1.1 BACKGROUND OF RESEARCH:

Our eyes are an important part of our health and work continuously. Eyes are important at all stages of life no matter how old are you. In fact the health of our eyes does vary according to time. There are many eye diseases that troubles eye health and if these diseases left untreated can be a serious problem. The corneal diseases are major reason for blindness in developing and undeveloped countries.1 As per World Health Organization, in world today after cataract and glaucoma, corneal diseases are the key causes for vision loss and blindness.2 In India, it is estimated that there are around 6.8 million people who have vision less than 6/60 in at least one eye due to corneal diseases; out of these, about a million have bilateral involvement.3 There is predictable that the number of individuals with unilateral corneal blindness in India will increase to 10.6 million by 2020.5

In relation to the National Programme for Control of Blindness estimates, there are presently 120,000 corneal blind people in the nation due to corneal disorders. According to this approximation there will be addition of 25,000-30,000 corneal blindness cases every year.6 In our country the load of corneal diseases is very high. This proves that 90% of the worldwide cases of ocular trauma and corneal ulceration are a major reason for corneal blindness in developing countries. After cataract and glaucoma conjunctivitis is also a threat to eye health which on ignorance leads to blindness. Conjunctivitis is common infection to the eye and which easily spreads, in other words it is ocular communicable disease.

Conjunctivitis is in early is normal but on ignorance unsafe, sight threatening ocular infection that needs instant ophthalmic care and management. Many people suffer from Conjunctivitis and inflict financial and social burdens. It is approximated that acute
conjunctivitis affect 6 million people yearly in the USA. The cost of treating bacterial conjunctivitis alone annually was around $377 million to $857 million. Bacterial conjunctivitis usually treated with antibiotics and the treatment duration will be for a week or longer than a week. This may result in poor patient compliance with traditional dosage forms due to greater frequency of drug administration that is 2–3 drops every 2–3h. Empirically broad-spectrum antibiotics are the drug of choice in the treatment of Bacterial conjunctivitis. So to treat such infections, broad-spectrum antibiotics are widely used, which are effective against both gram positive and gram-negative bacteria. Extensive effectiveness of Fluoroquinolone in the treatment of bacterial corneal ulcers, although many bacterial resistant strains are emerging have gained popularity in ocular therapy. Fluoroquinolones have been used regularly as modern active agents in routine bacterial conjunctivitis. Sparfloxacin is a new generation fluoroquinolone antibiotic which has been used efficiently to treat eye infections and provides better efficacy against chief ocular pathogens such as Staphylococcus aureus, Streptococcus pneumonia and Staphylococcus epidermis. In addition it is reported to be more prominent in vitro compared to ciprofloxacin against mycobacterium, gram positive bacteria. However, its main disadvantage as a therapeutic agent is its water insolubility.

1.2 REVIEW OF LITERATURE:

Conjunctivitis

Conjunctivitis is nothing but the inflammation to the conjunctiva. The conjunctiva is the thin transparent layer of tissue that lines the inner surface of the eyelid and covers the white part of the eye. In other words the conjunctivitis is termed as "pink eye," conjunctivitis is a common ocular disease, particularly in children. It may affect one or both eyes. Some forms of conjunctivitis are highly communicable and can easily spread
in schools and at home. While conjunctivitis is generally a slight eye infection but sometimes it can develop into a severe problem. A bacterial or viral infection causes conjunctivitis. The most common causes of conjunctivitis are viruses, bacteria, and allergens.

**Viral Conjunctivitis**

Viral conjunctivitis is nothing but the infection to the eye caused by virus. Viral conjunctivitis can be occur by many viruses, some of which are concomitant with a cold, or sore throat and upper respiratory tract infection. Viral infections begin in single eye and easily develop to the second eye within few days. It is very transmissible disease which spreads easily and rapidly.

**Bacterial Conjunctivitis**

Bacterial conjunctivitis is extremely infectious and caused by contamination to the eye with certain bacteria like *staphylococcus aureus*, *P. Aurgunesa*. Bacterial conjunctivitis usually begins in either eye or progress to the second eye. Bacterial conjunctivitis is a leading cause in the children. Earlier it is mild infection to the eyes but on ignorance it can be a severe damage to the eye and may cause blindness. However, bacterial conjunctivitis is generally cured with the topical.

