1. INTRODUCTION

1.1 OBJECTIVE OF THE INVESTIGATION

The present study was aimed at development of new delivery systems to improve the therapeutic efficacy of ciprofloxacin, a drug of choice for treating bacterial infections of the eye.

The conventional eye-drops suffer from certain limitations, such as, poor ocular bioavailability and lack of patient compliance. The eye ointments on the other hand cause significant blurring of vision. Hence, there is need for a new drug delivery system which can improve ocular bioavailability and thus reduce the dosing frequency and at the same time does not interfere with vision. Aqueous gel type formulations fulfill both of these requirements. Thus, the first part of our investigation was directed towards developing and evaluating a long-acting ophthalmic gel formulation of ciprofloxacin HCl. Co-administration of anti-inflammatory agents with antibiotics, enhances the therapeutic efficacy of the antibiotic. Hence, the second part of our investigation was directed towards development and evaluation of eye-drops containing a combination of ciprofloxacin HCl as well as dexamethasone, a potent anti-inflammatory drug. However, the conventional eye-drops and the eye-ointment are still widely used today despite their limitations and the technology for their manufacture is not disclosed, hence, the third part of our work was directed towards development of a technology for these as well.

1.2 NEW DRUG DELIVERY SYSTEMS: AN OVERVIEW

The process for the search of new drug entities is time consuming, expensive and the risk of failure is relatively high. On the other hand, development of **New Drug Delivery Systems (NDDS)** for established drugs, which have distinct advantages over their conventional counterparts have recently gained significant importance. This is because the time and cost of development of NDDS is comparatively far less and involves low risk potential and at the same time offers several other merits as well[1].

In addition to cost containment, NDDS can impart important advantages such as extending the duration of drug activity, which not only reduces dosing frequency and promotes patient compliance but also reduces the incidence of side-effects due to optimization of blood concentration-time profiles[2].

NDDS are also being developed for drug targeting, in which case an attempt is made to deliver the drug solely to the site of action, at a predetermined rate. However, it has not been possible to combine both these objectives in a single system. Another important application of NDDS being investigated, is the design of new systems to deliver proteins, peptides and other biotechnology based new moieties[3].

The earliest attempts at the development of drug delivery systems can be traced back to the 1960's. This was the period during

which events and changes took place that had a significant effect on the drug delivery world we are presently living in. The term 'biopharmaceutics' was first coined by Dr. Gerhard Levy in this period and subsequently the field of pharmaceutics was widened to encompass this as well. The most significant event, from the new drug delivery point of view, was the foundation of the Alza corporation by Dr. Alexandro Zaffaroni in 1968. The sole aim of this venture was to develop and commercialize innovative systems (NDDS) for established and yet to come drug entities. The successful utilization of NDDS in diverse therapeutic uses as well as the increasing demands posed by the recently developed biotechnology based pharmaceuticals has resulted in an increase in the number of pharmaceutical companies who have understandingly embraced this technology. Drug delivery became widely known to scientists outside pharmaceutics for the first time and many such individuals from the field of polymer sciences, physical chemistry, organic chemistry, biological sciences and other allied areas entered the field in large numbers, to help the pharmaceutical scientist in the cause of improved drug delivery[4].

During the last 5 years, there has been a remarkable increase in the market of NDDS, which was estimated to be US$ 15 billion in 1995, more than twice its 1990 value of US$ 6 billion. The market values of the various categories of NDDS were as follows: oral modified release dosage forms led the market with US$ 6 billion, site specific pulmonary delivery systems at US$ 5.1 billion,

transdermal therapeutic systems at US$ 1.9 billion, nasal site specific and modified release formulations at US$ 850 million and US$ 800 million, respectively. It is expected that the global market for NDDS will probably reach over US$ 50 billion by the year 2005[4,5].

The oral route of drug delivery is the most preferred route of administering drugs to patients and several delivery systems have been developed to deliver drugs more efficiently through the oral route[6,7], such as, coated pellets, ion-exchange resins[8] and hydrogels. Recent additions to the oral delivery systems include osmotic pumps, buccal adhesive systems and colonic delivery systems.

Oral osmotic pumps (OROS) developed by Alza Corporation, USA, may be used to tailor the delivery rate to that of a continuous intravenous infusion. The most simple osmotic pump consists of a semipermeable membrane that encases a drug core. A small hole is drilled in the membrane with the help of a laser beam. On being administered *in-vivo*, water diffuses into the system through the membrane and exerts a constant pressure that drives the drug out through the laser drilled hole. Examples of drugs incorporated in this form are ivermectin[9], phenylpropanolamine, salbutamol and nifedipine.

The ability of **mucoadhesive agents** to prolong the contact time of drug with buccal mucosa has also been explored[10,11]. The major advantage of this route is that it by-passes hepatic first-pass

metabolism and the time of onset is lesser than that of transdermal delivery systems. Polymers such as polyacrylic acid, carboxymethyl cellulose sodium, sialic acid and polycarbophil exhibit mucoadhesive properties. Mucoadhesives can be formulated in the form of a patch[12], gel or tablet. Oral mucosal delivery of drugs such as LHRH and morphine[13] is being currently evaluated. Two mucoadhesive products are commercially available in the UK, Buccastem^{R}, containing prochlorperazine, marketed by Reckitt & Coleman and Suscard Buccal^{R}, containing nitroglycerin, marketed by Pharmax[2]. Other mucoadhesive products containing buprenorphine and melatonin are currently under development in Germany[2].

The possibility of drug delivery specifically to the colon[14,15,16] has also been explored. Various approaches have been evaluated to target drug to the colon: (i) development of prodrugs, eg. azidosalicylate[17], which is metabolized by colon specific enzymes such as azoreductases; and dexamethasone-β-glucuronide[18], which is metabolized by β-D-glucuronidases, (ii) use of pH sensitive (pH > 7) polymers such as methyl acrylate-methyl methacrylate copolymers, or (iii) using polymers degradable by colon specific enzymes eg. azopolymers[19,20] and polysaccharides[21,22]. Above all, since the proteolytic enzyme activity in the colon is lower than in the small intestine, this approach may be exploited for the delivery of peptides and proteins too[23].

All drugs cannot be delivered by the oral route because of certain limitations of the drug, such as lack of permeability

across the gastrointestinal lumen, degradation by gut enzymes and microflora, as well as extensive first pass metabolism. Besides these, it is difficult to target drugs to the site of action by delivering them orally, hence alternative routes of drug delivery have to be developed, which can overcome the limitation of the oral route. The major alternate routes are the transdermal, pulmonary, nasal as well as parenteral.

Transdermal delivery systems[24] have probably been the most successful amongst the other systems. This can be judged by the number of drugs available in this form of delivery system. Seven transdermal products, containing estradiol, testosterone, clonidine, nitroglycerin, nicotine, fentanyl and scopolamine are already available abroad commercially. Two of these products containing estradiol and nitroglycerin are also available in India. A transdermal plaster containing ketoprofen (Ketolop^{R}) was launched in Korea in 1995[5], for the treatment of arthritis and rheumatism.

Transdermal iontophoresis or the use of electrical current to drive drugs across the skin has recently received much attention as a means of increasing skin permeation[25,26]. Existence of small charged pores on the skin creates the possibility of peptide delivery[27,28]. An iontophoretic system containing fentanyl aimed at developing a patient controlled analgesic system is in the early stages of clinical trials.

Nasal delivery is particularly convenient for local delivery of drugs, for treatment of allergic rhinitis and cold symptoms and

avoids hepatic metabolism. While proteins are not absorbed, small peptides such as somatostatin analogs and oxytocin can show excellent bioavailability[29,30]. Drugs may be administered intranasally in the form of aerosols, powders or small particulates[31].

Pulmonary delivery[32] offers a major opportunity for systemic delivery of macromolecules including proteins like interferon, cyclosporine, growth hormone, leuprolide acetate, calcitonin[33] etc. Delivery of a drug to the lung is highly dependent on particle size and the appropriate size is 1-2μm[34]. Proteins can also be delivered in the form of dry powders. Among the smaller molecules, the drugs most commonly delivered by this route are β-agonists, steroids[35], and bronchodilators. The major advantage of this route is a significant reduction in dose as well as site specific delivery, and also an improvement in the systemic bioavailability of drugs intended to be taken up by the systemic circulation. Long term toxicity of such delivery systems has yet to be studied.

New parenteral drug delivery systems have been prepared using biodegradable polymers[36-39]. These polymers degrade by hydrolysis to their respective monomers eg. poly-D,L-lactide-co-glycolide (PLGA)[40], polyanhydrides[41] and polyorthoesters[42]. Since the biodegradable system is absorbed over its life time, it is a significant advantage to the patient, provided that the degradation rate is reproducible. Commercially available

biodegradable PLGA based systems for treatment of advanced prostrate cancer are: Zoladex Depot^{R} manufactured by ICI Pharma, containing 3.6mg goserelin acetate an analog of LHRH, to be released over 28 days and Enantone LP^{R}, manufactured by Takeda Co., Japan, containing 7.5mg leuprolide acetate to be released over 28 days[43]. Biodegradable as well as non-biodegradable subcutaneous silicone implants containing norgestrel are also available.

Liposomes[44-46] too have finally made it to the market. Two products, manufactured by The Liposome Company, USA, are currently available in the market. The drugs encapsulated in liposomes for the purpose of targeting and significantly reducing their systemic toxicity are doxorubicin, an anti-cancer drug and amphotericin-B, a systemic antifungal agent. More recently, research has been diverted towards **long circulating liposomes**[47,48]. These are nothing but liposomes coated with a synthetic polymer such as polyethylene glycol. Using this approach, sterically protected long-circulating immunotargeted liposomes have been prepared which demonstrated good combination of longevity and targetability *in-vivo*.

