

1.4.3 Transscleral Drug Delivery:

At present, scleral drug delivery for various posterior disorders is attracting attention among academics and the pharmaceutical industry. The transscleral delivery route offers many advantages over other routes such as the corneal route: relative high scleral surface area (potential drug depot), high degree of hydration (enhanced diffusion of hydrophilic molecules), metabolically inactive and relatively high permeability to macromolecules [27, 28, and 29].
Drug delivery through the scleral route is normally achieved via periocular injections. Periocular injections (subtenon, subconjunctival, peribulbar and retrobulbar) are administered in the cavities around the eye or under the conjunctiva [30]. Figure 1.5 illustrates the periocular injection positions around the eye. Periocular injections provide high retinal and vitreous humour drug levels (approximately 0.01–0.1% of the applied dose) in comparison to topically administered eye drop (approximately 0.001% or less).

Sub conjunctival injections (volume up to 500 μL) are used to deliver drug to the anterior segment (through systemic circulation, trans-corneal and transscleral diffusion) and posterior segment of the eye (through transscleral route).

The subtenon (1-5 mL), peribulbar (8-10 mL), retrobulbar (2-3 mL) and posterior juxtascleral (0.5 mL) injections are mainly used to deliver injected drug to the posterior part of the eye through the transscleral route. These injections allow the dosing of large volumes and can be repeated as necessary [31] if required as per the patient need and condition and should be monitored very carefully.

1.4.4 Intravitreal Drug Delivery:

The intravitreal injection (maximum 0.2 mL volume) penetrates the sclera, choroid and retina, this route delivers the drugs directly to the vitreous humour (Figure 1.5). However, it is associated with many disadvantages such as endophthalmitis, retinal detachment, haemorrhage and the need for repetitive treatments. The puncture point of the needle is usually at the par planar which about 1.5 mm from the limbus is. The par planar is the ideal point for intravitreal injections and implants as the peripheral retina is less vascular at this point and it enables the needle to deliver the drug/implant deep in the vitreous humour. Despite all the associated disadvantages of the intravitreal technique, it is the most prevalent route for delivery of drugs to the posterior segment [32].

1.5 The Ocular Physiological Barriers:

In order to know the challenges and formidable obstacles facing delivery of drugs to the anterior and posterior segments of the human eye, the eyes physiological barriers must be explained. The next two sections (1.5.1 and 1.5.2) describe the precorneal and corneal barriers that any drug applied to the anterior segment of the
eye must overcome, whereas sections 1.5.3 detail the conjunctival and systemic barriers associated with drug delivery to the posterior segment of the eye.

**Figure 1.5 Schematic representations of periocular routes of administration [30].**

1.5.1 Lacrimal Fluid and Eye Movement (Precorneal) Barriers:

The tear film and eye lids are the first defensive barrier against any foreign materials entering the eye. Tear film (lacrimal fluid) is composed of three layers namely, lipid, aqueous and mucin, it has an approximate 3 μm thickness, 6-8 μL volume and 1.2 μL min⁻¹ turnover volume. The lipid layer is the superficial layer consists of wax esters, diglycerides, triglycerides and hydrocarbons. This layer reduces the surface friction related to blinking and eye movement and avoids the aqueous layer evaporation [31]. The aqueous layer consists of mainly water, proteins, glycoprotein, electrolytes and peptides. The mucin layer consists of glycoproteins which prevent bacterial adhesion, provide lubrication and function as antioxidants. The eye cul-de-sac can accommodate approximately 30 μL of administered drug without overflow. Drugs applied topically to the surface of the eye (typical eye drop volume 25-56 μL) [33] are subjected to rapid elimination via the lacrimal fluid and eye lid movement. The lacrimal drainage shows a quick restoration time (typically 2–3 min) to its original volume (6-8 μL) and the majority of the topically administered dose is washed away within just 15-30 Sec. after instillation due to lacrimal turn over, blinking (every 2-10 Sec. and induced lacrimation [31].
1.5.2 Corneal and Conjunctival Barriers:

Topically applied drugs can penetrate by two routes, namely the corneal and non-corneal pathways. The non-corneal pathways involve drug penetration through the conjunctival and scleral tissues before permeation into the inner intraocular tissues [31]. Drugs diffusing through the corneal route encounter the lipophilic corneal epithelial and endothelial layers and the hydrophilic stroma layer [34]. The epithelium and endothelium cell layers contain a 100-fold higher lipid content per unit mass than the stroma of cornea and the tight junctions in apical corneal epithelial cells (the estimated intercellular pore size is 60 Å or less) [35]. For corneal diffusion of hydrophilic and small ionic substance epithelial layer is the main rate limiting barrier, due to the presence of tight junctions which retard paracellular drug permeation of these molecules [36]. The endothelium layer is not a rate limiting barrier as it is approximately 200 fold more permeable than epithelium. After the epithelial layer drugs encounter the stroma (500 μm thick) this is hydrophilic in nature and acts as a main barrier for highly lipophilic drugs [25].

The conjunctiva has 5-15 layers of epithelia cells with tight junctions at the pointing end. Although the conjunctival tissue has more epithelia cell layers than the corneal tissue, it has larger pores and sixteen times greater pore density than the cornea [37]. Hence, the conjunctiva is more leaky and permeable to hydrophilic and large drugs than the cornea and allows drugs to diffuse through both paracellular and trans-cellular routes [38]. In humans, the conjunctiva possesses a relatively larger surface area (16-18 cm²) in comparison to the cornea (1 cm²). Due to this large surface area, the high vascularisation and lymphatic’s of the conjunctiva, topically applied drugs are quickly cleared into the blood circulation resulting in systemic distribution of the drug away from the eye [23].

The precorneal barriers and the short drug-cornea contact time are primary reason for the low percentage (typically 1%) of the topically administered drug to reach the intraocular tissues [39].

1.5.3 Blood Retinal Barriers:

This barrier affects drug delivery targeting the posterior part of the eye. Passive diffusion of materials into the retinal tissue is restricted by the blood retinal barrier (BRB) [40]. The BRB can be bifurcated into an inner and an outer BRB as
depicted in Figure 1.6. The inner BRB is located near the vitreous humour and is formed by endothelial capillary cells which have tight junctions. The outer BRB consists of the RPE and choroidal circulation.

The inner 2/3rd of the retina obtains nutrients from the retinal blood vessels which branch from the ophthalmic artery (inner BRB). These blood vessels are not fenestrated and have tight cellular junctions in between capillary endothelial cells of retinal. Remaining part retina obtains its nutrients from the choroidal circulation (outer BRB). The choroidal capillaries (choriocapillaries) have fenestrations of roughly 60 nm in diameter. They are leaky and relatively permeable allowing the exchange of waste products and nutrients [31].

**Figure 1.6 Schematic presentation of the inner and the outer blood–retinal barrier (BRB).**

Despite the high blood circulation to the retina and choroid (the average rate of the entire retina blood flow is 80 mL min⁻¹) the choroidal flow is ten times the retinal blood flow [31]. The tight junctions in the RPE and in the retinal capillaries restrict the motion of many substances and form a formidable barrier for drugs administrated systemically or periocularly (injections in the cavities between the eyeball and the eye orbit, Figure 1.5) [25].