**Allergic Conjunctivitis**

Allergic conjunctivitis is nothing but the body’s response to certain substances, to which it is hypersensitive, like pollen from grasses, plants, weeds and trees; dust mites; molds; dander from animals; lens solution; and cosmetic preparations.
Drugs used in the management of Conjunctivitis

1. Fluoroquinolone Derivative:

Ciprofloxacin, Ofloxacin, Basifloxacin, Moxifloxacin, Gatifloxacin, Gatifloxacin.

Sparfloxacin, Leofloxacin,

2. Aminoglycosides

Gentamycin, Tobramycin.

Polymyxin B Combinations

Neomycin, Bacitracin

3. Other

Azithromycin, Erythromycin

In a study, comparison of the effectiveness of selective antibiotic for reliving the conjunctivitis was studied. The study resolved that the sparflxacin is the drug of high-quality to treat the bacterial conjunctivitis. It is reported that it is high prominent in vitro than ciprofloxacin against mycobacterium, gram positive bacteria. But as said previously it suffers problem of ocular bioavailability. In direction to progress the ocular bioavailability of the sparflxacin various studies have been carried out earlier. Gupta H et al (2009) developed PLGA nanoparticles of Sparflxacin for extended ophthalmic drug delivery. In present work Gupta et al developed a new colloidal system, that is, poly (dl-lactide-co-glycolide) PLGA nanoparticles for sparflxacin ocular delivery, to increase precorneal holding time and ophthalmic penetration. Nanoparticles manufactured by nanoprecipitation technique and assessed for several properties such as particle size, zeta potential, in vitro drug release, stability. The developed nanosuspension showed extended release profile of sparflxacin, this study tells about the superiority of nanosuspension technology.  

16 Barrett MS et al (1996) reported in his comparative study between
sparfloxacin and other six compounds like ciprofloxacin, ofloxacin compared to
erthyromycin-resistant pneumococci (50 strains) for antimicrobial activity. He found
that sparfloxacin as a novel pyridine carboxylic acid fluoroquinolone derivative four-fold
more active than ciprofloxacin and ofloxacin. Sparfloxacin appears to have outstanding in
vitro activity against erythromycin-resistant S. pneumonia that was often very resistant to
beta-lactams. Bhatnagar A et al (2013) are the first to develop “nanoparticle loaded in
situ gel”, that is, poly lactic co glycolic acid nanoparticle included in chitosan in situ gel
for sparfloxacin ocular supply. The formulation was valued for many physicochemical
properties. The remark of acquired gamma camera pictures revealed good residence over
the intact precorneal area for sparfloxacin nanoparticle combined in situ gel (SNG) as
paralleled to commercial formulation. SNG formulation vanished very slowly and
remained at corneal surface for more time period as no radioactivity was observed in
systemic circulation. The developed formulation was found to be superior in combination
and can go up to the clinical evaluation and application. Ahmed MG et al (2013)
formulated sparfloxacin in situ gels built on the concept of pH triggered gelation systems
by using HPMC and carbopol polymers. Sol-to-gel conversion is occurred in the artificial
tear fluid at pH 7.4. Formulations were evaluated for drug content, gelling capacity,
viscosity, clarity and in vitro release study. The ocular gelation studies and in vivo
antimicrobial studies against Staphylococcus aureus using suitable animal models were
carried out. The formulations shown prominent satisfying efficacy and prolonged drug
release. These results reveal that the developed system is a choice over conventional drug
delivery system which may progress patient compliance. Khan N et al (2014) made an
attempt to prepare sparfloxacin ophthalmic in situ gel to avoid the limitations of
conventional formulations. The gelling comprised of sodium alginate as a gelling agent
and methylcellulose extended the drug release up to 24 h.\textsuperscript{20} Gawad et al (2013) made an attempt to increase the bioavailability of sparfloxacin by formulating thermo sensitive gel containing sparfloxacin-HP-β-CyD dried complex. Prepared sparfloxacin-HP-β-CyD complex were confirmed by DSC, FTIR and SEM. The prepared complex revealed a considerable progress in the water solubility of Sparfloxacin compared to the physical mixture. Ocular in-situ gelling formulations comprised of sparfloxacin-HP-β-CyD complex are found effective in ocular infection. \textsuperscript{21} Chethana SR et al (2015) formulated sparfloxacin and diclofenac sodium hydrogel contact lenses by using HEMA (hydroxyl ethyl methacrylate) and 4- Vinyl pyridine (monomers) for the treatment of ocular infections. Sparfloxacin is very effective against gram-negative and gram-positive microorganisms like \textit{S. aureus} and \textit{P. aureginasa}. Drugs are delivered by drenching the contact lenses in drug solution to load the drugs. In-vitro release study conclude that release rate of drug from hydrogel contact lenses dependent on concentration of 4-polyvinyl pyridine as it provided continuous release of the drug for the action of ocular infections.\textsuperscript{22} Sawant D et al (2016) developed sparfloxacin thermosensitive emulsomal in situ gel for ophthalmic delivery for the treatment of bacterial conjunctivitis First sparfloxacin emulsomes were prepared by thin film hydration. In the second step, the drug loaded emulsomal suspension was dispersed in Pluronic (PF127 and PF68)solution yielding the emulsomal in situ gel. The formulation was found non-irritant and showed promising in vitro and in vivo antibacterial action. A stability study indicates 4 ± 1 °C is suitable storage condition for the formulation. The study suggested that the novel emulsomal in situ gelling system could be a right alternative to traditional eye drops\textsuperscript{23}. In all above study an attempt was made to improve the solubility and ocular bioavailability of sparfloxacin.
CHAPTER 1