Besides these, there have been major advances in the drug delivery systems for the eye. The conventional drug delivery systems for the eye include the eye drops and the eye ointment. Both of these formulations suffer from major drawbacks, such as, the ocular bioavailability of drugs instilled in the form of

drops is extremely low, which necessitates frequent dosing. This leads to patient non-compliance and failure of therapy. The ointment on the other hand is extremely greasy and causes significant blurring of vision and is therefore best used at bed-time only, as adjunctive therapy to eyedrops. There is therefore a need to develop NDDS for the eyes which will overcome the limitations of the conventional dosage forms and improve their ocular bioavailability. This route of drug delivery has recently gained importance because of the better understanding of the ocular biopharmaceutics as well as pharmacokinetics provided by newly discovered non-invasive techniques such as gamma-scintigraphy[49] and fluorometry[50].

In order to understand the pharmacokinetic processes on administration of drug through the ophthalmic route, it will be helpful to know the ocular anatomy and physiology, hence it is being briefly discussed.

1.3 BRIEF DESCRIPTION OF OCULAR ANATOMY AND PHYSIOLOGY:

The ocular anatomy has been pictorially shown in Fig. 1. The outermost layer of the ocular surface is the tear film, which is more correctly called the **preocular tear film.** The tear film is a thin layer of fluid that covers the exposed part of the ocular globe, the cornea and the conjunctiva. This film is secreted by the lacrimal gland. The presence of a continuous tear film over the exposed ocular surface, is imperative for nourishing the corneal epithelium and facilitation of blinking[51,52].

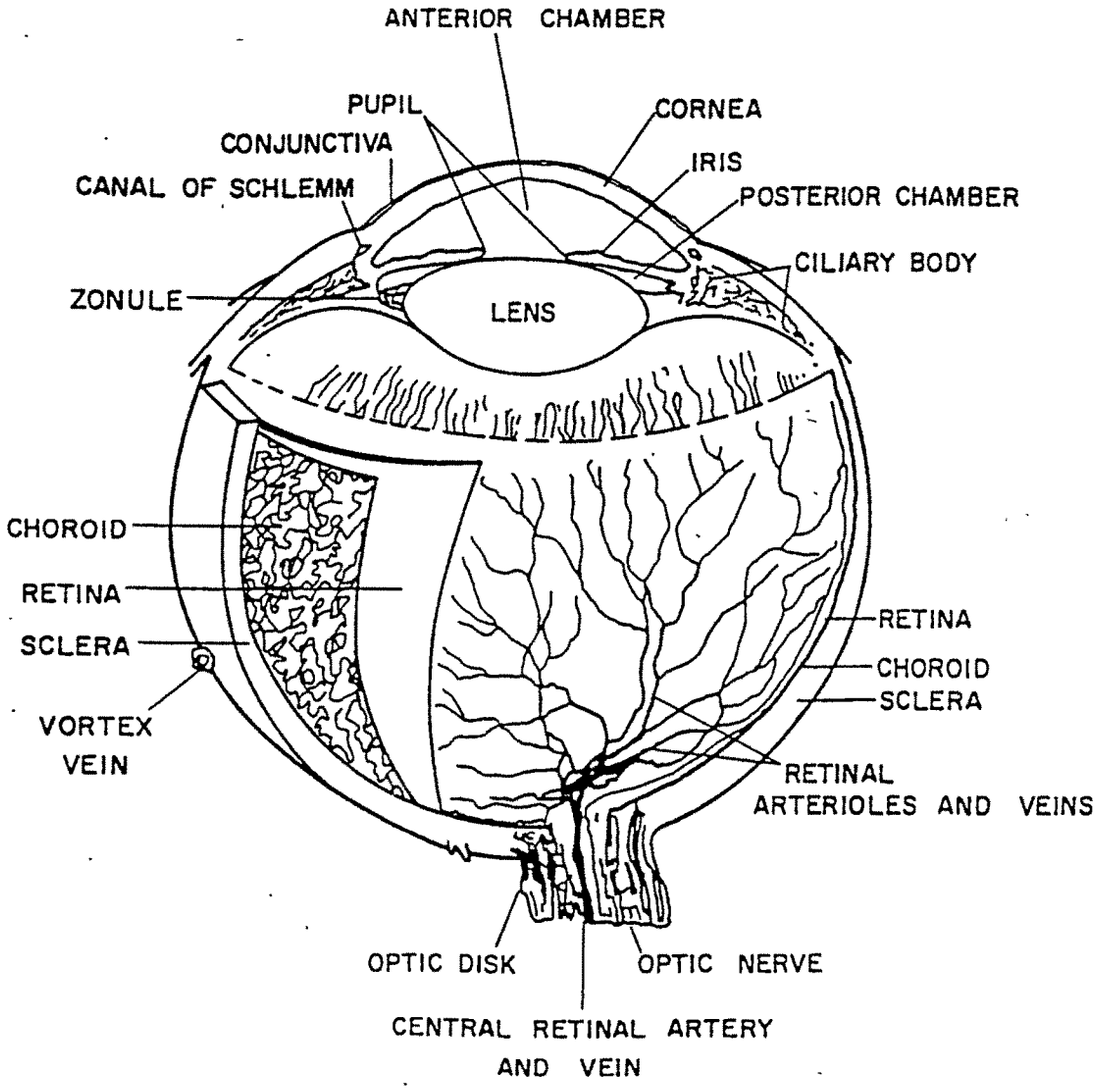


Fig. 1: Anatomical structures of the eye

The tear film is composed of two fluid layers. The major layer consists of aqueous portion secreted by the lacrimal gland and its thickness ranges between 6 to 10μm[51]. This layer is coated with a much thinner lipid layer, 0.1μm thick, which is secreted by the meibomian glands situated in the eye-lids[51]. This lipid layer consists mainly of waxy and cholesteryl esters. The tear film rests on a semisolid but fully hydrated mucin layer, which is about 10% of the total thickness of the tear film. The tear film has an osmotic pressure of about 310-350mOsm[53] and its pH ranges from 6.5-7.6[51].

The normal aqueous tears contain proteins such as lactoferrin, tear-specific prealbumin, immunoglobulin-A and lysozyme[54,55]. Glycoproteins are also found in tears. The total protein content in the tears normally is approximately 1%[54]. The normal tear volume of the human eye is 7-8μL and the tear turnover rate is approximately 61%/min[56]. Under normal conditions, a person blinks on the average 15 times per minute. With each blink, the tear film is pushed into the puncta, from where the tears overflow into the nasal canal (nasolacrimal drainage).

The cornea is an optically transparent, avascular tissue which covers approximately one-sixth of surface area of the anterior portion of the ocular globe. Since it is avascular in nature, it receives its nutrition from the physiological solutions in which it is bathed, tears on the anterior surface and aqueous humour on the posterior surface[51]. Additionally, blood vessels at the corneo-scleral junction, the limbus, also nourish the cornea.

The human corneal diameter is about 11.5mm with a radius of curvature of the anterior corneal surface of 7.8mm. Its thickness is about 0.5mm at the centre and about 0.7mm at the limbus.

The cornea is made up of the following 5 layers[51,57]:

(i) Epithelium: The corneal epithelium is made up of 6-7 layers of tightly packed cells which are continuous with the conjunctiva and is ≈50μm in thickness. It is the most important barrier to invasion by foreign substances, including drugs. The epithelium is lipophilic in nature but has small intercellular aqueous pores.

(ii) Bowman's membrane: It is 8-14μm in thickness and is acellular. It separates the epithelium and the substantia propria.

(iii) Substantia propria (stroma): The stroma forms about 90% of the cornea and is made of a modified connective tissue. Water constitutes ≈70% of the volume of stroma and the rest is made up largely of collagen.

(iv) Descemet's membrane: It is 10-15μm thick and lies between the stroma and endothelium. It is very elastic and remarkably resistant to proteolytic enzymes. It often remains intact even when the epithelium and stroma have been destroyed.

(v) Endothelium: It is made up of a single layer of cells, separated from each other by substantial intercellular space. Its barrier properties are not as good as those of the epithelium. The endothelium houses the water pump responsible for maintaining corneal thickness.

The conjunctiva is a vascularized mucous membrane that covers the anterior surface of the globe. At the limbus, it merges with the corneal epithelium and on the other side it merges with the epidermis of the lids. It has a surface area about 5 times that of the cornea. Mucus-producing goblet cells which are important for wetting and tear film stability are located in the conjunctiva[51].

The anterior chamber of the eye is bound anteriorly by the corneal endothelium, and posteriorly by the iris, a part of the anterior surface of the lens and the ciliary body. The ciliary body secretes the **aqueous humour** into the anterior chamber. The total volume of aqueous humour in humans has been shown to be ≈250µL, with a turnover of ≈1%/min[58].

The aqueous humour has low protein content and high ascorbate concentration as compared to the plasma. Additionally, lactate concentration of the aqueous humour is higher than that in plasma. The pH of aqueous humour is about 7.3-7.4[58]. The aqueous humour is responsible for generating the intraocular pressure and maintaining curvature of the cornea.

The canal of Schlemm is the conventional pathway of aqueous humour outflow from the anterior chamber of the eye. Aqueous humour leaves the anterior chamber to enter the canal of Schlemm. The canal of Schlemm is directly connected to venous plexus which drains the aqueous humour[51,58].

The sclera is the outermost vascularized fibrous coat of the eye and functions as a protective barrier. It occupies five-sixths the surface area of the globe. The sclera consists of dense bundles of collagen fibres. The scleral thickness varies from 0.6mm to 1.0mm[51].

1.4 PHARMACOKINETICS OF A DRUG APPLIED LOCALLY TO THE EYE:

When a quantity of drug is applied to the eye, generally the cul-de-sac, several factors immediately begin to affect the availability of drug contained in that quantity of dosage form[50,59-61]. First, the instilled dosage form mixes with the tear fluid and may induce reflex tearing due to its pH or inherent ability to cause irritation. Moreover, the volume of the instilled drop is usually 25-50µL and the eye can accommodate only 7-10µL, this leads to overflow of the tear fluid along with the drug out of the eye and partly into the nasolacrimal duct. The nasal canal is well vascularized and may absorb almost all of drug that overflows into it. Almost 80% of the instilled drug is lost in this way[59, 60, 62]. The second mechanism contributing to the loss of instilled drug is absorption of drug into the conjunctiva. Since the conjunctiva is vascularized, the absorbed drug is immediately lost into the systemic circulation[63]. Ionization of the drug in the tear fluid is yet another factor affecting ocular bioavailability of drug. Only the unionized drug is capable of being transported across the cornea and the ionized drug is left unabsorbed and subsequently washed out of

the tear film[64]. The normal protein content in the tear fluid is only 1% and hence any drug bound to protein would not be of much significance, however the tear protein concentration increases sharply during infection and inflammation and then drug protein binding could play a significant role in affecting ocular bioavailability. Enzymes present in the tear fluid may also metabolize the drug or it may undergo spontaneous degradation in the tear fluid.

