FORMULATION AND EVALUATION OF OCULAR IN SITU GELLING SYSTEMS OF CIPROFLOXACIN HYDROCHLORIDE

AUTHOR-MOHINI RAWAT, HARSH THAKUR

Himalayan Institute of Pharmacy and Research, Rajawala, Dehradun, 248001

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

In today's world, ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. Ophthalmic preparations (eye preparations) are sterile liquid, semi-solid or solid preparations that may contain one or more active pharmaceutical ingredients. Ophthalmic products are intended for application to the conjunctiva, the conjunctival sac, or the eyelids. The course of treatment may extend during several days. Although eye preparations contain a preservative, there is a probability of microbial contamination after the package sterility seal has been broken during the period of use.

Eye is a vital organ and unique. It is situated in the orbital cavity of the skull. It is well protected by bony walls of the orbit. Orbit also contains muscles of eyeball, their nerves, blood vessels, and lacrimal gland. It provides living organisms with vision, the ability to receive and process visual detail, as well as enabling several photo response functions that are independent of vision. Drug delivery to eye is tricky job because its typical anatomy restricts drug absorption into deep tissues. The structure of the eye can be classified into anterior and posterior segments. The anterior segment makes up the visible one-third portion of the eye which mainly consists of cornea, conjunctiva, aqueous humor, iris, ciliary body, and lens. The remaining two-thirds of the eye is known as the posterior segment or the back of the eye. The posterior segment mainly consists of vitreous humor, retina, choroid, and optic nerve. There are various ophthalmic drug delivery systems available as conventional dosage form such as eyes drops, eye ointments, eye lotions, and eye suspensions.

Optimize ophthalmic drug delivery systems the following characteristics are required;

- Sterility
- Iso-tonicity
- A good corneal penetration
- Minimum protein binding
- Less drainage tendency
- Easiness in installation and removal

1.1 ANATOMY OF EYE

For research and to develop an effective ophthalmic delivery system, a good knowledge of the structure and physiology of the eye is necessary. The structure of the eye is an important topic to understand as it is one of the important sensory organs in the human body. It is mainly responsible for vision, differentiation of colour (the human eye can differentiate approximately 10 – 12 million colours) and maintaining the biological clock of the human body. The human eye can be compared to a camera as both works by gathering, focusing and transmitting the light through the lens for creating an image of an object.. The human eye is comprised of layers and internal structures, each of which performs distinct functions.

The anatomy, physiology, and biochemistry of the eye render this organ highly impervious to foreign substances. Drug delivery to the eye can be broadly classified into anterior and posterior segments [Figure 1]. Conventional systems like eye drops, suspensions, and ointments cannot be considered optimal in the treatment of vision-threatening ocular diseases. . The anterior and

posterior segments of the eye are affected by various vision threatening diseases. A few diseases affecting the anterior segment could be, but not limited to, glaucoma, allergic conjunctivitis, and cataract. Some of the vision threatening diseases affecting the posterior segment of the eye includes age-related macular degeneration (AMD) and diabetic retinopathy macular edema [DME], proliferative vitreoretinopathy [PVR], posterior uveitis, and cytomegalovirus [CMV]). However, more than 90% of the marketed ophthalmic formulations are in the form of eye drops.

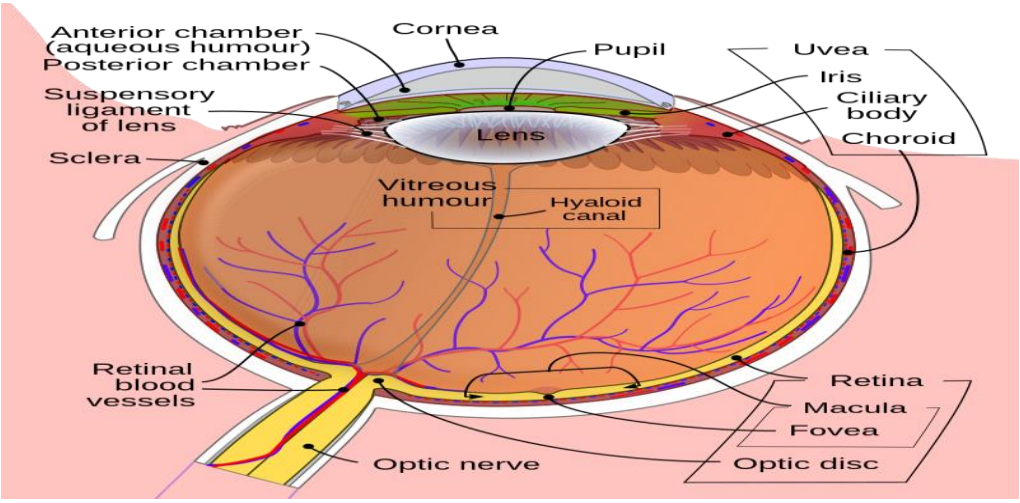


Figure 1.1: Schematic diagram of the human eye

COMPOSITION OF EYE

Water – 98%
Solid – 1.8%
Organic element
Protein – 0.67%
Sugar – 0.65%
NaCl – 0.66%
Other mineral elements : sodium, potassium, & ammonia – 0.79%

The structure of eye can be divided into two main parts: anterior segment and posterior segment [Figure 1]. Anterior segment of the eye occupies approximately one-third while the remaining portion is occupied by the posterior segment. Tissues such as cornea, conjunctiva, aqueous humor, iris, ciliary body and lens make up the anterior portion. Back of the eye or posterior segment of the eye include sclera, choroid, retina, optic nerve and vitreous humor.

Cornea- Cornea is a strong clear bulge located at the front of the eye. It has an important optical function as it refracts light entering the eye which then passes through the pupil and onto the lens. The cornea is described as the “window of the eye”. The cornea, a non-vascular structure gets the necessary nutrients from the capillaries that terminate in loops at its circumference. It is supplied by many nerves derived from the ciliary nerves. These enter the laminated tissue of the cornea. It is therefore extremely sensitive.

Conjunctiva- Conjunctiva is a thin transparent mucous epithelial barrier, lines of the eyelids and covers the anterior one-third of the eyeball. The respective portion of conjunctiva is referred to as the palpebral and bulbar conjunctiva. The conjunctiva is composed of two layers: an outer epithelium and its underlying stroma. It contributes to the formation of the tear film by way of secreting substantial electrolytes, fluids and mucins.

Iris- Iris is a thin circular contractile curtain located in front of the lens but behind the cornea. It forms the coloured, visible part of your eye in front of the lens. Light enters through a central opening called the pupil. The iris function is to adjust the size of the pupil to regulate the amount of light that enters your eye. Pupil generally appears to be the dark "centre" of the eye, but can be more accurately described as the circular aperture in the centre of the iris through which light passes into the eye.

Ciliary body– Ciliary body is the structure that produces aqueous humor. It is directly continuous with the choroid behind and with the iris in the front. Internally it shows a scalloped periphery where it is continuous with the choroid and retina. The ciliary body is concerned with the suspension of the lens and with accommodation. Contraction of the ciliary muscle change tension on the zonular fibres that suspend the lens and allows the eye to focus from distant to near objects. It is also a major source of aqueous fluid for the anterior segment of the eye.

Lens– Lens is a transparent structure enclosed in a thin transparent capsule which is located behind the pupil of the eye and encircled by the ciliary muscles. It helps to refract light travelling through the eye. It focuses light into an image on the retina. This adjustment of shape of the lens is called accommodation and is achieved by the contraction and relaxation of the ciliary muscles.

Choroid– Choroid layer is located behind the retina and absorbs unused radiation and nourishes the outer portion of the retina. It also contains a pigment that absorbs excess light so preventing blurring of vision. The choroid has one of the highest blood flows in the body. The choroid is loosely attached to the inner surface of the sclera by the lamina fusa. It is thicker posteriorly. Internally it is firmly attached to the retinal pigmented layer.

Aqueous humour –The total quantity of aqueous humour is small, filling the anterior and posterior chambers. The ciliary processes are responsible for its production. Aqueous humour is a jelly-like substance located in the outer/front chamber of the eye. It is a watery fluid that fills the "anterior chamber of the eye" which is located immediately behind the cornea and in front of the lens. The aqueous humour is very slightly alkaline salt solution that includes tiny quantities of sodium and chloride ions. It flows from the ciliary body into the anterior chamber, out through a spongy tissue at the front of the eye called the trabecular meshwork and into a drainage canal (dark blue region next to the trabecular meshwork).

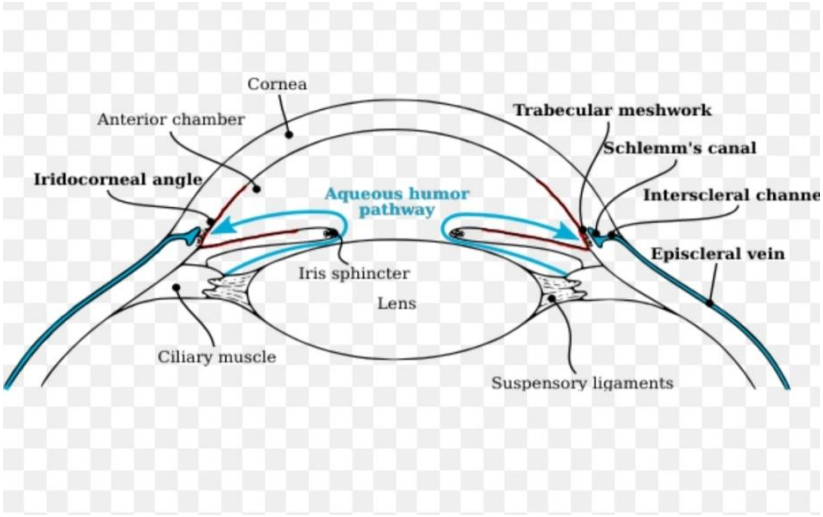


Figure 1.2: Aqueous humour pathway

Sclera–Sclera is the white part of the eye, a tough covering with which the cornea forms the external protective coat of the eye and maintains the shape of the eyeball. It is a firm fibrous membrane that maintain the shape of the eye as an approximately globe shape. It provides passage for nerves of the cornea and vascular autonomic nerves and attachment for extrinsic eye muscles. It resists intraocular pressure.

Pupil – Pupil is the dark "centre of the eye" but can be more accurately described as the circular aperture in the centre of the iris through which light passes into the eye. The size of the pupil (and therefore the amount of light that is admitted into the eye. It is regulated by the papillary reflex. These reflexes are also known as the light reflex.

Retina– Retina is located at the back of the human eye. The retina may be described as the "screen" on which an image is formed by light that has passed into the eye via the cornea, aqueous humour, pupil, lens, and finally the vitreous humour before reaching the retina. The function of the retina is not just to be the screen onto which an image may be formed but also to collect the information contained in that image and transmit it to the brain in a suitable form for the use by the body.

Optic nerve– Optic nerve is a bundle of more than 1 million nerve fibers and also known as the second cranial nerve. It is critical to your vision. It's an extension of our central nervous system, which includes our brain and spine. Optic nerve is responsible for transmitting nerve signals from the eye to the brain. These nerve signals contain information on an image for processing by the brain. The front surface of the optic nerve, which is visible on the retina, is called the optic disk.

Vitreous Humour– The vitreous humour is also known as the vitreous body. It is located approximately 80% of each eye in the body. It is perfectly transparent thin-jelly like substance that fills the chamber behind the lens of the eye. It is an albuminous fluid enclosed in a delicate transparent membrane called the hyaloids membrane.

1.2 CONDITIONS OF THE EYE

Emmetropia – It is the ideal state of an eye in which parallel rays of light entering the eye are focused on the retina, creating an image that is perceived as crisp and in focus. Emmetropic eyes are just the right length to allow light to reach the ideal spot on the retina to create crisp, clear vision. When a person has emmetropia in both eyes, the person is described as having ideal vision.

For example, on a Snellen chart test, Emmetropic eyes score at least "6/6"(m) or "20/20(ft) vision, meaning that at a distance of 20 ft (the first number) they see as well as a "normal" eye at a distance of 20 ft (the second number).

Myopia – It is also known as nearsightedness. It is a common vision condition in which a person can see near objects more clearly than distant objects. It occurs when the shape of your eye causes light rays to bend (refract) incorrectly, focusing images in front of your retina instead of on your retina. Myopia is caused by a refractive error. A refractive error occurs when our eye does not focus light correctly. Myopia, typically less than 4.00 to 6.00 diopters is the most common form.

Hyperopia– It is also known as farsightedness. It is a common vision condition in which a person can see distant objects more clearly than near objects. People experience hyperopia differently. Some people may not notice any problems with their vision, especially when they are young. Most commonly hyperopia is caused by a cornea (the clear layer at the front of the eye) that is not curved enough or by an eyeball that's too short. These two problems prevent light from focusing directly on the retina, which makes close-up objects look blurry. It is an eye focusing disorder, not an eye disease.

Presbyopia – It is a refractive error that makes it hard for middle-aged and older adults to see things up close. It happens because the lens (an inner part of the eye that helps the eye focus) stops focusing light correctly on the retina (a light sensitive layer of tissue at the back of the eye). The most significant risk factor for Presbyopia is age. Most people lose some ability to focus on close objects by age around 40.

Astigmatism – Astigmatism is often hereditary, which means it's passed down from your parents. It occurs when either the front surface of the eye (cornea) or the lens inside the eyes has mismatched curves. Instead of having one curve like a round ball, the surface is egg-shaped. This causes blurred vision at all distances. It can also be the result of eyelids putting pressure on the cornea, it can get better or worse over time. Sometimes, astigmatism happens after an eye injury or surgery.

Cataract - Cataract is also an abnormality of the lens in which it becomes partially or completely opaque so that no light gets into the eye. A cataract is a cloudy area in the lens of the eye that leads to decrease in vision. It often develops slowly and can affect one or both

eyes. Symptoms may include faded colours, blurry or double vision, halos around light, trouble with bright lights, and trouble seeing at night.

1.3 ABSORPTION AND BIOAVAILABILITY OF THE DRUG FROM THE EYE

The most common route for the treatment of ocular disease is topical medication. The application of drug via this route is preferred due to ease of administration and low cost. The drug solution in stilled as eye drop into the ocular cavity may disappear from the precorneal area of the eye. The drug from the eye is absorbed by two routes –

(a) Corneal route
(b) Non-corneal route.

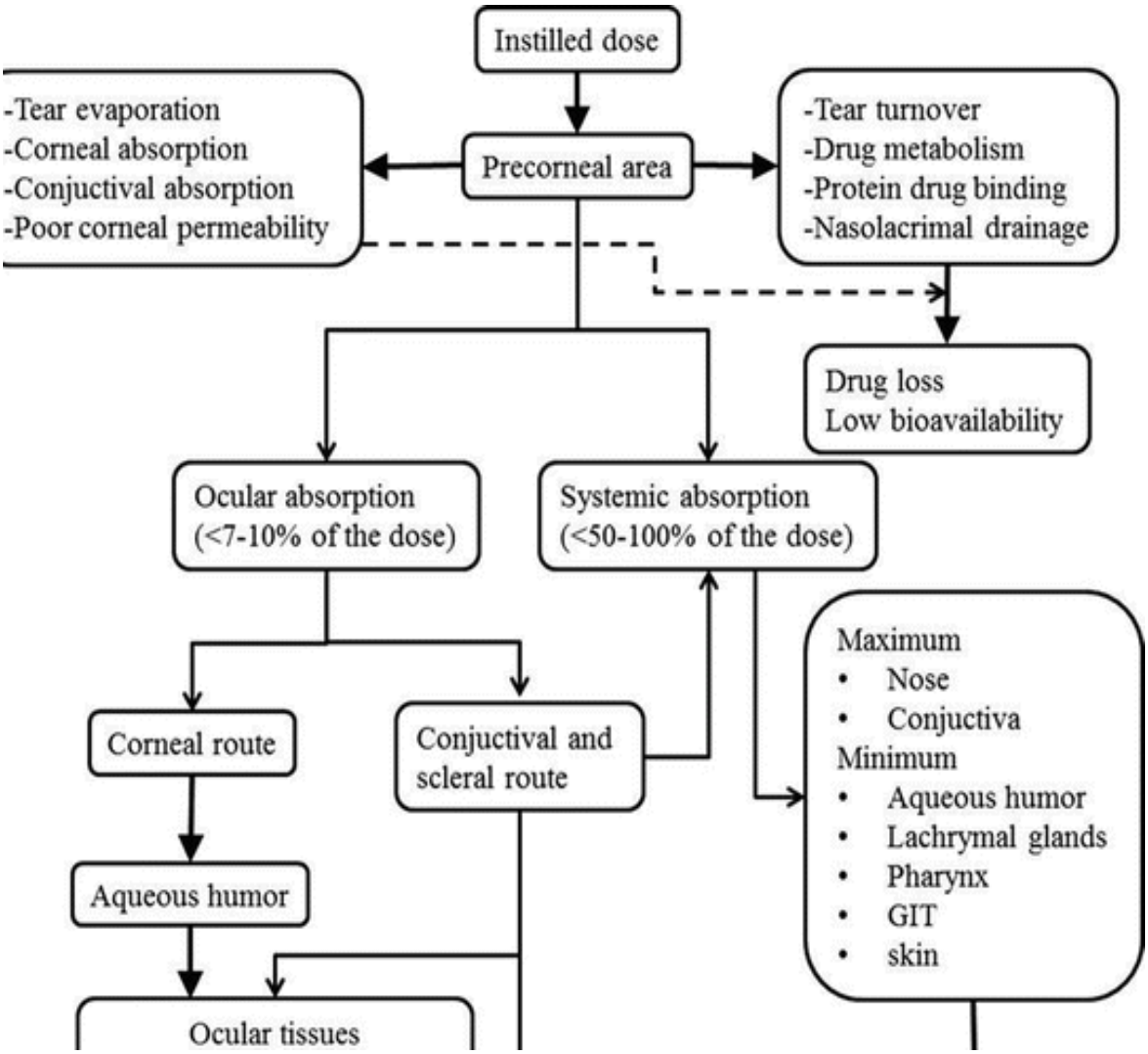


Figure 1.3 : Absorption of the drug from the eye

GLAUCOMA - Glaucoma is not one disease but rather a group of disorder characterised by increased IOP and the consequences of elevated pressure, optic nerve atrophy and peripheral visual field loss. It is a symptomatic condition of the eye where the IOP is more than the normal (above 25mm Hg). A proper balance between the rate of aqueous production and rate of aqueous reabsorption is necessary to maintain the IOP normal limits. When the rate of inflow is greater than rate of outflow, IOP can rise above the normal limits. If IOP remains elevated, permanent vision loss occurs. Untreated of glaucoma leads to permanent damage of the optic nerve.