Glaucoma

Glaucoma known as a group of eye disorders that cause to destruction to the optic nerve axons and progressive degeneration of retinal ganglion cells (RGCs)\textsuperscript{24-26}. Optic nerve is the nerve which takes visual information to the brain form the eyes. In some cases damage occurred to the optic nerve is due to the raised pressure in to the eye and it recognized as intraocular pressure.\textsuperscript{27} The prevalence on the glaucoma concludes that approximately 60 million people are affected with glaucoma worldwide. Glaucoma is major cause for the blindness and near about 7.5 million people are blind as it is a second most common cause of blindness. In India it affects 11 million people out of which 1.5 million people are blind. Glaucoma normally reasons no signs in early stages; it comes to know with only by regular eye examinations and screenings. Intraocular pressure rise when there is fluid is produced in the eye or the outflow of the aqueous humor to the eye become blocked. The person with glaucoma is at greater risk. Damage to the optic nerve causes glaucoma which sometimes leads blindness. Glaucoma is generally cured with eye drops, even though lasers and surgery can also be used. Most cases of glaucoma can be controlled well with these treatments to prevent the further loss of vision. More research into the reasons and effective treatment of glaucoma is being carried out throughout the world. Early diagnosis and treatment is the solution to preserving vision in people with glaucoma.

Causes of Glaucoma

The eye is full of vitreous humour and aqueous humour. Aqueous humour is the fluid in the anterior part of the eye. Vitreous humour is jelly-like, clear substances that fills the eye behind the lens and helps the eyeball to conserve its shape. In a normal eye, aqueous
humor is produced, uniformly circulates through the eye, and then lick out from the trabecular meshwork, which called as eye's filtration system. These are a chain of small channels adjacent the angle formed by the cornea, iris and the sclera. If there is any type of blockage in these channels, pressure increases inside the eyeball.

Types of Glaucoma

1. Primary Open Angle Glaucoma:

The most cases of the illness are primary open angle glaucoma. The primary open angle glaucoma is having more prevalence compared to the other types of glaucoma. There are numerous known danger elements for glaucoma, such as a raised intraocular pressure, high myopia, aging, family history, cardiovascular disease, systemic hypertension, peripheral vasospasm, migraine headaches, and prior nerve damage. The vision damage occurred from glaucoma is considered to be permanent. However, the glaucoma is treated if it is diagnosed at early stages. Blindness is finally induced by the damage of optical field caused by neuronal cell death.

2. Normal tension glaucoma:

This kind of glaucoma is occurs because of reduced blood supply to the optic nerve. This state is characterized by loss of peripheral vision and gradual optic-nerve damage even though intraocular pressures is in the below normal range. This type of glaucoma can be identified by repetitive screening by the ophthalmologist to detect the visual field or loss nerve damage.
3. Secondary open-angle glaucoma

It is another type of open-angle glaucoma resulted from the damage to the eye or the minor injuries occurred many years ago. Another reason for secondary glaucoma is cataracts, inflammation in the iris of the eye, the use of topical or systemic steroids. It could be coupled with a retinal detachment or retinal vein blockage.