1.6 Ocular Drug Permeation Pathways:
1.6.1 Anterior Segment:

Transport of drugs across corneal epithelial cells occurs via two pathways namely, trans-cellular and paracellular. The trans-cellular involves cell membrane diffusion, channel diffusion and carrier-mediated transport through the cells. The paracellular involves diffusion through intercellular spaces and tight junctions. The passive trans-cellular pathway, in which molecules emit through the lipid matrix of
the epithelial cells membrane, is believed to be the major mechanism of corneal absorption of topically administered ocular drugs [42]. The molecules ability to diffuse across epithelial cells through the trans-cellular pathway depends upon the molecules interaction with the constituents of the cell plasma membrane (lipids, cell receptors). The partitioning of molecules into the cornea is a crucial step to enter the cell. Consequently, the K o/w (partition coefficient) is a major factor in determining the flow across epithelium and stroma. A parabolic relationship between K o/w and the corneal permeability has been reported for many molecules [43, 44 and 45]. As a result of the lipophilic and hydrophilic nature of the corneal barriers (corneal epithelium and stroma), the optimal corneal permeability was reported to be in the range of 1-3 log partition coefficient [23, 35 and 44] indicating the absorption of neither extremely hydrophilic nor extremely lipophilic compounds is favored. Nonetheless, other factors such as molecular size and charge influence the corneal permeability [35].

The paracellular route is the preferred route of diffusion for small hydrophilic molecules across the corneal epithelium. However, the existence of three forms of junctional complexes namely, tight junctions (zonulaoccludens), intermediate junctions and spot desmosomes hinders the transport of the hydrophilic molecules [42].

The two dominant barriers encountered in topical ophthalmic delivery are the precorneal short residence time and the poor permeability across of the cornea. Prolonged ocular residence time is achieved using inserts, bioadhesives and via vehicle modification. Poor corneal permeability of drugs can be overcome by altering the integrity of the corneal epithelium either by using chemical penetration enhancers or physical enhancement techniques (e.g. iontophoresis) or by altering the physicochemical properties of the drug (prodrug).

Ocular penetration enhancers used topically affect either the paracellular or trans-cellular route. The paracellular enhancers alter reversibly the epithelial tight junction, increasing paracellular transport. Many ocular paracellular enhancers were reported in the literature, most commonly calcium chelating agents and cytochalasins (group of small molecules which bind to the actin microfilaments rendering the permeability of the tight junctions). The chemical trans-cellular enhancers disrupt the
cell membrane lipids and protein components, increasing drug trans-cellular permeability [23, 46].

1.5.2 Posterior Segment:

As scleral tissue is acellular and does not have epithelium and endothelium cell layers, the trans-cellular drug permeation pathway is absent. Scleral drug permeation can occur via the perivascular and the collagen network spaces and via the aqueous media of the mucopolysaccharides [24]. Due to the framework of scleral tissue (mainly the absence of the tight junctions), large molecules can diffuse passively via the tissue. Molecules of size up to 120 kDa were reported to diffuse into human sclera [47]. Since the porous nature of the sclera and the passive diffusion of penetrants between the free spaces of the collagen bundles, scleral drug permeability is influenced by drugs lipophilicity and solubility [48]. A converse correlation between the drug lipophilicity of six corticosteroids (triamcinolone, prednisolone, dexamethasone, fluocinoloneacetonide, triamcinolone acetonide, and budesonide) and scleral transport of bovine was reported [48]. The latter study also reported positive correlation between drug solubility and tissue concentration, which affected free drug levels and scleral drug transport. Similar to the cornea, the drug diffusion across the sclera can be enhanced using the prodrug approach. [49].

![Image of transcellular and paracellular pathways for drug transport across membrane.](Image)

The alteration of the BRB permeability is another method to enhance posterior drug delivery. The BRB permeability can be changed using a hyperosmotic solution (e.g. mannitol) which prompts shrinkage of the RPE cells and retinal capillaries.
ultimately opening the cellular tight junctions [23]. However, the aforesaid method is non-specific, prompting shrinkage of all blood capillaries and hence rendering them more permeable, making this method unsuitable for specifically increasing ophthalmic drug delivery.

1.6. 3 Factors affecting corneal transport:

Three factors are considered responsible for determining the transport efficiency of a particular drug species through the corneal membrane.

1) The physiochemical properties of drug substance (e.g., ionization constant, aqueous solubility, oil/water partition coefficient).
2) The dosage form in which drug is prepared (e.g., pH of solution, types and concentration of buffers, viscosity inducing agents and stabilizers).
3) The corneal structure and integrity.

