Chapter 1
Introduction
1. Introduction

The eye is a unique organ, both anatomically and physiologically, containing several widely varied structures with independent physiological functions that render the organ highly impervious to foreign substances. The cornea and crystalline lens are the only tissues in the body besides cartilage that have no blood supply. The inner and outer blood-retinal barriers which have no cellular components, separate the retina and the vitreous body from the systemic circulation therefore reduce the diffusion of molecules. This complexity of the eye imposes challenges in front of the pharmaceutical scientist to circumvent the protective barriers of the eye without causing any permanent tissue damage [1].

1.1 Anatomy of Eye [2]

The human eye is an anatomically and physiology unique and a sensitive organ. It offers many barriers to the entry of any foreign material. The eyes are protected by the blinking eyelids at the anterior and at the posterior are housed in a protective bony cavity, the orbit. The eyeball is ablate spheroid in shape.

Fig. 1.1. Anatomy of eyeball

The eyeball is composed of three layers:

i. Outer fibrous layer (Sclera and Cornea),

ii. Middle vascular layer, the uveal tract (Choroid, Ciliary body & Iris) and
iii. The innermost nervous layer (Retina).

i. Outer fibrous layer

The outer fibrous cornea-sclera layer comprises of the cornea and conjunctiva at the anterior and the sclera at the posterior segment. The white and opaque sclera a matrix composed of proteoglycans and collagen fibres, at the anterior portion is extended to a clear transparent mucosal surface, the conjunctiva. The most anterior portion of the outer part of the eye is the transparent, dome-shaped window, the cornea. It forms the strongest refractive medium of the eye offering resistance to the passage of most drugs.

The cornea is composed of five layers:

a. Stratified squamous epithelium,

b. Bowman’s membrane,

c. Thick, elastic network of connective tissue, the stroma (Substantia propria),

d. The fibrous collagen rich Descemet’s membrane, and

e. Endothelium (posterior epithelium).

Fig. 1.2. The corneal structure showing the sequential hydrophobic and hydrophilic barriers

The Bowman’s membrane ends at the cornea sclera junction, the limbus. In the limbus is present the Canal of Schlemm that drains aqueous humor (secreted in the chamber
between the lens and cornea) from the eye into veins of the sclera therefore, preventing the rise of intraocular pressure. The hydrophobic epithelium and the hydrophilic stroma prevent the entry of the hydrophilic and hydrophobic molecules respectively making cornea the rate limiting membrane for the absorption of drugs. Conjunctiva is the thin, transparent mucous membrane extending from the lateral margin of the cornea, across the sclera, covering the internal surface of the eyelids. It is composed of a stratified squamous columnar epithelium which contains many goblet cells, that rests on a lamina propria of loose connective tissue.

ii. The uveal tract
The uveal tract also known as the vascular tunic consists of the choroid, ciliary body and iris. The choroid forms the posterior five-sixths of the vascular tunic. It is the brown coloured membrane pigmented by the melanocytes which prevents scattering of light rays within the eye. The ciliary body is composed of ciliary muscles, thickened ring of tissue which functions for the visual accommodation by controlling the extension of lens. Iris is the circular, muscular, lightly pigmented diaphragm with a pupil in the center. The contraction and expansion of the muscles of the iris adjusts the size of the pupil in response to the light entering the eyeball.

iii. The innermost nervous layer
It is the retina consisting of the photoreceptor cells, bipolar neurons, ganglions and the pigment cells. The retina is also known as tunica nervosa due to the presence of the neurons. It is composed of two layers the pigmented layer which is a single layer of melanocytes and a neural layer which is a sheet of nervous tissue. It contains three main types of neurons: photoreceptor cells, bipolar cells and the ganglion cells. There are two types of photoreceptor cells; rod cells responsible for coloured vision and cone cells to impart vision in bright light. The ganglion cells function to process the electrochemical information transmitted from the photoreceptors.

The lens is a transparent, avascular, biconvex structure, suspended by the suspensory ligaments of the lens. It has three components:

i. The lens capsule, produced by anterior lens cells.
ii. A sub capsular epithelium, a cuboidal layer of cells that is only present on the anterior surface of the lens.

iii. Lens fibres, derived from the sub capsular epithelial cells, which lose their nuclei and organelles to become filled with proteins called crystallins.

A semi solid substance called the vitreous humor fills the posterior chamber of the eyeball behind the lens in direct contact with the retina. It is responsible for the transmission of light, provides support to the posterior surface of the lens and helps to maintain the intraocular pressure.

1.2 The Nasolacrimal Apparatus
It is responsible for the production of tears which lubricates the cornea and conjunctiva. The apparatus consists:

i. Meibomian glands (also known as sebaceous tarsal palpebral glands) secrete anterior layer composed of lipids, which retard the evaporation of the underlying water layers, serve to stabilize the film by increasing the surface tension and lubricate the eyelids.

ii. Lacrimal gland and the accessory lacrimal glands (Krause and Wolfring glands) secrete the middle aqueous layer which provides oxygen to the corneal epithelium, and

iii. The goblet cells of the conjunctiva secrete the posterior layer which is a mixture of mucins (glycoproteins) for hydrating the cornea. The stability of the tear film depends on the integrity of mucin. In the absence of mucin, the cornea becomes non wettable and the tear film becomes unstable and is subjected to breaking, with the formation of dry areas.