4. Angle Closure Glaucoma

Angle-closure glaucoma is a rare form of glaucoma in the Western countries but it is highly common in Asian countries. Angle-closure glaucoma might be acute or chronic glaucoma. The common part in both is that angle drainage partially or the entirely blocked, so that the aqueous humor inside the eye cannot even reach all part of the trabecular meshwork. In acute angle-closure glaucoma, the patient's intraocular pressure, which usually is common, can rise very rapidly. This unexpected pressure increase arises because the drainage angle becomes closed and blocks all the drainage channels. This type of glaucoma arises when the pupil dilates. As an outcome, the peripheral edge of iris can become bunched up against its corneal attachment, thereby blocking the drainage angle. Thus, the problem with angle-closure glaucoma is the difficulty with influx of the eye fluid to the trabecular meshwork. In this concern remember that the problem in open-angle glaucoma is congestion within the drainage system itself. In chronic open-angle glaucoma, shares of the drainage angle become blocked over a lengthy period. As more areas become blocked, the force inside the eye increases, repeatedly over age of months or years.
Drugs Used in the treatment of Glaucoma:

1. **Sympathomimetics:** 1) Apraclonidin 2) Brimonidine 3) Clonidine 4) Dipivefrine 5) Epinephrine

2. **Parasympathomimetics:**

   A. **Muscarinic:** Aceclidine

   B. **Muscarinic/Nicotinic:** 1) Acetylcholine 2) Carbachol

   C. **Acetyl cholinesterase inhibitors:** 1) Demecarium 2) Ecothiopate 3) Stigmine (Neostigmine. Physostigmine) 4) Paraoxon

3. **Carbonic anhydrase inhibitors:** 1) Acetazolamide 2) Brinzolamide 3) Diclofinamide 4) Dorzolamide 5) Methazolamide

4. **Beta blocking agents:** 1) Befunolol 2) Betaxolol 3) Carteolol 4) Levobunolol 5) Metipranolol 6) Timolol

5. **Prostaglandin analogues:** 1) Bimatoprost 2) Latanoprost 3) Travoprost

Bimatoprost is a hydrophobic drug having log P 3.2 believed to decrease intraocular pressure (IOP) in humans by raising outflow of aqueous humor through both the uveoscleral routes and trabecular meshwork. Bimatoprost decreases the pressure in the eye by imitating the action of a naturally-occurring prostaglandin. Prostaglandins are a group of chemicals originate in numerous places in the body. In the eye, they rise the drainage of the aqueous humor out of the eyeball. Bimatoprost is a synthetic compound related to one of the natural prostaglandins and works by increasing the outflow of
aqueous humor out of the eyeball. Bimatoprost might also lessen the rate of aqueous formation inside the eye. Both these effects reduce the pressure inside the eye. But the difficulty related with Bimatoprost is its water insolubility and short biological half life leads to low ocular bioavailability. To bypass this problem Faraco AAG et al. (2014) have developed Bimatoprost-loaded ocular inserts as sustained release drug delivery systems for glaucoma treatment. Chitosan polymeric inserts were formulated by using the solvent casting method. The inserts were assessed for their healing effectiveness in glaucomatous Wistar rats. In conclusion, eye drops were only effective throughout the daily treatment period. IOP results were revealed in RGC counting and optic nerve head cupping damage. BIM-loaded inserts seem to be a promising system for glaucoma management by providing the sustained release of BIM.

Barriers of Ocular Delivery and Reasons for Low Ophthalmic Bioavailability

1. Drug loss from the eye surface:

After ocular administration, the drug is removed from the ocular surface by the flow of lachrymal fluid. The excess volume of the instilled liquid goes to the nasolacrimal duct rapidly in a couple of minutes because lachrymal turnover rate is about 1 µl/min. Normally without blinking human eyes the volume of tear is 7 µl and with blinking accommodate 30 µl, the approximate drop volume of is up to 50 µl, 70 % of dose is remove from the ocular area by overflow.
CHAPTER 1

1. Systemic Absorption In place of opthalmic absorption

Another source of non-productive drug removal is its systemic absorption in place of ocular absorption. Systemic absorption may take place either after the fluid flow to the nasal cavity or directly from the conjunctival sac via local blood capillaries.

2. Lachrymal fluid-eye barriers:

Corneal epithelium causes restrictions of drug absorption from the lacrimal fluid into eyes. The corneal epithelial cells form tight junction’s that restrict the paracellular drug permeation. Hence, lipophilic drugs normally have at least a direction of magnitude greater absorptivity in the cornea than the hydrophilic drugs. In general the surface area of the conjunctival epithelium is about 20 times larger than that of the cornea is permeable epithelium than the cornea.