Figure 1.8 Schematic Illustration of the Ocular Disposition of Topically Applied Formulations

1.7. Drawback of traditional ophthalmic formulations [50]:

1. They suffers with poor systemic availability because of
   a. Rapid precorneal elimination
b. Conjunctival absorption

c. Solution drainage by gravity

d. Induced lacrimation

e. Normal tear turnover

2. Frequent instillation of ophthalmic drops is necessary to achieve a therapeutic effect.

3. Undesirable adverse events because of drugs systemic availability and drainage of additives through nasolacrimal duct.

4. During external application, the instilled drops are not uniform at all times and those delivers usually not correct. Due these reasons the amount of drug delivered may vary.

5. Presence of viscous vehicles may cause blurred vision.

1.8 Novel ophthalmic delivery systems:

To conquer the draw backs of conventional ocular dosage form, many progresses have been done to improve the pre-corneal drug absorption and minimize pre-corneal drug loss.

1.8.1 Mucoadhesives:

Mucoadhesives will be retained in the eye with the aid of non-covalent interactions established with the corneal conjunctival mucin for extending the pre-ocular residence time [51, 52].

1.8.2 Phase transition system:

These are dosage forms which change their physical state from liquid to gel or solid when applied in the cul-de-sac. After converting in to gel it remains in contact with the cornea for prolonged period of time due to which drug elimination is reduced [53, 54]

1.8.3 Niosomes:

Niosomes are the vesicles, containing non-ionic surfactants that can encapsulate hydrophilic/lipophilic drug substances either in aqueous layer or in lipoidal vesicular membrane [55]. It helps in preventing the metabolism of the drug by the enzymes present at the tear/corneal surface [56].

1.8.4 Liposomes:

Liposomes are microscopic vesicles composed of membrane like lipid layers
surrounding aqueous compartments. The lipid layers are comprised mainly of phosphorolipids [57]. Both hydrophilic and lipophilic drugs can be entrapped in the aqueous compartment and in the lipid bilayers respectively [58].

1.8.5 Nanoparticles:
Nanoparticles are solid particles of polymeric nature ranging in size from 10-1000 nm. The drugs are bound to small particles, which are then dispersed into aqueous vehicle [59]. Due to very small in size these are not washed away with tears quickly [60].

1.8.6 Contact lenses:
Contact lenses are substitutes for spectacles and are enjoying a certain degree of popularities. Use of soft lenses soaked in drug solution has been suggested for slow and prolonged drug delivery but particularly to corneal tissue [61].

1.8.7 Pharmacosomes:
Pharmacosomes are the vesicles formed by the amphiphilic drugs. Any drug possessing a free –COOH group can be esterified to the –OH group of a lipid molecule thus making an amphiphilic prodrug. These are converted to pharmacosomes on dilution with tear [62].

1.8.8 Ophthalmic inserts:
Inserts are slender disks or tiny cylinders made with suitable polymers and placed into the lower or upper conjunctival sac. Their long residence in preocular region can result in increased drug availability with respect to liquid and semisolid formulation [63].

1.9. Requisites of controlled ocular delivery systems [50]:
1. To conquer the adverse effects of pulsed dosing (frequent dosing and high concentration) of conventional systems.
2. To provide sustained and controlled drug delivery.
3. To enhance the ocular availability of drug by increasing corneal residence time.
   This can be done by effective coating or adherence to corneal surface, so that the released drug effectively reaches the anterior chamber.
4. Targeting the drug within the ocular globe thereby restricting the loss to other ocular tissues.
5. To overcome the ocular barriers like lacrimation, drainage and diversion of
exogenous substances into the systemic circulation by the conjunctiva.

6. Better patient compliance by having better therapeutic effect than conventional dosage forms.

7. To provide a better housing for the delivery system in the eye so as the drug loss to other tissues besides cornea is prevented.