1.4 TYPES

a) Open angle glaucoma – Usually bilateral, but one eye may be more severely affected than the other. The anterior chamber angle is open and appears normal. It develops gradually and painlessly and has no initial symptoms. If untreated, peripheral or side vision is slowly loss. It usually happens to older people (people over 50). There is no cure for it, ant it gets worse over time. The key is to get checked and treat it early. Once we get to know we have it, we can get medicine and surgery to slow it down and save your eyesight.

b) Angle closure glaucoma – Obstruction in aqueous humour outflow due to the complete or partial closure of the angle from the forward shift of the peripheral iris to the trabecula. The obstruction results in an increased IOP. It can rise within a matter of hours. It isn't as common as other types of glaucoma, which cause pressure buildup much more slowly over time.

c) Low tension glaucoma - Low tension (or normal tension) glaucoma is not as common as others. In these case, the eye pressure is in the normal range, but the optic nerve still gets damaged. The exact mechanism of damage is still unknown.

1.6 CAUSES AND RISK FACTOR

- Age
- Genetics -Family history of glaucoma
- Medical conditions – diabetes mellitus, cardiovascular disease
- Ocular hypertension - A condition where the pressure in your eyes, or IOP, is too high. Continually high pressure within the eye can eventually damage the optic nerve and lead to glaucoma or permanent vision loss.
- Physical injuries – Eye trauma is most commonly caused by blunt trauma, which is an injury that doesn't penetrate the eye, such as a blow to the head or an injury directly on the eye. This can lead to an increase in eye pressure, which can damage the optic nerve.
- Severe Myopia - It is associated with an increased risk of pathological ocular complications and may lead to blinding disorder like glaucoma.
- Ocular surgery - It can cause a change in the eye's pressure. Sharp increases in eye pressure are called "pressure spikes" and sometimes occur in patients after Cataract surgery. Often these pressure spikes are short term and can be treated with medicines.

- Migraine - Prolonged increased pressure can lead to visual loss if not corrected.
- Corticosteroids use - Long-term use of topical and systemic steroids produces secondary open-angle glaucoma similar to chronic simple glaucoma. The increased IOP caused by prolonged steroid therapy is reversible but the damaged produce by it is irreversible.

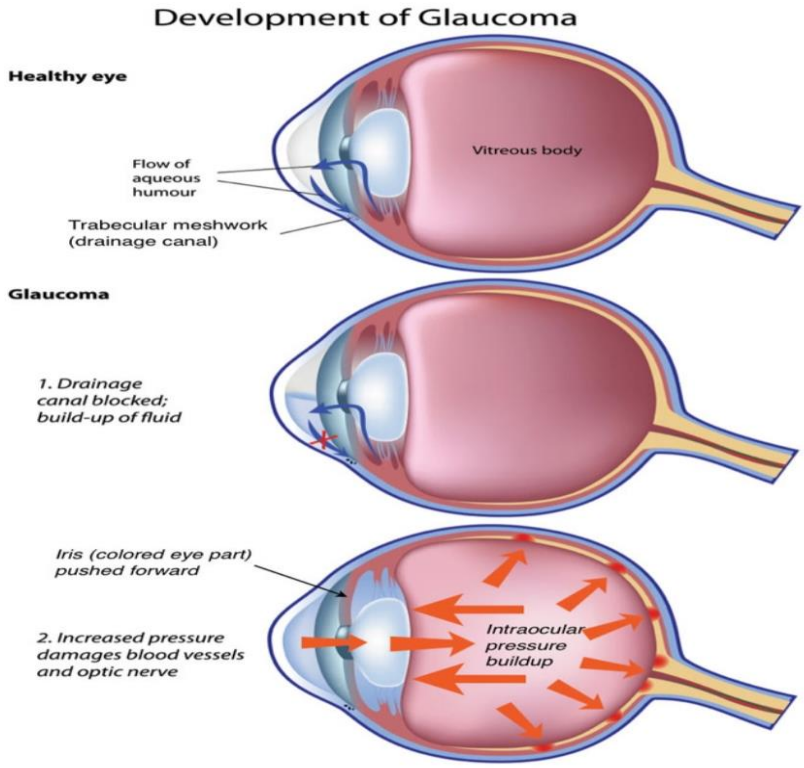


Figure 1.4 : Structure of Normal Eye and Glaucoma Eye

1.7 PATHOPHYSIOLOGY

IOP is a function of production of liquid aqueous humor by the ciliary processes of the eye and its drainage through the trabecular meshwork.

Aqueous humor is produced by the ciliary body and flow into the posterior chamber behind the iris.

The trabecular meshwork filters the aqueous humor into Schlemm's canal, where is picked up by the episcleral vessels and mixed with blood.

1.8 GLAUCOMA TESTS

Slit lamp & Gonioscopy - A special microscope called as a slit lamp is used to examine the structure of the eye. A gonioscopy lens may be used to view the drainage angle. It is the most important piece of equipment in the present day. Slit lamp are used in the examination of the anterior segment of the eye, or frontal structures of the eye, which includes ;

i. Eyelid
ii. Cornea
iii. Sclera
iv. Conjunctiva
v. Iris
vi. Anterior chamber
vii. Anterior vitreous

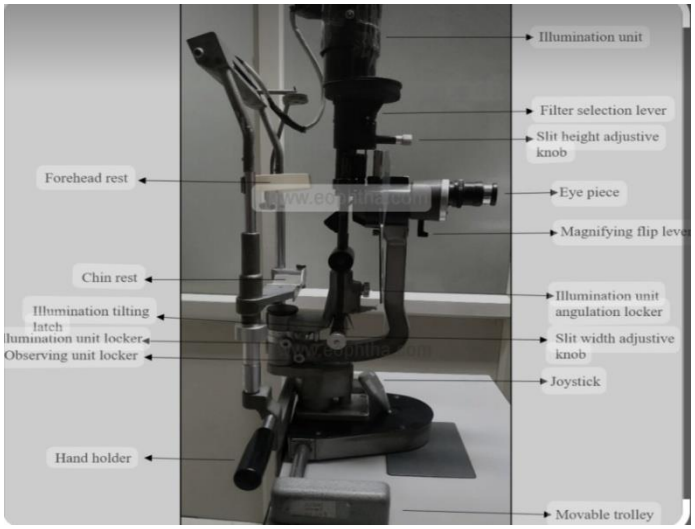


Figure 1.5 : Slit Lamp Microscope

Tonometry - Eye pressure is measured in this test with and instrument called Tonometer. It is performed to determine the intraocular pressure (IOP), the fluid pressure inside the eye. It is an important test in the evaluation of patients at risk from glaucoma. Normal pressure range is 12 to 22 mm Hg.

Ophthalmoscopy - Also called funduscopy, is a test that allows a health professional to see inside the fundus of the eye and other structures using an ophthalmoscope. It is done as part of an eye examination and may be done as part of routine physical examination. Special magnifying lenses are used to examine the retina and optic nerve for damage. Eye drops may be placed in the eyes to dilate the pupils. The pupil is a hole through which the eye's interior will be viewed. Opening the pupil wider (dilating it) is a easy and effective way to better see the structures behind it.

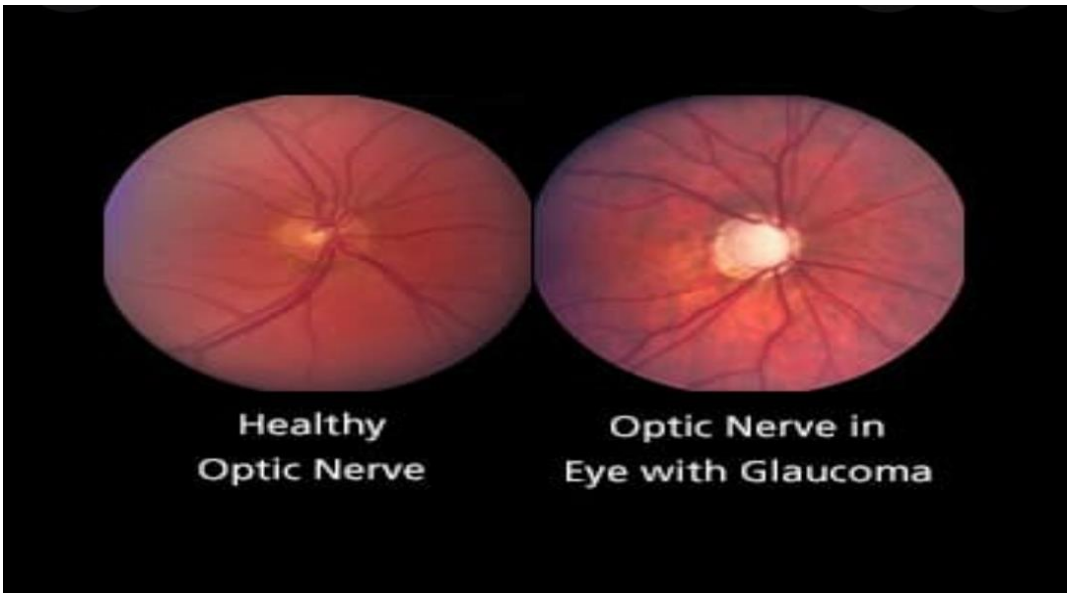


Figure 1.6 : Optic Nerve with Glaucoma

1.9 OCULAR DRUG DELIVERY SYSTEM

Eye drops – Eye drops are saline-containing drops used as an ocular route to administer. Eye drops are used only for anterior segment disorders of eyes because insufficient drug concentrations reached in the posterior tissues using ocular drug delivery system. The purpose of eye drops are;

- Treat infection
- To dialate or contract pupil
- Lubricate eyes
- To instill medication before examination or surgery to eyes
- To relieve pain, itching, discomfort etc.

Sprays - Eye spray are mainly used for pupil dilation or for cycloplegics (paralysis of the ciliary muscle of the eye) examination. Even ophthalmic sprays are not commonly used, but some patients with my driatics or cycloplegics use alone or in combination in the form of eye spray.

Ocular insert – This novel ophthalmic drug delivery as they show a much higher extent of controlled, sustained drug release as compared to the conventional forms. Ocular insert are solid and semi-solid dosage form placed into the conjunctival sac. The ocular insert represents a significant advancement in the therapy of eye disease. Ocular inserts are defined as sterile, thin, multilayered, drug-impregnated, solid or semisolid consistency devices placed into the cul-de-sac or conjuctival sac, whose size and shape are especially designed for ophthalmic application. They are composed of a polymeric support that may or may not contain a drug. The drug can later be incorporated as dispersion or a solution in the polymeric support.

Nanosuspension – Nanosuspensions are poorly water-soluble drug suspended in an appropriate dispersion medium stabilized by surfactants. Nanosuspensions have an advantage to improved solubility of the drug, enhanced bioavailability, and reduced irritation to the eye. These have higher bioavailability and cause lesser irritation. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200-600 nm.

In situ forming gels – Ophthalmic in situ gel system is most widely used. It is reduce the drainage of the drug from cornea due to increase viscosity hence, bioavailability automatically enhanced. In-situ gelling system can be effect by temperature, pH or ion activation.

1.10 IN-SITU GEL

Ocular in-situ gel are liquid dosage form suitable to be administered by simple conventional instillation into the eye, changes to the gel phase upon exposure to physiological conditions there by increasing the pre-corneal residence time of the drug enhancing the ocular bioavailability.

These ocular drug delivery systems consist of polymers that reveal sol-to-gel phase transition appropriate to change in specific physio-chemical parameters such as (pH, temperature) in their environment.

Depending on the technique engaged to cause sol-to-gel phase transitions on the eye surface the subsequent three types of systems are accepted, Ph triggered system, temperature dependent system and ion activated system.

1.11 IDEAL CHARACTERISTICS OF POLYMERS:

It should be biocompatible.
It should have good tolerance.
It should impact the tear behaviour.
It should be capable of adherence to mucus.
Polymer should be capable of decreasing viscosity with increasing shear rate there by lowering viscosity during blinking.

Table 1.1: Polymers used for ocular in situ gelling system

Polymer	**Origin**	**Charge**	**Solubility**	**Mucoadhesive capacity**
Carbomer	Synthetic	Anionic	Insoluble	+++
Poly-acrylic acid	Natural	Anionic	Insoluble	+++
Chitosan	Natural	Cationic	Soluble	++
Xanthangum	Natural	Anionic	Insoluble	+
Methylcellulose	Natural	Nonionic	Soluble	+
Xyloglucan	Natural	Anionic	Soluble	+
Poloxamer	Synthetic	Nonionic	Soluble	++
Sodium alginate	Natural	Anionic	Soluble	++
HPMC	Natural	Nonionic	Soluble	+
Mucoadhesive capacity : Excellent(+++), Good(++), Poor(+)				

1.12 ADVANTAGES OF IN SITU DRUG DELIVERY SYSTEM:

- Low dose is required for treatment
- Ease of application.
- Reduced recurrence of medication administration.
- Improved patient compliance and enhance therapeutic performance of drug.
- Improved bioavailability.
- To provide sustained and controlled drug delivery.
- Drug effect is prolonged, and hence, frequent instillation of drug is not required.

1.13 BARRIERS IN OCULAR DRUG DELIVERY SYSTEM:

Drug loss from the ocular surface

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 μl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity.

Lacrimal fluid-eye barriers

Corneal epithelium limits drug absorption from the lacrimal fluid into the eye. The corneal epithelial cells form tight junctions that limit the paracellular drug permeation. Therefore, lipophilic drugs have typically atleast an order of magnitude higher permeability in the cornea than the hydrophilic drugs. In general, the conjunctiva is leakier epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea.

Blood-ocular barriers

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the middle layer of the eye beneath the sclera. It consists of the iris, ciliary body, and choroid).

This barrier prevents the access of plasma albumin into the aqueous humour, and also limits the access of hydrophilic drugs from plasma into the aqueous humour. The posterior barrier between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries.

Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extra vascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia.

1.14 VARIOUS BARRIERS TO DRUG ABSORPTION

Non-corneal permeation

Primary mechanism of drug permeation is the sclera is likely to be diffusion across the intercellular aqueous media in the case of structurally similar corneal stroma. Therefore, the possibility of partitioning mechanism cannot be eliminated. Although like cornea, the conjunctiva is composed of an epithelial layer covering an underlying stroma, the conjunctival epithelium offers substantially less resistance than does the corneal epithelium.

Bioavailability of drugs administered to the eye is an important consideration. There are physiological factors, which can affect a drug’s bioavailability including protein binding, drug metabolism and lachrymal drainage.

