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INTRODUCTION

OBJECTIVE OF THE INVESTIGATION

The present study is aimed at the development of ophthalmic delivery systems of ketorolac tromethamine, a non-steroidal anti-inflammatory agent which has established its superiority over other anti-inflammatory agents for some important inflammatory conditions of eye. The investigation included the optimization of product and process development as well as evaluation, which could overcome the limitations of existing delivery systems, particularly in respect of stability and efficacy of the product and convenience and compliance of administration for the patient. This is in keeping with the present trend of R & D in pharmaceutical industry, to optimize the effect of clinically established drugs, rather than search for altogether new drug entities, the latter being a very costly and lengthy process.

1.1 IMPROVED DRUG DELIVERY SYSTEMS - A PERSPECTIVE:

The present trend in pharmaceutical industry is to develop improved delivery systems for existing as well new drug molecules because of many reasons. The conventional drug dosing may follow a "sawtooth" kinetic profile, in which the dose first greatly exceeds the desired therapeutic level, then falls to subclinical level, and on subsequent dosing rises to dangerously high values, falling again to ineffective concentrations, in continuous cycles of excessive-ineffective levels. The rationale for the improved delivery of drugs is to promote therapeutic benefits while at the same time minimizing toxic effects. These systems are also termed as controlled drug delivery systems (CDDS) or new drug

delivery systems (NDDS) or sustained drug delivery systems etc. Controlled, sustained drug delivery can reduce the undesirable fluctuation of drug levels, enhancing therapeutic action and eliminating dangerous side effects. The NDDS can impart important advantages such as extending the duration of drug activity, which allows greater patient compliance owing to elimination of multiple dosing schedules; addressing chronobiological issues by temporal optimization of dosing and by using feedback control self-regulatory polymers and other systems; and being the carrier of proteins and peptides and the vehicles for drug targeting. The localization through preprogrammed drug delivery methods of a drug in the vicinity of its target cells can prevent systemic or side effects involving other tissues.

Furthermore, over 250 million dollars and 15 to 20 years are usually spent to bring a new drug to the market whereas, marketing approval of a novel drug delivery system can be secured for as little as ten million dollars[1].

The realization of these advantages of improved drug delivery systems/new drug delivery systems, has led to their increased popularity. The value of the world market for drug delivery systems was US $ 486.6 million in 1986 and an estimated US $ 15 billion in 1995, more than double its 1990 value of US $ 6 billion[2]. Because of the uncertainty of FDA's rules & regulations and cost control, development of NDDS will flourish in the coming years.

Several approaches to the design of controlled release systems have been developed of which very few have been translated into commercially successful products. These include transdermal, parenteral, pulmonary site specific, pulmonary systemic,

pulmonary proteins and peptides, nasal, transmucosal, nasal proteins and peptides, implants and oral controlled release drug delivery systems[2].

Among the various available drug delivery systems, the major market share is occupied by oral dosage forms, a roughly US $ 6 billion. Sustained release products made their debut in 1952 when SK&F began marketing a spansule (wax-coated pellet) delivery system for dextroamphetamine sulfate, however, the introduction of Contac capsules in 1961 was great commercial success, leading to a widespread awareness of the sustained release concept.

The oral CR delivery systems using different mechanism of release (diffusion, erosion, osmosis and combination thereof) are classified as matrix system, pellet system, membrane controlled matrix, OROS and reservoir. The major products in the US market utilizing i) matrix technology include theophylline SR, verapamil SR, diltiazem SR, nifedipine SR ii) pellet technology include verapamil, diltiazem and cough & cold preparations, iii) OROS systems include nifedipine (Procardia^{R}) XL, phenyl propanolamine (Actrim^{R})[3].

With a greater understanding of the limitations in designing oral CR systems, several new approaches have been tried[4,5]. The potential of mucoadhesives to prolong the residence time of the dosage form in the oral cavity has been explored[6-11]. Two commercially available buccal adhesive formulation include Buccastem^{R} (Reckitt and Coleman) containing prochlorperazine maleate and Suscard Buccal^{R} (Pharmax) containing glyceryl trinitrate. Two mucoadhesive formulations developed by 3M pharmaceuticals are in the pipeline[12].

Sublingual dosage forms have been developed for nitroglycerine, ergotamine, nicotine, buprenorphine, methyl testosterone, and nifedipine. Oxytocin, a small peptide, has been shown to cross the buccal mucosa[1].

To prolong the residence time of oral CR dosage forms in the stomach many approaches have been made[13-15]. Some of the important investigations to prolong the residence time in the stomach include, development of bioadhesive systems incorporating mucoadhesive polymers such as polycarbophil[16,17], formulation of high density (> 1.4 g/ml) pellets[18], design of floating dosage forms using the ion-exchange resins Amberlite IRA-410 and Dowex 2X100[19].

Colonic drug delivery[20-24] has also been explored to provide preferential release of drug in the large intestine either through i) the development of prodrugs as in the case of sulphasalazine[25] (Azulfidine^{R}), olsalazine[26] (Dipentum^{R}) and budenoside ii) development of formulations using pH sensitive (pH > 7) biodegradable polymers such as methacrylic acid-methyl methacrylate copolymers[27,28] or using polymers degradable by colon specific enzymes, such as azopolymers[29,30] and saccharide polymers[31].

In the non oral routes of novel drug delivery systems, transdermal delivery of drugs has gained interest in the pharmaceutical industry. In the U.S, there are seven approved, systemically active transderamlly delivered drugs (scopolamine, nitroglycerine, clonidine, estradiol, fentanyl, nicotine and testosterone), given that the first 'patch' was not on the market until the early 1980s, these statistics reflect a rather successful history[1,32].

Biodegradable, controlled release microcapsules/microsphere formulation for parenteral administration constitute an exciting new technology for drug delivery[33-36]. The major polymers that have been studied are poly (lactides/glycolides), poly (orthoesters) and poly (ϵ-caprolactone). Nowadays, more than 10 products, mostly microspheres and inserts have been introduced in Japan, America and Europe. Among these one can mention, Zoladex^{R} containing goserilin (ICI), Decapentyl^{R} Retard (Ferring) containing Triptorelin, Enantone^{R} (Takeda) containing leuprolein, which are indicated fro prostate carcinoma, and Capronor^{R} (WHO, NIH) containing levonorgestrel, a contraceptive implant[37].

Other area of drug delivery which has gained interest recently is targeting especially by liposomes[38-42]. The targeting of drugs is aimed at achieving a desired pharmacological response at selected site without incurring undesirable interactions at other sites. A number of systems such as liposomes, nanoparticles, biodegradable microspheres, and cellular carriers have been investigated to target drugs. Liposomes containing amphotericin B (Ambisone^{TM}, Nexstar Pharma. Co.) is commercially available since 1996. The second generation liposomes are called as sterically stabilized or long circulating liposomes[42] are the lipid derivatives of the hydrophilic polymers for eg. poly-ethylene glycol have been evaluated to overcome the problems associated with classical liposomes. Several products containing PEG have already received US-FDA approval, including the enzyme PEG-adenosine deaminase and sterilcally stabilized liposomes containing entrapped doxurubicin (Doxil ^{TM}, Sequua pharma.), Daunorubicin (Daunoxome^{TM}, Nexstar Pharma. Co.).

Nasal[43-46] route has been studied to deliver peptides and proteins, which have poor oral bioavailability. To increase the

residence and penetration in the nasal cavity many polymers and surfactants have been studied. Metered dose aerosols for treating various disease conditions such as asthma, allergic rhinitis (budenoside) and pulmonary infections has also been explored.

Drug delivery through the eye, a very specialized organ, have been considered for local action as well as systemic use. The first zero order controlled drug delivery systems namely, Ocusert by Alza corp. has been developed for this route. The eye presents some unique opportunities as well as challenges for drug delivery. Therefore, to design effective ocular drug delivery, it is necessary to understand the relevant anatomical and physiological constraints that impede or modify ophthalmic drug and vehicle disposition.

1.2 OVERVIEW OF OCULAR ANATOMY AND PHYSIOLOGY RELEVANT TO OPHTHALMIC DRUG DELIVERY :

The eye is a specialized organ that is relatively secluded from systemic access by the blood-retinal, blood-aqueous and blood-vitreous barriers[47]. The structure of the eye (Fig.1) can be described under two broad headings; extraocular and ocular structure.

1.2.1 EXTRAOCULAR STRUCTURES :

The eye is protected by the eyelids and by the orbit. Understanding ocular and orbital anatomy is important for consideration of safe periocular drug delivery, including subconjunctival, sub-Tenons's and retrobulbar injections.