1.10. Approaches to ophthalmic drug delivery:

Three factors have to be reviewed in developing the ophthalmic dosage form. Firstly, how to cross the blood eye barrier (systemic to ocular) or cornea (external to ocular) to reach the site of action; secondly, localizing the pharmacological action of drug at required site and finally, how to extended the duration of drug action so that the frequent drug administration is minimized [64].

1.11. Nano Size Drug Delivery Systems:

The European commission define nano-materials as “A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.”. However, the range of nano-size drug delivery systems in nano-pharmaceuticals as per the European Science Foundation ranges from 1-1000 nm. Nano-size drug delivery systems, broadly referred to as nanocarriers, have a number of exploitable properties for drug delivery, due to their small relative size such as, high surface area: volume ratio, the effects of Brownian motion and low settling velocity [65]. Aqueous nano-size drug delivery systems below 1000 nm in size have higher Brownian motion velocity (a 1000 nm particle has a speed of 1716 nm.s⁻¹) than the settling velocity (a 1000 nm particle has a speed of 430 nm.s⁻¹) and hence the colloidal solutions are stable and have a long shelf-life [65]. Colloidal solutions precipitate upon agglomeration or the formation of particles larger than 2 μm.

1.11.1 Nanoparticles in Ocular Drug Delivery:

The use of nano-sized delivery systems is an emerging concept with promising potential to allow ophthalmic drugs to overcome the eye’s physiological barriers by enhancing penetration, prolonging drug residence time and allowing the delivery of high drug concentrations to ocular tissues, with relatively fewer side effects such as
toxicity in comparison to drug suspension and conventional formulations [23, 42]. Nano-size drug delivery systems are of special interest in ophthalmic drug delivery due to their small size (typically 20-500 nm), which not only enables the foreign body sensation in the eye to be avoided but also influences their permeation through ocular barriers. For example the uptake of small PLGA nanospheres (100 nm) was found to be higher than larger particles (800 nm and 10 μm) in primary cultured rabbit conjunctival epithelial cells [66].

![Diagram of nanostructures](image)

**Figure 1.9 Types of nano-size delivery systems used in drug delivery.**

Nano-size drug delivery systems have been reported to enhance drug bioavailability in the eye compared to drug alone solutions/suspensions. For example after topical instillation of an indomethacin chitosan nano-emulsion in rabbits the levels of indomethacin in the aqueous humour were found to be 30 times higher than topical administration of an indomethacin solution [67]. Similarly, the levels of prednisolone in the aqueous humour of a rabbit eye following topical administration of polymeric micelles were similar to those found after administration of a tenfold dose of a commercial suspension of the drug [68]. NDDS not only offer enhanced corneal permeation and high drug entrapment, but can also reduce drug related side
effects. Poly-ε-caprolactone nanocapsules with oily core containing metipranolol evaluated in rabbit’s eyes lowered the cardiovascular side effects in comparison to commercial drops [69].

Due to the high Brownian motion velocity and low settling velocity of NDDS in comparison to microparticles, NDDS can offer prolonged vitreal half-life. NDDS injected in the vitreous humour (static fluid) stays suspended for a prolonged period in comparison to microparticles before coming in contact with the surrounding tissue (retina) and hence limits contact of the drug with cells capable of engulfing and clearing the NDDS. Sakurai et al. reported decreased fluorescein vitreal half-life with an increase in size of polystyrene nanospheres containing fluorescein. The author reported fluorescein vitreal half-life of 5.4 ± 0.8, 8.6 ± 0.7 and 10.1 ± 1.8 days for 2 μm, 200 nm and 50 nm particles, respectively [70].

A plethora of reports demonstrated the capability of NDDS to sustain drug release in the posterior segment [71, 72] and to enhance corneal permeability, via trans-cellular, paracellular routes or by a combination of both.