Competing with all these mechanisms of drug removal is the **transcorneal absorption** of the drug, the route effective in bringing drug to the anterior portion of the eye, via absorption[64]. The cornea is the target tissue for most antimicrobials and steroids.

The rate of drug penetration into the cornea depends upon various factors[65-68], such as: (i) surface interactions of the drug between the tear film and mucosal layer that is in contact with the corneal epithelium. (ii) transport of drug across the multilayered cornea by trans- and pericellular routes[69,70]. Some small molecules can penetrate the cornea through the small intercellular aqueous pores in the epithelium, but this route is not of any clinical significance[69]. (iii) interactions of drug with components of the cornea such as protein binding and (iv) metabolism of drug by enzymes such as esterases present in the cornea.

Transcorneal absorption of drugs is better studied *in-vitro* rather than *in-vivo*, as in the *in-vivo* situation disposition of

the drug is complicated by tear production, tear drainage, corneal transport and elimination from the aqueous humour. In animal models as well as in clinical studies, drug concentration can be estimated in the cornea at a given time after topical application of the dosage form. In animal studies it is done by sacrificing the animal and removing the cornea for assay of drug, whereas in the clinical studies, corneas of those patients undergoing corneal transplant can be used. Since the drug equilibrates between the cornea and the aqueous humour, the aqueous humour can be repeatedly used as a convenient sample to estimate drug concentration in the cornea[66,67].

A schematic diagram[71] depicting all these processes is shown in Fig. 2. One of the key parameter in ocular absorption is the corneal permeability co-efficient (CPC)[58]. For a series of molecules of similar size it was shown that the permeability increases with increasing partition co-efficient (≤1 to 2), until a plateau is reached. This is because the lipid soluble drugs can penetrate the lipophilic corneal epithelium with great ease, but their diffusion through the hydrophilic stroma will become the rate limiting step in the absorption process. The endothelium is very thin and porous as compared to the epithelium and can be ignored in calculating the corneal flux. If the aqueous pores in the epithelium are disregarded due to their insignificant contribution in the absorption process, the steady state corneal flux J_s can be written as:

$$J_s = \frac{P\ C_w}{P\ l_s/D_s + l_e/D_e}$$

C_w= concn. of drug in the tear film
l_s= stromal thickness
l_e= epithelial thickness
P= partition co-efficient
D_s= diffusion co-efficient across cornea
D_e= diffusion co-efficient across epithelium

and

$$CPC = J_s/C_w$$

Distribution and elimination

Once the drug is past the cornea, it enters the anterior chamber which is filled with aqueous humour, secreted by the ciliary body. The aqueous humour is in contact with the iris-ciliary body and the lens, hence the drug that has entered the aqueous humour easily distributes into these tissues. The iris-ciliary body are target tissues for drugs like mydriatics, miotics and anti-glaucoma agents. Some drugs such as timolol, pilocarpine and atropine bind strongly to melanin, the pigment present in the iris and their elimination is therefore retarded. This factor has to be considered when dealing with ocular pharmacokinetics[65]. The iris-ciliary body are vascularized, hence drug taken up by these structures is emptied into the systemic circulation. From the lens the drug can distribute to the vitreous humour and subsequently to other vascularized structures of the eye such as the retina and choroid. The aqueous humour which still contains some part of the drug slowly empties into the canal of Schlemm from where it drains into the systemic circulation.

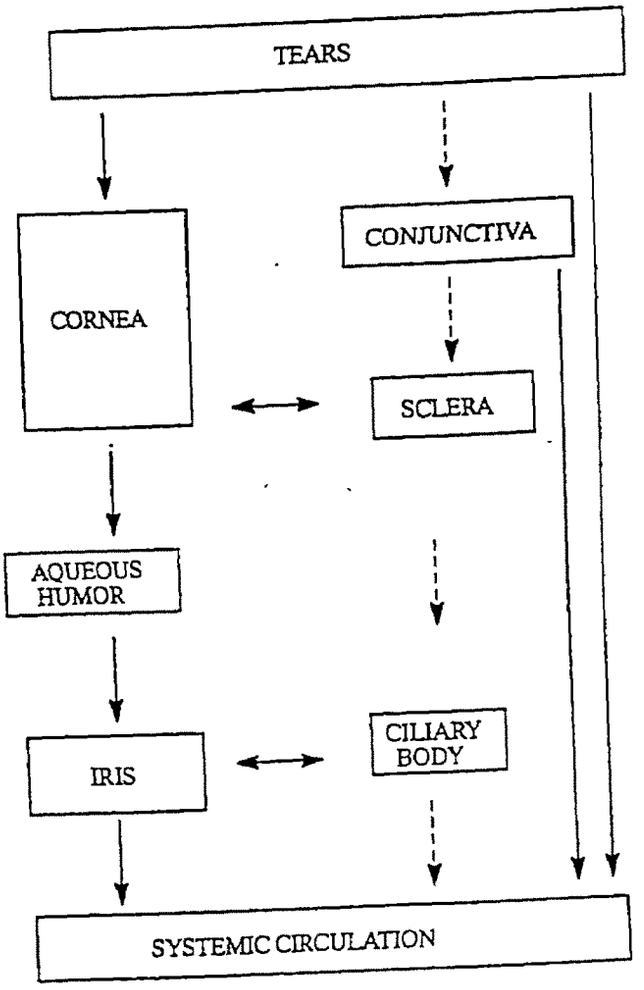


Fig. 2: Schematic diagram showing disposition of drugs instilled into the eye.

These are the various mechanisms by which an ocularly applied drug is absorbed and disposed, and give the pharmaceutical technologist useful information on how to design ophthalmic drug delivery systems.

1.5 OPHTHALMIC DRUG DELIVERY SYSTEMS:

Drugs are applied topically to the eye to treat or diagnose a number of complications of the external eye such as: (i) infective keratitis, conjunctivitis, blepharitis, which are treated with antimicrobials; (ii) Non-infectious inflammatory conditions of the cornea resulting from physical injury or ophthalmic surgery, which are treated with anti-inflammatory drugs and analgesics; (iii) glaucoma, which is treated largely with carbonic anhydrase inhibitors such as acetazolamide; (iv) dry eye syndrome (aphakia), which are treated with medicated as well as non-medicated eye-drops to keep the eyes moist; (v) mydriatics and miotics; (vi) local anaesthetics which are used during removal of foreign body from the eyes; (vii) antihistaminics and decongestants which are used to treat allergic conjunctivitis; (viii) diagnostic purpose, for which fluorescein sodium eye-drops are widely used to detect foreign body on the corneal surface[72].

External eye diseases are treated with local application of drugs to the eye so as to minimize exposure of rest of the body to the drug thereby minimizing the incidence of side-effects[73]. Additionally, local treatment utilizes a much lesser amount of

drug as compared to systemic therapy and hence also significantly cuts down the cost of therapy.

An ideal drug delivery system is one which can deliver requisite amount of drug for the prescribed period of time without leading to undesirable side-effects. With the recent advances in non-invasive techniques such as gamma-scintigraphy and fluorometry[61,74-78] for studying drug absorption and disposition in the body it has now become possible to design effective drug delivery systems, even to the complex organs of the body such as the eye. This has led to the development of new ophthalmic delivery systems such as long-acting gels, inserts, particulates, etc. Even though new delivery systems are being evolved, the conventional delivery systems, namely the eye-drops and eye ointments still lead the market of ophthalmic drug delivery systems.

1.5.1 CONVENTIONAL SYSTEMS IN OPHTHALMIC DRUG DELIVERY:

1.5.1.1 Ophthalmic solutions and suspensions:

A majority of the ophthalmic therapeutic agents are water soluble[79] or can be formulated as water soluble salts, those drugs that are insoluble can be suspended in a finely divided form in a suitable aqueous vehicle. A solution because of its homogeneity offers greater assurance of uniformity of dosage[80] and bioavailability and at the same time also simplifies large scale manufacture. Hence isotonic aqueous vehicles are widely used to dispense ocular drugs[81]. Additionally aqueous vehicles do not cause blurring of vision. To build up drug concentration in

the cornea, very frequent dosing would be required or the use of an ophthalmic solution of greater strength would probably help. Thus, the effective dose of medication administered locally to the eye may be varied by the strength of medication administered, the retention time of the medication in contact with the surface of the eye and the frequency of administration[85]. In the case of suspensions the particle size of the suspended drug is of utmost importance. Very small particles will overflow into the nasolacrimal duct and larger particles will cause considerable eye irritation. Selection of an ideal particle size should also take into consideration the rate of dissolution of the drug. The BP 1993 specifies the limits on particle size to be incorporated in ophthalmic suspensions. It specifies that in 10µg of the solid substance, NMT 20 particles should have a maximum dimension above 25µm, NMT 2 particles should have a maximum dimension above 50µm and no particle has a maximum dimension above 90µm.

In order to simulate natural tears, eye drops containing lipids have also been evaluated[86,87].

1.5.1.2 Ophthalmic ointments:

The principal semi-solid dosage form used in ophthalmology is an anhydrous ointment with a petrolatum base[87-89]. The ointment vehicle is usually a mixture of mineral oil and white petrolatum[87]. The mineral oil is added to reduce the melting point and modify the consistency. The principal advantages of petrolatum bases are their blandness and their anhydrous inert nature, which make them suitable vehicles for moisture-sensitive

drugs. They are used as lubricants in dry-eye syndromes and are most commonly used as adjunctive night-time therapy with eye-drops administered during the day. The anhydrous petrolatum base may be made more miscible with water through the use of an anhydrous liquid lanolin derivative.