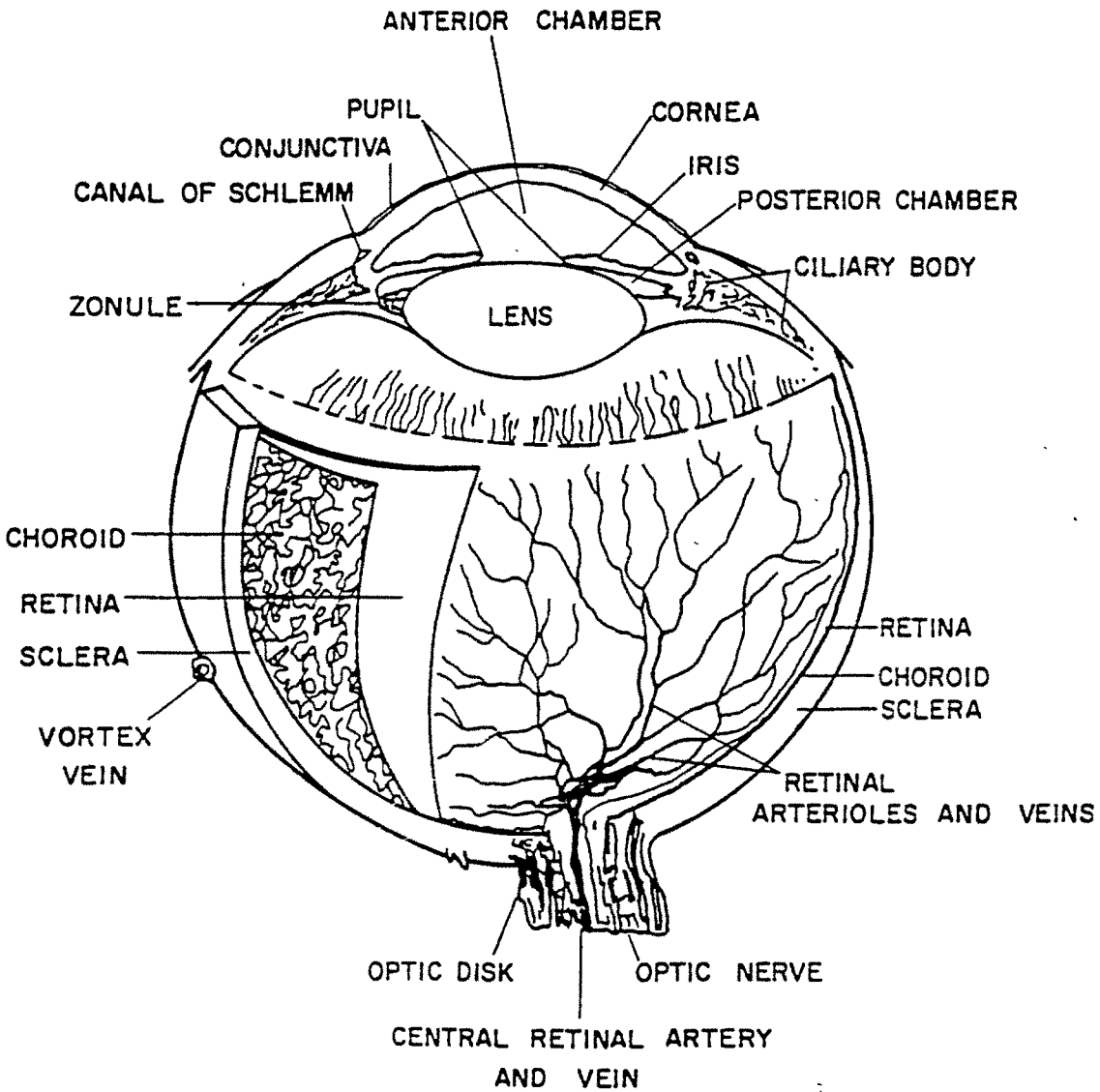


Fig. 1: Anatomical structures of the eye

The eyelids serve a variety of specialized functions including, protection of the eye from mechanical and chemical injuries. In human beings, the average blink rate is 15 to 20 times per minute, which has great influence on the bioavailability of drugs that are administered as semisolids[48].

The **conjunctiva** is a vascularized mucous membrane that covers the anterior surface of the globe with the exception of the cornea. The conjunctiva has great potential combating infection because of presence of immunocompetent cells and different cells types located within the conjunctiva can initiate and participate in inflammatory reactions. The anatomy and biochemistry of conjunctival cells enable it to phagocytize and neutralize foreign particles[49,50].

The more common causes of pain in and about the eye include inflammation, hypoxia, denudation and deformation or compression of receptors. The last two causes are especially difficult to deal with when designing a drug delivery system that is intended to remain in contact with the eye for extended periods of time. Topical medication usually are placed in the inferior fornix, also known as the inferior cul-de-sac[51].

The **lacrimal system** consists of secretory glandular and excretory ductal elements. The conjunctiva and cornea is covered by a thin fluid film, the so-called preocular tear film, which is formed and maintained by lacrimal apparatus. The tear film is a trilamilar structure (lipid-aqueous phase-mucin) with each layer distinctive in its own composition. The osmotic pressure of the tear film is ~ 311-350 mOsm in normal eyes and is regulated by principal inorganic ions Na^+, K^+, Cl^+, HCO_3^- and proteins[52].

The cul-de-sac normally holds 7-9 µl of tears but can retain up to approximately 20-30 µl without blinking. Under baseline conditions, the normal tear flow rate and tear film thickness are 16 %/min and 4-9 µm, respectively[53]. The tear drainage system starts through small puncta located on the medial aspects of both the upper and lower eyelids. With blinking, tears enter the puncta and continue to drain through the canaliculi, lacrimal sac, nasolacrimal duct and then into the nose[54,55]. Since nose is lined by a highly vascular epithelium, the topically applied medications that pass through this nasolacrimal system have direct access to the systemic circulation. This drainage system is the main reason for low ocular bioavailability and systemic toxicity of topically applied drugs. The normal tear contains approximately 1 % of proteins, which increases during ocular inflammation. The pH of the normal tears is ~ 6.5-7.6.

1.2.2 OCULAR STRUCTURE:

Anatomically, the eye is divided into anterior and posterior segments as shown in Fig. 1.

Anterior segment :

The anterior is bounded in front by the cornea and a small portion of the sclera, posteriorly the anterior chamber is bounded by the iris, lens and ciliary body (Fig. 1).

The cornea is an optically transparent and avascular tissue organized into five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium.

The corneal epithelium represents the most important barrier to invasion by foreign substances, including drugs[56]. This epithelium is a reasonably hydrophobic tissue and therefore useful ocular drugs intended to cross the cornea should have an

oil/water partition coefficient of greater than one and preferably 10-100.

The stroma comprises approximately 90% of the corneal thickness is a hydrophilic layer and this acts as barrier for hydrophobic molecules[57]. Descemet's membrane is a very elastic and remarkably resistant to proteolytic enzymes. The endothelium is a monolayer of cells of hydrophobic nature and maintains corneal thickness by active transport processes[58]. Hence, for issues of drug absorption across the cornea, one must consider the trilamellar hydrophobic-hydrophilic-hydrophobic domains of the various anatomical layers.

The trabecular meshwork and schlemm's canal form the conventional pathway of aqueous humor outflow from the anterior segment of the eye. Once aqueous humor percolates through the trabecular meshwork, it enters into Schlemm's canal[59]. From the canal of Schlemm, fluid drains into an episcleral venous plexus and eventually into the systemic circulation.

The **iris**[48] separates the anterior and posterior chambers and permits the free flow of aqueous from the posterior to the anterior chamber. Individual variation in the number of melanocytes may be an important consideration for ocular drug distribution due to drug-melanin binding.

Aqueous humor is produced both by active and passive secretion from the ciliary processes. It has been estimated that the active secretion accounts for 80-90% of total aqueous humor formation. The aqueous has low protein and high lactate & ascorbate concentration relative to the plasma[60].

Posterior segment :

Because of the anatomical and vascular barriers to both local and systemic access, drug delivery to the eye's posterior pole is more challenging. The outermost coat of the eye, the sclera, covers the posterior portion of the globe. Clinically, ophthalmologists have used injection into Tenon's capsule for prolonged release of drug[48].

The retina is a thin, transparent, highly organized structure of neurons, glial cells and blood vessels. The pigments of the retina play important role in drug toxicity. The vitreous is a clear medium that makes up about 80% of the eye's volume. It is composed of 99% water bound with collagen type II, hyaluronic acid and proteoglycans as well as glucose, ascorbic acid, aminoacids and a number of inorganic salts[61].

1.3 FACTORS AFFECTING BIOAVAILABILITY OF TOPICALLY APPLIED DRUGS:

After topical instillation of a drug, the rate and extent of absorption are determined by the following: the time the drug remains in the cul-de-sac and precorneal tear film; elimination by nasolacrimal drainage; drug binding to tear proteins[62-64]; drug metabolism by tear and tissue proteins; and diffusion across the cornea and conjunctiva. Possible absorption pathways of an ophthalmic drug following topical application to the eye is shown in Fig. 2.

The penetration of drugs into the eye occurs through cornea and/or an alternative route, the so called noncornel route[65,66]. The noncorneal route has been shown to be important for delivery of drugs that are poorly absorbed across cornea (inulin)[67]. There is no much evidence about the usefulness of this route. Further studies are needed to fully characterize and understand its utility.