The utility of nanoparticles in ocular drug delivery may depend on [73]

a. Optimizing lipophilic-hydrophilic properties of the polymer-drug system.

b. Optimizing rates of biodegradation in the precorneal pocket.

c. Increasing retention efficiency in the precorneal pocket.

1.11.2. Nanoparticles preparation techniques [74]:

The choice of the appropriate method for nanoparticles preparation depends on the physicochemical characteristics of the drug to be loaded and the polymer. The methodologies are classified as follows:

1. Amphiphilic macromolecule cross linking
   a. Heat cross linking
   b. Chemical cross linking

2. Polymerization based methods
   a. Polymerization of monomers in situ
   b. Emulsion (miceller) polymerization
   c. Dispersion polymerization
   d. Interfacial condensation polymerization
   e. Interfacial complexation
3. Polymer precipitation methods
   a. Solvent extraction/evaporation
   b. Solvent displacement (nanoprecipitation)
   c. Salting out

**a) Solvent evaporation:**

Solvent evaporation is one of the most widely and frequently used method for nanoparticles preparation. In this method, suitable polymer is dissolved in an appropriate organic solvent and the emulsions are prepared. In the past, chloroform and dichloromethane [75] were extensively used as organic solvents, but are now substituted with ethyl acetate because of its less toxicity and is accepted by FDA. Nanoparticles are formed on evaporation of an organic solvent; by its diffusion across continuous aqueous phase of emulsion. In the normal methods, two main approaches are being adapted for the preparation of emulsions, the formation of single-emulsions (o/w) or double-emulsions (w/o/w). High speed homogenizer or probe ultrasonicator is used to reduce the dispersed droplets size to nano size range. The organic solvent was removed either by continuous magnetic stirring or under negative pressure. There after nanoparticles are collected by ultracentrifugation and washed with distilled water. However this method can be applied to liposoluble drugs and limitation are imposed by the scale up issue.

**b) Emulsification/solvent diffusion (ESD):**

This method is a altered version of solvent evaporation technique [76]. The encapsulating polymer is solubilized in a partially water miscible solvent such as propylene carbonate or ethyl acetate. Subsequently, the polymer solvent is emulsified in stabilizer containing aqueous phase, resulting in solvent diffusion to the aqueous phase and the formation of nanospheres or nanocapsules, as per the oil-to-polymer ratio. Finally, the solvent is removed by applying a negative pressure or by continuous magnetic stirring at room temperature. This technique has number of advantages, like high encapsulation (generally >70%), high batch-to-batch reproducibility, simplicity, easy scale-up and narrow size distribution. Disadvantages are, a large volume of water has to remove from the suspension and escape of hydrophilic drugs into the external aqueous phase during emulsification process, reducing entrapment [76, 77].
1.11.3 The general advantages of nanoparticles are [76, 77 and 78]:

1. Small & relatively narrow size distribution which provide biological opportunities for site specific drug delivery by nanoparticles.
2. Controlled release of drug over extended can be achieved.
3. Entrapped drug is protected against chemical degradation.
4. Possible sterilization by gamma irradiation/autoclaving.
5. They can be spray dried as well as lyophilized.
7. Ease of industrial scale production by hot dispersion technique.
8. Side effect can be minimized.
9. Surface alteration can be done easily & hence can be used for site specific drug delivery system.
10. They offer better therapeutic effectiveness and overall pharmacological response/unit dose.
11. Possess better stability and high drug entrapment efficiency as compared to microsphere.

In spite of these advantages, nanoparticles do have limitations [77]

1. Because of their small size, physical handling of nanoparticles is difficult in liquid and dry forms.
2. In addition, small particles size and large surface area readily result in limited drug loading and burst release.
3. Presents bio-acceptability restrictions.
4. Difficult to manufacture in large scale.