The ointments are used almost exclusively to administer antimicrobials and steroids. The chief disadvantages[81] of the use of ophthalmic ointments is their greasy nature and blurring of vision they produce. Expulsion of ointment from the eye is also one of its major drawbacks. However, ointments do remain in the precorneal area for a relatively longer duration as compared to conventional solutions, because of their viscous nature[90]. The limits of particle size of the drug are the same as in the case of ophthalmic suspensions.

Thus conventional drug delivery systems may lead to systemic toxicity because of too frequent instillations or may fail to produce therapeutic effect if dosing is not done at regular intervals, hence the margin between therapeutic effect and toxicity is not really wide. There is therefore a need to improvise the approach to drug delivery, which would deliver the drug to the site of action at the desired concentration, for the desired period of time and would not cause discomfort to the patient. Hence, new ophthalmic drug delivery systems have been developed which have one or more distinct advantages over their conventional counter-parts.

1.5.2 NEW OPHTHALMIC DRUG DELIVERY SYSTEMS:

Utilization of the principle of controlled release offers an attractive approach to the difficult problem of prolonging precorneal drug residence time. This is because the ultimate goal of a long acting ocular drug delivery system is to maintain an effective drug concentration in the target tissues and yet minimize the required number of applications, which is in agreement with the function of controlled release systems.

The various approaches investigated to design new ocular drug delivery systems are as follows:

1> Long-acting ocular aqueous gels

2> Ocular inserts, which are further divided into a) erodible and b) non-erodible inserts.

3> Particulates, which include nanoparticles and liposomes

4> Ocular iontophoresis

1. Long-acting ocular aqueous gels:

It is well known that conventional solutions upon instillation into the eye get rapidly drained out into the nasolacrimal duct. On the other hand the ointment because of their semisolid form resist drainage, however it causes considerable blurring of vision. Hence it was realized that if the aqueous solution were presented to the ocular surface in a semisolid (gel) form, it would be able to remain in the precorneal area for a longer duration and at the same time would not cause blurring of vision because of its aqueous nature. Many hydrogels have been studied for their ability to prolong the residence time of the drug in

the precorneal area, such as: carboxymethyl cellulose sodium, carbomer, methylcellulose, hydroxypropyl methylcellulose, polyacrylamide, polymethacrylate, polycarbophil, polyoxyethylene and poloxamers[91-93].

Some of the polymers have a unique ability of forming gels *in-situ* i.e. the polymer solution gels on coming in contact with the tear fluid. Three types of polymers have been investigated for this purpose. **Carbopol** is a polyacrylic acid polymer and is insoluble in water as such, however, it undergoes hydration at pH values around 7 and forms a transparent gel. A drop of aqueous slurry of Carbopol would gel on instilling into the eye because of neutralization of the polymer by the tears. The incorporated drug would then be gradually released from this gel[94-97]. **Gelrite**^{R} is an ion sensitive gel forming polysaccharide. It gels in the presence of metallic cations such as Na^{+}, and Ca^{+2}. A fine dispersion of the polysaccharide in aqueous non-ionic isotonic vehicle when instilled into the eye, gels because of the presence of cations in the tear fluid[98,99]. **Poloxamers** are polyoxyethylene-polyoxypropylene block coploymers. These polymers form thermoreversible gels in water, i.e. they liquefy on cooling and gel at body temperature. All of these polymers have the advantage of being dispensed and instilled as conventional drops and additionally providing sustained release of the drug incorporated therein[100-103].

Two such polymeric gel formulations containing timolol and pilocarpine for once daily administration are already available in the USA.

2. Ocular inserts:

Ocular inserts[104,105] are solid drug delivery systems intended to be placed in the cul-de-sac from where they release the drug at a predetermined rate. Inserts can be broadly categorized into two types (a) non-erodible inserts which include contact lenses and Ocusert^{R} and (b) erodible inserts such as Lacrisert^{R}, soluble ophthalmic delivery insert (SODI), novel ophthalmic delivery system (NODS) and collagen shields.

(a) Non-erodible inserts:

Apart from being used for correction of vision, **contact lenses**[104-106], can also be used as drug delivery systems. The use of hydrophilic contact lenses, presoaked in drug solution, for ocular drug delivery has therefore been extensively examined for a variety of drugs. These included antibiotics, antiglaucoma agents and polypeptides. However, drug release from the contact lenses is extremely rapid, most of the drug being released in the first 30 min. Alternatively, drugs can be incorporated into the monomer mix which is to be polymerized, in this way, drug release can be retarded for upto 180 hours. Recently, there was a report on evaluation of a non-erodible system for long-term release of cyclosporine[107].

The Ocusert^{R} is a flat elliptical device consisting of three layers. The outer two layers of ethylene-vinyl acetate enclose the inner core of pilocarpine gelled with alginate[104,108]. The Ocusert is marketed in two sizes, Pilo-20 and Pilo-40, which release pilocarpine at the rate of 20µg/h and 40µg/h, respectively. Both systems are used for the continuous delivery

of pilocarpine for a week for the treatment of glaucoma. The major drawbacks of the Ocusert are feeling of a foreign body sensation in the eye, expulsion, difficulty in handling and insertion and specialized technology for its manufacture[104].

(b) Erodible inserts: Over the years, several erodible drug delivery systems have been conceived and tested for ophthalmic use[104,105,109-111]. These have included pilocarpine-containing wafers and polyvinyl alcohol discs or rods. Also wafers of collagen containing gentamicin sulphate have shown some promise in extending precorneal residence time as compared to conventional drops. Despite these efforts, there are only three erodible systems that have been marketed to date, the Lacrisert, the Soluble Ocular Drug Insert (SODI) and collagen shields.

The Lacrisert, developed by Merck Sharp & Dohme, is a sterile rod shaped device made up of hydroxypropyl cellulose without any preservatives and is used for the treatment of dry eye syndrome. The device weighs 5mg and measures 1.27mm in diameter with a length of 3.5mm. The insert was to be placed in the cul-de-sac. However, there have been reports of significant eye irritation and the use of cellulosic polymer caused matting of the eyelids[104].

The SODI developed by Diversified Tech Inc., USA, is a small oval wafer of polyacrylamide impregnated with drug. Its dimensions are 9mmX4.5mm with a thickness of 0.35mm. Clinical tests have indicated that the SODI impregnated with pilocarpine or tetracycline compares favourably with the conventional drop

treatment, for glaucoma and trachoma[104].

Corneal collagen shields were developed by Svyatoslav Fyodorov for use as corneal bandage after corneal surgery[112]. The shield was fabricated from porcine scleral tissue, which has a collagen composition closely resembling that of the human cornea. It forms a thin film approximately 0.1mm in thickness, has a diameter of 14.5mm and a base curve of 9mm. When the shield is hydrated it conforms to the corneal surface and dissolves slowly due to the action of proteases in the tear fluid. The shields marketed in the USA by Bausch & Lomb, Bio-Cor12, Bio-Cor24 and Bio-Cor72 dissolve in the tear fluid over a period of 12, 24 and 72 hours, respectively[113]. The time of dissolution can be extended by increasing the degree of cross-linking that is provided to collagen during the manufacturing process, by exposing to UV radiation. Many researchers have evaluated the efficacy of antibiotic soaked collagen shields versus conventional antibiotic eye-drops in animal models of eye infection and they found the shields to be superior to the drops in eradicating the infection[114-117].

The NODS was launched in the UK by Smith & Nephew Research Ltd. It consists of a water soluble drug loaded flag approximately 4mm long and 6mm wide. It is attached to a water soluble handle film by means of a thin soluble membrane. All of these are made up of polyvinyl alcohol. For administration, the flag is placed in the conjunctival sac and on contact with the tear fluid, the thin membrane rapidly dissolves releasing the flag. Tropicamide, chloramphenicol and pilocarpine have been evaluated in the NODS.

The NODS releases almost 70% of the drug in about 10min[118].

3. Particulates:

Among the first particulate system with pilocarpine that were developed was a cellulose acetate phthalate (CAP) pseudolatex formulation[119]. The pH of this formulation was maintained at 4.5, which upon instillation into the eye rose to 7.4 causing dissolution of the polymeric particles. The resulting polymer solution had high viscosity which prevented washout of drug from the eye. Recently however, the most frequently used polymers for preparation of ocular nanoparticles are the polyalkylcyanoacrylates[120,121]. These have the advantage that their polymerization can be carried out in an aqueous phase at room temperature without the use of high energy irradiation. The drugs may be added before, during or after the polymerization process. The nanoparticles on instillation into the eyes also face rapid drainage into the nasolacrimal duct. However some of these get trapped in the conjunctiva and some penetrate the first two cell layers of the cornea. Here the polymeric matrix undergoes either enzymatic hydrolysis due to esterases or spontaneous non-enzymatic hydrolysis to release the drug. The rate of biodegradation depends on the chain length of their ester side chain. The ocular uptake of these nanoparticles is increased during inflammation hence these could significantly improve the therapeutic efficacy of a number of antiinflammatory agents, antibiotics or antivirals. Other drugs delivered to the eye in the form of nanoparticles include hydrocortisone-17- butyrate-21-

propionate and metipranolol[121,122].

Generally smaller particles are tolerated better by the patients than larger particles and for this reason ophthalmic nanoparticles may represent a very comfortable prolonged action drug delivery system. Additionally, polybutylcyanoacrylate nanoparticles have been shown to adhere preferentially to the inflamed eye[123], therefore they may represent very promising class of drug carriers for the inflamed eye.

The use of **liposomes**[124] as an ophthalmic drug delivery system was first considered in the animal studies of Smolin *et al*[125] who reported that in the treatment of acute and chronic herpetic keratitis in rabbits, idoxuridine entrapped within the liposomes was more effective than a comparable therapeutic regimen of untrapped drug. It was later shown that the corneal penetration of idoxuridine was significantly enhanced after liposomal entrapment. Other drugs whose corneal penetration was improved by entrapment into liposomes include norfloxacin[126] and pilocarpine[127]. The precise mechanism by which liposomes interact with the cells and prolong drug release is not completely understood, however, the following mechanisms are suggested: intermembrane transfer, contact release, adsorption of liposomes onto cell surface, fusion of liposomes with cell membranes and endocytosis of the liposomes by the cell. The potential use of liposomes as drug delivery systems is limited by their short shelf life and limited drug loading capacity. Nevertheless they offer the advantage of being completely biodegradable and

relatively non-toxic.