Protein bound drugs are incapable of penetrating the corneal epithelium due to the size of the protein drug complex. Because of the brief time in which an ophthalmic solution may remain present in the eye (due to lachrymal drainage), protein binding of a drug substance could quickly negate its therapeutic value by rendering it unavailable for absorption. One of the major problems encountered with conventional ophthalmic solutions is the rapid and extensive elimination of drugs from the precorneal lachrymal fluid.

Nasolacrimal drainage system

The nasolacrimal drainage system consists of three parts: the secretory system, the distributive system and the excretory system. The secretory system consists of basic secretors that are stimulated by blinking and temperature change due to tear evaporation and reflex secretors that have an efferent parasympathetic nerve supply and secrete in response to physical or emotional stimulation.

The distributive system consists of the eyelids and the tear meniscus around the lid edges of the open eye, which spread tears over the ocular surface by blinking, thus preventing dry areas from developing. The excretory part of the nasolacrimal drainage system consists of: the lachrymal puncta, the superior, inferior and common canaliculi; the lachrymal sac; and the naso-lachrymal duct.

In humans, the two puncta are the openings of the lachrymal canaliculi and are situated on an elevated area known as the lachrymal papilla. It is thought that tears are largely absorbed by the mucous membrane that lines the ducts and the lachrymal sac; only a small amount reaches the nasal passage.

1.15 IN SITU GEL DRUG DELIVERY SYSTEM

In situ gel drug delivery system is a drug delivery system which is in a solution form before the administration in the body, but it converts in to a gel form after the administration. These are aqueous polymeric solutions characterized by a low viscosity before administration and, once administered, form a gel at the application site, able to control the release of the loaded drug.

The sol–gel transition depends on one or more stimuli, which can be exogenous as ultraviolet irradiation or endogenous like temperature variation, pH change, ionic cross-link formation, and solvent exchange. The advantages of these formulations encompass ease of application and prolonged permanence at the administration site, as well as protection of the drug from environmental conditions, modulation of drug release and, depending on the polymer used and the application site, capability to interact with the biological substrate.

Gels are semi-solid systems consisting of a suspension of small-sized inorganic particles (such as aluminum hydroxide or silicates) or of organic macromolecules (polymers) being interpenetrated by a liquid. Depending of the nature of the liquid, gels are classified as hydrophobic (organogels) or hydrophilic (hydrogels).

Among gels, hydrogels, based on the employment of hydrophilic polymers, have gained increasing interest in the pharmaceutical field, since a proper choice of polymer physical–chemical properties (namely type, grade, cross-linking degree, etc.) enables easily tailoring their rheomechanical properties in order to achieve good spreadability on mucosal surfaces, accompanied by a controlled drug delivery.

Although organogels have been known for decades, their potential as drug delivery systems is newer. This is justified by the use of biocompatible and biodegradable organic solvents and organogelators that make these hydrophobic gels more pharmaceutically-acceptable and environmentally friendly.

1.16 ADVANTAGES

- Improved drug absorption
- Easy in administration
- Improved local bioavailability
- Reduced dosing frequency
- Better patient compliance and comfort
- Reduced dose concentration
- Simple formulation and
- Cost effective

1.17 CLASSIFICATION OF IN SITU GEL DRUG DELIVERY

These are classified in two categories as listed below;

- Based on Mechanism of gelatin
- Based on Route of administration
- Based on Mechanism of gelatin
- pH sensitive gel
- Presence of ions
- Gel sensitive to electrical current
- Enzyme sensitive
- Thermosensitive gel
- Based on Route of administration
- In situ forming polymeric systems for ocular delivery
- In situ forming polymeric systems for oral administration
- In situ forming polymeric systems for rectal and vaginal delivery
- In situ forming nasal drug delivery systems
- In situ forming injectable drug delivery systems

1.18 APPLICATION OF OCULAR IN SITU DRUG DELIVERY SYSTEM

Natural polymers such as gellan gum, alginic acid and xyloglucan are most commonly used polymers.

Drug released from gellan gum based in situ gels is prolonged due to longer precorneal contact times of the viscous gels compared with conventional eye drops.

Conventional delivery systems often result in poor bioavailability and therapeutic response because high tear fluids turn over and dynamics cause rapid elimination of the drug from the eye. So, to overcome bioavailability problems, ophthalmic in situ gels were developed.

Antimicrobial agents, anti-inflammatory agents and autonomic drugs used to relieve intraocular tension in Glaucoma.

Miyazaki et al. attempted to formulate in situ gels for ocular delivery using Xyloglucan (1% w/w) as the natural polymer.

1.19 FACTOR AFFECTING OF IN SITU DRUG DELIVERY SYSTEM

- Temperature
- Modulation
- pH change
- Presence of ions
- UV radiation
- Solvent exchange

1.20 VARIOUS BIODEGRADABLE POLYMERS USED

- Gellan Gum
- Poloxamer
- Pectin
- Chitosan
- Poly (DL lactic acid)
- Poly (DL lactide-co-glycoside)
- Alginic acid
- Xyloglucan
- Poly-caprolactone

1.21 APPROACHES OF IN SITU GEL DRUG DELIVERY

There are certain broadly defined mechanisms used for triggering the in situ gel formations of biomaterials;

Physiological stimuli : Example – Temperature and pH

Physical mechanical changes in biomaterials: Example – swelling and solvent exchange-diffusion

Chemical reactions: Example –Ionic, Enzymatic and photo-initiated polymerization

1.22 PHYSIOLOGICAL STIMULI

These are thermally triggered systems. Temperature-sensitive hydrogels are probably the most commonly studied class of environment sensitive polymer systems in drug delivery research. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature, is an attractive way to approach in-situ formation useful system should be tailorable to account

for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity.

They are classified into;
Positively Thermosensitive - A positive thermosensitive hydrogels is having upper critical solution temperature [UCST], such hydrogels contract upon cooling below this UCST.
Negatively Thermosensitive – A negative thermosensitive has lower critical solution temperature [LCST], contracts upon heating above LCST. E.g.: poly(N-isopropylacrylamide) (PNIPAAm)
Thermally reversible gel – Mostly used thermally reversible gels are prepared from Pluronics and Tetronics. Pluronics are poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) triblock co-polymer that are fluid at low temperature, nut forms thermo responsible gel when heated as a consequence as a disorder-order transition in micelle packing which makes these polymers suitable for in-situ gelation.

1.23 PHYSICAL MECHANICAL CHANGES IN BIOMATERIALS

Swelling – It may occur when material absorbs water from surrounding environment and expand to cover desired space.

Solvent exchange-diffusion – It involves the diffusion of solvent from polymer solution into surrounding tissues and results in precipitation or solidification of polymer matrix.

1.24 CHEMICAL REACTIONS

Iconic cross-linking – Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones.

Enzymatic cross-linking – In situ formation catalyzed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators.

Photo initiated polymerization – It is commonly used for in situ formation of biomaterials. A solution of monomers or reactive macromers and initiators can be injected into the tissues site and the application of electromagnetic radiation used to from gel.

CHAPTER 2

LITERATURE REVIEW

2.1 LITERATURE REVIEW

Nagesh et.al, (2017), design and characterization of pH triggered In-Situ Gel containing Ciprofloxacin for ophthalmic delivery. In-situ gelling system of Ciprofloxacin with pH sensitive polymer. IR studies revealed that the drug and excipients were compatible with each other. Preparations were found to be clear, pH and drug content of all the preparations were found within the acceptable ranges. These formulations showed pseudo plastic flow behavior. The results of sterility test confirmed that all the formulations were sterile.

Makwana et.al, (2015), development and characterization of in-situ gel of ophthalmic formulation containing ciprofloxacin hydrochloride. Ciprofloxacin hydrochloride was successfully formulated as in-situ gel forming eye drops using sodium alginate and HPMC. The mixture of HPMC and alginate used as in-situ gelling vehicle to enhance ocular bioavailability and patience compliance. Physiochemical property and *in-vitro* drug release studies indicated that the developed formulation is ease of administration with improved patient compliance.

Mandal et.al, (2012), formulation and evaluation of an in-situ gel-forming ophthalmic formulation of ciprofloxacin hydrochloride. Ciprofloxacin hydrochloride was successfully formulated as in-situ gel-forming eye drops using Sodium alginate as a gelling agent in combination with HPMC as a viscosity enhancing agent. Thus, the developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer pre-corneal residence time and ability to sustain drug release.

Al-bazzaz et.al, (2018), ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. Ciprofloxacin was successfully formulated as thermo-sensitive ophthalmic in-situ gel using Poloxamer 407 as a gelling agent in combination with HPMC as a viscosity-enhancing agent. Upon instillation into the eye as drops. The formed gel expressed prolonged in-vitro drug release over 8-hour period. The ease of administration and the expected reduced frequency of administration will result in better patient compliance.

Sopyan et.al, (2018), application and characterization of in-situ gel. Evaluation of in-situ gel is determined to ensure that the prepared preparation meets the standard and is safe. In the chemical evaluation in-situ gel determined the diffusion of the active substance of a compound by measuring its concentration. Ocular irritation studies – Draize Test in an animal (mice) and determination of visual appearance, clarity, and pH is required.

Tinu et.al, (2013), polymers used in ophthalmic in-situ gelling system. Polymers play a vital role in the delivery of drug from its dosage form. Polymeric in-situ gelling system provides prolonged release of drug as compared to conventional delivery system. Use of polymers for in-situ gel formulation makes acceptable and controlled drug delivery system.

Lin HR et.al, prepared in situ gelling of alginate/pluronic solutions for ophthalmic delivery of pilocarpine. The optimum concentration of alginate solution for the in-situ gel forming delivery systems was 2% (w/w) and that for pluronic solution was 14% (w/w). The mixture of 0.1% alginate and 14% Pluronic solutions showed a significant increases in gel strength in the physiological conditions; this gel mixture was also found to be free flowing at pH 4.0 and 24 degrees C. Both in vitro release and in vivo pharmacological studies indicated that the alginate/Pluronic solution retained pilocarpine better than the alginate or Pluronic solutions alone.

Kumar et.al, (2015), review advance technique in ocular drug delivery system. The latest available targeted drug delivery systems focus on the localized delivery of the drugs as well as certain macromolecular substances like proteins, genes like DNA, RNA to the internal parts of the eye.

Dhruvi et.al, (2017), innovation in ocular drug delivery system. In this scenario, drug delivery to the posterior part of the eye becomes all the more challenging due to the anatomical and physiological barriers that separate the posterior and anterior segments. These need shave generated interest and development in the novel techniques of ophthalmic drug delivery systems.

Kumari et.al, (2019), ocular drug delivery system approaches to improve ocular bioavailability. In the therapy of eye disease, it provides many advantages such as; improve patient compliance by reducing the frequency of dosing, provide sustained and controlled drug delivery and reduce the dose consequently, in- situ forming gel, and ion to phoresis for effective delivery and to further enhance ocular absorption and reduce side effects.

Noriyuki et.al, (2011), recent advances in ocular drug delivery systems. Due to transparent ocular mediums, intraocular tissues are relatively easy to be observed without invasion, and various administration approaches including intravitreal could be developed in the ophthalmic field. Certainly, we should further consider the most efficacious combinations of optimal drugs, dose, route, and drug release pattern according to the pathophysiology and progressive courses of the targeted disease.

Bobde et.al, (2013), design and evaluation of in-situ gelling systems of ciprofloxacin hydrochloride in the treatment of bacterial eye infections. The revised formulation met the objectives and all the expected targets of this research study. The developed formulations were found to be efficacious, stable, and non-irritant and thus a viable alternative to conventional eye drops by virtue of its ability to sustain drug release over a 12 hour period.

Al-Kassas RS et.al,(2020) developed ophthalmic controlled release in-situ gelling systems for ciprofloxacin based on polymeric carriers. Carbopol and alginates polymers were used to confer gelation properties to the formulations. Hydroxypropyl methylcellulose and methylcellulose were combined with carbopol to increase the viscosity of the gels and to reduce the concentration of the incorporated carbopol.

K.Kavitha et.al,(2021) prepared sustained release In situ ocular gels of levofloxacin hemihydrates using gel rite as gel forming polymer, which is used in treatment of various bacterial infections. Formulations were evaluated for physical parameters like clarity, pH, drug content, gelation, Rheological studies, sterility test, in vitro drug release study and ocular irritancy. The formulated gels were transparent, uniform in consistency and had spreadability with a pH range of 7.1 to7.4 studies. A maximum of90.2% drug release was observed in in vitro studies. Further in vivo results conclude that it is be possible to formulate in-situ ocular gel containing Levofloxacin Hemihydrates.

Sultana et.al,(2022) developed ophthalmic delivery system for perfloxacin-mesylate based onin-situ gel of gel rite and evaluated for rheological characterizations, antimicrobial efficacy, in-vitro release pattern. The developed formulation showed better therapeutic efficacy than marketed preparation.

Liu Y et.al, (2023) developed and evaluated an ion-activated in situ gel, the rheological properties of polymer solutions, including gel rite 0.3% (w/w) and that for alginate solution was 1.4% (w/w). The mixture of 0.2% Gel rite and 0.6% alginate solutions shows significant enhancement in gel strength at physiological condition. The Gel rite/alginate solution had the better ability to retain drug than the gel rite or alginate solutions alone.

Cao F et.al,(2023) developed a method for ophthalmic delivery of azithromycin by Poloxamer/ carbopol-based in situ gelling system. Addition of Carbopol 974P (CP 974P) to the gelling systems could increase the solubility of ATM by salt effect and enhance the Mucoadhesive property of the systems. Gelation temperature of these systems ranged from 31.19 to 36.30 degrees C depending on

The ratio of P407 and P188. Mucoadhesion force of the system composed of P407/P188/CP 974P (21/5/0.3%, w/v) was 2.3-fold that without carbopol 974P. the formulation exhibited a 24-hour sustained release of ATM.

CHAPTER 3

NEED OF STUDY

3.1 NEED OF STUDY

- Ciprofloxacin belongs to the class 4 of the BCS systems of drugs.
- **Ciprofloxacin is only indicated in infections caused by susceptible bacteria.**
- **It is indicated for bacterial corneal ulcers and conjunctivitis.**
- Ocular in-situ gel overcomes the problems associated with eye drops (i.e. rapid drainage of drug).
- Improved the precorneal residence time.
- Ocular in-situ gel prolongs the contact time of the vehicle at ocular surface and also slows down the elimination of drug.
- Improve the bioavailability.

CHAPTER 4

MATERIAL AND METHODOLOGY

4.1 MATERIAL AND METHODOLOGY

MATERIAL

4.1.1 Drug profile

Ciprofloxacin hydrochloride is selected as an active pharmaceutical ingredient (API) for the preparation and formulation of ophthalmic in-situ gelling drug delivery system.

Ciprofloxacin has good anti-bacterial effect because it belongs to a broad spectrum anti-biotic that results in the death of the bacteria. Ciprofloxacin was patented in 1980 and introduced in 1987.

It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. It is available as a generic medication and is not very expensive. It functions by inhibiting DNA gyrase, and a type II topoisomerase, topoisomerase IV, necessary to separate bacterial DNA, thereby inhibiting cell division.

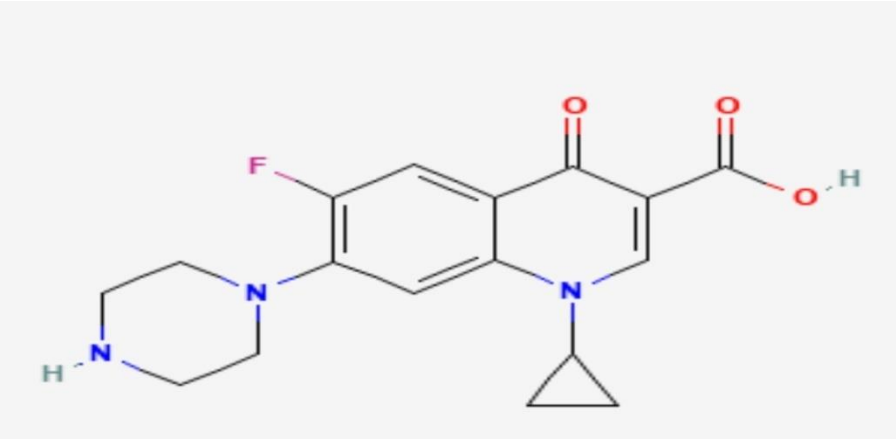


Figure 4.1: Structure of Ciprofloxacin hydrochloride

API Name – Ciprofloxacin Hydrochloride

Chemical Formula-$C_{17}H_{18}FN_3O_3$

Molecular mass-331.346g/molg·mol^{-1}

IUPAC name - 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic

Bioavailability – 70%

Solubility – Water, dilute (0.1N) hydrochloric acid

pH – 1.0% aqueous concentrates in vials is 3.3 3.9

Protein binding – 20% - 40%

Metabolism – Liver

Elimination half life - 3.5hours

Excretion – Kidney

Route of administration – By mouth, IV, local (eye drops)

BCS class of ciprofloxacin – Ciprofloxacin hydrochloride belongs to class IV i.e low solubility and low permeability.