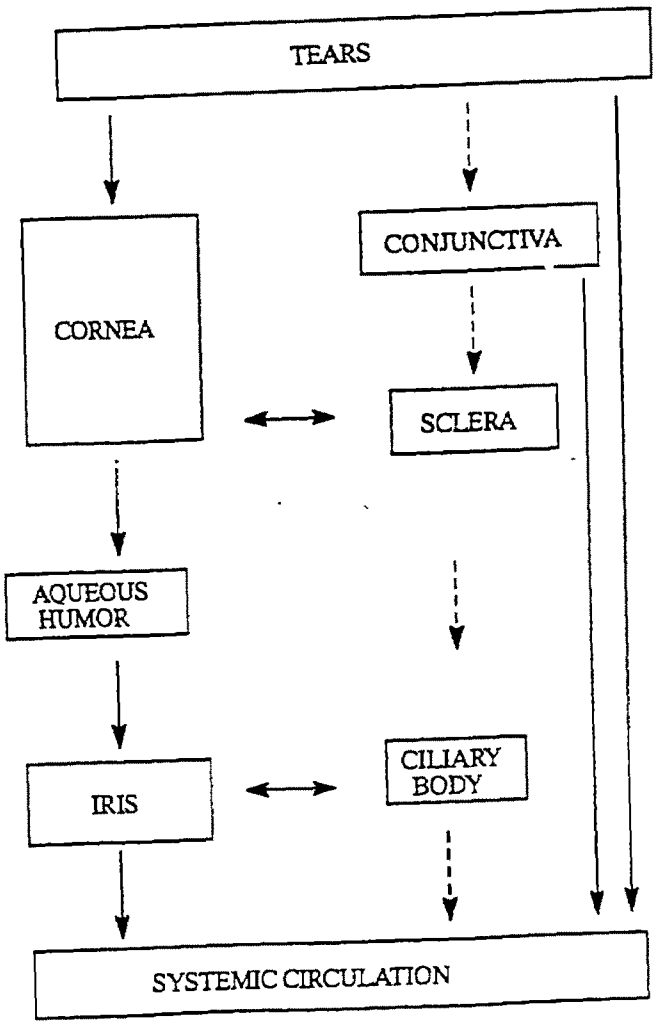


Fig. 2: Schematic diagram showing disposition of drugs instilled into the eye.

One of the major noncorneal route of drug absorption is through conjunctiva. The conjunctiva possesses two important features that render it more effective in competing with the cornea for drug absorption i) a 17 times larger surface area[68], and ii) a 2-30 times greater permeability to drug[69]. It is therefore, not surprising that drug uptake by conjunctiva is as important as solution drainage loss in reducing the fraction of drug available for corneal absorption[70]. However, conjunctival drug absorption can be reduced by increasing formulation pH, lowering solution tonicity, and lowering the percentage of EDTA and benzalkonium chloride in the formulation.

Transcorneal and transepithelial absorption are the desired routes for localized ocular drug effects[71]. Transcorneal route is the one which brings drug into the anterior chamber of the eye. The drug concentration gradient between the tear film and the cornea and conjunctival epithelium provides the driving force for passive diffusion across these tissues. Other factors that affect a drug's diffusion capacity are the size of the molecule, chemical structure, and steric configuration. A drug with both hydrophilic and lipophilic properties is best suited for transcorneal absorption, because of trilamellar "fat-water-fat" nature of the cornea. Transcorneal drug movement has been postulated for steroids, ß-blockers[72], timolol ester[73,74] drugs and n-alkyl p-aminobenzoate esters[75]. However, it has been shown that an aqueous diffusion space is available for small hydrophilic compounds such as water and butanol. For some-what larger hydrophilic molecules (glycerine and mannitol), a cation-sensitive aqueous diffusion pathway also exists.

The transport of drugs across the cornea has been studied both *in vivo* and *iv vitro*[57]. The *in vitro* studies are simpler to analyze than the *in vivo* absorption studies, which are complicated by tear flow, tear drainage, corneal transport, and elimination from the aqueous humor. One of the key parameters in ocular absorption is the corneal permeability coefficient (CPC). The CPC form majority of ophthalmic drugs is on the order of 0.1 to 4.0×10^{-5} cm/s[76]. Drug penetration into the eye is approximately linearly related to its concentration in the tear film. When the corneal epithelium is absent, the degree of penetration enhancement can be as high as 10-30 fold, as is the case for 5-fluorouracil, which penetrates the cornea poorly because of its hydrophilicity. Ocular inflammation also changes the corneal epithelial permeability to such drugs as cyclosporine and dexamethasone phosphate[77,78].

Benzalkonium chloride and other cationic surfactants have been shown to enhance the ocular absorption including pilocarpine[79], carbochol[80] and prednisolone[81].

Following transcorneal absorption, the aqueous humor accumulates the drug, which is then distributed to other intraocular structures as well as potentially to the systemic circulation via trabecular meshwork pathway. Drug binding to melanin pigments[82-85] can affect the bioavailability of topically applied drugs both positively (atropine's mydriatic activity) and negatively (ephedrine mydriatic activity). Another clinically important consideration for drug-melanin binding involves the retinal pigment epithelium. Accumulation of chloroquine causes a toxic retinal lesion known as "bull's-eye" maculopathy, after binding to retinal pigment epithelium.

Enzymatic biotransformation of ocular drugs may be significant since local tissues in the eye express a variety of enzymes, including esterases, oxidoreductases, lysosomal enzymes, peptidases, glucoronide and sulfate transferases and many more[86-90]. The esterases have been of particular interest because of the development of prodrugs for enhanced corneal permeability; for eg. dipivefrin hydrochloride is a prodrug for epinephrine and alkyl ester prodrugs of timolol.

Elimination of drug from the eye occurs over the entire concentration-time profile. Topically applied ocular drugs presumably are eliminated by the liver and kidney after systemic absorption.

1.4 OPHTHALMIC DRUG DELIVERY SYSTEMS :

Today, topical ophthalmic application is considered to be the preferred way over systemic administration to achieve therapeutic levels of drug agents used to treat ocular diseases for obvious reasons including the systemic toxicity of many ophthalmic drugs, the rapid onset of action, and the smaller dose required compared to the systemic route.

Efficient delivery of a drug for a desired length of time, while minimizing its systemic and/or local side effects is the key to the treatment of ocular diseases. The unique anatomy and physiology of the eye offer many challenges toward developing effective ophthalmic drug delivery systems. Currently, the body of knowledge in this field is rapidly expanding. An increase in the knowledge of ocular drug absorption and disposition mechanisms has led to the development of many of new delivery systems like gels, inserts, corneal collagen shields,

particulates and iontophoresis. To date, ophthalmic drugs have been marketed in three different conventional dosage forms including aqueous solutions, suspensions, ointments and three new delivery systems viz. solid ocular insert, gels and implants. However, conventional delivery systems are the leaders in the market.

1.4.1 CONVENTIONAL SYSTEMS IN OPHTHALMIC DRUG DELIVERY :

The earliest accounts of ophthalmic treatment on record date back to the Mesopotamian era, circa 3000-4000 B.C. These were mostly prophylactic. One of the first therapeutic delivery systems, the collyrium, noted in the writings of Celsus (20 B.C to A.D. 50), was apparently introduced by the Romans and Greeks. However, improvement in ocular delivery preparations and treatments did not occur for many centuries[91].

The conventional formulations for this route fall into several categories: solutions, suspensions and ointments.

1.4.1.1 Aqueous solutions:

A majority of ocular formulations consist of a specifically formulated aqueous medium which acts as the drug carrier vehicle. A majority of the ophthalmic therapeutic agents are water soluble or can be converted as water soluble salts. The selection of the appropriate salt form depends on its solubility, the therapeutic concentrations required, the ocular toxicity. The most common salt form used are the hydrochloride, sulfate, nitrate, tromethamine, and phosphate. The drug must be in a form that is not only stable within the formulation but well absorbed at the precorneal site. A homogeneous solution offers the potential of greater assurance of uniformity of dosage. The eye drops are simple to manufacture and administer, relatively inexpensive and do not obscure vision[91,92].

1.4.1.2 Suspension :

The drugs that are sparingly soluble in water are often formulated as suspensions[93]. The drug is present in a micronized form, generally less than 10 μm in diameter, suspended in a suitable aqueous vehicle. It was assumed that drug particles from suspensions persist in the conjunctival sac and give rise to a sustained release effect[94]. However, studies attempting to assess the *in-vivo* performance of the ocular suspensions are limited, providing no data on the actual residence profile of the suspended particles[95,96].

Increase in drug particle size were shown to influence bioavailability. Ocular suspensions, however, have the following disadvantages: shaking is required which can lead to inconsistency in the administered dose if not done properly; a fine sediment may form which can be difficult to disperse with gentle shaking; and seldomly occurring, but of serious consequences, is a polymorphic change in the suspended drug to form a less soluble or inactive form of the drug. In addition, suspensions are prone to elimination by the physiological mechanisms[97].

1.4.1.3 Ointments :

Ophthalmic ointments are useful as drug carriers for improving bioavailability and sustaining drug release. In a text-book of the Indian Sanskrit literature, the "Ayur-Veda of Susruta" (500 A.D), a cream- and butter-based ointment is mentioned (1). Vaseline, introduced in 1876 by Piffard in New York and was used immediately afterwards in Europe in dermatology, rapidly became the favorite base for ophthalmic preparations. Liebreich, in 1885 advocated the use of lanolin in ophthalmic ointments. At present the most common vehicles for drug in ointments are simple

petrolate bases, such as petrolatum and mineral oil, with or without hydrophilic lipids such as lanolin or polyethylene glycols.