4. Iontophoresis:

Iontophoresis or the use of small amounts of electrical currents has also been evaluated as a means to deliver drugs into tissues. A prerequisite for iontophoresis is that the drug must undergo ionization, preferably close to physiological pH. The cornea has been used as the target tissue to deliver several drugs in the form of their anions or cations. A small cup is placed on the cornea and the drug solution is filled in it, one of the two platinum electrodes having the same charge as the drug molecule, is dipped into this solution. The other electrode with opposite charge is attached to the body surface eg. the ear pinna. A small current, typically 2mA is passed in between the electrodes and the ionized drug molecules are pushed into the cornea as they are attracted by the oppositely charged electrode[128]. This process should be continued for not more than 10 min. The main advantage of this system is that large amounts of drug can be delivered to the cornea, which is especially useful in cases of acute sight-threatening infections where it is essential to control the growth of microbes as quickly as possible. Many antibiotics have been delivered to the cornea by means of iontophoresis such as gentamicin, tobramycin, ciprofloxacin and vancomycin[128,129]. Transscleral iontophoresis has also been carried out to deliver drug into the vitreous humour eg. foscarnet[130]. After 10 min. of transcorneal iontophoresis, mild epithelial disruption and corneal oedema are observed. Additionally, iontophoresis can only

be carried out in the hospital by trained staff, and these facts limit its regular use and hence its clinical importance is not so well established.

1.6 CONSIDERATIONS IN FORMULATION OF OPHTHALMIC DRUG DELIVERY SYSTEMS:

Conventional ophthalmic dosage forms have come a long way and hence most of the factors that need to be considered in their development are published officially. These dosage forms when applied to the eye, remain in the eye for a very short period of time, whereas the ocular NDDS do remain in contact with the eye for a much prolonged period of time. If these NDDS are not formulated with utmost precision and accuracy, they may lead to potentially destructive results e.g. NDDS usually contain a much larger dose than that present in a single dose of the conventional dosage form and if the release rate controlling mechanism fails, all of the drug may be dumped into the eye. Such a dose dumping was recently reported from the pilocarpine Ocusert^{R}. In order to get an insight into the various aspects of ophthalmic formulation development, it is important to review the factors that are to be considered in the development of a safe and effective drug delivery system.

(i) Raw materials:

The official guideline in the USP for the manufacture of sterile dosage forms states that they should be prepared from

presterilized ingredients. This holds good even for ophthalmic ointments, since no standard terminal sterilization method for these exists as yet. In case of ophthalmic solutions, suitable methods of sterilization of the solution are mentioned. As for ophthalmic suspensions, the suspended drug needs to be in a micronized and sterile form, prior to being incorporated in the sterile vehicle.

The USP recommends the membrane filtration method for sterilization of ophthalmic solutions. However, the level of assurance of sterility is relatively low as compared to terminal sterilization by autoclaving. This level of assurance can be greatly increased if the formulation were prepared from sterile ingredients, however, this may add significantly to the product cost. In order to avoid this, concentrated solutions of the drug and additives can be presterilized before mixing and this solution can be resterilized by membrane filtration, at the time of aseptic filling[131,132].

(ii) Sterility:

All dosage forms intended for ophthalmic use must be sterile. Ophthalmic solutions are generally sterilized by steam sterilization, at 121°C and 15 psi for a period of 15-20 minutes, or heating with a bactericide at 100°C. In case the drug or any of the additives are thermolabile, cold sterilization method such as membrane filtration (0.22µm pore size) is used. In case of suspensions, the suspended drug is separately sterilized before incorporating into the sterile vehicle. The finely powdered drug

by the USFDA, hence their use is also restricted[135].

The choice of preservative depends upon the type of drug, type of container closure, pH of the formulation and method of sterilization. Combination of preservatives may also be used to increase the spectrum of antimicrobial activity.

(iv) Tonicity:

It is desirable for all ophthalmic solutions and suspensions to be isotonic so as to prevent discomfort to the eye upon instillation[139]. However, it has been shown that the eye can tolerate tonicity in a range from 0.5-1.8% w/v of sodium chloride. Methods of obtaining acceptable limits of tonicity have been well documented in the literature. Commonly used tonicity adjusters are sodium chloride, potassium chloride, dextrose, mannitol, and glycerol[140]. Traditionally, aqueous isotonic buffered vehicles were used, in which the active ingredient as well as the preservatives were dissolved. The buffers were chosen so as to cover a range of different pH values: (a) The Sorenson buffer system also known as isotonic phosphate vehicle consisted of a mixture of 0.066M solutions of sodium acid phosphate monohydrate and anhydrous disodium hydrogen phosphate. These two buffers were mixed in various proportions so as to obtain pH in the range of 5.9-8.0. Sodium chloride was added to adjust the tonicity[141]. (b) Boric acid vehicle, which was nothing but a solution containing 1.9% boric acid and chlorbutanol as a preservative. It had a pH of 5. If drugs that are easily oxidized were to be incorporated in this isotonic vehicle, addition of 0.1% sodium bisulfite was recommended. (c) The sodium borate-

boric acid vehicle had a pH slightly over 9.0. This was commonly used for dispensing sulphonamide salts. (d) Self-sterilizing saline vehicle consisted of 0.9% sodium chloride and a strong microbicide, such as phenyl mercuric nitrate. These isotonic solutions were used for the extemporaneous preparation of eye drops and are now obsolete. The final tonicity of the ophthalmic solution is adjusted after calculating the contributions of each of the additive to tonicity.

(v) Additives:

These include stabilizers such as disodium EDTA, creatinine, surfactants, anti-oxidants such as sodium metabisulfite, ascorbic acid, etc.[81]. Non-ionic surfactants such as Tween 80 and Cremophor EL (polyethoxylated castor oil) are often used to stabilize certain ophthalmic formulations of NSAIDs. In the preparation of suspensions, these surfactants aid in dispersing the drug powder in the aqueous vehicle. Cationic surfactants such as benzalkonium chloride are used with dual purposes, to serve as a preservative and also as a corneal penetration enhancer[142]. Polymers such as polyvinyl alcohol, methylcellulose, hydroxypropyl methylcellulose and polyacrylates have been used to increase the viscosity of ophthalmic solutions with an aim to prolong the contact time of drug and the cornea so as to improve ocular bioavailability. These polymers are used in suspensions as suspending agents for the dispersed drug. Ophthalmic drops are with a few exceptions, aqueous in nature, however at times oily vehicles may be used. Castor oil is instilled in eyes during

inflammation so as to provide a lubrication in the eyes. Other oils that can be used include olive oil, sesame oil, arachis oil and mineral oil.

In the case of ocular NDDS, the important additives are the polymers which are used to modify the release characteristics of the drug. Natural and synthetic polymers have been used for this purpose. These can be further classified into water soluble and water insoluble polymers. Among the natural water soluble polymers gellan gum[78,92,99] and sodium alginate[143] have been used. Hyaluronic acid[97,144] gel which has high optical clarity and is also a component of the body tissues was recently introduced into the market, recently. Collagen, which too is a component of the body tissues, is a water insoluble polymer has been used for the manufacture of medicated corneal shields. Such medicated corneal collagen shields have been recently launched abroad[113]. Synthetic polymers are more widely used because they carry a limited bioburden, and are less expensive than their natural counterparts. The water soluble synthetic polymers most commonly used are: cellulose based polymers, such as methylcellulose[145], HPMC[146], polyvinyl alcohol[97,147], poloxamers[101], and carbomers[96,97,148]. The polymers may be ionic or non-ionic in nature. Water insoluble synthetic polymers such as acrylates and ethylene-vinyl acetate (EVA) are used. The pilocarpine Ocusert^{R} utilizes EVA as the release retarding polymer. Acrylates are used in the manufacture of medicated soft contact lenses[149].

(vi) Buffering and pH adjustments:

It would be ideal if all ophthalmic solutions and suspensions could be adjusted to a pH 7-7.4, which is that of tears. However, most drugs used in ophthalmic practice are either not soluble in this pH range or are unstable, hence they need to be formulated at a pH other than the physiological pH[81,88]. The eye can tolerate pH from 3.5-10, because of the buffering capacity of tears, which quickly neutralize the instilled drop. However, the buffering capacity of tears is weak, hence if buffers with strong buffering capacity are used in the formulations, they would tax the buffering capacity of tears and cause marked irritation and lacrimation. To ensure that the pH of the formulation does not change appreciably with storage, adequate buffering is required. The most commonly used buffering agents for ophthalmic use are phosphate, borate, acetate, and glucuronate[81,88,139-141,150,151].

(vii) Stability:

The chemical stability of the drug in an ophthalmic solution is of utmost importance. In cases of ophthalmic suspensions, physical stability also needs to be assessed eg. aggregation of particles, caking, etc. Drug stability depends upon chemical nature of the drug substance, pH of the formulation, method of sterilization, nature of additives and type of container-closure[134]. In addition to optimal pH if oxygen sensitivity is a problem, stabilization may be achieved by addition of suitable antioxidants such as ascorbic acid or sodium metabisulfite, if metal ions catalyze degradation, addition of chelating agents such as EDTA and 8-hydroxyquinoline would help in stabilizing the

formulation[74,81]. If the drug or the preservative is sensitive to light, use of amber coloured vials can adequately protect the product from harmful rays of light.

The stress here is on total product stability and not just stability of the active ingredient. A total stability programme should include assay of drug, degradation products and the preservative used in the formulation. The minimum acceptable period of accelerated stability studies is 6 months. During the period of accelerated stability studies physicochemical as well as microbiological parameters need to be evaluated e.g. pH of the product, particle size distribution, clarity, sedimentation rates, viscosity, *in-vitro* drug release profile, discolouration etc. as well as sterility and antimicrobial effectiveness test. According to the USP, the product is stable if the percentage of degradation products remains within the specified limits or if the decrease in potency of the drug is < 5%.

(viii) Clarity of product:

As a basic requirement, ophthalmic solutions should be free from particulate matter[74,81,131]. Clarity is usually achieved by membrane filtration. It should be ensured that the container and closure do not contribute any particulate matter.