3. Blood-ocular barriers

The eyes are endangered from the foreign matter in the blood stream by blood-ocular barriers. These walls are divided in two parts: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier present in the uvea and is comprised of the endothelial cells. This barrier limits the entry of hydrophilic drugs from plasma into the aqueous humor and also prevents the entry of plasma albumin into the aqueous humor. The barrier between eye and blood stream is called as posterior barrier which is comprised of retinal pigment epithelium (RPE) and the tight junctions of retinal capillaries. Unlike retinal capillaries the vasculature of the choroid has broad permeable walls and blood flow. Drugs easily get entree to the choroidal extravascular space, but thereafter distribution into the retina is restricted by the retinal endothelia and RPE.  

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Various factors responsible for disposition of ophthalmic drugs

Bioavailability of drugs inserted to the eye is a main consideration. There are physiological factors, which can disturb a drug’s bioavailability including drug metabolism, lacrimal drainage and protein binding.

1). Protein binding:

Protein bound drugs are unable of penetrating the corneal epithelium because of the increased size of the protein drug complex. Because of the concise time in which an ophthalmic solution may remain existent in the eye, protein binding of a drug substance could quickly contradict its therapeutic value by rendering it occupied for absorption.

2). Lachrymal drainage:

One of the key problems related with conventional ocular solutions is the quick and high amount of drug removal from the precorneal lacrimal fluid. It must be careful that this high removal rate from eye is cause of its capacity to maintain its residence volume at 7–10 μl permanently, whereas volume of topically administered dosage range is from 20–50 μl. In fact in vivo it has to be verified that 90% of the dose was drained within 2 min for an instilled volume of 50 μl and within 4 min for an administered volume of 10 μl. Since, the ocular dwelling time of conventional formulations is limited to a few minutes and the overall absorption of a topically administered drug is limited to 1–10%. In addition to the many factors touching ocular bioavailability, Physiological factors also have major effect on ocular bioavailability, other factors such as the physicochemical characteristics of the active pharmaceutical ingredient and product formulation are important. Because the eye cornea is a membrane-barrier comprises both lipophilic and hydrophilic layers, drug
substances having both lipophilic and hydrophilic characteristics are easily permeated. It is beneficial for corneal penetration to adjust the pH of the formulation to increase the proportion of unionized drug in the eye.  

3). Nasolacrimal drainage system

The nasolacrimal drainage system comprises of three different parts: the secretory system, the system and the distributive system. The excretory system composed of fundamental secretors that are stimulated by temperature change and blinking due to tear evaporation and reflex secretors which have an efferent parasympathetic nerve supply which secrete and respond physical stimulation. The distributive system comprises of the tear meniscus and eyelids in the region of the lid ends of the open eye, which by blinking spread tears over the ophthalmic surface and prevents developing dry areas. The excretory region of the nasolacrimal drainage system poised of the lacrimal puncta, the superior, inferior and common canaliculi; the lacrimal sac; and the nasolacrimal duct. In human beings, the two puncta are the beginnings of the lacrimal canaliculi and are located on an eminent area known as the lacrimal papilla. It is considered that tears are broadly absorbed by the mucous membrane that lines the lacrimal sac and the ducts; only a small amount reaches the nasal channel.
MECHANISM OF OPHTHALMIC DRUG ABSORPTION

Drugs administered by instillation required to penetrate to the eye and do so first and foremost through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion throughout the conjunctiva and sclera and look to be very important for drugs that are poorly absorbed through the cornea.

Fig 1: Ophthalmic Drug Absorption

1. Corneal permeation

The permeation of drugs throughout the corneal membrane occurs from the precorneal region. Thus, the mixing of the drug substance and the kinetic behaviour of drug characteristics in tears has a direct influence on the efficiency of drug absorption into the internal eye. The dynamic absorption of maximum ophthalmic drugs results from diffusional route across corneal membrane. The effective progression in absorption is a role of rate and extent at which the transport process occurs. The flux of any drug molecule around the biological membrane depends on the physicochemical
characteristics of the permeating molecule and its dealings with the membrane. The level to which the transport or absorption progression occurs is also function of physiological mechanism of precorneal fluid drainage or turnover. The cornea, in relations of transcorneal drug permeation can be considered to involve of three primary layers, stroma, epithelium and endothelium. The epithelium and endothelium enclose on the command of a 100 fold greater amount of lipid material equated to the stroma. As a result, depending on the physicochemical properties of a diffusing drug, the resistance obtained by the individual layers varies significantly. Epithelium, as a lipoidal layer, characterises a diffusional barrier proposing high resistance to ionic or other aqueous soluble or polar species. In comparison, compounds with relatively low polarity meet a greater diffusional battle in the hydrophilic stroma layer. This commonly cited theory of drug penetration athwart the corneal membrane is referred to as “differential solubility concept”.