An important feature of the ointments is that they remain in the conjunctival cul-de-sac, forming a reservoir of the drug. Moreover, the disappearance from the precorneal area of a drug administered in an ointment vehicle is very slow (0.5% per minute) when compared with the elimination by the normal lacrimal turnover (about 16% per minute). No impediment to epithelial or stromal wound healing was exhibited by currently used ophthalmic ointments. However, interference with vision and aesthetic considerations are obvious disadvantages associated with the use of ocular ointments. They are most often used as adjunctive nighttime therapy, with eye drops administered during the day[98,99].

1.4.2 IMPROVED/NOVEL OPHTHALMIC DRUG DELIVERY SYSTEMS:

One of the major problem encountered with the conventional topical delivery of ophthalmic drugs is the rapid and extensive precorneal loss caused by the drainage and high tear fluid turnover[100,101]. Lacrimation and blinking are actually efficient protective mechanisms which keep the eye free of foreign substances, but they prevent efficient ocular therapy. As a consequence, the ocular residence time of conventional eye drops is limited to a few minutes and the ocular absorption of a topically applied drug is reduced to approximately 1-10 %.

A typical time course of drug release in the eye from an conventional ophthalmic solutions follows a pulsed entry, i.e. peak and valley pattern. It initially shows a very high drug concentration followed by rapid decline. The transient peak can

represent an overdose of the applied ocular drug, whereas, the valley generally represents a period of underdosing. Usually the eye drops are need to be administered four to six times a day, which leads to patient non-compliance[102-104].

Various approaches that have been attempted to increase the bioavailability and the duration of therapeutic action of ocular drugs can be divided into two categories.

I) Maximizing corneal drug absorption and minimizing precorneal drug loss.

II) Drug-delivery systems which provides the controlled and continuous delivery of ophthalmic drugs to the pre- and intraocular tissues.

1.4.2.1 MAXIMIZING CORNEAL DRUG ABSORPTION AND MINIMIZING PRECORNEAL DRUG LOSS:

i) Viscosity-Imparting Agents :

There have been several claims of improvement in ocular bioavailability of drugs based on the increased residence time in the eye due to the addition of viscosity-building soluble polymers to aqueous formulations[105-109]. It is reasoned that the solution drainage would be reduced by the increased solution viscosity[110-113]. The polymers used include polyvinyl alcohol (PVA),polyvinylpyrrolidine, methylcellulose, hydroxyethyl-cellulose, and hydroxypropyl methylcellulose (HPMC). Chrai and Robinson conducted studies in rabbits and found that over a range of 1.0 to 12.5 cP viscosity there is threefold decrease in the drainage rate constant and a further threefold decrease over the viscosity range of 12.5 to 100 cPs[114]. Most of the commercial eye drops have their viscosities adjusted to be with in range 15 to 25 cPs by the addition of HPMC or PVA.

However, as studies to date indicate, this approach has only limited value as the formulations are liquid and therefore subject to elimination from the eye by all of the factors discussed previously[93].

ii) Semisolid Ophthalmic Hydrogels :

Many researchers have recently turned their attention towards aqueous gels, in the attempt to improve the bioavailability of hydrophilic and lipophilic drugs and the acceptability of pharmaceutical semisolid dosage forms[115-117]. Aqueous gels may offer several physiological advantages, such as: a generally good tolerability; the formation of a protective film on the cornea; and a protection against conjunctival adhesion.

The ophthalmic hydrogels can be classified as follows:

a) preformed gels

b) *in situ* forming gels.

a) PREFORMED GELS:

The success of ocular drug-delivery systems depends on their retention in the eye. Preformed gels usually utilizes polymers such as carbopol (polyacrylic acid), polyvinyl alcohol, methyl cellulose, to name but a few.

Carbopols are of interest among synthetic polymers, and have been indicated in many publication as suitable ingredients for semisolid hydrophilic ophthalmic dosage forms[116-119]. Carbopol has the property to attach to conjunctival mucin by noncovalent bonds and remain in contact with the precorneal tissue until eliminated by mucin turnover[120]. Carbomer based gels are easily extruded from tubes and can be administered without any loss. Among the various carbopols, Carbopol 934 and 940 have been studied extensively for ophthalmic use, and it has been reported

that Carbopol 940 was better tolerated than Carbopol 934[116]. Bottari and co-workers demonstrated that carboxyvinyl polymer based gels were better vehicles for lidocaine and benzocaine than aqueous solutions or paraffin suspensions[121]. Moreover, the gels showed optimal release characteristics, as indicated by a prolonged effect[122]. A prolonged activity has been reported for many drug such as prednisolone-acetate[123], betamethasone[124], phenylephrine[124], pilocarpine[125], prednisolone-phosphate[123], etc, when administered in a carbopol gel form. Suitability of carbopol as an ophthalmic vehicle has been investigated by evaluating physico-chemical and toxicological properties[116].

Presently, carbopol is the most widely used polymer in ophthalmic sustained delivery hydrogels and it is used for the controlled release of, among others, pilocarpine in pilopine HS[R][122], and fusidic acid[126-128] is Fucithalmic[R].

b) *IN SITU* FORMING GELS:

A major recent advance in the field of ocular drug delivery is the development of several polymeric systems that undergo phase transition (sol-to-gel)[129-132]. These system are liquid under certain conditions and therefore are easy to administer as eyedrops. Such a phase transition can be mediated by a change in temperature[133], a change in pH[134], or electrolyte or ion activation[135]. Because of increase in the viscosity after administering in to the eye, these systems have been evaluated to improve the bioavailability of topically applied drugs. Various polymers have been investigated to design such systems.

Poloxamer 407 is a thermally reversible gel-forming polymer. Poloxamers are block polymers consisting of polyoxyethylene-polyoxypropylene-polyoxyethylene units. Their relatively low

toxicity and capacity to form clear gels make them particularly suitable for pharmaceutical applications, such as ophthalmic as well as in the area of other controlled drug delivery systems[136-138]. The poloxamers are reported to be well tolerated and non-toxic even though large amounts (20-30 %)of polymer are required to obtain a suitable gel. At low concentration (10^{-4}-10^{-5} %) they form monomolecular micelles, but at higher concentrations multimolecular aggregates with hydrophobic central core are formed while their hydrophobic central core are formed while their hydrophilic polyoxyethylene chains face the external medium. In concentration of 20 % w/v and higher, aqueous solutions of poloxamer-407 remains as a liquid at low temperature (<15°C) and yield a highly viscous semisolid gel upon instillation into the cul-de-sac. Miller et al. examined a temperature sensitive solution of Poloxamer-407 in delivering the miotic agent pilocarpine[139]. Delivery of many other drugs to the eye by incorporating in Poloxamer gel has been investigated.

Gelrite[140-143] is a cation-selective heteropolysaccharide, that undergoes phase transition to a clear gel in the presence of mono- and divalent cations. Gellan 0.6 % w/v, forms a gel in the cul-de-sac within few seconds due to the diffusion of cations present in tears. In rabbits, the gel so formed increased the bioavailability of timolol maleate, compared with an equiviscous solution of the more conventional vehicle hydroxy-ethylcellulose[144].

A pseudo-latex of cellulose acetate phthalate has a sufficiently low buffering capacity that it will gel when instilled as a drop, with a pH of 4.4, into the cul-de-sac, with a pH of 7.2[145].
More recently, in an attempt to reduce polymer content, Kumar et al., used a combination of methylcellulose and Carbopol, the

former exhibiting thermal gellation and the latter pH dependent gellation[130].

iii) Particulate Polymer Drug Delivery Systems :

One of the possibilities of decreasing the elimination rate from the eye and hence increasing the amount of ocular absorption is drug delivery with particulate polymeric drug delivery systems. Particulate polymeric drug delivery systems include micro and nanoparticles[146,147]. The discrimination between micro- and nanoparticles which differ in their particle size is useful because of their biological and physicochemical behaviour. The uppersize limit for micropartciles for ophthalmic administration is about 5-10 µm, above this size, a scratching feeling in the eye can result after ocular application. The manufacturing methods for the production of microparticles are very numerous, however, they have been extensively reviewed by a number of authors[148,149].

Nanoparticles for ophthalmic drug delivery have been mainly produced by emulsion polymerization. The materials that have been so far mainly used for ophthalmic nanoparticles are the polyalkylcyanoacrylates. Pilocarpine as well as timolol were successfully incorporated into nanoparticles[150].