(ix) Packaging:

The most preferred container for dispensing ophthalmic solutions is colourless or amber type I glass vials. The advantages offered are: low reactivity with the contents, no leaching of

contents of the container, good clarity and autoclavability[88,140]. Plastic containers made of LDPE can be used but the pharmacist must bear in mind that it is permeable to oxygen and volatile chemicals. Additionally, LDPE is known to adsorb many drugs and preservatives. With the recent developments in technology, products with LDPE containers can be made by the form-fill-seal (FFS) technique.

Closures available are made from natural or synthetic rubber, silicone elastomer coated or teflon coated rubber or LDPE. Latex rubber stoppers are widely used as closures for ophthalmic solutions, however they are incompatible with some drugs and preservatives such as benzalkonium chloride. Among the rubber stoppers, the most commonly used are grey butyl and grey bromobutyl stoppers. The grey bromobutyl rubber stoppers are slightly expensive but are extremely inert and show excellent resistance to autoclaving. LDPE stoppers are available in presterilized form, however they cannot withstand autoclaving because of their low melting point.

Apart from formulation considerations, the development of a NDDS for ophthalmic use calls for assurance of safety and efficacy of the product and hence the development programme needs to include ocular toxicity, ocular irritation and efficacy evaluation of the product under development.

(i) Ocular toxicity:

Any drug which is intended to be delivered to the eye, whether it

be a new chemical entity or a drug approved for administration by other routes, has to undergo ocular toxicological evaluation. This is also known as safety evaluation. This step involves exposing several species of animals to increasingly larger doses and durations of exposure with the test compound. This continuum begins with short-term studies (one dose upto several instillations per day), continues with intermediate-term studies (upto one month) and finally long-term studies (6 to 12 months). These studies evaluate changes in animal health or behaviour, as well as extensive gross and microscopic pathologic characteristics[152]. Acute and subacute studies are required before testing of the new drug in humans.

(ii) Ocular irritation:

The ocular surface is extremely sensitive to external stimuli and especially the cornea as it is highly innervated. Since most ophthalmic solutions are formulated at slightly acidic pH, they may cause transient irritation and lacrimation, or the drug may be inherently irritant to the eye eg. flurbiprofen. It is therefore essential to evaluate the ocular irritation potential of newly prepared ophthalmic formulations. As a part of the Federal Hazardous Substances Act (FHSA), a modified Draize test has been adopted for eye irritancy. Albino rabbits are used to evaluate the irritation potential of ophthalmic formulations[153,154]. The USP too has suggested guidelines for a 72 hour eye irritation test in rabbits in the final dosage form.

(iii) Efficacy studies:

Once a product is found to be stable and safe, it has to be

evaluated for its efficacy. Efficacy studies are routinely carried out in animal models, by experimentally inducing the required disease in the animals[152]. Animal disease state models of human ocular disease are an integral part of ophthalmic product development process. 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.

Animal models for bacterial keratitis:

Quantitative models of bacterial keratitis have been developed in rats, rabbits and guinea pigs. Since the rabbit eye resembles the human eye quite closely, anatomically and physiologically, it is the most widely used ocular model[155].

Infection in the corneas can be induced by merely abrading the epithelium and exposing the stroma. Additionally, local application of infected material to the abraded cornea can lead to infection. However, in quantitative studies[156], it is essential to know the number of viable bacteria that were inoculated. Hence a better method of developing infection is to inject 10-20µL of a suitable bacterial broth culture with a fine needle (29 or 30 gauge), directly into the stroma. Once infection is established, treatment with the given antibiotic and controls is initiated. The number of viable bacteria remaining after a period of treatment or at the end of treatment can be found by carefully dissecting out the cornea with a sterile trephine, homogenizing it and incubating it in suitable microbiological media to get the number of colony forming units per mL

(cfu/mL)[156]. Alternatively the progress of infection, in terms of the inflammatory tissue response, before and after starting treatment can be estimated using a standard Draize scale.

The most commonly used bacteria for inducing bacterial keratitis are *Staphylococcus aureus*[157,158], *Pseudomonas aeruginosa*[159,160] *and Streptococcus pneumoniae*[161]. These bacteria are the most destructive ocular pathogens. As these bacteria grow in the corneal stroma, they secrete a variety of endotoxins and antigenic proteins which lead to tissue destruction and inflammation. The main pro-inflammatory bacterial product of *S. aureus* are α-toxin and protein-A[162], that of *P. aeruginosa* is exotoxin-A[163] whereas that of *S. pneumoniae* is teichoic acid[161]. Hence, the same models of infection have also been used to evaluate combinations of antibiotics and steroids.

The most common infections of the external eye occur in the cornea or the conjunctiva[164]. Among these too, viral infections are the most common followed by bacterial infections and then fungal infections. Most viral infections of the eye are usually self limiting, but are treated with antibiotics so as to prevent bacterial superinfection. Bacterial infections are usually caused due to abrasion of ocular surfaces by non-sterile material, foreign particles or occur following ocular surgery. In such cases antibiotics have to be applied locally to the eye. Fungal infections of the eye are quite rare, and are most commonly seen if the patient is severely immunologically compromised or is on prolonged steroid therapy. However, these too can be treated with

topically applied antifungals.

Thus bacterial infections of the external eye appear to be the ones that demand maximum attention and the most common among them are bacterial keratitis and bacterial conjunctivitis.

1.7 BACTERIAL KERATITIS AND CONJUNCTIVITIS:

Bacterial keratitis[165] (inflammation of the cornea) is an opportunistic infection of the avascular corneal stroma, initiated by a breakdown of the epithelial barrier. Corneal abrasion with infected material, extended-wear contact lenses, eyelid disease and disorders of the ocular surface are predominant contributing factors. Rapid diagnosis and treatment of bacterial keratitis are essential to prevent corneal scarring and minimize visual loss. The tear film is colonized by saprophytic bacteria which coexist with the host with some mutual advantages. For the host, the bacterial flora provides protection against colonization by other virulent bacteria, thereby becoming a part of the eye's defence against infection. The presumed benefits for the flora are a habitat and resources to grow. This stable relationship exists as long as the normal flora does not proliferate beyond its usual numbers or location. The tear film constantly dilutes and flushes free-floating bacteria from the surface of the eye. Lactoferrin, antibodies, lysozyme, betalysins, ceruloplasmin, orosomucoid and components of the complement system all play a role in the control and elimination of bacteria on the ocular surface[54,55].

Acute bacterial conjunctivitis[165] occurs when sufficient bacteria

are introduced into the fornices to overwhelm the normal bacteriostatic and flushing mechanisms. Chronic conjunctivitis is associated with a constant adnexal source of bacteria and resolves rapidly when the source is successfully treated.

Bacterial adhesion on the corneal surface is inhibited by the epithelial glycocalyx and although bacteria are tolerated on the ocular surface, keratitis results if they infiltrate the corneal stroma. With excessive proliferation of the normal flora, its toxic by-products accumulate and the response to this insult is an inflammatory reaction (conjunctivitis or kerato-conjunctivitis)[55].

Staphylococcus epidermidis and diphtheroids predominate among the flora isolated from the healthy eyes, although species of greater virulence such as *S. aureus, Streptococcus pneumoniae, P. aeruginosa* and even meningococci have been isolated. It has been suggested that these more virulent species of bacteria represent transients and their presence does not represent true colonization of the eye. Given the proper conditions, virtually any strain of bacteria can cause colonization of the conjunctiva or the cornea. The proper conditions of infection usually involve a breakdown of host defences e.g. epithelial abrasion, or introduction of pathogenic organisms in sufficiently large numbers to overwhelm host defences[164].

The most commonly used antibiotics for the treatment of bacterial conjunctivitis and keratitis have been gentamicin, neomycin, polymyxin-B, chloramphenicol, erythromycin and tobramycin[166]. Recently the fluoroquinolones comprising of

norfloxacin, pefloxacin, ofloxacin and ciprofloxacin have also been included in the treatment of ocular infections[167-170].

1.8 CHOICE OF DRUGS:

The commonly used antibiotics for treating bacterial infections of the eye have been tetracycline, erythromycin, neomycin, polymyxin-B, chloramphenicol etc[166]. However, these suffer from major limitations such as, having low potency and a narrow spectrum of activity. Moreover many bacterial strains resistant to these have emerged. Recently, the fluoroquinolones, which have a much wider spectrum of activity have found use in ophthalmic practice[167-170]. The most commonly used fluoroquinolones for this purpose are norfloxacin, ciprofloxacin, ofloxacin and pefloxacin. These act by inhibiting the bacterial enzyme DNA gyrase and hence impair cell division[171]. Due to this unique mode of action, resistance develops at an extremely slow rate[171,172]. Amongst these, ciprofloxacin is the most potent agent and is particularly active against *Pseudomonas aeruginosa*, the most destructive ocular pathogen. Typical MIC values of ciprofloxacin are in the range 0.1-2.0µg/mL, which is one-half to one-fifth the MICs of the other fluoroquinolones. These advantages make ciprofloxacin an excellent choice for the empirical treatment of external ocular infections. Additionally, ocularly applied ciprofloxacin was found to be non-toxic and safe in preclinical studies[173,174].