The secreted tears flow through the superior and inferior punctum lachrimal followed by their passage into the superior punctum canaliculus, lachrimal sac. The eyeball can accommodate approximately 7 µL of fluid. Instillation of more than 10 µL leads to reflex blinking of the eyelids. The reflex blinking of the eyes in response to the entry of any
foreign matter like the topically administered formulation leads to tear secretion and drainage through the nasolacrimal duct to the nasal cavities.

![Fig. 1.3. Anatomy of nasolacrimal apparatus](image)

Fig. 1.3. Anatomy of nasolacrimal apparatus

Through the highly vascular mucosa of the nasal epithelium, it is absorbed into the systemic circulation. Tears dilute the drug remaining in the *cul-de-sac*, which reduces the transcorneal flux of the drug.

1.3 Mechanisms and route of transport of drug molecules

The membrane, whether it is plasma membrane or a membrane encompassing cellular organelles, imposes a barrier to the free movement of molecules. There are two general processes of drug absorption across barrier membranes. The first type is called "the transcellular transport process," where drug molecules have to go through the barrier cells to reach the circulation. Transcellular transport is typically a two step process, starting with drug uptake into the cells, and ending with drug efflux out of the cells. The second type is called "the paracellular transport process," where the drug molecules travel between the cells (or in the gaps) to reach the circulation.
Fig. 1.4. Transcellular and paracellular pathways for drug transport across membrane

The transport of most of the molecules is contributed by both pathways. Lipophilic molecules and molecules with specialized transport processes prefer the transcellular route; whereas hydrophilic molecules lacking membrane transport processes prefer the paracellular route. The mechanism of transcellular transport includes simple diffusion, facilitated diffusion, active transport, endocytosis and exocytosis. Simple or passive diffusion is a process that does not require cellular energy, but requires a chemical gradient for the molecule to be transported. Facilitated diffusion is similar to simple diffusion not requiring energy and follows chemical gradient, but it requires a membrane transporter or carrier to facilitate the transport; two primary mode of facilitated transport have been recognized in the biological system [3,4].

The paracellular pathway is an aqueous route involving diffusion of the molecule between adjacent cells restricted by the presence of a series of junctional strands known as tight junction, gap junction & desmosomes.

Mechanism of Ocular (Topical) Drug Absorption

Drugs administered by instillation must penetrate the eye and do so primarily through the cornea and followed by the non-corneal routes. These non-corneal routes involve drug
diffusion across the conjunctiva and sclera and appear to be particularly important for drugs that are poorly absorbed across the cornea.

Corneal permeation: The penetration of drugs across the corneal membrane occurs from the pre-corneal space. Thus, the mixing and kinetic behaviour of drug disposition in tears has a direct bearing on efficiency of drug absorption into the inner eye. The productive absorption of most ophthalmic drugs results from diffusional process across the corneal membrane. The efficiency of absorption process is a function of rate and extent at which the transport processes occur. The flux of any drug molecule across a biological membrane depends on the physicochemical properties of the permeating molecule and its interaction with the membrane. The extent to which the transport or absorption process occurs is also a function of physiological mechanism of pre-corneal fluid drainage or turnover.

Factors affecting corneal transport:

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

1) The physiochemical properties of drug substance (e.g., ionization constant, aqueous solubility, oil/water partition coefficient).

2) The formulation 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.

Non-corneal permeation: Primary mechanism of drug permeation in the sclera is likely to be diffusion across the intercellular aqueous media as in the case of structurally similar corneal stroma. Therefore the possibility of partitioning mechanism cannot be eliminated. Although, like cornea, the conjunctiva is composed of an epithelial layer covering an underlying stroma, the conjunctival epithelium offers substantially less resistance than does the corneal epithelium [5, 6].
1.4 Approaches to ocular drug delivery

Topical administration is more direct, but conventional preparations of ophthalmic drugs, such as solutions or suspensions are relatively inefficient as therapeutic systems. A large proportion of the topically applied drug is immediately diluted in the tear film and excess fluid spills over the lid margin and the remainder is rapidly drained into the nasolacrimal duct. A portion of the drug is not available for therapeutic action since it binds to the surrounding extra orbital tissues. In view of these losses frequent topical administration is necessary to maintain adequate drug levels. This results in transient periods of over and under-dosing. Three factors have to be considered when drug delivery to the eye is attempted.

Firstly, how to cross the cornea (external to ocular) to reach the site of action; secondly, how to localize the pharmacodynamic action at the eye and minimize drug action on other tissues and finally, how to prolong the duration of drug action such that the frequency of drug administration can be reduced [7].

Initial attempts to overcome the poor bioavailability of topically instilled drugs (in solution forms) typically involved the use of ointments based on mixtures of white petrolatum and mineral oil. Ointment ensures good drug bioavailability by increasing the
contact time with the eye, minimizing the dilution of tears, and resisting nasolachrymal drainage. But these vehicles has major disadvantage of providing blurred vision, they are nowadays mainly used for either night time administration or for treatment on the outside edges of the eyelids [8].