The ocular distribution of nanoparticles have been studied extensively. First attempts to address the problem of precorneal elimination of particles were made by Sieg and Triplett[151]. They have used ^{141}Ce-labeled polystyrene sphere of sizes of 3 and 25 µm. The elimination and ophthalmic distribution of ^{14C}carbon-polyhexylcyanoacrylate nanoparticles in rabbits was extensively studied by Wood et. al[152]. Some of the other drugs that have been studied to deliver to the eye with particulate systems

included ^{3}H-labeled hydrocartisone- 17-butyrate-21-propionate, betaxolol[153]. Although this approach shows promise for delivering lipophilic drugs, it would not likely be beneficial for incorporating drugs that are water soluble as these drugs are released from the nanoparticles themselves are eliminated from the precorneal cavity.

iv) Liposomes:

Liposomes are biocompatible and biodegradable phospholipid microcapsules. Smolin et al. were among the first to demonstrate the usefulness of a liposomal ocular delivery systems for enhancing corneal drug absorption. They reported that idoxuridine entrapped in liposomes was more effective than a comparable therapeutic regimen of idoxuridine[154] alone in the treatment of acute and chronic herpetic keratitis. The literature survey suggested that ocular delivery via liposomes may benefit lipophilic compounds to a greater extent than hydrophilic compounds. The bioavailability of indoxol[155], triamicinolone acetonide[156], atropine base[157], pilocarpine[158] and dihydrostreptomycin[159] doubled when these drugs were applied topically in liposomal form. Liposomal are a potentially useful ocular drug delivery system, but suffer form the disadvantage of instability due to the hydrolysis of phospholipids normally used in their preparation, limited drug loading capacity, and technological difficulties in obtaining a sterile liposomal preparation.

v) Prodrugs/Soft Drug Approaches :

Prodrugs are chemical drug derivatives which are sensitive to chemical or enzymatic cleavage. The advantages of prodrug approach is improving the corneal permeability of a less permeable drug and transforming an inactive or marginally active

molecule into a clinically useful agent.[160-162] An example of commercially available ocular prodrug is dipivalyl epinephrine (Propine, Allergan Pharmaceuticals). Owing to increased lipophilicity, epinephrine penetrability has been improved about 10 fold.[163]

Successful use of soft drug approach[164] has led to soft-β-blockers as safe antiglaucoma agents, soft anti-cholinergics as short acting mydriatic agents, and soft corticosteroids as a type of novel, safe anti-inflammatory agents, which due to their unique design, do not elevate IOP and do not produce other systemic and local side effects.

1.4.2.2 OCULAR CONTROLLED AND CONTINUOUS DRUG DELIVERY SYSTEMS:

i) OCULAR INSERTS:

Solid-state ocular inserts represent another category of dosage form. These systems can achieve therapeutic action with a smaller dose and fewer systemic and ocular side effects. For a controlled-release device to be considered satisfactory for use as an ocular insert, it must not only be capable of reproducible release kinetics but also must meet a unique set of criteria namely; comfort, lack of expulsion during wear, ease of handling and insertion, noninterference with vision and oxygen permeability, applicability to variety of drugs, sterility, stability and ease of manufacture[165].

The marketed ocular inserts can be classified into two categories:

(A). Nonerodible systems and (B). Erodible systems.

(A) Nonerodible systems:

A.1. Contact lenses: Therapeutic soft contact lenses are often used to aid corneal wound healing[166]. The use of presoaked hydrophilic contact lenses for ocular drug delivery has been extensively examined for a variety of drugs[167]. These have included antibiotics, antiglaucoma agents, and polypeptides. Unfortunately, the residence time of drugs using commonly available presoaked lenses is not significantly prolonged. Moreover, contact lenses fall short of the ideal in the areas of comfort and difficulty of handling and insertion.

A.2 Ocusert systems:

It is a membrane-reservoir type device, containing drug and alginic acid in the core reservoir, which is placed between a transparent, lipophilic ethylene-vinyl acetate rate-controlling membrane. In 1975, the first controlled release topical dosage form was marketed in the USA by the Alza corporation. The drug in the first ocusert is pilocarpine[168].

The Ocusert pilocarpine system is an elliptical membrane which is soft and flexible and designed to be placed in the cul-de-sac to release pilocarpine continuously at a steady zero order rate of 20 or 40 µg/hour around the clock for 7 days[169].

They are less popular and useful among elderly who have difficulty in insertion and do not retain the device well. Many patients feel a foreign body sensation. A significant drawback of Ocusert therapy is its high cost. Finally, Ocusert is a nonbiodegradable system and must therefore be removed at the end of its therapeutic life[165].

(B). Erodible systems:

Over the years several erodible drug delivery systems have been conceived and tested for ophthalmic use. These have included pilocarpine-containing carboxymethylcellulose wafers[165] and polyvinyl alcohol discs[170] or rods. Also, wafers of collagen containing gentamicin sulfate have indicated some promise in extending its ocular residence time as compared to conventional treatments. Despite all these efforts, there are only three erodible devices that have been marketed to date[165].

B.1 The lacrisert: The lacrisert is a sterile, rod-shaped device made of Hydroxypropyl cellulose without any preservatives that is used for the treatment of dry eye syndromes. It was introduced by Merck, Sharp & Dohme in 1981[171]. The device weighs 5 mg and measures 1.27mm in diameter with a length of 3.5mm[172]. The Lacrisert is useful in the treatment of patients with keratitis sicca whose symptoms are difficult to control with artificial tears alone.

B.2 The SODI: The SODI (soluble ocular drug insert) is a small oval wafer which was developed by Soviet scientists for cosmonauts who could not use eye drops in weightless conditions[173]. It is a small oval wafer of polyacrylamide impregnated with drug. Its dimensions are 9 mm X 4.5 mm with a thickness of 0.35 mm. Clinical tests have indicated that SODI impregnated with pilocarpine or tetracycline has compared favorably with the conventional drop treatments used for the management of glaucoma and trachoma, respectively[165].

C. Collagen shields:

Collagen shields were originally developed by Fyodorov in the Soviet Union as bandage lenses to promote healing after radical keratotomy. A collagen molecule consists of three polypeptide chains, called α-chains, which form a helix connected by interchain hydrogen bonds. The collagen shield was designed to be a disposable, short-term therapeutic bandage lens for the cornea. It conforms to the shape of the eye, protects the corneal surface, and provides lubrication as it dissolves[165].

One commercially available shield, first introduced in 1986, is made from porcine scelral tissue. There are presently three different types of BioCor collagen shields marketed by Bausch & Lomb Pharmaceuticals. These are BioCor-12, 24 and 72, representing different times of dissolution (in hours). Antibiotics such as tobramycin[174] and gentamicin & vancomycin[175] have been shown to have their residence time in the rabbit eye increased as compared to the traditional eye drops. In addition, steroids such as prednisolone-acetate[176] and dexamethasone[177] have demonstrated similar results.

ii). OCULAR THERAPEUTIC SYSTEMS MINIDISCE:

Bawa et al[178] developed a controlled-release device for the eye, known as the minidisc, a monolithic matrix-type device. Its principal component is α,w-bis(4-methacryloxybutyl)- polydimethyl siloxane. Minisdisc can be made hydrophilic or hydrophobic. In three volunteer subjects, sulfisoxazole was released from the hydrophilic minidisc over three days[165].

iii). NEW OPHTHALMIC DRUG DELIVERY SYSTEMS (NODS):

This system was developed by Smith and Nephew research company, is another example of an ophthalmic insert designed to deliver a precise amount of an ocular medication for an extended period.

The NODS is a method for presenting drugs to the eye within a water-soluble, drug-loaded film. It provides for accurate, reproducible dosing in an easily administered preservative-free form.

The device consists of a water-soluble drug loaded flag approximately 4 mm long and 6 mm wide with an area of 21 mm^2 and weight of approximately 500 µg which is attached to a water-soluble handle film via a soluble membrane approximately 0.7mm in length. All three regions of the device is made of water-soluble polyvinyl alcohol[179].

The ocular clearance rates for NODS was compared in human volunteers with a solution using the technique of τ-scintigraphy and employing a gamma camera. It has been shown that $t_{1/2}$ of water soluble drug was only 3 seconds from aqueous solution, and 7 minutes from NODS[180].

The NODS have been studied in humans for both ease of administration/comfort and efficacy. Kelly[181] et al demonstrated that the ocular bioavailability of pilocarpine is eightfold higher when administered with the NODS compared to a pilocarpine solution administered in a conventional way. Tropicamide and chloramphenicol also showed improvement in the bioavailability from NODS as compared to solution. The NODS have been well received by both patient and physicians in terms of ease of administration, comfort, and effectiveness[179].

1.4.3 Ocular iontophoresis

Ocular iontophoresis offers a drug delivery system that is fast, painless, safe and in most cases, results in the delivery of a

high concentration of the drug to a specific site[182]. Iontophoretic application of antibiotics such as tobramycin[183], ciprofloxacin[184] may enhance their bactericidal activity and reduce the severity of disease; similar application of other types of drugs, such as anti-inflammatory agents, could prevent or reduce vision- threatening side effects. Clinically, however, aside from ocular iontophoresis of fluorescein in patients to study anterior segment fluid dynamics, few studies have been done in humans. One reason is that most ocular drugs can be effectively delivered without the use of iontophoresis. At this time, however, a role for iontophoresis in clinical ophthalmology remains to be identified.

1.5 IMPORTANT FACTORS TO BE CONSIDERED IN THE DEVELOPMENT OF OPHTHALMIC DOSAGE FORMS:

In addition to physico-chemical factors to be considered in the development of a dosage form, the ophthalmic product demands a special attention to the following factors.