2. Non-corneal permeation

Primary mechanism of drug permeation, the sclera performs very key role in the diffusion across the intercellular aqueous media in the case of structurally analogous corneal stroma. Consequently the chances of partitioning mechanism cannot be eliminated. Even though like cornea, the conjunctiva is encompassed of an epithelial layer jacketing an underlying stroma, the conjunctival epithelium offers substantially less resistance compared to the corneal epithelium.

Nanosuspensions (NS) are colloidal dispersions of pure drug particles in an outer liquid phase with a mean particle diameter ranging between 100 and 1000 nm. The important
and common features of NS are their ability to enhance the saturation solubility and consequently the dissolution rate of specific drug \(^{36}\).

Nanosuspensions offer many advantages firstly, the chemical and physical stability of drugs in the nanosuspension can be improved as they are actually in the solid state; secondly, dose and toxicity can be decreased and the higher drug loading can be achieved. Finally, it is valuable for those molecules which are insoluble (BCS Class II Drugs) benefits in terms of increased bioavailability. \(^{37}\) It is also useful for molecules with, poor permeability, poor solubility or both, which poses an important task for the formulators. The decreased particle size condenses the chances of permeability of poorly water soluble drugs. The nanosuspensions can also be spray dried or lyophilized and the nanoparticles of a nanosuspension can also be merged in a solid matrix. Apart from this, it has all other advantages of a liquid dosage form over the solid dosage forms.

**Important parameters of Nanosuspension** \(^{38-40}\)

1). **Particle size and size distribution**: It is the most essential parameter in the evaluation of the suspensions as it is having significant effect on the dissolution rate and solubility and the physical stability of the formulation. The mean particle size and particle size can be analysed by Photon Correlation Spectroscopy (PCS), laser diffraction and coulter current multisizer.

2). **Particle charge (Zeta Potential)**: The particle charge also has an importance in the stability of the suspensions. Generally, zeta potential ±40mV or more are thought to be required for the stabilization of the colloidal dispersions. For electrostatically stabilized nanosuspension a least zeta potential of ±30mV is required and in occasion of mutually electrostatic and steric stabilization it should be at least of ±20mV zeta potential is essential.
3). **Particle Morphology:** It is essential to identify that is maximum possibility of the polymorphism during the storage of the nanosuspensions. Therefore, it is mandatory to learn the surface morphology of the drug or nanoparticles in nanosuspension. Transmission electron microscopy (TEM) is most regularly used for such studies.

4). **Saturation solubility:** Nanosuspension prominently enhances the dissolution velocity and saturation solubility. Size reduction of the drug particles up to the nano level leads to major increase in the dissolution pressure. An enhanced increase in solubility that occurs with moderately low particle size reduction may be principally due to a change in surface tension leading to increased saturation solubility.