Now use of suspensions ophthalmic delivery system relies on the assumption that particles may persist in conjunctival sac. The efficiency of suspension has shown high variability, which occurred as a result of inadequate dosing, probably mainly due to the lack of patient’s compliance in adequately shaking the suspension before administration [9]. These disadvantages led to other formulation approaches being investigated or simply into the development of dosage formulation that are divided into the ones that can affect the precorneal parameters, and those that provide controlled and continuous delivery to the pre and intraocular tissues.

The system discussed involves:

a) The newer system involved include, phase transition hydrogel systems, use of cyclodextrins to increase solubility of various drug, vesicular systems, particulate system, and chemical delivery systems such as the prodrugs.

b) The developed and under-developed controlled /continuous drug delivery systems including ocular inserts, collagen shields, ocular fits, disposable intraocular lenses, other new ophthalmic drug delivery systems, and

c) The newer trends directed towards the combination of drug delivery technologies for improving the therapeutic response of a non-efficacious drug.

1.4.1 Conventional ocular delivery system

Eye drops/lotion - Eye drops may be solutions (aqueous and oily) are comparatively convenient, safe, immediately active and acceptable to patients. Depending on the disease condition, eye drops may contain steroids, prostaglandins, anesthetics, antihistamines, sympathomimetics, parasympathomimetics, β-receptor blockers, parasympathomimetics, and non-steroidal anti-inflammatory drugs (NSAID). Eye drops also contain various inactive ingredients such as viscosity enhancers (hydroxypropyl methylcellulose (HPMC), carboxy methylcellulose (CMC), polyvinyl alcohol (PVA), and carbopol), preservatives
(benzalkonium chloride and chlorobutanol), demulcents (polyethylene glycol 400 and polypropylene glycol), buffers (disodium phosphate, sodium borate, sodium citrate) and tonicity adjusting agents (sodium chloride).

Fortunately, many therapeutic agents used in eye products are water-soluble compounds or can be formulated as water-soluble salts, and a sufficiently high solution concentration can be achieved in the administered dose. In comparison with more sophisticated multiphase systems, solution products are preferred because they are generally easier to manufacture and potentially provide better dose uniformity and ocular bioavailability.

The absorption of drugs from the eye is dependent upon the complex interplay of physiological, physiochemical and formulation factors. Formulation of an optimal ophthalmic dosage form requires a balancing act between the ocular irritation, corneal permeation and stability. In this context, effect of formulation factors on corneal permeation from aqueous drops and oily drops have been investigated earlier for different NSAIDs like diclofenac [10,11], ketorolac [12,13] and flurbiprofen [14] and fluoroquinolones like gatifloxacin [15], moxifloxacin [16,17].

Aqueous or oily suspension eye-drop formulations may be considered for drugs that are poorly water soluble, or because of poor aqueous drug stability. The drug particle size must be reduced to less than 10 µm levels to avoid irritation of the eye surface, leading to blinking and excessive lachrymation. Suspensions may also be used to overcome chemical instability of the drug, but at the same time may pose physical instability problems. For example, there may be an increase in particle size with time (Ostwald ripening), or difficulties in resuspension after periods of storage. The latter may result in problems with homogeneity and dose uniformity. Although eye drops in the solution and suspension forms have been the most common formulation approach for the treatment of anterior segment diseases yet they have several drawbacks.

In a study it was reported that a large number of patients found it difficult to instill the drop [18]. The secretion and drainage of tears at the rate of 1µL/min leads to tear turnover at the rate of 16% which dilutes the drug solution and eventually increases its drainage [19, 20]. The rates of drainage increases with increase in volume of the solution
instilled in the eye resulting in reduced bioavailability of the drug [21]. The amount of drug absorbed cannot be estimated due to the limited volume holding capacity of the cul-de-sac. The most widely used preservative benzalkonium chloride causes peeling of the corneal epithelium cells at their borders, inhibits the growth of the cells and enlarges the intercellular spaces in the superficial cells of the cornea [22-24]. Corneal calcification can occur due to the phosphate buffer present in the eye drops [25, 26].

Eye ointments are sterile semi-solid preparations intended for application to the conjunctiva. They are attractive because of their increased contact time and better bioavailability compared to solutions but their use is limited as they interfere with vision and are not suitable for use during the day time [27-29].

1.4.2 Hydrogel

Aqueous gel (hydrogels) consists of high molecular weight, cross linked polymers that form a three dimensional network in water. Hydrogels are based on the addition of hydrocolloids to aqueous drug solutions. Currently, two groups of hydrogels are distinguished, namely preformed and in situ forming gels.

Preformed hydrogels can be defined as simple viscous solutions which do not undergo any modifications after administration. Preformed hydrogels for topical administration in the eye can be based on natural, synthetic, or semisynthetic polymers (like cellulose derivatives, polyvinyl alcohol, carbomer, hyaluronic acid).

The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery or as tear substitutes. They do not allow accurate and reproducible administration of quantities of drugs and, after administration; they often produce blurred vision, crusting of eyelids, and lachrymation. A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the latter. Thus, in situ hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye [30].
**In situ gels**

In situ gels are the low viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological environment change (pH, ion activated or temperature). The viscous gels reduce the drainage and improve the contact time than the conventional eye drops. Gellan gum, Gelrite®, xanthan gum, chitosan, sodium alginate, carbopol, pluronic, poloxamers and pluronic copolymers are reported to exhibit the sol-gel transition [31-35].