1.5.1 STERILITY:

Every ophthalmic product must be manufactured sterile and proved sterile before release of the product to the marketplace[185,186]. The USP XXIII has listed five methods of sterilization: steam sterilization at 121°C, dry-heat sterilization, sterilization by filtration, gas sterilization (ethylene oxide and propylene oxide), and sterilization by ionizing radiation. Currently, the British pharmaceutical Codex suggests only three methods of sterilization: sterilization by autoclaving, sterilization by filtration, and sterilization by heating to 80°C with a bactericide.

1.5.2 PRESERVATION AND PRESERVATIVES:

In 1953 the US-FDA required that all ophthalmic solution be manufactured sterile. The bioburden in unopened products is minimized by the sterilization methods used during manufacturing; however, maintenance of sterility during the use of multiple-dose depends upon the inclusion of appropriate antimicrobial compounds. The in-use contamination of eye drops with a variety of micro-organisms, is a well recognized problem. For this purpose various preservatives have been evaluated. However, choice of preservatives is limited to only a few chemicals that have been found over the years to be safe and effective for this purpose[187-189].

The BPC XII[99] lists quarternary ammonium compounds, organic mercurial compounds, aromatic or chlorinated alcohols. Many times it is necessary to design the formula to fit the requirement of the preservative agent (s) desired.

The most widely used antimicrobial is benzalkonium chloride (BKC), which is a present in over 70 % of all commercial ophthalmic products. The literature on benzalkonium chloride has been summarized by many authors. It is well tolerated in the eye at a concentration upto 0.02%. It is non-volatile and is stable to autoclaving. The compound is bactericide against a wide range of Gram-negative and Gram-positive bacteria at the pH of the ophthalmic solutions. It is the most reliable ophthalmic solution preservative, because it was a broad antimicrobial spectrum of activity. It has been found that a preservative mixture of BKC (0.01%) and di-NaEDTA (0.01-0.1%) is effective against most resistant strains of *Ps. aeruginosa*[187].

Because of its cation, BKC is incompatible with nitrates, salicylates, anionic soaps, and fluorescein. It is usually advisable to design the formula to avoid these incompatible anions rather than to substitute a less effective preservative. The usual concentrations of benzalkonium chloride used in eye drops is 0.01 %, with a range of 0.004 to 0.01 %.

Organic mercurials[187] are the next most commonly used antimicrobial agents but these have been losing favour over recent years and are being phased out, where possible, by some manufacturers. These mercurials are brought under the umbrella of the mercury toxicity alarm that occurred in the early 1970s. The organic mercurials such as phenylmercuric nitrate (PMN), phenylmercuric acetate (PMA), or thiomersal is used when benzalkonium chloride can not be used. These agents exert an inhibitory effect against a wide range of bacteria and fungi over a wide range of pH values. Since the organic mercurials offer an alternative to BKC at this time, and since adequate preservation of ophthalmic solutions is essential, it is hoped that the benefit-to-risk will be considered before a ban is imposed on their use. The usual concentration ranges are 0.002 to 0.004 % for PMN and PMA and 0.005 to 0.02 % for thiomersal.

The other categories of antimicrobial preservatives such as parabens, chlorobutanol and chlorhexidine are rarely used in ophthalmic formulation.

1.5.3 ADDITIVES:

Additives or therapeutically inactive ingredients in ophthalmic solution, suspension or gel dosage forms are necessary to perform one or more of the following functions: adjust tonicity, buffer

and adjust pH, stabilization, impart viscosity, increase solubility and act as the solvent[91].

1.5.3.1 Vehicles and viscosity imparting agents:

Ophthalmic formulations are, with few exceptions, aqueous fluids using purified water as the solvent. Water for injection is not required as in case of parenterals. Oils are used as vehicles for several topical eyedrop products which are extremely sensitive to moisture. Diisopropyl fluorophosphate has been supplied as a 0.1 % sterile solution in anhydrous peanut oil. Vegetable oils such as olive oil, castor oil, and sesame oil have been used extemporaneously for this purpose.

In case of ointment formulation the most common bases are simple petrolate bases, such as petrolatum and mineral oil, with without hydrophilic lipids such as lanolin or PEGs. An ointment should become a liquid with low viscosity at the temperature of the eye in order to spread readily as a thin film on the surface of the tear film. In general, only water-in-oil emulsions are appropriate for ophthalmic use as the oil-in-water type are irritating. The ointment bases selected should not cause any irritation to the eyes and be able to sterilize by an acceptable sterilization techniques[92].

Polymers are frequently added to improve viscosity of aqueous formulations as well as for the preparation of semisolid gels. The polymers selected should not damage the tissue or produce unwanted side effects on the eye, and be relatively inexpensive. The polymers should have less bioburden and be sterilizable by the acceptable sterilization technique.

1.5.3.2 Tonicity and Tonicity adjusting agents[99,190]:

In the past a great deal of emphasis was made that the ophthalmic solutions should be isotonic with the tear fluids. However, it has been shown that 0.5 to 2.0 % NaCl equivalency does not cause a marked pain response and that a range of 0.7 to 1.5 % should be acceptable. Tonicity adjusting ingredients usually used include sodium chloride,potassium chloride, buffer salts, dextrose, glycerine and propylene glycol.

1.5.3.3 pH adjustment and buffers[192]:

The pH and buffering of an ophthalmic solution is probably of equal importance to proper preservation since the stability comfort, safety and activity of most commonly used ophthalmic drugs is largely controlled by the pH of their environment. Product with extreme pH may cause irritation and tend to be flushed from the eye, and hence a more rapid loss of medication may occur. Ideally, every product would be buffered to a pH of 7.4, which is considered the normal physiological pH of tear of fluid. The argument for this concept is that the product would be comfortable and possibly have optimum therapeutic activity. A number of experiments, primarily in rabbits, have shown an enhanced effect when the pH was increased due to the solution containing a higher concentration of the nonionized lipid-soluble drug base. This would not be the case if the drug were an acidic moiety. If buffers are required, their capacity is controlled so as to be as low as possible, thus enabling the tears to bring the pH of the eye back to the physiological range. The acceptable pH range for ophthalmic products can be 3.5-8.0.

1.5.3.4 Stabilizers and suspending agents[99]:

Stabilizers are added to decrease or prevent decomposition of the active ingredients via specific mechanisms. These include

antioxidants, complex forming agents, and surfactants. The stabilizers used should not have any adverse effect on the eye. The major antioxidants used in ophthalmic products are sodium metabisulfite or metabisulfite in concentration upto 0.3 % (epinephrine HCl and bitartarate solutions) and ascorbic acid upto 0.1 %. Non-ionic surfactants such as tween 20 and 80, tyloxapol, polyoxyl 40 stearate, cremophor-EL, octoxynol-40 etc, have been used in many ophthalmic formulations for increasing the solubility, stability, or clarity. Recently, complex forming agent namely hydroxypropyl ß-cyclodextrin has been studied extensively for ophthalmic use to improve stability, solubility, or bioavailability.

Various surfactants and polymers such as cellulose derivatives and polyacrylic acids have been utilized as stabilizers and /or suspending agents in ophthalmic suspensions. The suspending agents/stabilizers should not cause any changes in physicochemical properties of drug during storage and should be non-irritant to the eye.

1.5.4 PACKAGING[91,99]:

Eye drops have been packaged almost entirely in plastic dropper bottles since the introduction of the drop-Tainer dispenser in the 1950s in western countries. However, glass vials are still in use because of special stability considerations. The plastic bottle and dispensing tip is made of low-density polyethylene (LDPE). The LDPE resins used have been found to be compatible with a very wide range of drugs and formulation components. Their one disadvantage is their sorption and permeability characteristics. The LDPE resin used for the bottle the dispensing tip cannot be autoclaved and they are thus gas sterilized.

The glass bottles are mainly used for products which are extremely sensitive to oxygen or contain permeable components that are not sufficiently stable in plastic. The glass used should be type 1 form maximum compatibility with the sterilization process and the product. Like most of consumer product today, some form of tamper-evident packaging is mandatory to ensure product safety.

Rubber is a common component of stoppers and part of dropper assemblies. Like plastics, elastomers can be found as a wide range of basic materials (natural rubber, synthetic polyisoprene, neoprene, butyl including bromo- and chlorobutyl), which are further extended by specific formulations. The application of an epoxy lining to the rubber closures reduces the amount of leached extractives but essentially has no effect on the sorption of the preservative from the solution. Teflon-coated rubber stoppers may prevent most of the sorption and leaching.

Ophthalmic ointments and gels are packaged in metal tubes with an ophthalmic tip. Such tubes are sterilized by autoclaving or by ethylene oxide. To prevent metal reactivity or incompatibility, tubes lined with epoxy or vinyl plastic may be obtained.

1.5.5 STABILITY[91]:

The stability refers to total product stability and not just the stability of a single product component. The stability of an ophthalmic product depends on the chemical nature of the drug substance, method of preparation, compatibility with preservatives and other additives, and packaging. The attainment of optimum stability most often imposes compromises on the formulator, such as the pH may be lower than the preferable for

product comfort. Stabilizers may be required and convenient packaging may diminish shelf life of the product. A well-planned stability programme will consider and evaluate the chemical stability of the active ingredient, chemical stability of the preservative substance, continuing preservative efficacy, sterility and organoleptic properties.