4.1.2 Selection of vehicle

Distilled water is selected as a vehicle for this formulation because it is economic, non-toxic and compatible with the drug ciprofloxacin. Distilled water is water that has been boiled into vapor and condensed back into liquid in a separate container. Impurities in the original water that do not boil below or near the boiling point of water remain in the original container. Thus, distilled water is one type of purified water.

4.1.3 Selection of polymers

For preparation of an ideal in-situ gelling system, the main factor is the selection of an appropriate polymer which is compatible with the drug, vehicle and other excipients in the ophthalmic formulation as well as it should have good gelling property and viscosity.

For this we first selected Guar gumas a polymer because it's a natural polymer and hence, inexpensive that would have economic for the formulation. But after many trials **Guar gum** showed incompatibility with the drug ciprofloxacin and resulted in formation of clumps of guar gum and the drug. Then after some literature review, we selected Hydroxypropyl methylcellulose (HPMC) and Sodium Alginate as the beneficial polymer for the formulation.

Hydroxypropyl methylcellulose (HPMC), a semi-synthetic polymer which is mostly used in ophthalmic preparations. It is non-toxic, compatible with the vehicle and the drug and yet economic as well. It is used as a thickening agent. Two types of HPMC grade were used for the preparation of the formulation and different concentrations. HPMC K4M has less gelling capacity than HPMC E50LV. Two grades of HPMC used are; HPMC K4M and HPMC E50LV.

Hydroxypropyl methylcellulose (HPMC) K4Mis a high purity, water-soluble cellulose derivative. It has a viscosity range of 2,700 to 5,040 cps and particle size of 170 to 250 micrometers. Hydroxypropyl methylcellulose (HPMC) E50LV has little less purity than K4M, but it is water-soluble cellulose derivative. It has a viscosity range of 35 to 65 cps.

Sodium Alginate, was selected because these semi-synthetic polymers will give the formulation ion-activated sustained release of drug. It is also compatible with the drug and vehicle as well as non-toxic to the eye. It also gives the gelling properties needed

4.1.4 Selection of preservative

Benzalkonium chloride (BKC) is selected as an appropriate preservative for this formulation because this preservative has been used in ophthalmology since the 1940s, and is found in up to 70% of eye drops.

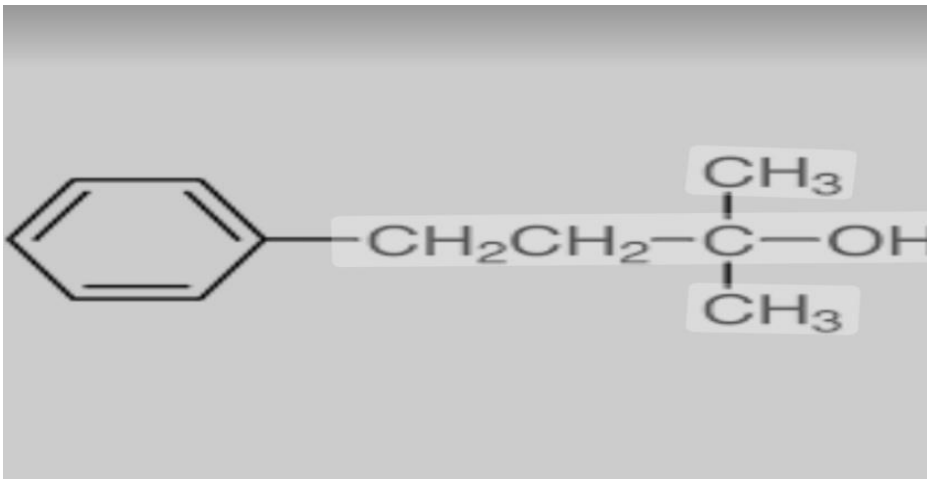


Figure 4.2 : Structure of Benzalkonium Chloride

Benzalkonium chloride is also known as alkyldimethylbenzylammonium chloride and by the trade name Zephiran. It is a type of cationic surfactant. BKC is a quaternary ammonium compound, acts as a detergent, lysing cell membranes, and killing microorganisms.

This makes it very effective as a preservative. Preservatives are used in ophthalmic medications because they are cost effective. A large bottle of eye drops can last an entire month when a preservative. Although BKC is a preservative with an excellent antibacterial spectrum, it has also been shown to induce toxic effects to the ocular surface.

Several studies have indicated that BKC may also have altering effects on the bacterial flora of the conjunctiva.

- Molar mass - variable
- Density - 0.98 g/cm3
- Solubility in water - very soluble
- Flash point - 250°C (482°F) (if solvent based)
- ATC code - D08AJ01 (WHO)

4.2 METHODOLOGY

4.2.1 PREPARATION OF IN-SITU GEL

Polymer solution was prepared by dispersing sodium alginate in distilled water at room temperature. Drug solution was prepared by dissolving ciprofloxacin into mixture of HPMC and distilled water. Drug solution was mixed with polymer solution in magnetic stirrer and allows to hydrate overnight. Benzalkonium chloride was added which acted as preservative. The prepared in-situ gels were filled in glass vials closed with rubber closures and sealed with aluminum caps.

4.2.2 Analytical method development

4.2.3 Determination of λ_{max} of ciprofloxacin hydrochloride

For the determination of absorption maxima, stock solution (1000 µg/ml) was prepared by weighing 100 mg (0.1 g) drug and dissolving it in 100 ml volumetric flask and making the volume to the mark with Methanol.

10 ml of standard stock solution was taken in 100 ml volumetric flask and making the volume to the mark with methanol to make 100 µg/ml of ciprofloxacin. Serial dilutions with concentrations 2, 4, 6, 8 and 10 µg/ml were prepared by transferring 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the stock solution in 10 ml volumetric flask and makeup the volume with phosphate buffer 7.4 up to the mark.

The resulting solution was scanned between 200 and 400 nm using UV–visible spectrophotometer UV 1400, Shimadzu.

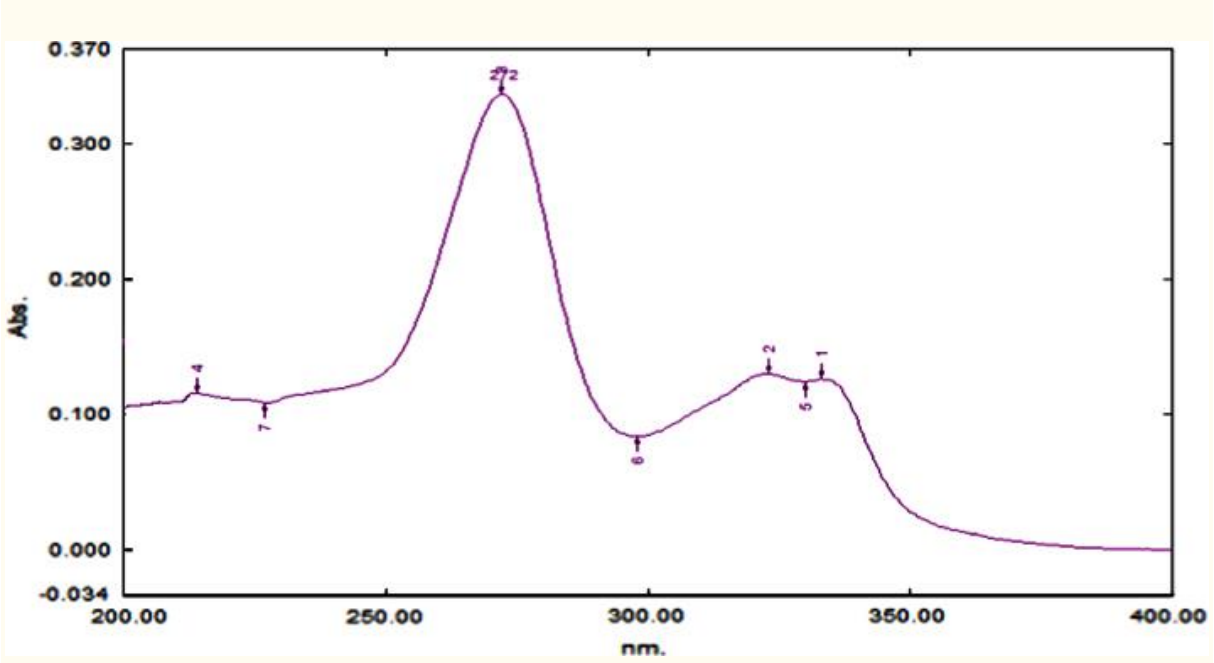


Figure 4.3 : UV visible spectra of ciprofloxacin hydrochloride at 272 nm.

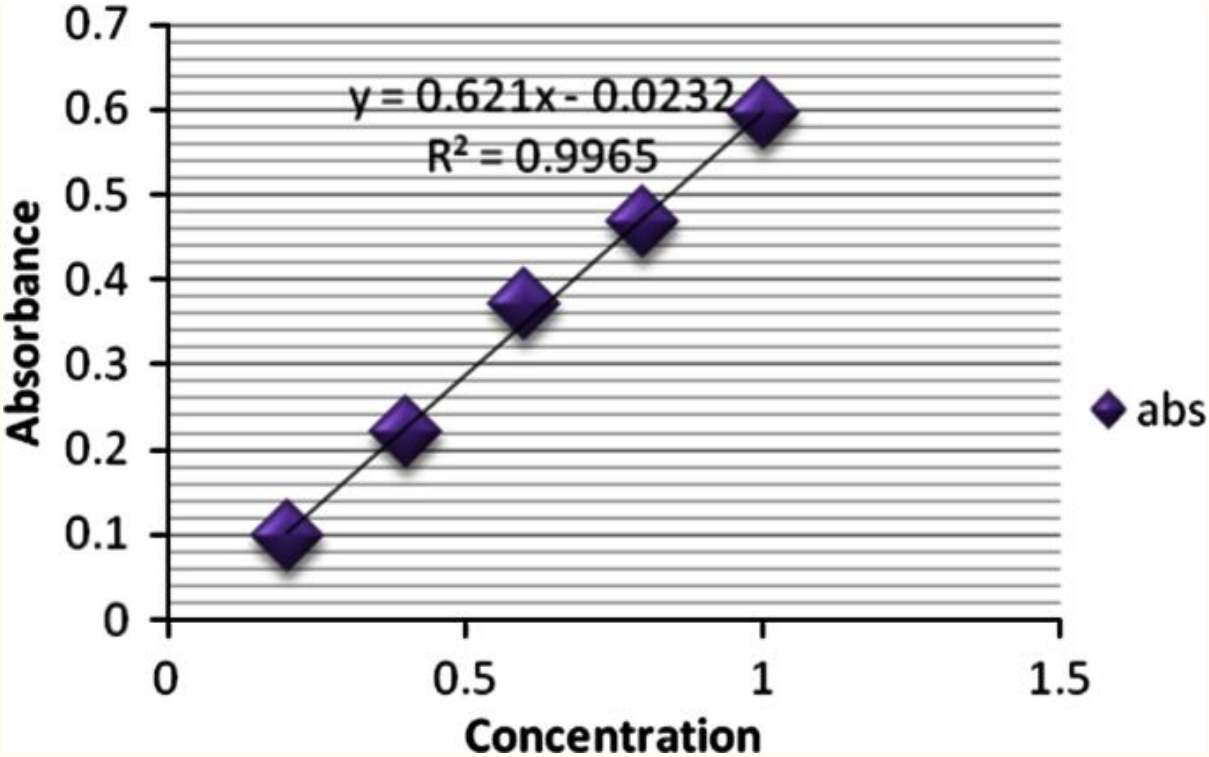


Figure 4.4 : Calibration curve of Ciprofloxacin Hydrochloride.

4.2.4 FT-IR studies

The IR spectra were recorded on Thermo Nicolet, Avatar 370. FTIR spectra of sodium alginate, HPMC and the drug (Ciprofloxacin HCl) were obtained. Spectral scanning was done in the range between 4000 and 500 cm^{-1} . FT-IR study of drug and polymer mixture along with water (in situ mixture) is shown in Figure.

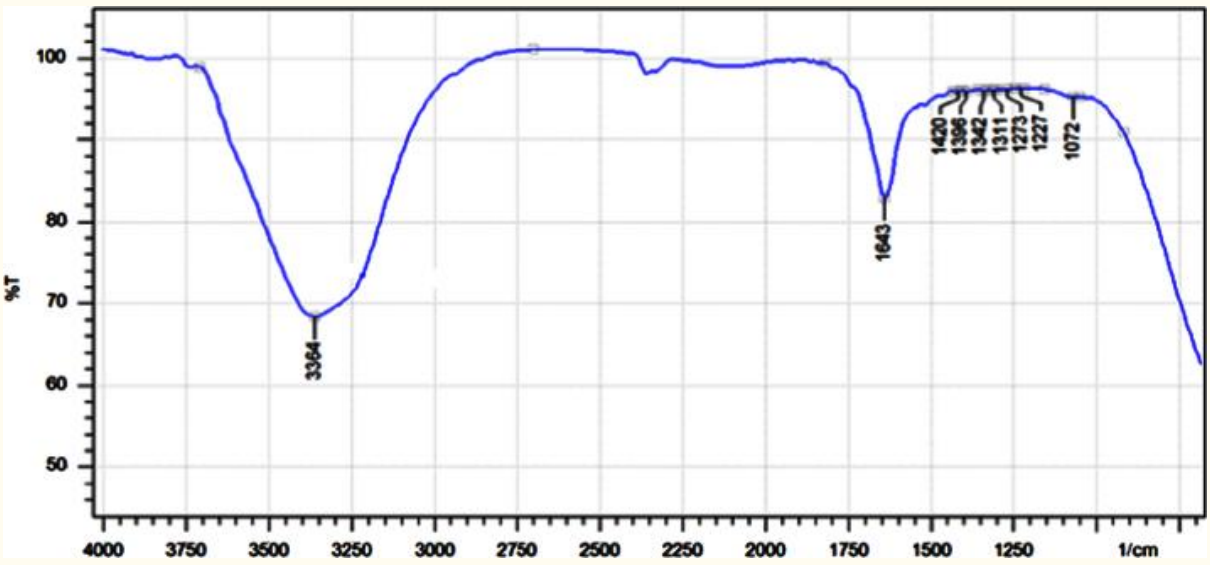


Figure 4.5 **:** FT-IR study of drug and polymer mixture along with water (in situ mixture).

4.2.5 DSC Studies

The DSC curve for pure ciprofloxacin exhibits , which represent water loss and melting of the sample, respectively. DSC studies of drug ciprofloxacin and its mixture was done using Shimadzu thermal analyzer (DSC-60) where it was found out that there was no incompatibility between the drug and physical mixture by giving yields sharp endothermic peak at 120.1ºC and 227.2ºC of the drug and the excipients respectively.

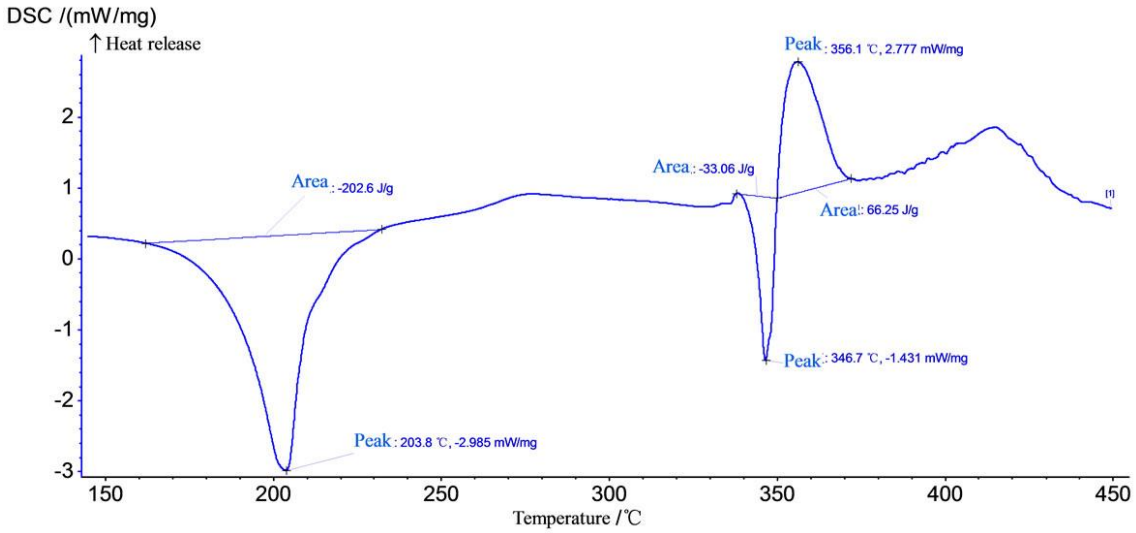


Figure 4.6**:** DSC study of drug and polymer mixture along with water (in situ mixture)

Table 4.1: Formulation table of Ciprofloxacin in-situ gel preparation

S.NO	INGREDIENTS	F1	F2	F3	F4	ROLE
1.	Ciprofloxacin	0.3%	0.3%	0.3%	0.3%	Antibacterial. Antimicrobial.
2.	Sodium alginate	0.5%	1%	0.5%	1%	Gelling polymer
3 .	HPMC K4M	0.5%	0.5%	-	-	slow release Mucoadhesive polymer.
4 .	HPMC E50LV	-	-	0.2%	0.4%	Viscosity modifier slow release Mucoadhesive polymer
5 .	Methyl Paraben	0.01%	0.01%	0.01%	0.01%	Preservative
6 .	Distilled water	q.s. upto 100 ml	q.s. upto 100 ml	q.s. upto 100 ml	q.s. upto 100 ml	Vehicle

4.3 Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a beaker containing 50 ml of freshly prepared concentrated calcium chloride solution and was visually observed for gelling time. Coding for the gelling capacity described in below table.