**1.4.3 Ocular inserts**

Solid erodible (soluble) or non-erodible (insoluble) inserts have been commercially available for some time as a means of prolonging the release of drugs in to the eye. The Ocusert™ (nonerodible system developed by Alza Corporation) and SODI™ (Soluble Ophthalmic Drug Insert - erodible system) are commercially available formulations. Ocular inserts are the solid ophthalmic devices usually of 8 mm diameter with drug dispersed in the polymer reservoir or matrix system intended to increase the precorneal contact time, increase bioavailability and maintain a therapeutic drug concentration in the target tissues. The release rate and the mechanism can be controlled by the use of different natural and synthetic polymers like sodium alginate, collagen, gelatin, Eudragit RS-100 and RL-100, p-HEMA, polyethylene oxide, cellulose acetate phthalate and hydroxypropylmethyl cellulose (HPMC) [36-41].

To improve the intraocular retention of inserts, mucoadhesive thiolated ocular inserts comprising of polyacrylic acid-cysteine conjugate as polymeric matrix, containing either diclofenac sodium or diclofenac-tris (hydroxymethyl)-amino methane were prepared and evaluated. *In-vitro* release studies conducted using simulated lachrymal fluid revealed a significantly lesser release of drug from diclofenac-tris inserts compared with diclofenac sodium inserts. The inserts were successful in providing a controlled drug release with release rate approximating zero order release kinetics [42].

In another study reservoir type ocular inserts of ketorolac were formulated using HPMC or methylcellulose and povidone as polymeric films, and ethylcellulose film as rate
controlling membrane [43]. The ocular inserts casted using HPMC (4%) and ethyl cellulose (3%) was found to sustain ketorolac tromethamine release by zero order kinetics for 22 h.

Ocular inserts of indomethacin were fabricated using a combination of low and high molecular weight polyvinyl alcohol. The ex-vivo permeation of drug from ocular inserts was evaluated through excised goat cornea, and it was observed that physical cross linking of inserts by freeze-thaw cycling and increase in the proportion of low molecular weight polyvinyl alcohol decreased the apparent corneal permeability of indomethacin [44].

In another study, ocular inserts/films of aceclofenac were prepared using glycerol-gelatin polymer and evaluated for physicochemical parameters and drug release profiles. Ocular irritation of the developed formulation was also checked by HET-CAM test and efficacy of the developed formulation against prostaglandin-induced ocular inflammation in rabbit eye was determined. The results of the study showed that the cross-linked ocular insert proved to be significantly better in various evaluating parameters such as tensile strength, swelling potential, drug release and pharmacodynamic activity [45].

1.4.4 Contact lenses

Use of soft contact lenses as a carrier for drug delivery is gaining attention. Sedlacek, in the year 1965 described [46] the use of contact lenses to increase the residence time (typically days) of the drug in the eye, patient comfort and to limit the drug loss by drainage, lacrimation or conjunctival absorption [47]. Medicated soft contact lenses can be used to treat ocular diseases in addition to the correction of refractive deficiencies. Contact lenses loaded by soaking technique [48] are not therapeutically effective due to a very low uptake of drug by the lens and a rapid release might lead to low residence time. The other limitations like the limited drug incorporation in lens matrix, incompetent to offer slow and extended drug release, wastage of large fraction of drug during loading procedure have limited its use in ODDS. The uptake and release of the drug depends on the material of the lens (as a function of ionicity, water and silicone content), loading solution, time during which is placed in the loading solution and molecular weight of the
drug incorporated [49]. Novel techniques molecular imprinting and supercritical solvent impregnations have surmounted all these issues. Molecular imprinting produces synthetic macromolecular networks template-mediated polymerization mechanisms with specific affinity, capacity and selectivity for the diffusion of drug molecules. A therapeutically relevant amount of drug can be loaded and released over an extended period of time and this technique is applicable to daily-wear and extended-wear contact lenses. In the supercritical fluid impregnation technique, drug is impregnated and dispersed in a polymer matrix by dissolving in compressed high volatile fluids (like carbon dioxide) at temperatures and pressures near or above their critical temperatures and pressures and bringing the resultant mixture in contact with polymer matrix [50-52].

A method for preparation of soft contact lenses of poly (hydroxyethyl methacrylate) (PHEMA) using molecular imprinting technique for norfloxacin delivery has been described [51]. PHEMA hydrogels are used as soft contact lenses. Hydrogel is a polymeric material which swells in water and retain large quantity of water within its structure but does not dissolve. Due to high water content hydrogel is soft and rubbery and offers low frictional irritation to the body tissue. PHEMA hydrogels on immersion in drug solution takes up the drug which can be applied to eye. PHEMA is synthesized by polymerization. If HEMA is polymerized with a functional monomer like acrylic acid which can interact with cationic drug by non-covalent interaction, the affinity of the lens for drug could be increased. However, if the lens is synthesized by conventional polymerization method, a random distribution of functional monomer is obtained. In the molecular imprinting technology, the drug is polymerized with the monomers, so that the functional monomers arrange themselves around the drug molecule, according to their interaction capacity. The polymerization and cross-linking fix the spatial sequence and after removal of the template molecule, recognition cavities complementary in shape and functionality of the drug are obtained. In the said study, acrylic acid and ethyleneglycol dimethacrylate (cross-linker) were dissolved in 2- hydroxyethyl methacrylate (HEMA). Norfloxacin was added to the solution and 2,2'-azo-bis (isobutyronitrle) was added as a polymerization initiator. The solution was put in a mould and exposed to 50°C for 12h
and 70°C for 24h. After polymerization, the gel was immersed in boiling water for 15 min to remove unreacted monomers and treated with HCL and water to remove norfloxacin and subsequently the polymer was dried. Similarly, a non-imprinted polymer was made without addition of norfloxacin. The imprinted polymer when immersed in norfloxacin solution loaded greater amounts of norfloxacin than the non-imprinted ones. Imprinted polymeric hydrogel, synthesized with norfloxacin: acrylic acid in 1:3 and 1:4 molar ratios, showed greatest ability to control the release process sustained for >24h.