1.6. *IN-VIVO* CONSIDERATIONS:

Animal experimentation is an essential part in the research development of ocular drug delivery systems. The nonclinical or preclinical studies conducted should be able to support the pharmacological rationale for the intended use of the drug. The animal toxicology data to assess the safety risks for human exposure, and, if possible, systemic and ocular pharmacokinetic data are useful in getting the product approval for clinical trials from the FDA.

1.6.1 Ocular Toxicity and Irritation:

Assessment of the ocular irritation potential of ophthalmic dosage forms represents an extremely important step in the development of both over-the-counter and prescription pharmaceuticals. Excellent reviews have appeared in recent years in regard to the evaluation procedures utilized[91]. The historical evaluation of these procedures can be traced through the literature, as can an understanding of the mechanisms of ocular response to irritants, based on examination of the conjunctiva, cornea or iris[192,193]. Significant advances have been made, resulting in greater reliability, reproducibility, and predictability.

Albino rabbits are currently used to test the ocular toxicity and irritation of ophthalmic products[194]. A modified Draize test was adopted as the official method for eye irritancy evaluation. If the delivery system contains a new component, such as polymer or surfactant to prolong ocular residence and/or enhancing bioavailability, then additional safety testing may be required for the new component if the supplier has not already provided this information. At present US-FDA does not have specific requirements or guidelines to answer the often asked question - how much animal safety data do I need for ophthalmic IND?. In general, to begin clinical testing US-FDA will require at least the same duration of testing in animals as proposed for human exposure[195,91].

Current guidelines for toxicity evaluation ophthalmic formulations involve both single and multiple administration. The multiple applications extend over a 21-day period and involve both irritation and systemic toxicity studies.

1.6.2. Pharmacokinetic/Pharmacodynamic Studies:

Pharmacokinetic studies of topically administered ophthalmic drugs are usually carried out in animals. Human ocular pharmacokinetic experimentation is limited to the noninvasive observation of fluorescent, or gamma-scintigraphic probes to the determination of drug concentration from the aqueous humor during cataract surgery.

The ophthalmic literature contains many reports of drug concentrations measured in eye tissues over time. However, when classic pharmacokinetic approaches have been applied to ophthalmic drugs, a number of limitations have been found to restrict the usefulness of pharmacokinetics in the practice of ophthalmology[196-198].

Major advances in the ability to prevent, diagnose, and treat diseases of the eye during the last two decades have been possible owing to animal studies. Rabbits are being widely used to develop these disease state models as compared to other animal species[199]. **Since the present thesis is focussed on inflammation of the eye,** various mediators involved, various anti-inflammatory agents available for treatment and different pharmacological models used for inflammation are discussed in brief.

1.7 OCULAR INFLAMMATION

Ocular inflammation[200], like inflammation in other areas of the body, is a complex process involving the actions and interactions of several chemical mediators on ocular as well as non-ocular tissues and cells in the initiation, maintenance and termination of the inflammatory process. The inflammatory process can be elicited by numerous stimuli, namely infectious agents, antigen-antibody interaction, and thermal or other physical injury. Inflammation of the eye is the final common pathway responsible for most blindness in the world today. Inflammation may affect virtually any of the ocular structures, and such affliction, may, if not controlled, eventually lead to functional loss of vision.

Inflammation is classically defined as tissue response to injury, with attempted elimination of noxious stimuli and repair. The five classical signs of inflammation include *erythema, heat, pain swelling, and loss of function* were described by Cornelius Celsus and john Hunter. Some of these signs may not be observable when one is dealing with special structure, such as the eye,.

1.7.1 Mediators of Inflammation[201,202]:

Inflammation is really a molecular phenomenon, with molecules or "mediators" producing the observable signs. These mediators of inflammation originate predominantly from cells, typically leukocytes. These inflammatory cells include the polymorphonuclear leukocytes (PMNs), mononuclear phagocytic cells, lymphocytes, plasma cells, and mast cells. The neutrophils contain two principle enzyme-containing granules namely, specific granules and azurophilic granules. These granules release lysozymal enzymes. In addition, reactive oxygen metabolites are generated after neutrophil membrane perturbation. These oxygen metabolites produce profound degrees of cytotoxicity through both membrane and intercellular component damage.

Finally, arachidonic acid generation by phospholipase A action on membrane phospholipids follows synthetic pathways involving lipoxygenase and cyclooxygenases and results in prostaglandin and leukotriene mediator generation by neutrophils. The mediators (prostaglandins, thromboxanes, leukotrienes and hydroxyeicosatetrnoic acid) which are called as eicosanoids, can result in massive amplification of the inflammatory reaction. As leukocytes, including neutrophils, migrate into an inflammatory focus, they are influenced by a large number of mediators[203].

Histamine was one of the earliest mediators of the inflammatory process identified. Bradykinin and 5-HT also may play a role in mediating inflammation.Prostaglandins are 20-carbon metabolites of arachidonic acid that are biosynthesized by ocular tissues and are involved in human intraocular inflammation. When released in large concentration following trauma, intraocular surgery, or in association with uvetis, they may contribute to the disruption of

the blood-aqueous barrier, miosis and cystoid macular edema[204]. To prevent the damage done by the above mediators of inflammation, many pharmacologically effective agents have been evaluated. There are many experimental animal models available for evaluating anti-inflammatory efficacy of these agents.

1.7.2 Pharmacological Models of Corneal Inflammation:

Corneal inflammation, ulceration and vasularization are often coincident in corneal disease. Several experimental models have been developed to study these individual events. The following corneal inflammatory models are generally used to investigate various aspects of the anti-inflammatory agents.

Alkali Burns: Alkali burns of the eye are among the most disastrous of ocular injuries. Characteristically, such burns result in blinding corneal opacification followed by intense vascularization, chronic irritation, conjunctival overgrowth and persistent morbidity. A standardized experimental model, using filter paper disc soaked in different concentrations of NaOH has been reported. Anti-inflammatory agents have been evaluated using this model[205-207].

Corneal Inflammation: In a rabbit model, intracorneal injection of laboratory-grade clove oil (about 0.03 ml) induces an inflammatory keratitis which can be suppressed by topical steroids or NSAIDs. Corneal inflammation is also induced by intrastromal injection of human serum albumin and lipopolysaccharide into the rabbit cornea[208].

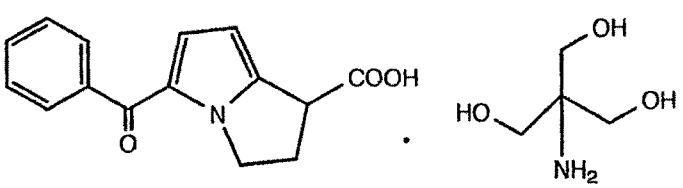
Other methods of inducing inflammation[209,210] in the eye include paracentesis, angiogenesis, intravitreal injection of endotoxins, cauterization, topical administration of allergens (Kentucky bluegrass, short ragweed, cat hair and dander).

1.7.3. Treatment of Ocular Inflammation:

Two major categories namely corticosteroids and non-steroidal anti-inflammatory agents (NSAIDs) are available for the treatment of various inflammatory conditions of the eye. Topical corticosteroids were considered as standard therapy for ocular inflammation, till the introduction of NSAIDs. The NSAIDs have recently been introduced in clinical ophthalmology due to the side effects of corticosteroids therapy, such as cataract formation, steroid-induced glaucoma, enhanced microbial proliferation, decreased stromal healing rates and rebound inflammation. The mechanism of action of NSAIDs include inhibition of cyclooxygenase pathway of prostaglandin biosynthesis, a major metabolic pathway of arachidonic acid[211]. There are many clinical studies providing evidence that NSAIDs ophthalmic preparations are potentially useful in the management of post-operative inflammation, cystoid macular edema (CME) following surgery and other common inflammations of the eye[212]. The US-FDA has approved several topical NSAIDs for clinical use in ophthalmology, but these approvals have been limited solely to specific indications. For eg. flurbiprofen (Ocufen^{R}) and suprofen (Profera1^{R}) are only approved for the prophylaxis of surgical miosis, diclofenac sodium (Voltaren^{R}) has been approved for post-cataract inflammation. But, most of these are found to be irritating to the eye. However, ketorolac tromethamine (KT) was found to be less irritating. Furthermore (KT) is the only drug that has been approved by US-FDA for the treatment of itching caused due to seasonal allergic conjunctivitis and recently for the prophylaxis and reduction of inflammation and associated symptoms following ocular surgery. And also KT was found to be promising and superior to corticosteroids in treating CME following surgery, ocular inflammation caused by fungal, viral and bacterial infections.

1.8 KETOROLAC - A DRUG PROFILE :

ketorolac was developed by Syntex research Corp. in the early 1980's. It is chemically (±) 5-benzoyl 1,2-dihydro-3H-pyrrolo-[1,2a] pyrrole-1-carboxylic acid with 2-amino-2 (hydroxymethyl) -1,3,propane-diol (1:1). Its molecular formula is $C_{15}H_{13}NO_3 \cdot C_4H_{11}NO_3$[213]. The molecular weight is 376.4. Ketorolac is used in the form of ketorolac tromethamine to improve aqueous solubility[214]. Ketorolac tromethamine bulk drug, tablets and injections are official in USP XXIII[215]. The elemental composition of ketorolac is C 70.58 %, H 5.13 %, N 5.49 % and O 18.8 %. Ketorolac tromethamine (KT) is an odourless, white to off-white crystalline powder with melting point in the range of 160-161°C.