Since severe inflammation occurs secondary to bacterial infection, the eye needs to be treated with both, an

antibacterial and an anti-inflammatory agent. During an infection the host immune reaction causes migration of neutrophils to the infected tissue, these neutrophils contain histolytic enzymes which are released when they rupture and cause tissue destruction[175]. Steroids are the most potent inhibitors of such immune reactions[176] and need to be administered along with antibacterial agents. Since antibacterial agents can restrict bacterial proliferation only, the presence of steroids will help in controlling the host immune reaction[177]. The most commonly used steroids for ophthalmic use are dexamethasone, hydrocortisone, prednisolone and triamcinolone[73,178]. Amongst these, dexamethasone is the most potent steroid with predominant anti-inflammatory activity (glucocorticoid) and almost no mineralocorticoid activity. Moreover, dexamethasone is about 25 times as potent as hydrocortisone and about 5 times as potent as prednisolone and triamcinolone[73,179]. Thus, combining ciprofloxacin with a potent anti-inflammatory drug such as dexamethasone would increase the clinical efficacy of ciprofloxacin in treating bacterial keratitis. The use of such a combination is indicated where there is a risk of superficial ocular infection or where there is an expectation that potentially dangerous numbers of bacteria will be present in the eye. Other antibiotic - steroid combinations for ophthalmic use are available in the market and some of these have also been evaluated in suitable animal models[180,181]. Antimicrobials can sometimes overcome the depressed resistance to infection caused by corticosteroids, depending on the type of infectious agent,

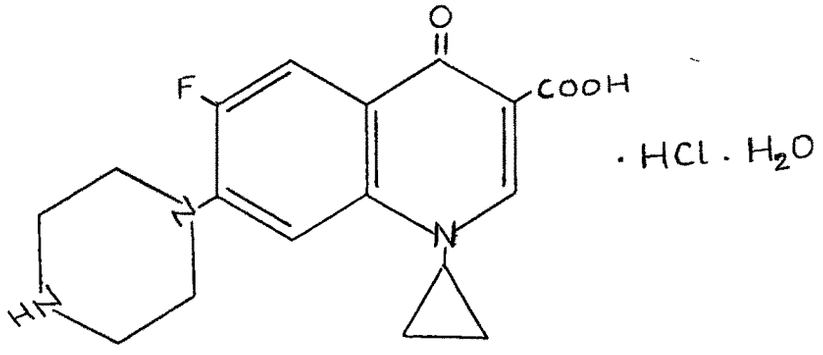
the resistance of the host to it and the efficacy and toxicity of the antimicrobial drug. The combined use of corticosteroids with effective antimicrobial agents has been proved to be life saving in certain cases of tuberculous meningitis and caseous pneumonia.

The formulation and performance of drug delivery systems is largely influenced by the physicochemical properties of the drug and also its pharmacokinetic characteristics. Thus, to develop a successful drug delivery system, the formulator should have a complete knowledge of the important physicochemical and biological properties of the drug and the way in which they influence its performance[182]. For example, the pKa values of the drug would indicate at what pH the drug would be predominantly unionized, since this is the form in which the drug would be absorbed by the body tissues. The pH solubility profile and the pH stability profile give valuable information on selection of pH of the final formulation and are of great importance in the manufacture of liquid dosage forms. Information on factors detrimental to drug stability help the formulator in preparation of a stable formulation.

All such valuable information pertaining to the drug which can be utilized by not only the formulator, but also the analytical chemists, pharmacologists and all those who are involved directly or indirectly in formulation development is systematically documented as a comprehensive database, which is commonly known as the drug profile.

1.9 DRUG PROFILE OF CIPROFLOXACIN:

Ciprofloxacin belongs to a new generation of fluorinated quinolones structurally related to nalidixic acid. It was developed in the early 80's by Bayer A.-G., Germany, and was then known as Bay o 9867. Chemically it is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl)-3-quinoline carboxylic acid. It is also available as the hydrochloride salt[183]. Both, the base and the salt are official in USP XXIII as well as IP '96. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and the structural formula is



Ciprofloxacin HCl occurs as a faintly yellowish to yellow crystalline powder.

Elemental composition of the base is C:61.62%, H:5.48%, O:14.49%, N:12.68%, F:5.73%[183]. The base melts at 270-275°C whereas the HCl salt decomposes at 315-320°C[184]. Ciprofloxacin is zwitterionic in nature and has two pKa values: pKa_1:6.09, corresponding to the piperazinyl -NH- group and pKa_2:8.74, corresponding to the -COOH group. The isoelectric point, pI, where the drug exists as

positively as well as negatively charged ion, is at pH 7.42[185]. The apparent partition coefficient (log P_{app}) at isoelectric point is -1.08[186,187]. The base has an intrinsic solubility of 0.0792mg/mL at 25°C in water[184]. Ciprofloxacin base has a solubility of 16mg/100mL in ethanol and 55mg/100mL in dichloromethane. The HCl salt has a solubility of about 3.6mg/mL in water at 25°C. The solubilities of the HCl salt, at pH 5, 7 and 9 were found to be 3.46mg/mL, 0.09mg/mL and 0.28mg/mL at 25°C, respectively[184]. It is practically insoluble in ethanol and absolutely insoluble in dichloromethane. The pH of 1 in 40 solution of ciprofloxacin hydrochloride in water is 3.0-4.5[188]. The UV spectrum exhibits some shifts in wavelength of maximum absorbance with change in pH of the solution, 271 nm in 0.1N NaOH, 274 nm in pH 7.4 phosphate buffer and 277 nm in 0.1N HCl.

Ciprofloxacin is very stable in the solid state as well as in aqueous solution[189]. However degradation occurs on prolonged exposure to light. Ultraviolet light causes maximum degradation with loss in antibacterial activity[190-193].

The principal degradation product is desethylene ciprofloxacin[194] although others do exist, their chemical identities have not been completely established[190]. Maximum stability occurs in the pH range 3-4 and degradation is accelerated at pH > 6[193]. Complexation with cyclodextrins significantly reduces photodegradation of ciprofloxacin[195].

Ciprofloxacin HCl solution is compatible with normal saline and 5% dextrose solution[196], it is also compatible with most acidic

salts, however immediate precipitation occurs when the solution is mixed with alkaline solutions of drugs such as heparin sodium, aminophylline, amoxicillin sodium, clindamycin phosphate, furosemide, dexamethasone sodium phosphate, hydrocortisone sodium succinate, etc[197].

Ciprofloxacin is a broad spectrum synthetic antibacterial agent. It exhibits excellent activity against gram negative bacteria and the presence of a 6-fluoro group enables it to extend its spectrum of activity to gram positive bacteria as well. The 7-piperazinyl group confers anti-pseudomonal activity and the N-cyclopropyl group facilitates its penetration into the bacterial cell[198]. Gram negative bacteria that are sensitive include *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Neisseria gonorrhoeae, Neisseria meningitidis, Vibrio cholerae, and Enterobacter cloacae*[199,200]. Typical MIC values for these are in the range 0.0125-0.5µg/mL. Gram positive bacteria that are sensitive include *Staphylococcus aureus (penicillinase producing and penicillinase non-producing), Staphylococcus epidermidis and Streptococcus faecalis.* Typical MIC values for gram positive bacteria range from 0.5µg/mL to 2µg/mL. Its spectrum of activity also extends to other bacteria such as *Proteus vulgaris, Proteus mirabilis, Providencia stuartii, Morganella morganii, Citrobacter freundii, Campylobacter jejuni, Shigella flexneri and Shigella sonnei.* Most of the other strains of *Streptococci* are moderately sensitive, as are *Mycobacterium tuberculosis, Mycobacterium*

fortuitum, Chlamydia trachomatis and *Mycoplasma pneumoniae.* Anaerobic bacteria are generally resistant, however, those that are moderately sensitive include species of *Actinomyces, Peptococcus and Peptostreptococcus, Propionibacterium.* Bacteria exhibiting MIC values ≥4μg/mL are considered to be resistant to ciprofloxacin. Ciprofloxacin is indicated in the treatment of infections, caused by sensitive organisms, affecting the lower respiratory tract, urinary tract, skin & skin structures, bone & joint. It is also indicated in the treatment of infectious diarrhoea as well as to treat external infections of the eye and ear by local application in the form of drops or ointment[201]. Ciprofloxacin HCl is administered orally in the form of tablets and intravenously as the lactate salt[197]. For the treatment of urinary tract infections, the usual adult oral dosage is 250mg every 12 hours[199,201,202]. The usual adult dosage for lower respiratory tract, skin & skin structure or bone & joint infections and infectious diarrhoea is 500 mg every 12 hours, for a period of atleast 1 week. A dose of 750 mg every 12 hours may be needed in case of severe infection of the bones and joints and treatment is prolonged, generally 4-6 weeks[199,201].

Ciprofloxacin exerts its antibacterial effect by inhibiting the bacterial enzyme DNA gyrase, which is responsible for the successful replication of DNA. Inhibition of this enzyme impairs cell division and eventually cell death occurs. Ciprofloxacin is bactericidal in nature. The minimum bactericidal concentration (MBC) is 1-4 times higher than the MIC, although the MBC may

occasionally be as much as 8 times higher[171].

The absolute bioavailability of oral ciprofloxacin is approximately 70%[203]. Food has been shown to prolong the time to reach maximum plasma concentration, but this does not seem to be of any clinical significance. Following oral administration of single doses (250-750 mg) of ciprofloxacin to healthy volunteers, mean Cmax values ranging from 0.8-3.9µg/mL were reached within 1-2 hours. Following intravenous administration of ciprofloxacin 200 and 400 mg, Cmax values ranged from 2.8-3.8µg/mL and 3.4-6.7µg/mL, respectively[199,201,204,205].

Following oral administration, ciprofloxacin distributes well into most tissues and body fluids and concentrates in some of these. It has a volume of distribution of 2.1-5.0L/Kg, indicating a high degree of tissue distribution[199,201,202,204,205]. In the bile, urine, kidneys, gall bladder and liver tissues, the drug achieves concentrations almost 6 times those of the corresponding plasma concentrations. It also penetrates well into the lung tissue and selectively concentrates in prostatic tissue, fluid and semen. Ciprofloxacin is not concentrated in ocular tissues. Concentrations in aqueous humour were less than 25% of the corresponding serum concentration[206,207]. Ocular trauma however, increased delivery of ciprofloxacin to the eye, following oral or i.v. administration[208]. Thus local instillation of drug in the eye is required to attain therapeutic concentrations in the fluids and tissues of the external eye.

After an oral dose, four metabolites have been identified in human urine[209,210], which together account for approximately 15% of the administered dose. The metabolites are M1: desethylene ciprofloxacin, M2: sulphociprofloxacin, M3: oxociprofloxacin and M4: N-formylciprofloxacin. The metabolites have antibacterial activity but are less active than ciprofloxacin[211]. After a 250 mg oral dose, urine concentrations usually exceed 200µg/mL during the first 2 hours and are about 30µg/mL, 8-12 hours after dosing. The elimination half life ($t_{\frac{1}{2}\beta}$) is about 3-5 hours. Urinary ciprofloxacin excretion is virtually complete within 24 hours after dosing. Renal clearance is ≈300mL/min. Urinary elimination occurs by active tubular secretion[199,201,202,204,205]. In patients about 20-35% of an oral dose is recovered from faeces[212].