Many researchers has done extensive research to rise the bioavailability of poorly soluble drugs bypassing the limitations of conventional drug delivery system and still basic investigation is going on in this area, **Ige PP et al., (2013)** formulated Fenofibrate nanosuspension by using probe sonicator and characterized for different physicochemical parameters. The pharmacokinetic study of optimized nanosuspension in New Zealand white rabbits revealed the four fold rise in relative bioavailability paralleled to pure drug. This improved dissolution and bioavailability of fenofibrate nanosuspension seem to be the promising approach for oral delivery of hydrophobic drug.\(^{41}\) **Hany S.M. Ali et al.,(2011)** prepared hydrocortisone nanosuspensions for ocular delivery by using microfluidic wet milling and nanoprecipitation methods. HC nanosuspension of around 300nm particle size was produced by modifying investigational conditions. The Comparative study is done between two methods by characterizing the prepared nanosuspension for particle shape, size and zeta potential.\(^{42}\) **Jithan A.V. et al (2014)** has prepared nevirapine nanosuspensions to increase the dissolution rate. Nevirapine nanosuspensions were formulated by using nanoedge method. The nanosuspension
formulations were stabilized by using stabilizers like, Poloxamer 407, Lutrol F 127 and hydroxypropyl methyl cellulose. The nanosuspension was accessed for particle size analysis, PDI, surface morphology, in vitro dissolution and pharmacokinetics studies in rats after oral administration. The obtained results that developed nanosuspensions improved the solubility and bioavailability of nevirapine.43 Bhatta R. S. et al (2014) have developed natamycin nanoparticles to progress the corneal penetration and to lessen the dosing frequency and dose of the natamycin. The developed nanoparticles were characterised for pharmacokinetic and Pharmacodynamic study and compared with the commercial formulation of natamycin. The results reveal that the 1/5th dose reduction of nanoformulation found effective equated to the dose of commercial formulation.44 Lewis S. et al (2015) developed nanosuspension based on combinative technology to improve the intestinal absorption of Olmesartan medoxomil, a potent antihypertensive agent with poor oral bioavailability. In the current study two combinative methodologies were applied and then characterised. The prepared nanosuspension showed the optimal particle size and zeta charge. In ex vivo intestinal absorption, then permeability and absorption of nanosuspension were detected to be improved compared to pure drug. This proves that there was better reduction in size resulting in greater surface area which enhances the permeation and ultimately improved absorption.45 Shi S. et al (2015) formulated diclofenac ophthalnic nanosuspension comprised of chitosan and methoxy poly (ethylene glycol)-poly (ε-caprolactone) (MPEG-PCL. The prepared nanosuspension was characterized for Fourier transform infrared, X-ray diffraction and differential scanning calorimetry. The nanosuspension observed very stable at 4 °C and 25 °C for 20 days. Enhanced pre-corneal holding and penetration of the nanosuspension was observed in pharmacokinetics studies in rabbit, it is also observed that higher concentration of
diphenoxylate and better bioavailability compared with marketed
diclofenac eye drops.\textsuperscript{46} Mishra B. et al (2015) formulated naproxen nanosuspensions
stabilized by hydroxy propyl methyl cellulose using combination of ultrasonication
technology and precipitation. The prepared preparations were evaluated for zeta potential,
particle size and drug dissolution studies. The combination approach of probe sonication
and precipitation found effective in improving the drug dissolution. It may provide in the
advancement of an ideal pharmaceutical formulation with high drug content and greater
dissolution velocity to overcome the poor bioavailability of naproxen.\textsuperscript{47}
The literature review reveals that nanosuspension found to be promising drug delivery to
increase the bioavailability of hydrophobic drugs by growing the saturation solubility.

1.3 JUSTIFICATION FOR THE STUDY

Solubility is a vital asset for the drug in formulation and their effectiveness. The main
problem related with the new molecular entities of drug formulation is its poor solubility.
At present about 40\% of drugs in the development pipelines and nearly 70\% of drugs
coming from synthesis are poorly soluble in aqueous media, many as well in organic
solvents\textsuperscript{48}. Poor solubility creates delivery problems such as low bioavailability and
erratic absorption. The problem is even more extreme for drugs belonging to BCS class II,
as they are poorly soluble in both organic and aqueous media and for drugs having a log P of \(>2\). Such drugs often have a highly variable bioavailability because their
performance is dissolution-rate limited.\textsuperscript{49}

Bioavailability of opthalmic drugs from traditional eye formulas (i.e. solution,
suspension and ointment) is often poor due to precorneal loss resulting from removal
mechanisms, transient residence time and non-productive absorption in the \textit{cul-de-sac}
CHAPTER 1

INTRODUCTION

and the relative impermeability of the drugs through corneal epithelial membrane\textsuperscript{50}. Poorly water soluble drugs are a challenging in ocular formulation; generally, ocular effectiveness is closely allied to ocular drug bioavailability, which may be improved by increasing corneal drug permeation, extending precorneal drug dwelling time and increasing the saturation solubility of poorly water soluble drugs\textsuperscript{51}.

Model drugs Selected:

- Bimatoprost is prostamide a synthetic structured analogue of prostaglandin with ocular hypotensive activity.
- Sparfloxacin is a newer-generation hydrophobic fluoroquinolone used in the management of bacterial conjunctivitis.

Table 1 Ideal Drugs selected for the Nanosuspension of ophthalmic delivery

<table>
<thead>
<tr>
<th>Name of the API</th>
<th>Activity</th>
<th>Log P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bimatoprost</td>
<td>Anti-Glaucoma</td>
<td>3.2</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>Antibacterial</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Problem allied with selected drugs:-

The selected drugs sparfloxacin and bimatoprost belonging to Biopharmaceutical Classification Scheme Class II (BCS CLASS II) as classified by BCS System as they are poorly soluble in both aqueous media and for those drugs having a log P of greater than 2. The effectiveness of these drugs is dependent on dissolution rate (for Class II and III drugs). Dissolution rate water insoluble drugs are depending upon the particle size as well
as the particle shape. Therefore reduction in particle size results in a growth in dissolution rate.\textsuperscript{52}

Bimatoprost and Sparfloxacin are very effective in the management of glaucoma & bacterial conjunctivitis respectively but these two drugs are belonging to hydrophobic category so the bioavailability is a major problem. With conventional drug delivery more frequent dosage required to gain the therapeutic activity.