1.4.5 Collagen Shield
Collagen is the most abundant primary fibrous protein present in the animal tissues. The fibrils of the connective tissue in skin, bones, eyes, tendons and ligaments mainly comprise of collagen. Stroma, which forms 90% of the cornea, and the Bowman’s layer are lamellas of collagen, which imparts mechanical strength to the eyeball [53, 54]. Collagen derived from porcine sclera and molded into clear thin pliable sheets was fabricated as a potential vehicle to promote the healing of the de epithelialized corneal conditions, to eliminate the painful use of contact lenses and to achieve a sustained delivery of drugs [55, 56]. Bio-Cor® marketed by Bausch and Lomb was the first commercially available porcine collagen shield. Glucan/collagen therapeutic eye shields were the patented products of Biosource Genetics Corporation used to deliver glucan through the collagen shields [57]. A pre-soaked collagen shield of ofloxacin applied before surgery appeared safe and provided higher concentration of ofloxacin in aqueous humor (0.96 µg/mL) of the human eye compared to 0.3% w/v ofloxacin aqueous drop (0.29 µg/mL) [58].

1.4.6 Microspheres
Microspheres are monolithic particles possessing a porous or solid polymer matrix, whereas microcapsules consist of a polymeric membrane surrounding a solid or a liquid drug reservoir. The application of such systems in the ophthalmic field has been extensively reviewed by Kreuter and colleagues [59-60]. Upon topical instillation of a particulate suspension in the cul-de-sac, the drug is slowly released in the lachrymal pool by dissolution and mixing, diffusion, or mechanical disintegration or erosion of the
polymeric matrix (60). The upper size limit for microparticles for ophthalmic administration is about 5-10 µm. The ophthalmic administration of particles of higher size can result in an itching sensation and can induce lachrymation, with the possible consequence of reducing drug bioavailability. Modification with the use of mucoadhesive and viscous polymers can be used to improve the corneal retention and bioavailability of the formulation [61].

1.4.7 Nanoparticles

Nanoparticles are polymeric colloidal particles ranging in size from 10 to 1000 nm. Depending on the method of the preparation, nanospheres or nanocapsules can be obtained. Nanospheres have a matrix-like structure, wherein active compounds can be firmly adsorbed onto their surface, entrapped, or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In this case the active substances are not only dissolved in the core but may also be adsorbed onto the nanocapsule surface. Colloidal systems in the sub-micron range can be incorporated in the eye drops as an alternative to the conventional dosage forms [62, 63].

Pignatello et al formulated nanoparticles with Eudragit RL and RS using a modified quasi-emulsion solvent diffusion technique and solvent evaporation method [64]. Upon administration of these nanoparticles in rabbits, sustained release and increased absorption of the incorporated NSAIDs (ibuprofen and flurbiprofen) were observed. Furthermore, no signs of inflammation or discomfort were detected in the rabbits' eyes, suggesting a local bio tolerance of these nanoparticles [65, 66].

To increase the ocular availability, ketorolac was entrapped in N-isopropylacrylamide, vinyl pyrrolidone and acrylic acid based copolymeric nanoparticles. The particles were spherical and had a size of 35 nm. In-vitro permeation studies through excised cornea revealed twofold higher permeation of drug from nanoparticle formulation, compared with an aqueous suspension of the drug. Pharmacodynamic evaluation of nanoparticle formulation in PGE2-induced ocular inflammation in rabbits showed a significantly higher ocular anti-inflammatory activity for 5 h compared with the aqueous suspension,
which has been attributed to the small size of the particles and mucoadhesiveness. The nanoparticle formulation did not show any corneal damage during in-vitro study [67].

Adibkia et al also studied a NSAID-loaded Eudragit nanoparticle formulation. The piroxicam based Eudragit RS100 nanoparticles were prepared by solvent evaporation/extraction technique. These nanoparticles were used to control inflammatory symptoms in rabbits with endotoxin-induced uveitis, where a microsuspension of piroxicam was used as control. The in-vivo examinations revealed that the inflammation could be inhibited by the nanoparticle formulation more significantly than the pure piroxicam microsuspension, improving ocular delivery for local inhibition of inflammation [68].