Table 4.2 : Coding for the gelling capacity

Observation	**Coding**
No gelation	–
Gelation occurred in few minutes and remained for few hour	+
Gelation immediate, remained for few hour	++
Gelation immediate, and for extended period	+++
Very stiff gel	++++

4.4 Rheological studies

The primitive ophthalmic solution, suspension, and ointment dosage forms are clearly no longer sufficient to combat these diseases, and current research and development efforts to design better therapeutic systems are the primary focus of this research work.

The aim of the present investigation is to formulate an in situ gel and from our prior knowledge we know that gels show thixotropic behaviour, so rheological studies are to be performed.

The viscosity measurements were carried out using Brookfield viscometer model DVII. The developed formulations were placed in the sampler tube using spindle no. 4.

Viscosity of the prepared formulations was measured by using Research Rotator and Oscillatory Rheometer. The gel under study was placed in the small sample holder and the spindle was lowered perpendicularly into it. The spindle was rotated at varying speeds and the suitable speed was selected . Rheological studies of formulation are shown below.

Table 4.3 : Rheological studies of formulation

Formulation code	**Viscosity of solution (Pa s)**	**Viscosity of in situ gel (Pa s)**
F-1	0.0163	89.5
F-2	0.0447	266
F-3	0.189	856
F-4	0.00466	0.147

4.5 *In* vitro dissolution studies

Dissolution studies of samples were performed using Franz diffusion apparatus and phosphate buffer (pH=7.4) as a dissolution medium. Phosphate buffer with pH 7.4 will simulate the lachrymal fluid . The temperature was maintained at 37±0.5 °C with the speed of rotation maintained at 100 rpm. The samples were withdrawn at various time intervals and analysed spectrophotometrically for the drug content.

4.6 Evaluation Studies

4.6.1 Organoleptic characteristics of drug

It is a faintly yellowish to light yellow crystalline substance, odorless and has characteristic taste.

4.6.2 Organoleptic characteristics of sodium alginate

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder. Melting point is less than 300C. Dissolves slowly in water, forming a viscous solution, insoluble in ethanol and ether.

4.6.3 Organoleptic characteristics of Hydroxypropyl methylcellulose (HPMC)

It’s an odorless, tasteless, white or creamy white fibrous or granular power.

It is soluble in water, most polar organic solvents, and the appropriate proportion of ethanol/water, propanol/water and dichloroethane, but insoluble in diethyl ether, acetone, and anhydrous alcohol.

In cold water, it will swell into a clear or slightly turbid colloidal solution.

4.6.4 Organoleptic characteristics of Benzalkonium chloride

It’s colorless, viscous oily liquid with characteristic odor. Benzalkonium chloride is readily

Benzalkonium chloride is readily soluble in ethanol and acetone. Dissolution in water is slow. Aqueous solutions should be neutral to slightly alkaline. Solutions foam when shaken. Concentrated solutions have a bitter taste and a faint almond-like odor.

4.6.5 Organoleptic characteristics of ophthalmic in-situ gel formulation

Table 4.4 - Characteristics of formulation.

S.NO	TEST	OBSERVATION			
		F1	**F2**	**F3**	**F4**
1	Colour	whitish	whitish	Whitish	whitish
2	Odour	odourless	odourless	odourless	odourless

4.6.6 pH of the in-situ gel formulation

The pH of ophthalmic formulations was determined by using pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions. The formulation temperature was maintained at 25±3ºC. The pH meter electrode was immersed in formulation and the pH was recorded.

Table 4.5 : pH of the formulation

S.N	**FORMULATIONS**	**pH (observed)**
1	F1	6.53
2	F2	6.49
3	F3	6.58
4	F4	6.55

4.6.7 Gelling capacity of the in-situ gel formulation

Gelling capacity of formulations was evaluated in order to identify the formulations suitable for use as in-situ gelling systems. Gelling was determined by mixing the formulation with simulated tear fluid and examined visually and numerical scores assigned depending upon, quickness of gel formation and time taken for collapse of gel structure on shaking the vials.

Simulated Tear Fluid (STF) was prepared below

Table 4.6 : Composition of STF

INGREDIENTS	QUANTITY
Sodium chloride	0.670 g
Sodium bicarbonate	0.200 g
Calcium-chloride dihydrate	0.08 g
Distilled water	100 g

4.7.8 OBSERVATIONS

Table 4.7: Gelling capacity of the formulation

S.N	Formulations	Grade	Observed
1	F1	-	No phase transition
2	F2	+	Formation of gel after 60 seconds and gel collapsed within 1 hour.
3	F3	++	Formation of gel after 60 seconds and gel collapsed within 3 hour.
4	F4	+++	Formation of gel within 60 seconds and gel remained stable for more than 5-6 hour.

4.6.8 Rheological studies of the in-situ gel formulation

Rheological studies are carried out with the help of Ostwald viscometer. Viscosity describes the liquid's resistance to the flow. Mathematically viscosity is the ratio of the shearing stress to the velocity gradient in a fluid.

Ostwald viscometer, also known as U-tube viscometer or capillary viscometer is a device used to measure the viscosity of the liquid with a known density. The method of determining viscosity with this instrument consists of measuring the time for a known volume of the liquid (the volume contained between the marks A and B) to flow through the capillary under the influenceof gravity. Ostwald viscometers named after the German chemist Wilhelm Ostwald (1853-1932).

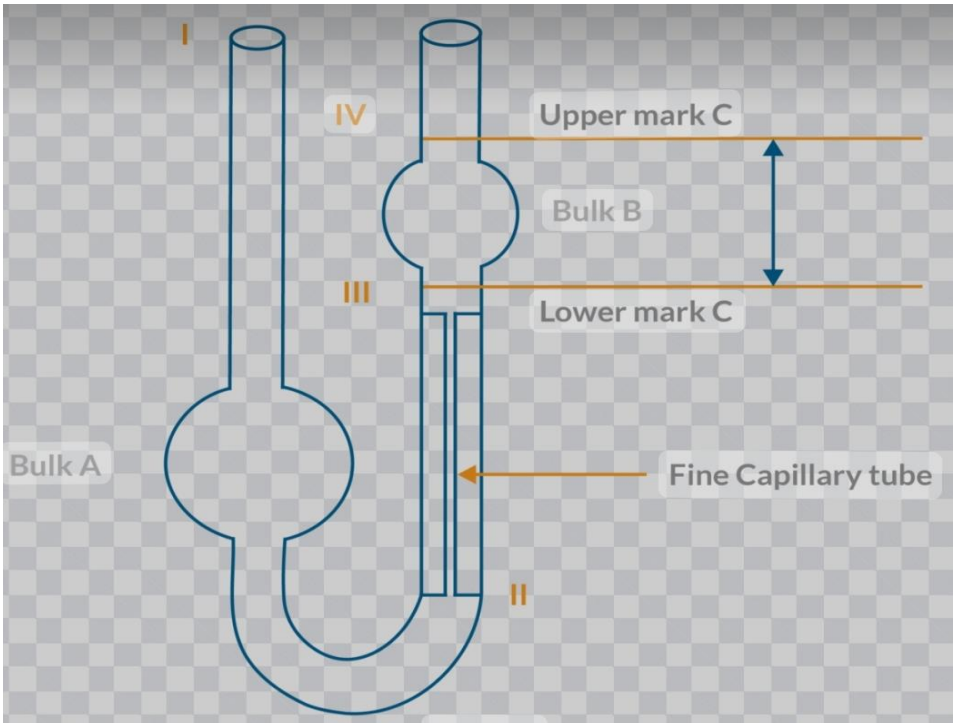


Figure 4.7: OSTWALD VISCOMETER

Table 4.8 : Viscosity of various formulations.

S.NO	FORMULA	VISCOSITY OF SOLUTION AT 25·C	VISCOSITY OF IN-SITU GEL AT 37·C
	F1	0.016	56.09
	F2	0.024	76.07
	F3	0.076	98.94
	F4	0.125	121.09

4.6.9 Clarity test of the in-situ gel formulation

Formulations were examined visually for colour and clarity against white background and for the presence of particulate matter any if present. Clarity examination involves the visual assessment of formulation in suitable lighting on white background. It is performed for liquid forms and in-situ gels.

Table 4.9: Clarity observation of the formulation

S.N	**FORMULATION**	**OBSERVATION**
1	F1	Clear
2	F2	Clear
3	F3	Clear
4	F4	Clear

4.6.10 Antimicrobial/Antibacterial efficacy of the in-situ gel formulation

Antimicrobial efficacy was determined by the agar diffusion test employing ‘Bore-well method. “Sterile solutions of Ciprofloxacin and the developed formulations (test solution) were poured in to wells bored into sterile nutrient agar previously seeded with test organisms (E.coli) after allowing diffusion of the solutions for hour. Agar plates were incubated at 37C for 24 hour. The zone of inhibition (ZOI) measured around each well was compared with that of control. The entire operation except the incubation was carried out laminar airflow unit.

For the Zone of Inhibition measurement, Cup-Plate method was used and it was measured
by the formula,

$C_A/C_B = Z_A/Z_B$

C_A = Concentration of Sample 'A'.

Z_A = Zone of inhibition of Sample 'A'.

C_D = Concentration of unknown sample.

Z_D = Zone of inhibition of unknown sample.

Note: Concentration of sample or known sample i.e. marketed Ciprofloxacin eye drop is 0.3 µg/ml.

4.6.11 Stability study

Stability study was carried out on optimized formulation F4 at 30±2°C temperature and 60±5% RH for 0,30,60 and 90 days. At a periodic interval, sample was withdrawn and was observed for clarity, pH, viscosity and drug content at the interval days. All the measurements were performed after allowing the samples to equilibrate at 25°C.

CHAPTER 5

RESULT AND DISCUSSION

RESULT AND DISCUSSION

5.1 Determination of drug (Ciprofloxacin)

The drug obtained was determined whether it is Ciprofloxacin, by conducting melting point analysis of the chemical organic compound because every organic compound has unique melting point or melting point range.

Table 5.1 : Determination of drug

S.No	Melting point (observed)	Melting point (as per I.P)
1	316°C- 319°C	320°C

5.2 Clarity and pH of the in-situ gel formulation

The clarity and pH of the ophthalmic in situ hydrogel was tested by against white background and pH meter respectively. The results of clarity and pH were compatible with consideration to in situ ophthalmic hydrogels.

The ophthalmic in situ hydrogels should be clear without any particulate matter as well as should have pH between range of 6-7.

Table 5.2 : pH and Clarity

S.N	Formulations	Clarity	pH
1	F1	Clear	6.49
2	F2	Clear	6.43
3	F3	Clear	6.51
4	F4	Clear	6.57

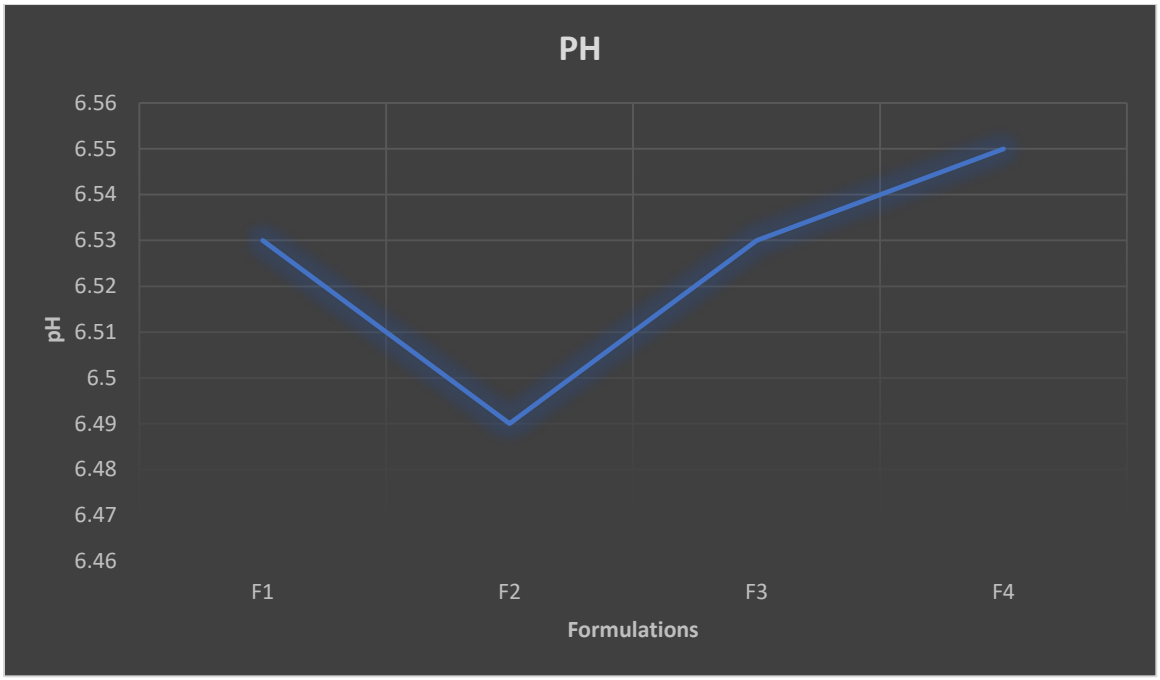


Figure 5.1: Graphical pH representation of the various formulation.

5.4 Gelation and Rheological studies of the in-situ gel formulation

The gelation and rheological study of the ophthalmic in situ hydrogel was tested by simulated tear fluid (STF) and Ostwald Viscometer respectively. The results of gelation and rheological study was compatible with consideration to in-situ gel.

Table 5.3: Gelation and Rheological study

S.NO	Formulations	Gelation Property	Rheological property
1	F1	-	$184.63x10^{-4}$ cm^2/s
2	F2	+	$188.67x10^{-4}cm^2/s$
3	F3	++	$18.98x10^{-4}cm^2/s$
4	F4	+++	$33.90x10^{-4}cm^2/s$

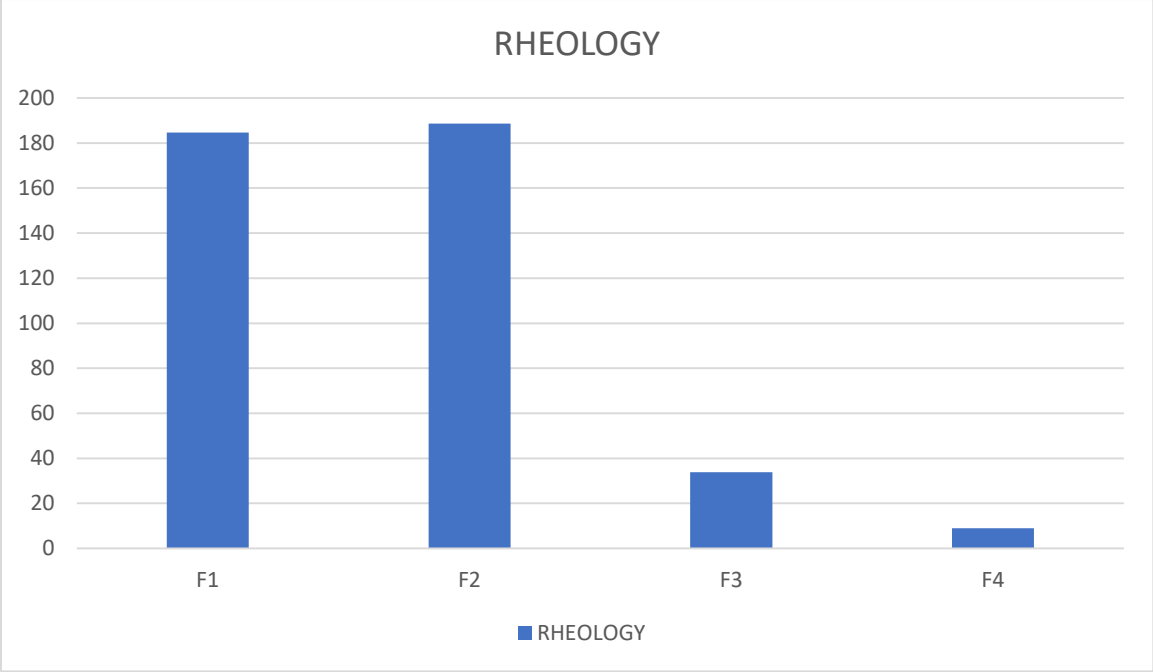


Figure 5.2 : Graphical representation of formulations showing rheology.