1.12. Topical ocular delivery of Non steroidal anti-inflammatory drugs (NSAID’s) [1]:

NSAID’s comprise of several chemically heterogeneous class of agents which possess potent cyclooxygenase inhibitory activity. However, the topical use of NSAID’s in ophthalmology is limited to relatively water soluble phenyl acetic acid, phenyl alkanoic acid and indole derivatives. Most of the NSAID’s are weakly acidic drugs, which ionize at lachrymal fluid pH and therefore permeate less. Decreasing the formulation pH raises the unionized fraction of the drug which enhances permeation.
NSAID’s being acidic are inherently irritant and reducing the pH of formulation further increases their irritation potential. In addition, being anionic NSAID’s tend to form insoluble complexes with cationic quaternary ammonium preservatives, such as benzalkonium chloride (BAC). Thus, it has proved difficult to formulate topical NSAID formulations that are comfortable when applied topically to the eye.
Table 1.1 List of NSAID’s used in ocular delivery

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>pKa</th>
<th>Mol wt</th>
<th>Log P</th>
<th>Relative aqueous humor bioavailability/conc</th>
</tr>
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<tbody>
<tr>
<td>SALICYLIC ACID DERIVATIVE</td>
<td></td>
<td></td>
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<tr>
<td>Aspirin</td>
<td></td>
<td>3.5</td>
<td>180.2</td>
<td>-1.1</td>
<td>1.00 (Topical)(^{a})</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.28 (IV)</td>
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<td></td>
<td></td>
<td></td>
<td>3.07 (Iontophoresis)</td>
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<tr>
<td>INDOLE ACETIC ACID DERIVATIVES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (Aq. soln.)(^{b})</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td>4.2</td>
<td>357.8</td>
<td>4.5</td>
<td>3 (Nanoparticle)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 (Nanocapsule)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 (Nanoemulsion)</td>
</tr>
<tr>
<td>Bendazac</td>
<td></td>
<td>---</td>
<td>282.3</td>
<td>---</td>
<td></td>
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<tr>
<td>ARYL ACETIC ACID DERIVATIVES</td>
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<td></td>
<td></td>
<td></td>
<td>1.0 (Aq. soln.)(^{c})</td>
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<tr>
<td>Diclofenac</td>
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<td>296.1</td>
<td>4.5</td>
<td>2.1 (Liposomes)</td>
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<td></td>
<td></td>
<td></td>
<td>1.0 (Aqueous)(^{b})</td>
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<tr>
<td>Ketorolac</td>
<td></td>
<td>3.49</td>
<td>376.4</td>
<td>2.32</td>
<td>3.16 (Soybean oil)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3.26 (Sesame oil)</td>
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<tr>
<td>Nepafenac</td>
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<td>254.2</td>
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<tr>
<td>Bromfenac</td>
<td></td>
<td>4.29</td>
<td>383.1</td>
<td>3.22</td>
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<tr>
<th>Drug</th>
<th>Structure</th>
<th>pKa</th>
<th>Mol wt</th>
<th>Log P</th>
<th>Relative aqueous humor bioavailability/conc</th>
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<td>Tolmetin</td>
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<td>1.0 (Conventional)</td>
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<td></td>
<td></td>
<td></td>
<td>2.6 (Mucoadhesive)</td>
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<tr>
<td>ARYL PROPIONIC ACID DERIVATIVES</td>
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<tr>
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<td>206.3</td>
<td>4.0</td>
<td>1.0 (Conventional)</td>
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<td></td>
<td></td>
<td>1.6 (Nanosuspension)</td>
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<td>244.3</td>
<td>4.2</td>
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<td></td>
<td>0.7 (0.3% w/v soln.)</td>
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<tr>
<td>Ketoprofen</td>
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<td>Naproxen</td>
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<td>230.3</td>
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<td>Oxaprozin</td>
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<td>293</td>
<td>4.8</td>
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<td>Pranoprofen</td>
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<td>Suprofen</td>
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<td>260</td>
<td>2.8</td>
<td>1(0.5% w/v soln.)</td>
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<td></td>
<td></td>
<td>2(1.0% w/v soln.)</td>
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<tr>
<td>ENOLIC ACID DERIVATIVE</td>
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