In another study with PLGA, Vega et al applied an experimental design to optimize a formulation of flurbiprofen-loaded nanoparticles. The particle surface was coated with Poloxamer 188 (BASF SE, Ludwigshafen, Germany), resulting in zeta potential values of approximately -25 mV and a particle size of 230 nm. Drug entrapment efficiencies of over 90% were obtained, and in-vitro release studies showed a burst release, followed by a slower release, completed after 90 min. In-vivo studies performed in rabbits demonstrated that the formulations did not induce toxicity or irritation. Nanoparticle formulations were compared with the commercial eye drops (Ocoflur Allergan, Belgium) after induction of inflammation by instillation of sodium arachidonate. The results indicated a very good anti-inflammatory efficacy for flurbiprofen-loaded PLGA nanoparticles providing controlled and continuous drug delivery [69].

1.4.8 Solid lipid nanoparticles

SLNs were first patented by Muller and Lucks (1996). Since then they have attracted scientists as a widely used, stable, non-toxic, and reliable particulate drug delivery vehicle. Lipid nanoparticles consist of solid lipid spheres, with average diameters between 50 and 1000 nm, dispersed in aqueous medium [70-72]. These particles are similar to those described as polymeric nanoparticles, being in this case composed of a solid lipid core matrix, stabilized by surfactants. As such, these lipid nanoparticles will have high encapsulation efficiency and loading capacity for lipophilic molecules.
Surfactants used for their production include biological membrane lipids (lecithin, pure phospholipids), bile salts (e.g., sodium taurocholate), biocompatible nonionic molecules (e.g., ethylene oxide/propylene oxide copolymers, sorbitan esters, and fatty acid ethoxylates). When optimized, lipid nanoparticles show high physical stability, protection of incorporated labile drugs against degradation and excellent in-vivo tolerability. However, these systems generally exhibit a low drug payload and have the tendency to expulsion of drug during storage due to the transition of highly ordered lipid particles [73, 74]. These disadvantages can be alleviated by using structured lipid matrices in SLN formulations and surface modification of the particles [75]. The sodium diclofenac loaded lipid nanoparticles were prepared by Attama et al using the combination of homolipid from goat (goat fat) and a phospholipid, applying the hot high-pressure homogenization method [76]. Administration of this formulation in bioengineered human cornea showed a sustained release of the drug. Furthermore, permeation of sodium diclofenac through the cornea construct was improved by surfacing nanoparticles with phospholipid, which showed the better performance of this formulation for ocular application.

1.4.9 Liposomes

Liposomes are membrane-like vesicles consisting of one or more concentric bilayers, produced from natural nontoxic phospholipids and cholesterol, alternating aqueous and lipophilic compartments. Because of their size, amphiphilic properties, and biocompatibility, liposomes are promising systems for drug delivery. They can be classified according to structural features as multilamellar vesicles, small unilamellar vesicles, and large unilamellar vesicles, depending on their size and the number of lipid bilayers. With respect to their electrical charge, liposomes can have a positive, negative, or neutral surface charge, depending on their chemical composition (type of phospholipids, drug substances, and other lipid molecules). They can accommodate both hydrophilic and lipophilic drugs, respectively, into the aqueous compartment or the lipid bilayer [77].

The use of phospholipid vesicles to increase the precorneal residence time and as carriers for water soluble and lipid soluble drugs has been reported [78, 79]. Positively charged
liposomes showed a higher binding affinity to the anionic corneal and conjunctival mucoglycoproteins and better entrapment efficiency than the neutral and negatively charged vesicles [80]. Mixed brain gangliosides were incorporated into the membranes of phosphatidylcholine liposomes to provide receptor sites for wheat germ agglutinin, a lectin that binds strongly to corneal epithelium, led to enhanced topical drug flux [81]. Collasomes, liposomes coupled to collagen matrices and PAMAM (Polyamido amine) dendrimers-coated puerarin liposomes were prepared to increase the adhesion capacity and penetration of liposomes [82, 83]. In another study, pre-corneal retention of diclofenac sodium has also been reported to be increased by use of liposomes. The cationic liposomes of diclofenac sodium prepared by reverse phase evaporation were observed to provide a 211% increase in aqueous humor concentration of drug compared with conventional aqueous eye drop formulation [84]. Niosomes were found to be better vesicle system to deliver hydrophilic and lipophilic drugs to eyes due to their better stability than the liposomes [85, 86]. Large disc shaped vesicles, discomes were derived from niosomes by the addition of a non-ionic surfactant Solulan C24 [87]. The large size of discomes fit in cul-de-sac thereby preventing its drainage into the systemic pool as well as disc shape [88].

1.4.10 Ocular minitablets
These are the bioerodible tablets weighing about 6 mg and 8 mm (4×2mm) in diameter. Minitablets with matrix system were prepared by direct compression of the powdered blend of drug, polymer and other excipients. The drug release occurs due to swelling of the polymer followed by dissolution in the tear fluid. High drug concentration can be maintained in tear fluid using minitablets for a prolonged time period. On insertion, the tablet absorbs the tear fluid and sustains the drug release on being hydrated [89, 90].