Many present approaches has been used to improve the solubility of the hydrophobic drugs like micronization, polymorphism, complexation, and prodrug etc., but at rest, these all are not broadly efficient because of their boundaries \textsuperscript{53}. The application of nanotechnology based drug delivery systems such as, nanosuspensions, microemulsions, nanoparticles, solid lipid nanoparticles, niosomes, dendrimers and liposomes found as key solution for variety of solubility related problems of water insoluble drugs, such as, tacrolimus, dexamethasone, acyclovir and so on \textsuperscript{54–57}. The majority of the ocular formulations are available like solution and suspension as it has shown well patient compliance. Nanosuspension gives many advantages over traditional ocular dosage forms, including extended drug release time, reduction in the dose, fall in systemic toxicity of drug, longer residence time of nanoparticles on the corneal surface, elevated drug concentrations in the targeted tissue and feasibility with poorly water-soluble drugs \textsuperscript{58, 59}. Hence, nanosuspension is a promising drug delivery to increase the bioavailability of water insoluble drugs by increasing the saturation solubility. Many of the researchers has developed Sparfloxacin formulations in different form like in situ gel, emulsion and PLGA- nanoparticles composed of polymers or stabilizers HPMC, eudragit and mucoadhesive polymer like chitosan. The HPMC and other polymers used were observed that those are less efficient compared to chitosan because chitosan is a polymer with
additional mucoadhesive property which causes adherence to the targeted area, and by altering the enhanced residence time, it also prolongs the drug release. But the key problem with chitosan is its poor solubility in water; it is soluble only in acidic environment and needs more processing time which limits its application in drug delivery. After carboxylation, an added derivative of chitosan is N-Carboxymethyl chitosan (water soluble chitosan) came into picture which is non-toxic, biodegradable and biocompatible. The core objective of current work is to prepare a technology-centric formulation which can bypass the problems associated with API as well as conventional ophthalmic dosage forms. In the current work, attempts were made to formulate nanosized sparfloxacin and bimatoprost ophthalmic dosage form by using mucoadhesive, water soluble grade chitosan and HPMC E5 to increase the drug residence time. The nanosuspensions were created by using combinative technology based methods i.e. precipitation method followed by probe sonicatıon. Prepared formulations were stabilized by optimizing the concentration of Poloxamer-407 and Kolliphor P188 in combination and characterized for zeta potential, particle size, particle surface morphology drug entrapment, in vitro diffusion, antibacterial assay, stability studies and cell viability study. The newness of this work is that different strategies and approaches are taken into concern to formulate the resourceful drug delivery that overcomes the drawbacks of present drug delivery systems.
1.4 AIM, OBJECTIVES AND PLAN OF WORK

**Aim of the study:**
Formulation and evaluation of Bimatoprost and Sparfloxacın Nanosuspensions for ocular use.

**Objectives of the study:**
1. To increase the solubility of selected hydrophobic drugs having different log p values.
2. To study the potential of nanosuspension formulation in ocular bioavailability enhancement.
3. To evaluate the impact of polymers in drug release from the ophthalmic nanosuspension.

**Plan of work:**
1) Preformulation studies.
   a. Identification Tests.
      • Solubility study.
      • Melting point determination.
      • FTIR analysis.
      • Analytical method development and validation.
   b. Compatibility studies by FTIR Spectroscopy and DSC.
2) Optimization studies for formulation of Nanosuspension
   • Polymer concentration.
   • Surfactant concentration.
3) Formulation of ophthalmic nanosuspension.
4) Characterization of ophthalmic nanosuspension.
   • Particle size analysis by Nanotrac.
- Determination of Entrapment Efficiency by ultracentrifugation method.
- Determination of Zeta Potential by Malvern zeta meter.
- Surface Morphology Study by Transmission Electron Microscopy (TEM).

5) *In vitro* drug release and *ex vivo* drug permeation study of nanosuspension through goat eye cornea using Franz Diffusion Cell.

6) *In vitro* eye irritation test (HET CAM Test).

7) *In Vivo* eye irritation tests (Draize Test).

8) *In vitro* Cell line study.

9) *In vitro* anti-microbial study.

10) *In vivo* anti-microbial study in rabbit.

11) Isotonicity study.

12) *In vivo* Pharmacokinetic study.

13) *In vivo* evaluation of Anti-glaucoma effect in rabbit.

14) Stability studies.