Formulation F3 has good gelation property i.e. formation of gel is within 60 seconds and gel remained stable for more than 5-6 hour and **Formulation F4** also has good rheological property i.e. $33.90x10^{-4}cm^2/s$ which is a good rheological value with consideration to in situ ophthalmic hydrogels.

5.3 IN-VITRO DRUG RELEASE

The in-vitro drug release was carried out in pH 7.4 in PBS with a modified paddle method (Hanson Research SR8-Plus Dissolution Test Station, Chatsworth, CA, USA) was used to measure the drug release.

The release studies were carried out in 100 mL of the PBS medium at 37 °C. The paddle was rotated at 100 rpm. Then, 5 mL samples were taken manually from the buffer solution after 5, 10, 15, 30, 60, and 90 min.

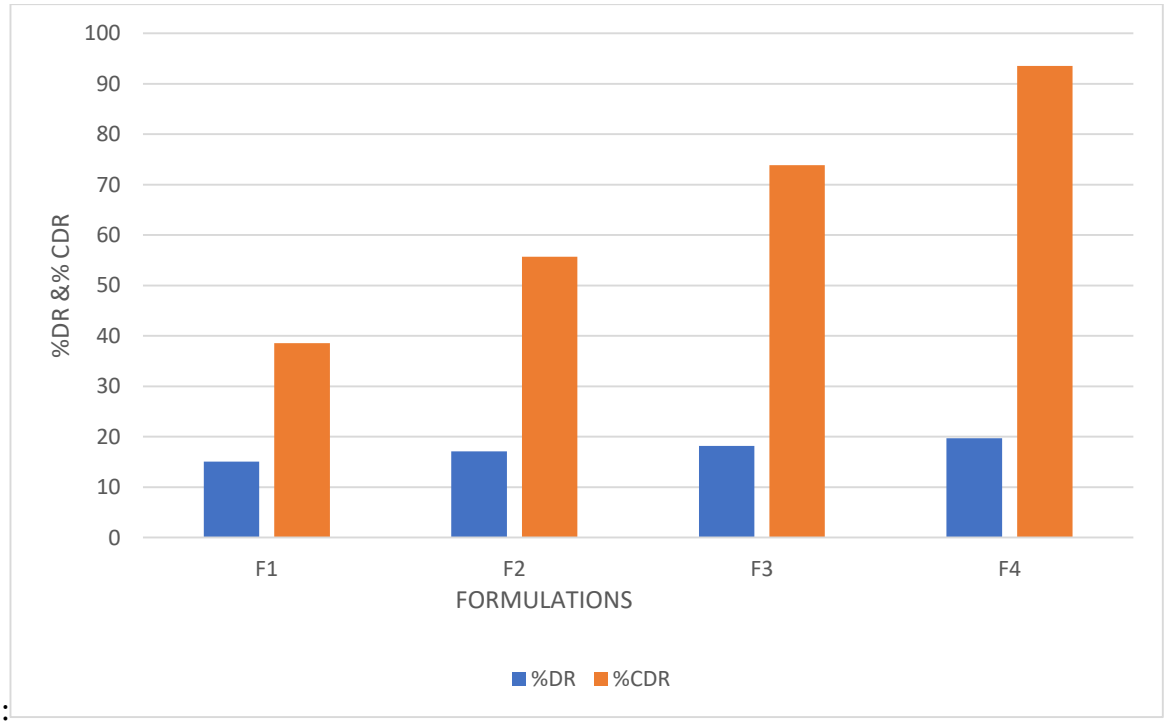
After sampling, the volume was replaced with fresh PBS. The amount of drug present in the aliquots was determined with UV-Vis spectrophotometry (ABL&E-Jasco UV/VIS Spectrophotometer V-730, Budapest, Hungary) at a λ_{max} of 275 nm.
The cumulative drug release was calculated using the calibration curve of CIP in pH 7.4 PBS.
Each experiment was performed in triplicate, and the average values and graphs are reported.
Percentage drug release in case of in situ gel of Ciprofloxacin hydrochloride was found to be 93.55%(F4) release in 1 h.
Thus the in vitro dissolution test indicated the sustained release nature of in situ gel of Ciprofloxacin hydrochloride.

Table 5.4: In-vitro drug dissolution chart

S.NO	FORMULATION	TIME (MIN)	LOG TIME	ABS.	CONC.	% D.R	%C.D.R	SQUARE ROOT OF TIME IN HOURS
	F1	15	1.17	0.008	0.83	15.04	38.57	30
	F2	30	1.47	0.01	1.78	17.12	55.69	42.42
	F3	45	1.65	0.04	3.76	18.15	73.84	51.96
	F4	60	1.77	0.05	4.25	19.71	93.55	60

5.5 Study of Drug-Release Kinetics and Mechanism

The release kinetics of CIP from the electrospun nanofiber and physical mixture was compared with the dissolution kinetics of the CIP powder. Five different mathematical models (zero order, first order, Higuchi, and Korsmeyer–Peppas model) were fitted with the obtained cumulative drug release vs. time curves to describe the kinetics.



:

Figure 5.3 : Formulations Showing D.R & C.D. R

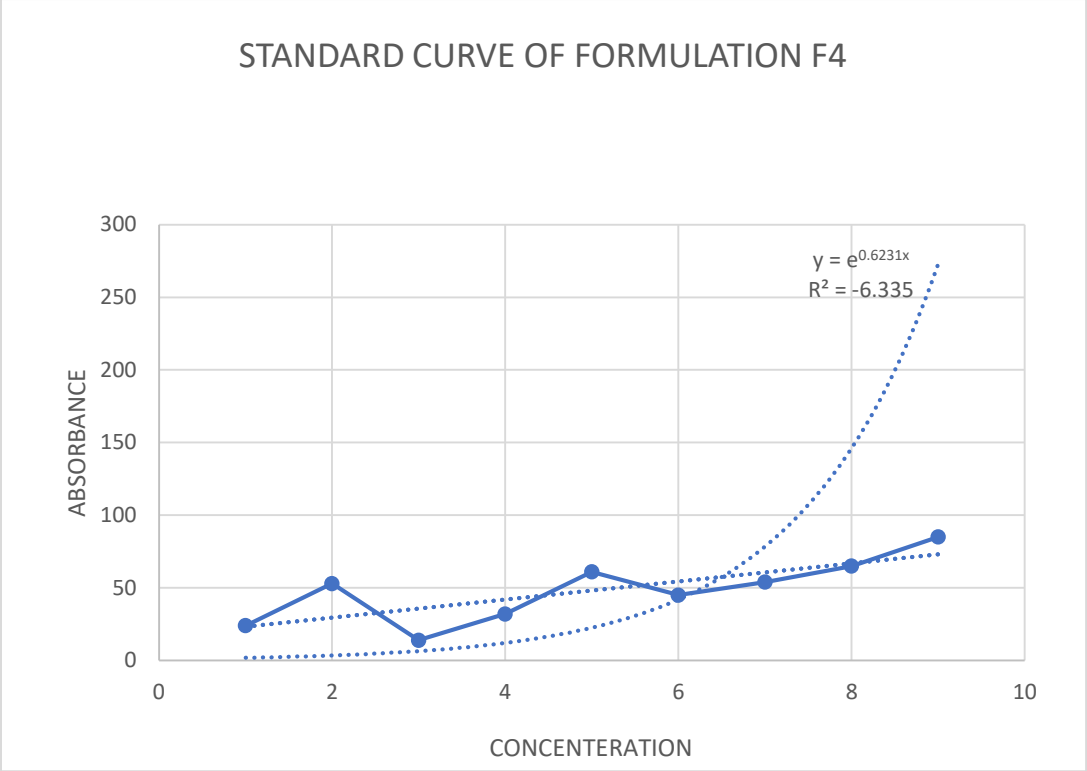


Figure 5.4 : Standard Curve of Formulations F4

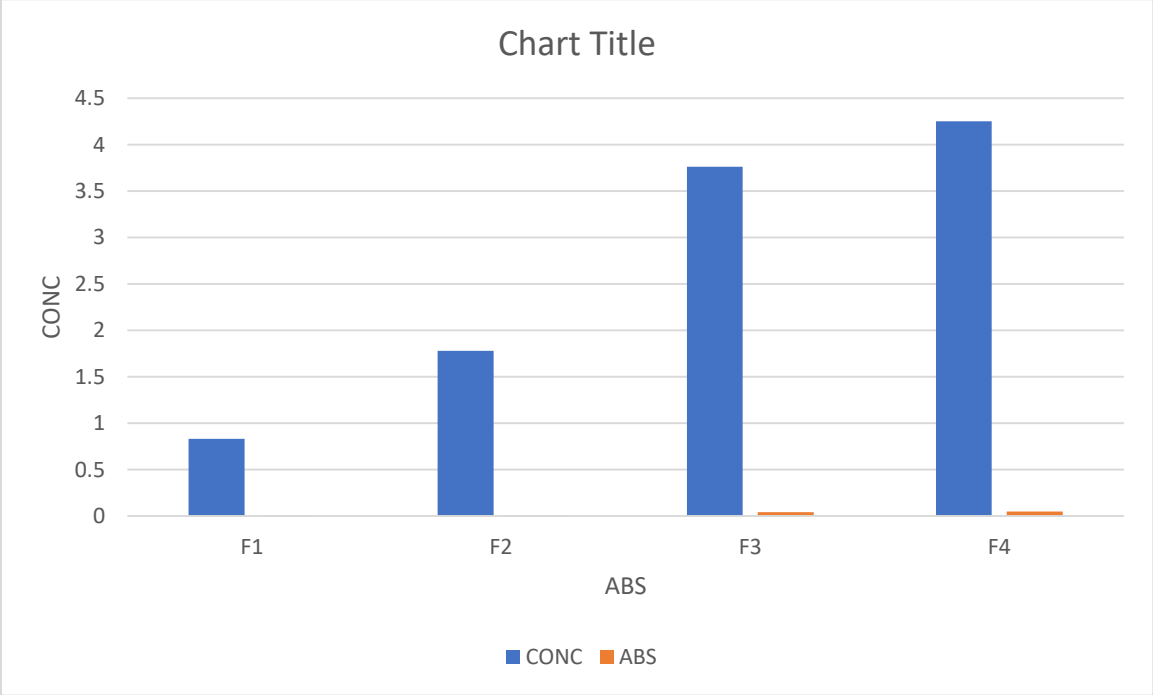


Figure 5.5 : Graphical representation of F4

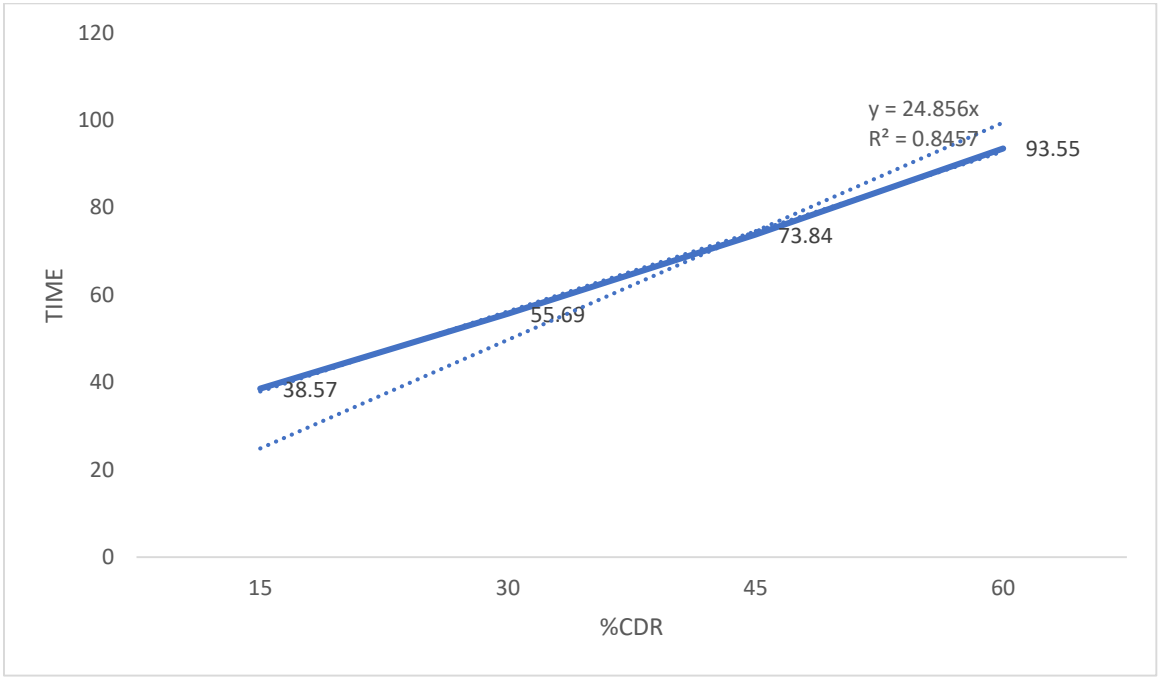


Figure 5.6 : Standard curve of F4 showing zero order release

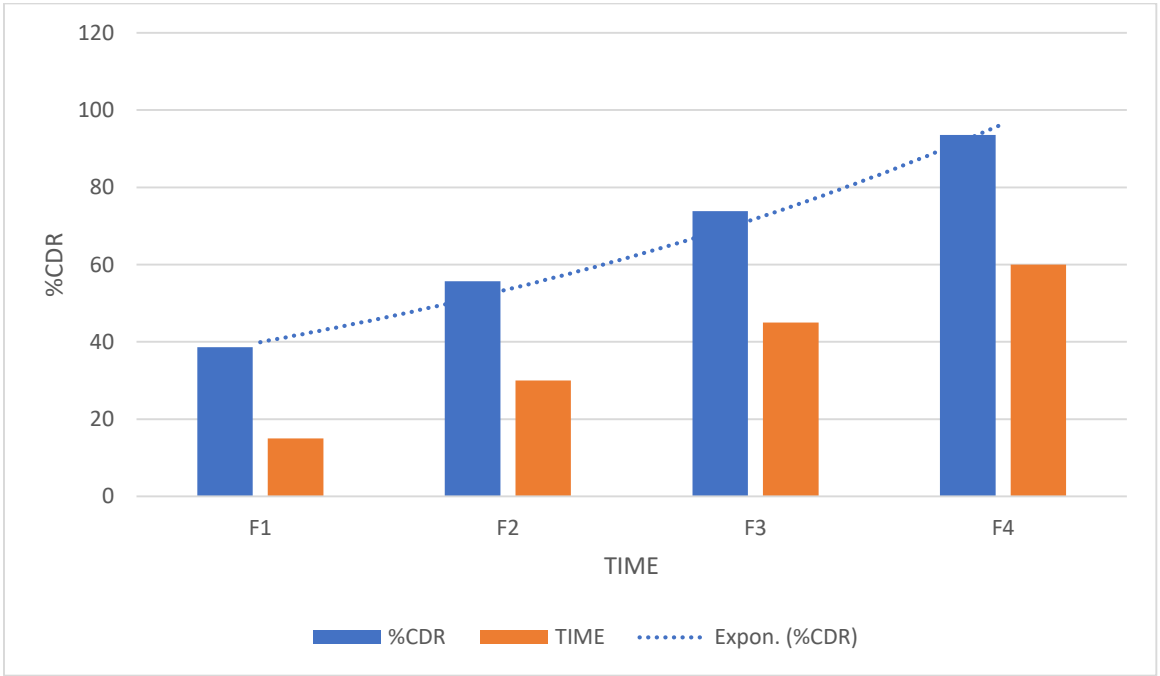


Figure 5.7 : Graphical Representation of F4 showing zero order release

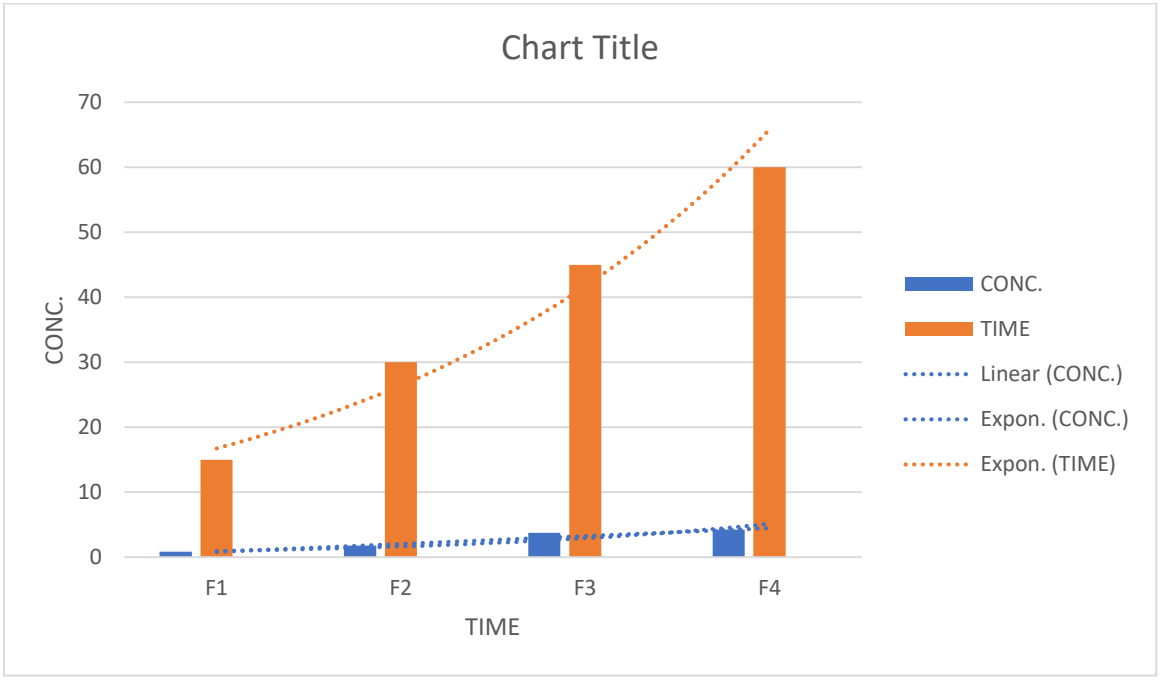


Figure 5.8: Graphical Representation of F4 showing first order release

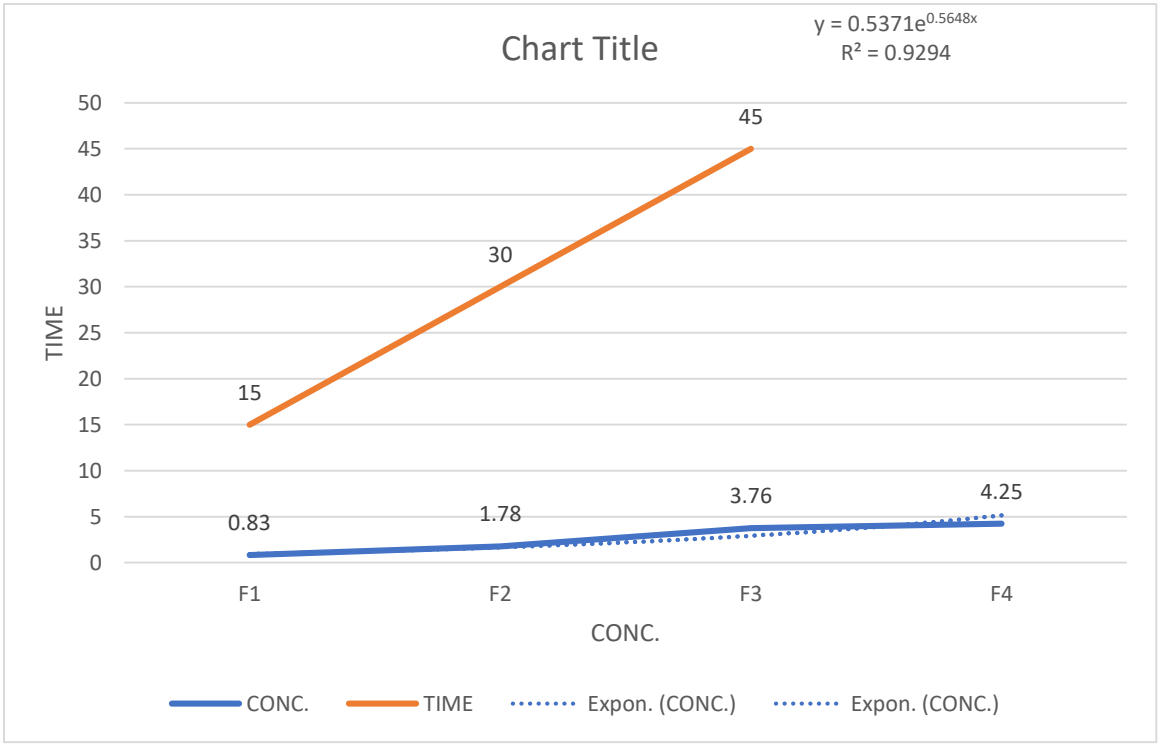


Figure 5.9 : Standard curve of F4 showing first order release

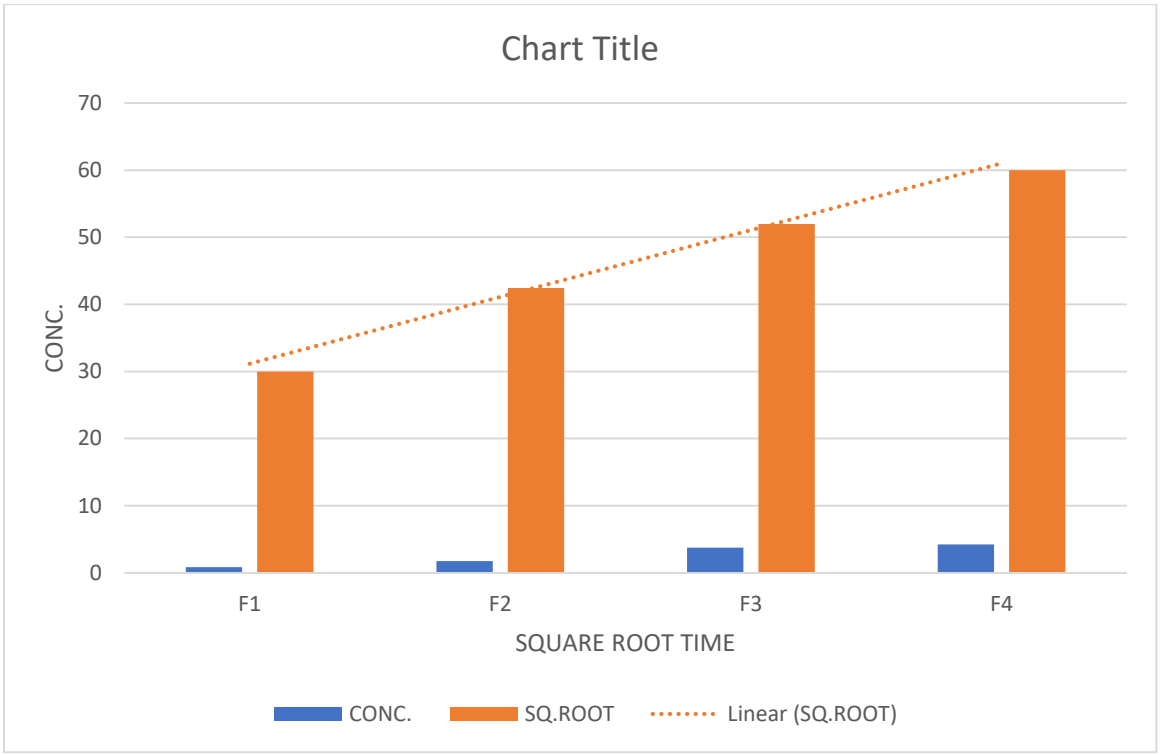


Figure 5.10: Graphical Representation of F4 showing Higuchi order release.

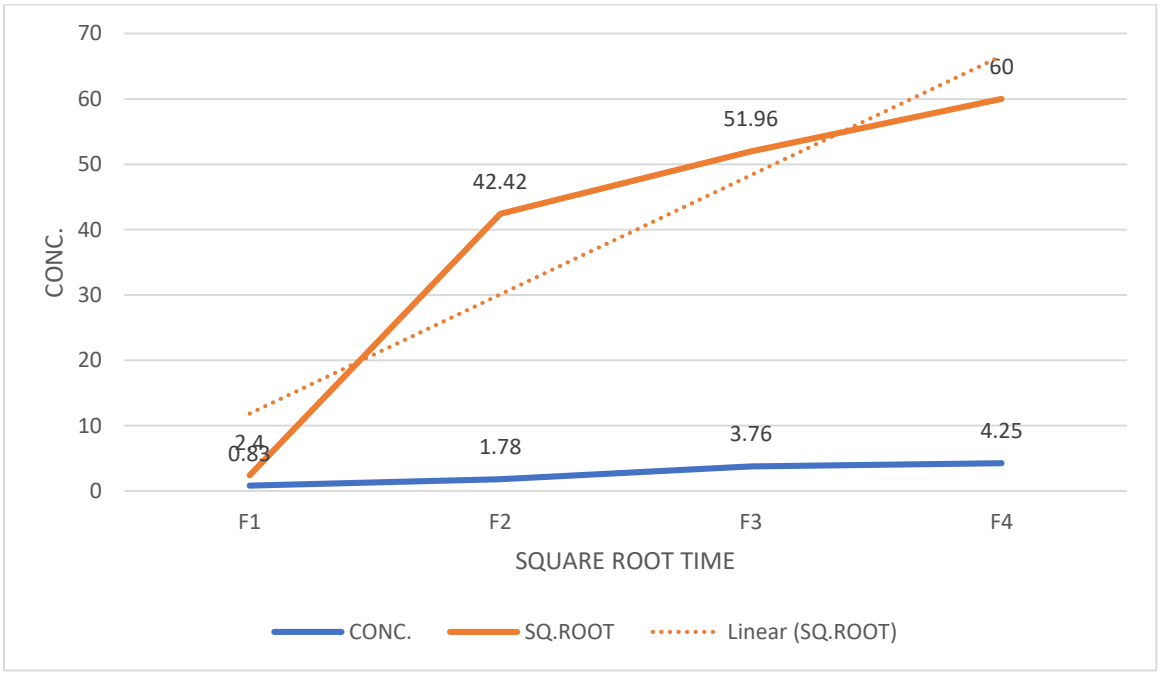


Figure 5.11: Standard curve of F4 showing Higuchi order release

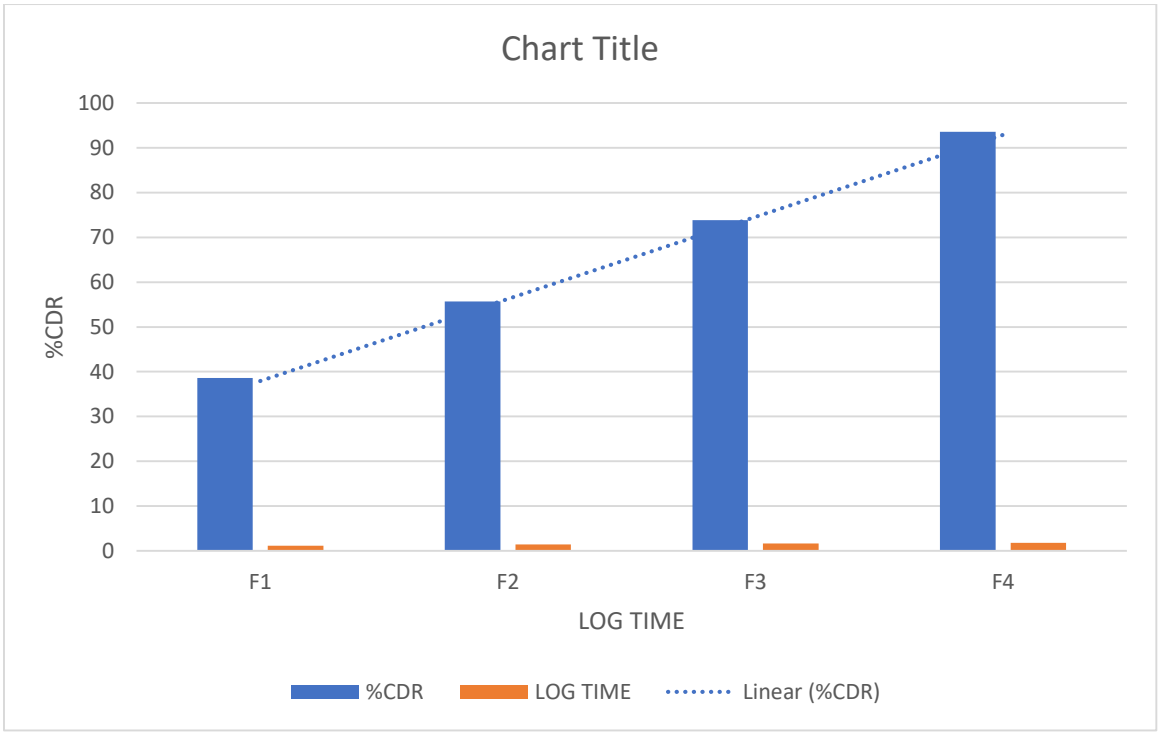


Figure 5.12 : Graphical Representation of F4 showing Korsmeyer–Peppas model release.

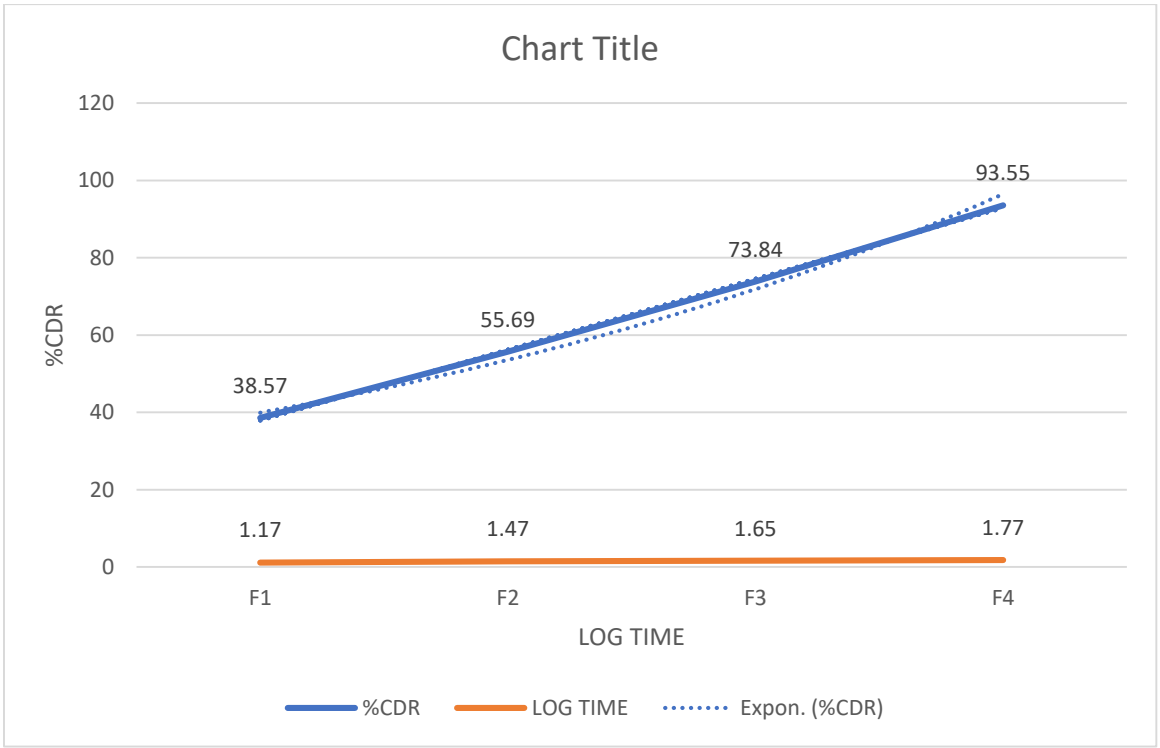


Figure 5.13: Standard Curve of F4 showing Korsmeyer–Peppas model release.