1.4.12 Ocular iontophoresis
It is an advanced non-invasive technique to avoid the complications associated with administration of frequent, high dose sub-conjunctival injections or eye drops which require hospitalization of the patient [91-93]. Direct current (DC) of low intensity 0.2 mA to 4 mA for 10-20 min is applied that drives charged molecules across the cornea, sclera
and the adjacent tissues [94-98]. It is limited to drug molecules which can ionize and have low molecular weight. Singh et al., proposed a new efficient technique MacroesisTM (patent filed by Buckeye Pharmaceuticals, Beachwood, OH, U.S.) which uses alternating current instead of direct current. Therapeutic concentrations were achieved in the ocular tissues in less time period and it is possible to deliver the drug to the posterior segment of the eyeball [99].

1.5 Ophthalmic inflammatory disorders and treatment

The most common disease affecting the eye is inflammation. Inflammatory conditions of the eye are designated according to the tissue affected. Conjunctiva and cornea are constantly exposed to various types of physical, chemical, microbial (bacteria, fungi, viruses), and allergic agents and hence prone to develop acute, sub-acute, and chronic inflammations. Conjunctivitis, keratitis, retinitis, uveitis, endophthalmitis, dacryocystitis, dacryoadenitis and iritis are the inflammatory disorders of the eye parts. The disease ranges in severity from mild forms, which can still interfere significantly with quality of-life, to severe cases characterized by potential impairment of visual function. Ocular inflammation is also a common result of cataract surgery, producing pain and photophobia in many patients and potentially leading to serious complications including increased intraocular pressure, posterior-capsule opacification, cystoid macular edema, and decreased visual acuity.

Inflammation is manifested as a cellular and vascular response to the injury, infection, ischemia and excessive or inappropriate operation of immune mechanism. The response is amplified by activation of inflammatory cells and production of chemical mediators like acidic lipids e.g. prostaglandins, thromboxanes, leukotrienes, vasoactive amines, cytokines etc. The acidic lipids are produced through arachidonic acid metabolism. Arachidonic acid is released from the phospholipids component of cell membrane by the action of phospholipase A2. The arachidonic acid is fed into the cyclooxygenase and lipoxygenase pathways, resulting in production of pro-inflammatory prostaglandins and leukotrienes [100].
Ocular actions of PGs are manifested in three ways [101]. Firstly, they act on intraocular pressure (IOP). PGE1 & E2 increase the IOP by local vasodilation and increased permeability of blood aqueous barrier. On the other hand PGF2α lowers the IOP which is attributed to increased uveoscleral out flow. Secondly they act on iris smooth muscle to cause miosis. Thirdly, PGs cause vasodilation and increase the vascular permeability resulting in increased aqueous humor protein concentration.

The pharmacological approach of management of inflammation involves administration of anti-inflammatory agents. Topical administration of drugs is the most preferred route for management of ocular inflammations as it provides higher ocular drug concentrations, avoiding the systemic side effects associated with the oral administration. However due to the physiologic constraints of the eye only few of the anti-inflammatory agents which possess certain physicochemical properties can be formulated into a suitable dosage form effective for the management of ocular inflammations. Corticosteroids used to be the mainstay of topical therapy in the management of ocular inflammations [102], but their anti-inflammatory effect was outweighed by serious adverse effects like elevation of intraocular pressure, progression of cataracts, increased risk of infection and worsening of stromal melting [103]. Corticosteroids, the potent anti-inflammatory agents elicit their action by blocking the enzyme phospholipase A2 to inhibit arachidonic acid production, thereby preventing the synthesis of all the PGs, thromboxanes and eicosanoids. On the other hand non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory action by inhibiting the enzymes cyclooxygenase (COX 1 & COX 2).

As a class, NSAIDs have proven to be a safe and effective alternative to corticosteroids in the topical management of ocular inflammations [104]. Currently these drugs are used topically very widely in inhibition of intra-operative miosis, management of post-operative inflammation, treatment of seasonal allergic conjunctivitis, prevention and treatment of cystoid macular edema and in the control of pain after photorefractive keratectomy [105]. NSAIDs have also been found to be useful in decreasing bacterial colonization of contact lenses and prevent bacterial adhesion to human corneal epithelial cells [106].
NSAIDs comprise of several chemically heterogeneous class of drugs which possess potent cyclooxygenase inhibitory activity. However, the topical use of NSAIDs in ophthalmology is limited to relatively water soluble salicylic acid, indole acetic acid, aryl acetic acid, aryl propionic acid and enolic acid derivatives. Most of the NSAIDs are weakly acidic drugs, which ionize at the pH of the lachrymal fluid and therefore have limited permeability through the anionic cornea which has an isoelectric point (pI) of 3.2 [107]. Reducing the pH of the formulation increases the unionized fraction of the drug which enhances permeation. Being acidic, NSAIDs are inherently irritant and reducing the pH of formulation further increases their irritation potential, as well as decreasing their aqueous solubility. In addition, the anionic nature of NSAIDs lends to the formation of insoluble complexes with cationic quaternary ammonium preservatives, such as benzalkonium chloride [10, 14]. Thus; it has proved difficult to formulate topical NSAID formulations that are comfortable when applied topically to the eye.

There are four NSAIDs currently approved by the US Food and Drug Administration for the treatment of postoperative inflammation after cataract surgery, namely kerotolac, bromfenac, diclofenac and nepafenac.