1H-Pyrrolizine-1-carboxylic acid, 5-benzoyl-2,3-dihydro, (±)-,compound with 2-amino-2(hydroxymethyl)-1,3-propanediol (1:1)

Ketorolac (acid form) has a pKa of 3.54 and log partition coefficient of 2.72. Ketorolac is an enantiomeric compound, which is commercially available as racemeic mixture with optical rotation of +173° (+form) and -171° (- form). The tromethamine salt form is soluble in water (more than 500 mg/ml) and (3 mg/ml) in alcohol at 23°C. The pH of 1.0 % w/v aqueous solution is between 5.7 and 6.7.

Several analytical methods have been reported to analyse ketorolac in its native form[216-218], dosage form[219,220] and biological samples[221,222]. The drug has characteristic UV absorption spectrum with two absorption maxima, at 319 nm and 249 nm. The USP suggests a HPLC method for the assay of the drug from bulk drug, tablets and injections. A UV, HPLC and titrimetric methods have been reported to analyse ketorolac from tablets. HPLC as stability indicating method has also been reported in the literature[219,223]. A number of analytical methods for the detection of ketorolac from biological samples have recently been published[224-232]. HPLC methods are used to analyse the drug in plasma and serum samples.

Aqueous and ethanolic solution of KT are found to decompose rapidly under laboratory black light (350 nm) to yield CO_2, decarboxylated product and oxidation products[223]. KT undergoes thermal degradation in aqueous solution[233]. The apparent degradation is catalyzed both by acid and base. The major degradation product at pH 7.4 and 1.1 was the keto analogue of ketorolac.

Many approaches have been made to improve the stability of ketorolac in powder blends including varying salt form, altering the excipient ratios, and adding antioxidants or pH modifiers to the formulation. Addition of propyl gallate (1.0 %) and pH modifiers caused a modest improvement in the stability of ketorolac[234].

Ketorolac undergoes racemization in aqueous buffered solution and the reaction has a U shaped pH rate profile at 80 °C with the maximum stability occurring in the region of pH 3-7.5[235].

Ketorolac tromethamine (30 mg/ml) injection has been reported to be compatible with commonly used i.v. infusion solution and also with administrative set components like polyvinyl chloride bags and glass bottles[219].

The interaction of KT powder with tablet excipients such as starch, magnesium stearate, lactose, and surfactants have been studied in depth by Z.K. Chowan et al[236-241].

Ketorolac is a non-steroidal agent with moderate anti-inflammatory and more pronounced analgesic activity[242]. The mechanism of action is thought to be principally inhibition of cyclooxygenase[243]. Ketorolac is administered as the tromethamine salt when used clinically, but in animal experiments, the free acid has mainly been used. Ketorolac's analgesic and anti-inflammatory activity is thought to be due to inhibition of prostaglandin biosynthesis i.e. acting at the cyclooxygenase pathway of the arachidonic acid metabolism[222].

The acute oral LD_{50} in mice is found to be approximately 200 mg/kg. The relative analgesic activity of Ketorolac has been demonstrated to be 180 to 800 times that of aspirin in animal models. Ketorolac has been tested systemically in rat paw edema and polyarthritis models of inflammation[244].
Ketorolac tromethamine solution (0.5 % w/v) when applied topically to the eye, was found to be non-irritating in animals and man[245]. It significantly inhibited corneal neovascularization induced by silver nitrate cauterization in rats and ocular inflammatory responses in rats and rabbits[246].
KT (0.5 % solution) suppressed the inflammation in the eyes of rabbits infected with *candida albicans*[247], herpes simplex virus[248] type 1 and *pseudomonas aeruginosa*[249] with out exacerbating the underlying infection in contrast to

corticosteroids.

Results of recent meta-analysis illustrated that ketorolac 10 to 20 mg provided significantly better analgesia than diclofenac 50 to 100 mg, aspirin 600 to 650 mg, etodolac 50 to 200 mg, suprofen 200 to 400 mg or diflunisal 250 to 1000 mg[222].

The pharmacokinetic properties of ketorolac has been studied extensively in animals as well as in man[222,250]. When administered orally or intramuscularly as the tromethamine salt, ketorolac is well absorbed[251]. The maximum plasma concentrations (C_{max}) are attained between 30 to 60 minutes after single dose of oral, rectal, subcutaneous or intramuscular administration of ketorolac. Mroszezak[250] et. al. reported absorption half-life of 3.8 minutes following oral administration of ketorolac tromethamine. The oral bioavailability has been reported to be between 80 and 100 %; bioavailability was reported to be similar with intramuscular and intravenous administration[252]. Food appears to reduce the rate, but not the extent of absorption of the drug[253].

The pharmacokinetics of ketorolac are linear over the usual oral and parenteral dosage range (10 to 30 mg). The relationship between plasma drug concentration and analgesic activity has not been reported in humans[222]. As with other NSAIDs, ketorolac is extensively bound to plasma proteins (more than 99 %). Thus it would be expected, the apparent volume of distribution (V_d) is small (0.3 L/kg)[253].

The major metabolite of ketorolac is acyl-glucoronide, which account for approximately 75% of the oral dose, is excreted in urine. The other major metabolite include p-hydroxy-ketorolac

(12 %). The metabolites have no significant analgesic activity.[253,254]

After single dose administration, the mean terminal elimination half-life of ketorolac in healthy volunteers and patients with hepatic impairment[255] is about 5 hours but was prolonged to 6-7 hours in the elderly and 9-10 hours in those with renal impairment[253]. Urinary excretion is the major route of elimination, with approximately 90 % of the dose in humans being recovered in urine, and the balance in feces. Intermittent or continuous intravenous administration of ketorolac 150 mg over 24 hours to post-operative patients[256] or volunteers[257] resulted in plasma clearance rates of 0.026 to 0.028 L/h/kg. Martinez et al have reported that elimination of ketorolac was reduced in patients with renal impairment[253].

KT shows a pH-dependent penetration of rabbit cornea and also exhibits penetration in both the ionized and unionized forms in *in-vitro* studies[258]. It has been shown that KT and tobramycin penetrate rabbit cornea when given in combination, and penetration was found to improve in presence of benzalkonium chloride and EDTA[259].

The ocular bioavailability[260] of topically applied (50 µl) 0.5 % w/v of KT was 4 % in anesthetized rabbits, and aqueous humor had 13 times higher concentration compared to plasma, with mean tmax of 3.4 hrs., Cmax of 1.9 µg. eq/ml and half life of 3.8 hr. The concentrations were highest cornea and scelra and lowest in the lens. KT subconjunctival injection in rabbits have been shown to be beneficial in controlling the inflammation in experimental uvetis[261].

Ketorolac ophthalmic solution has been assessed for the treatment of chronic aphakic and pseudophakic cystoid macular edema in patients[262]. In patients undergoing extracapsular cataract extraction and posterior chamber intraocular lens insertion, ketorolac 0.5 % (w/v) topical solution administered before and 3 times daily after surgery markedly reduced the breakdown with the vehicle solution[263]. Ketorolac tromethamine 0.5 % (w/v) solution preserved the blood-aqueous barrier to a greater extent than did a 0.1 % dexamethasone sodium phosphate solution following ocular surgery in patients[264]. Ketorolac may represent an alternative to topical corticosteroids in patients undergoing extracapsular cataract extraction[265]. Ketorolac tromethamine has been proven clinically effective for the treatment of itching due to seasonal allergic conjunctivitis[266,267].

In general, pre-, intra- or postoperative parenteral administration of a single dose of ketorolac, 30 or 60 mg appears to be an effective alternative to parenterally administered opiod agents such as fentanyl 50 to 100 µg, pethidine 100 mg, dezocine 6 mg or other NSAIDs such as diclofenac 75 to 100 mg (i.m or rectal), indomethacin 100 mg(rectal) and piroxicam 40 mg (oral) after various outpatient laparoscopic or orthopedic procedures associated with mild to moderate pain[253].

The major adverse effects with ketorolac involve the GI tract and range from mild upset to serious ulceration and hemorrhage. Topical administration causes transient stinging[222]. Serum lithium concentrations increased, and resulted in toxic effects, in 2 patients after coadministration of lithium and ketorolac[253].

Ketorolac 10 to 30 mg may be administered intravenously or intramuscularly as a single dose or every 4 to 6 hours; the usual oral dose is 10 mg every 4 to 6 hours. Total dosages should not exceed 120 mg/day for parenteral administration and 40 mg/day for oral therapy[253].

At present the marketed ketorolac tromethamine (0.5 % w/v) ophthalmic solution is administered (one drop) four times a day for treating itching due to seasonal allergic conjunctivitis[214]. One drop three times daily starting 24 hours pre-operatively and continuing upto three weeks post-operatively for the prophylaxis and reduction of inflammation and associated symptoms following ocular surgery.