As a result of age related decline in renal function, renal clearance of ciprofloxacin in the elderly is lower which leads to elevation of Cmax and AUC, which necessitates dose reduction[213]. Hepatic dysfunction appears to have little effect on the disposition and elimination of the drug. Elimination of ciprofloxacin in children aged 1-5 years, is rapid as compared to infants, shorter dosing intervals of 8 hours are required in children.

The major side-effects occurring due to oral or intravenous intake of ciprofloxacin are headache, dizziness, agitation, sleep disorders, convulsions in rare instances, gastrointestinal discomfort, skin rash or pruritus, and crystalluria[198].

Crystalluria occurs due to the low solubility of quinolones at physiological pH. The best way to avoid this is to keep the patient well hydrated and additionally, acidification of urine is also helpful. In rare cases, photo-toxicity has been reported during treatment with ciprofloxacin.

Co-administration of ciprofloxacin with aluminium or magnesium containing antacids, milk, yogurt and other dairy products, ferrous or ferric salts as well as zinc salts leads to a significant decrease in absorption of ciprofloxacin[214, 215]. This is due to the chelation of metal ions by ciprofloxacin resulting in formation of a large chelate complex which is difficult to absorb.

Ciprofloxacin reduces the clearance of caffeine and theophylline by inhibiting their metabolism. There are many case reports of enhanced theophylline toxicity when co-administered with quinolones[214,215].

Ciprofloxacin also inhibits the metabolism of the anticoagulant warfarin and hence patients on coumarin anticoagulant therapy need to be closely monitored when quinolones are co-administered.

There is evidence which suggests interaction between ciprofloxacin and NSAIDs, at the GABA receptor level. Among the NSAIDs, fenbufen and its metabolite biphenyl acetate produced marked inhibition of the GABA post synaptic receptor and may lead to convulsions. In patients with a history of epilepsy concomitant use of quinolones and NSAIDs should be avoided[214,215].

Infections of the external eye caused by susceptible bacteria are best treated by topical application of ciprofloxacin ophthalmic solution or ointment, since only sub-therapeutic levels are attained in ocular fluids and tissues following oral or i.v. dosing[206,207]. In clinical studies it has been shown that a 3 day course of ciprofloxacin 0.3% ophthalmic solution was highly effective in eradicating or reducing pathogenic bacteria causing acute conjunctivitis[216]. All bacterial species isolated from patients were sensitive to ciprofloxacin. Ciprofloxacin 0.3% was at least as effective as tobramycin 0.3%, a potent aminoglycoside widely used to treat ocular infections and significantly more effective ($P < 0.001$) than placebo. While carrying out clinical studies of ciprofloxacin ophthalmic solution in patients with acute bacterial keratitis[217], it was found that symptoms and signs of infection resolved or improved during the course of treatment and clinically successful outcomes were obtained. More than a 90% success rate was obtained among all the patients with bacterial keratitis. At present ciprofloxacin is the only ophthalmic antibacterial that has been shown in a large clinical study to be highly effective at its commercially available concentration as initial therapy for bacterial keratitis[218,219].

When applied locally to the human eye in the form of 0.3% w/v eye-drops, mean aqueous humour concentration of 0.072µg/mL was achieved within 90 min. Very high intersubject variability in aqueous humour drug concentration have been reported. Instillation of ciprofloxacin solution after removing corneal

epithelium results in significantly higher aqueous humour drug concentrations as compared to those obtained with intact epithelium, indicating that corneal epithelium is a barrier to absorption of ciprofloxacin. Even after repeated instillations in the eye, no drug could be detected in the plasma[220,221].

On topical instillation into the eye, mild burning sensation occurs due to the acidic pH of the eye-drops. In some cases, a white precipitate of ciprofloxacin is seen in the tear film or on a corneal ulcer. This is because of the low solubility of ciprofloxacin at physiological pH and the precipitate disappears rapidly on discontinuation of treatment.

Several analytical methods have been reported for the assay of ciprofloxacin. The various methods reported are colourimetry[222-224] and HPLC[225-227]. Ciprofloxacin forms coloured complexes with Fe^{+3}, methyl orange, MBTH, and bromocresol blue. The official method in the USPNF, for assay is based on HPLC separation of ciprofloxacin ethylenediamine RS (degradation product) from ciprofloxacin. The limit for this degradation product present in the bulk drug is 0.4%. Since ciprofloxacin has three amine groups, they strongly adhere to the chromatographic column material and cause severe tailing. Hence all mobile phases contain ion-pairing agents such as triethylamine, tetra-alkyl ammonium halides or alkylsulphonic acid salts, which minimize this interaction and generate sharp peaks. .pa

To study the dissolution of ciprofloxacin HCl form tablets, using water as the dissolution medium, the USP XXIII recommends use of

direct spectrophotometric measurement at 276 nm. The assay of drug from tablets is, however, carried out using HPLC.

The official method[188] for assay of ciprofloxacin from ophthalmic solution is also based on HPLC separation from its degradation product, hence the official method for assay itself is stability-indicating.

Ciprofloxacin has been assayed from plasma and other body fluids by HPLC. Some analysts have taken advantage of the native fluorescence of ciprofloxacin[228-231]. Using a fluorescence detector for quantitation of ciprofloxacin makes the method more sensitive and selective as most of the plasma constituents are non-fluorescent. On the other hand, some analysts have also used UV detectors for quantitation of ciprofloxacin[232-235].

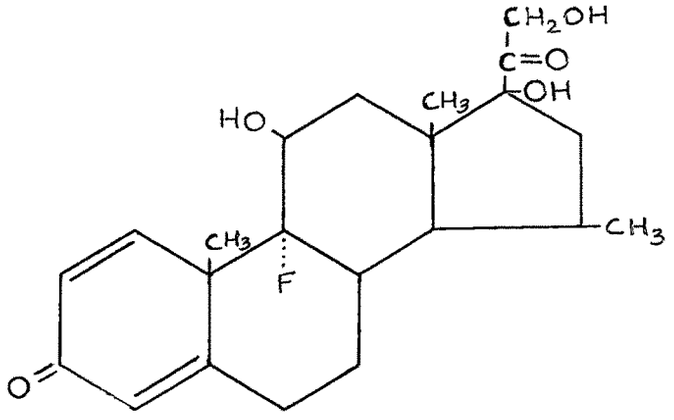
A TLC method for identification of drug and related impurities is official in the IP '96 and USP 23.

NMR and mass spectroscopy have been used to study structure of degradation products[190] and metabolites of ciprofloxacin[210].

Ciprofloxacin is presently available in the form of tablets, oral suspension, intravenous infusion, ophthalmic solution and ophthalmic ointment.

DEXAMETHASONE: A DRUG PROFILE (INFORMATION RELEVANT TO ITS OPHTHALMIC USE)

Dexamethasone is a synthetic fluorinated corticosteroid. Its molecular formula is $C_{22}H_{29}FO_5$ and has a molecular weight of 392.5. Chemically it is 9α-fluoro-11β, 17α, 21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione[236]. It has the following structural formula:



Dexamethasone occurs as a white crystalline powder which melts at 250-253°C with decomposition. A solution in dioxan is dextro rotatory. It has a log P value of 1.8, indicating that it is moderately lipophilic in nature. Its solubility in different solvents are: 1 in 10,000 in water, 1 in 42 in ethanol, 1 in 165 of chloroform. It is sparingly soluble in acetone, dioxan and methanol. It is very slightly soluble in ether. The drug is sensitive to light[237].

Dexamethasone is used topically to treat inflammatory conditions of the eye such as ophthalmitis following cataract surgery, retinal vasculitis, scleritis, bacterial or immunogenic conjunctivitis or keratitis as well as for the prevention of

rejection of corneal graft[192,238,239]. For ophthalmic disorders or for topical application, in the treatment of various skin disorders, dexamethasone or the sodium phosphate ester is usually employed; concentrations are usually expressed in terms of dexamethasone or dexamethasone phosphate and are commonly 0.05-0.1% for eye drops or eye ointments and 0.1% for topical skin preparations[192].

Corticosteroids act by indirect inhibition of the enzyme phospholipase A_2, which is involved in prostaglandin synthesis. Prostaglandins are metabolites of arachidonic acid and are the mediators of pain and inflammation. The corticosteroids inhibit not only the early phenomena of an inflammatory process[240] (oedema, fibrin deposition, capillary dilatation, migration of leukocytes into the inflamed area and phagocytic activity) but also later manifestations (proliferation of capillaries initially leading to neovascularization, followed by deposition of collagen and eventually leading to cicatrization). Topical ocular steroids are indicated in inflammatory conditions of the palpebral and bulbar conjunctiva, cornea and anterior segment of the globe where the inherent risk of steroid use in certain infective conditions is accepted to obtain a diminution in oedema and inflammation. They are also indicated in chronic anterior uveitis and corneal injury from chemical, radiation or thermal burns or penetration of foreign bodies.

Dexamethasone can be administered orally in doses of 0.5-9mg, daily in divided doses, for the treatment of various inflammatory conditions and adrenal hyperplasia as well as for the diagnosis of Cushings syndrome. For parenteral use, the soluble sodium phosphate ester is used. It is usually administered intravenously for the treatment of severe shock and cerebral oedema. A solution of the sodium phosphate form can be injected into soft tissue, lesions as well as intra-articularly, intraocularly, and intramuscularly. The ocular penetration of dexamethasone has been studied following topical application[241,242]. Attempts have been made to solubilize dexamethasone using cyclodextrins and such solutions have been evaluated in laboratory animals[243].

The most commonly used method of analysis for dexamethasone is the 'tetrazolium assay of steroids', which results in the formation of a wine red coloured complex whose absorbance is measured at about 550nm. This is also the official method of analysis for the bulk drug in the IP '96. The USP XXIII specifies the use of an HPLC method for bulk drug as well as from its various official formulations.