Aceclofenac, 2-[(2-[(2, 6-dichloro phenyl) amino] phenyl) acetyl] oxy] acetic acid, is a NSAID of the phenyl acetic acid group which is structurally related to diclofenac. It possesses good anti-inflammatory and analgesic activities, while maintaining better gastric tolerance in comparison with other NSAIDs such as indomethacin and diclofenac. Aceclofenac acts as such by inhibiting the secretion of tumor necrosis factor (TNF-α) and interleukin-1 along with preferential selective cyclooxygenase-2 (COX-2) inhibition after conversion into active metabolite [108-110]. The present study gives an insight into various approaches used in topical ocular delivery of aceclofenac.
Drug profile [110-112]

**Aceclofenac**

Chemical Structure

![Chemical Structure](image)

**Drug Category**: Anti-inflammatory  
**Chemical Name**: 2-[(2, 6-Dichlorophenylamino) phenyl]acetoxy acetic acid  
**Molecular Formula**: C_{16}H_{13}Cl_{2}NO_{4}  
**Molecular Weight**: 354.2  
**Melting Point**: 149° to 150 °C  
**Description**: A white to almost white crystalline powder.  
**Solubility**: It is practically insoluble in water; soluble in alcohol and methyl alcohol; freely soluble in acetone and dimethylformamide.  
**Partition Coefficient**: 2.9  
**Elimination Half Life**: 4 h  
**Dissociation Constant**: 4.7 [pKa]
Pharmacokinetics

Absorption: Aceclofenac is well absorbed after oral administration.

Metabolites: It is metabolised in hepatocytes and microsomes to 4-hydroxyaceclofenac, which can undergo further conjugation. Other metabolites also identified include diclofenac and 4-hydroxydiclofenac, and their hydroxylated derivatives.

Distribution: Aceclofenac penetrates into synovial fluid and concentrations here reach approximately 57% of those in plasma.

Volume of distribution: Apparent volume of distribution is approx. 25 L

Protein binding: >99% plasma protein bound.

Clearance: 70% of an administered dose is excreted in urine as glucuronides of aceclofenac and diclofenac

Mechanism of action: Preferential selective cycloxygenase-2 (COX-2) inhibition after conversion into active metabolite, aceclofenac acts as such by inhibiting the secretion of tissue necrosis factor (TNF-α) and interleukin-1.

Dosage: A usual dose of 100 mg twice daily is administered with a reduction to 100 mg daily for patients with hepatic impairment.

Adverse Effects: Dizziness, vertigo, puritus, rash and dermatitis

Drug Interactions: Interaction with anticoagulants, cyclosporins, diuretics, quinolones, digoxin, methotrexate and lithium

Usage and administrations: It is used in the management of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis, in usual oral doses of 100 mg twice daily. Reduced doses should be used in patients with hepatic impairment.

Proprietary names: Airtal; Barcan; Biofenac; Difucrem; Falcol; Gerbin.
polymer profile [113]

Eudragit RL 100 / Eudragit RS 100

**Nonproprietary Name**

"Ammonio Methacrylate Copolymer Type A“Ph. Eur.

"Ammonio Methacrylate Copolymer Type B“Ph. Eur.

"Ammonio Methacrylate Copolymer, Type A and B” USP/NF

"Aminoalkylmethacrylate Copolymer RS" JPE

**Commercial form**

Solid substances Eudragit RL 100 (Type A) and Eudragit RS 100 (Type B)

**Chemical structure**

Eudragit RL 100- Poly (ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) 1 : 2 : 0.2.

Eudragit RS 100- Poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) 1 : 2 : 0.1.

Eudragit RL 100 and Eudragit RS 100 are copolymers of acrylic and methacrylic acid esters with a low content in quaternary ammonium groups. The ammonium groups are present as salts and make the polymers permeable. The average molecular weight is approx. 150,000.
**Description**

Eudragit RL 100 and Eudragit RS 100: colourless, clear to cloudy granules with a faint amine-like odour. Eudragit RS and Eudragit RL are copolymers synthesized from acrylic acid and methacrylic acid esters, with Eudragit RL (Type A) having 10% of functional quaternary ammonium groups and Eudragit RS (Type B) having 5% of functional quaternary ammonium groups. The ammonium groups are present as salts and give rise to pH independent permeability of the polymers. Both polymers are water-insoluble, and films prepared from Eudragit RL are freely permeable to water, whereas, films prepared from Eudragit RS are only slightly permeable to water.

**Solubility**

1g of the substances dissolves in 7g aqueous methanol, ethanol and isopropyl alcohol (containing approx. 3% water), as well as in acetone, ethyl acetate and methylene chloride to give clear to cloudy solutions. The substances are practically insoluble in petroleum ether, 1 N sodium hydroxide and water.

**Safety**

Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical formulations. They are also used in topical formulations and are generally regarded as nontoxic and nonirritant materials. A daily intake of 2 mg/kg body-weight of
Eudragit (equivalent to approximately 150mg for an average adult) may be regarded as essentially safe in humans.

Applications in Pharmaceutical Formulation or Technology
Eudragit RL 100 and Eudragit RS 100 are used to form water-insoluble film coats for sustained-release products. Eudragit RL films are more permeable than those of Eudragit RS, and films of varying permeability can be obtained by mixing the two types together.
References


73. Souto EB, Mehnert W, Muller RH. Polymorphic behaviour of Compritol 888 ATO as bulk lipid and as SLN and NLC. J Microencapsul 2006; 23: 417-33.