5.6 Microbiological Study

The antimicrobial effect of CIP in the prepared ocular in-situ gel and the antimicrobial effect of the marketed ciprofloxacin eye drops were shown in table 4. The results clearly shows that CIP retain its antimicrobial effect after formulation as in-situ gel, with a higher ZOI observed in most data that might result from the slow and prolong diffusion of the drug from the prepare in-situ gel.

The antimicrobial effect of CIP from the optimum formula was compared to that of the commercial ciprofloxacin eye drop using agar diffusion test. In order to do that, five different phytopathogenic microorganisms were used in this test, out of these microorganisms, two are Gram-negative bacterium (Escherichia coli (E. coli) and Acinetobacter) and three are Gram-positive bacterium (Streptococcus, Staphylococcus aureus and Staphylococcus epidermidis) were collected from privet microbiology laboratory, Iraq.

The pure cultures of these organism were in lyophilized or freeze-dried form, therefore, they were reconstituted by the addition of sterile water. This resulted in required suspension of microbial cultures. Sterility was also maintained during inoculation and for this purpose sterile loops were used to transfer the cells to liquid broth medium.

The next step involved the incubation of the liquid cultures to ensure the optimum replication and growth of bacterial cells. Finally, they were stored in refrigerator for subsequent usage. Time given for incubation was 24 hours and thick spread of microbes was obtained for assays. The medium used for testing and maintaining the bacterial strains was Nutrient agar (NA).

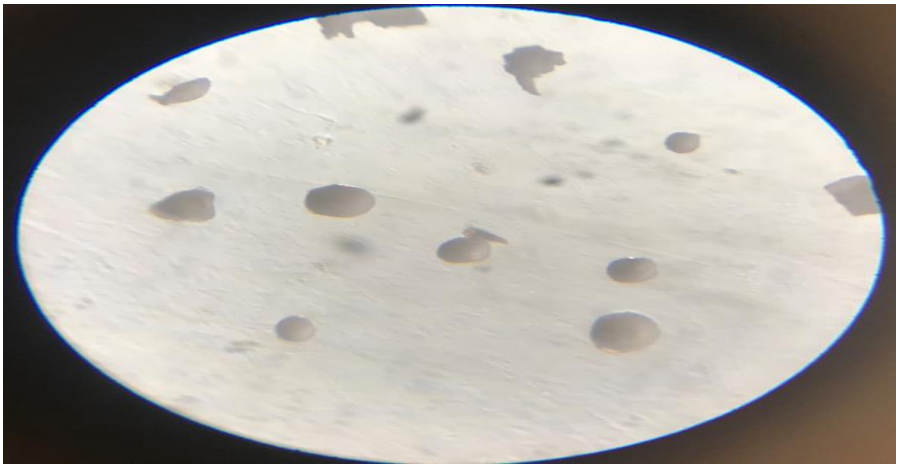
This was followed by pouring a suitable dilution of sterile CIP solution (from both optimum formula and the commercial CIP eye drop) into a pressed well in the previously prepared agar media seeded with the test microorganisms. These solutions were allowed to diffuse from the cell for 2hrs, followed by incubation period of 24hr at 37ºC. The inhibitory effect of CIP from the optimum formula was evaluated by measuring the zone of inhibition (ZOI) and compared to that of the marketed CIP eye drop.

Table 5.5 : Antimicrobial efficacy study

S.N	FORMULATIONS	Zone of inhibition of known sample (in diameter)	Zone of inhibition of known sample (in diameter)
1	F1	46mm	45mm
2	F2	47mm	44mm
3	F3	46mm	47mm
4	F4	50mm	43mm

Table 5.6 : Antimicrobial effect of Ciprofloxacin on marketed and optimized in-situ ocular gel formulation.

TYPE OF BACTERIA	TYPE OF MICROBES	MINIMUM INHIBITORY CONCENTERATION (MG/ML)	MINIMUM ZONE OF INHIBITION OF MARKET FORMULATION	MINIMUM ZONE OF INHIBITION OF MARKET FORMULATION
GRAM +VE	STREPTOCOCCUS PNEUMONIA	2	8	14
	STAPHYLOCOCCUS AUREUS	1	9	11
GR5AM -VE	E.COLI	2	12	16
	ACENOBACTER	2	10	13



A

Figure:5.14: Antibacterial activity of Streptococcus Pneumonia(A)

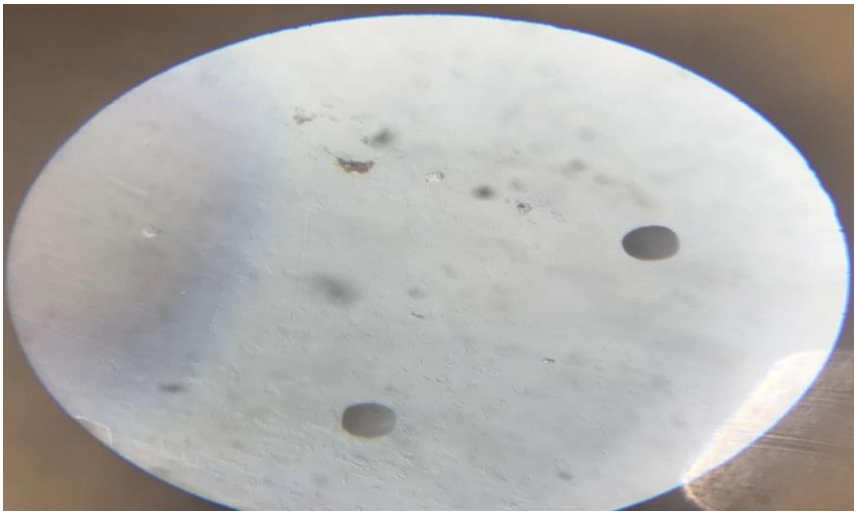
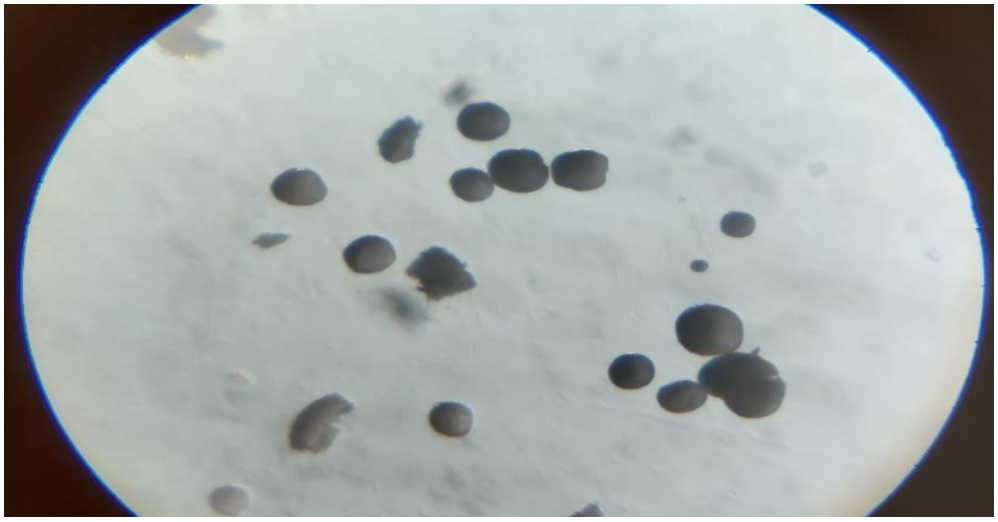


Figure:5.15: Antibacterial activity of Staphylococcus Aureus(B).



C

Figure 5.16: Antibacterial activity of E.COLI (C).

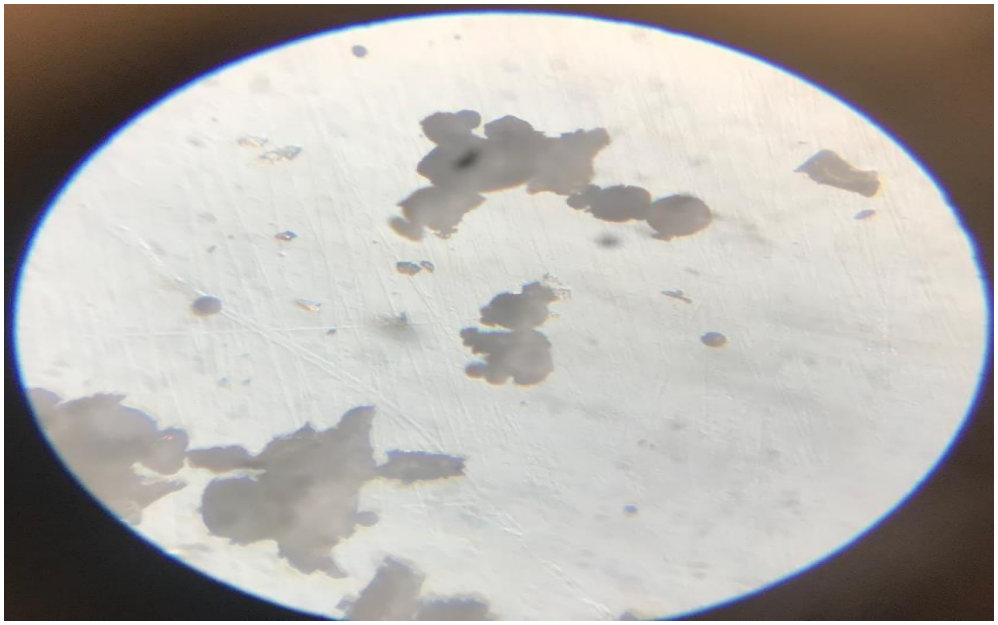


Figure 5.17: Antibacterial activity of ACENOBACTER (D).

5.7 STABILITY STUDY

The stability study carried out on optimized formulation F4 at 30±2°C temperature and 60±5% RH for 90 days. The formulation was showing good stability with no significant change in gelation and physicochemical properties and in vitro drug release profile as shown in Table 6. At a periodic interval sample were withdrawn and were observed for clarity, pH, viscosity, syringeability, and drug content at the interval of 30 days. All the measurements were performed after allowing the samples to equilibrate at 25°C.

Table 5.7: Stability data of F4 Formulation

S.NO	PARAMETERS	STORAGE PERIOD (30) DAYS AT 30±2·C AND 60±5 % RH			
		0	30	60	90
1	APPEARANCE	CLEAR WHITE	CLEAR WHITE	CLEAR WHITE	CLEAR WHITE
2	DRUG CONTENT (%)	99.35±.01	99.21±.04	99.11±-06	99.01±-08
3	GELATION STUDY	+++	+++	++	++
4	IN-VITRO DRUG RELEASE (%)	98.08±.02	97.05±.05	97.01±.04	96.04±.01

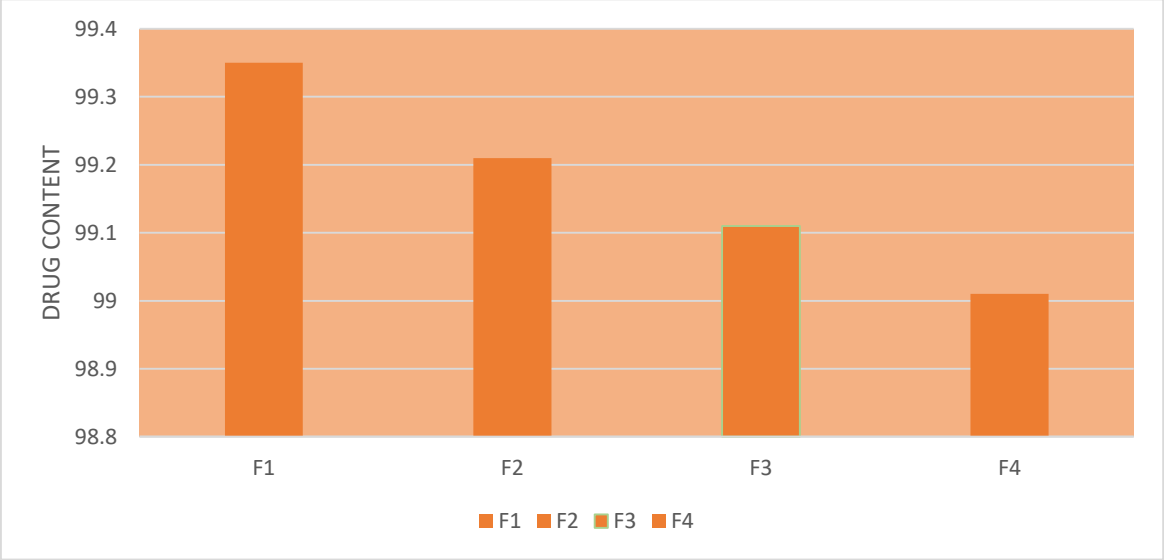


Figure 5.18 : Graphical representation of Drug content during stability study.

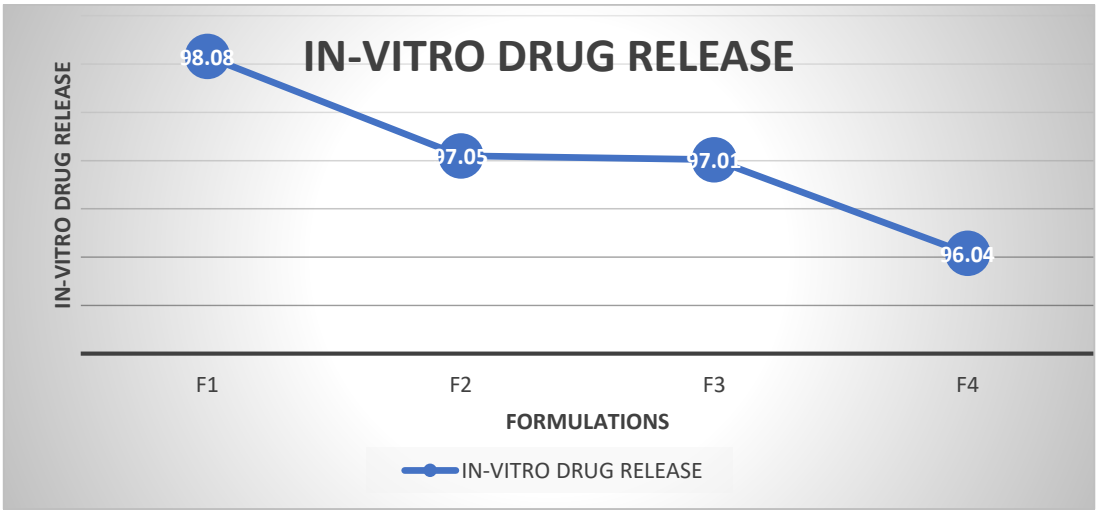


Figure 5.19 : Graphical representation of In-vitro Drug release during stability study.

CHAPTER 6

CONCLUSION

6.1 CONCLUSION

Ciprofloxacin, a broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as in situ gel-forming eye drops using Sodium alginate as a gelling agent in combination with HPMC as a viscosity enhancing agent. Thus, the developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance. It showed good gelation, rheological properties and exhibited better ability to retain drug. Based on results it was observed that the prepared Ciprofloxacin in-situ gel **(F4) can** overcome limitations of the conventional ocular dosage form and shows **93.55 %** cumulative drug release. This technique helped to increase patient compliance. They offer many advantages like easy installation, improves ocular bioavailability, prolongs the duration of contact with corneal tissue, lessens frequency of administration.

CHAPTER 7

REFRENCES

7.1 REFERENCE

1.AcharyaAnkit,GoudanavarPrakash,ChittiRenukaradhyaandDinnimathBasavarajM.,2019.PreparationofGellanGumandChitosanbasedInSituGelofTimololMaleateforOphthalmicdrugdeliveryandevaluationofphysicochemicalpropertiesanddrugreleaseprofile,*ActaScientificPharmaceuticalScience*,Volume3Issue2February2019. Page no. 68-78.

2. Nanjawade B., Manvi F., Manjappa A., 2007. In situ forming hydrogels for sustained ophthalmic drug delivery. J. Of controlled release, 122: page no. 119-131.
3. Ghate D., and Henry F., Ocular drug delivery. Expert Opin. Drug Delivery (2), page no.275-286.
4. NG NANJUNDSWAMY, FATIMAS DASANKOPPA, HN SHOLAPUR, A review on hydrogel and its use in situ ocular drug delivery, Indian journal of novel drug delivery.
5. Al-bazzazFadiaYassir,Al-KotajiMyasar.,2018.Ophthalmicin-situsustainedgelofciprofloxacin,preparationandevaluationstudy,*Internationaljournalofapplied pharmaceutics*,Volume 10,Issue4,2018, page no. 153-161.
6. Mathiowitz E., Wiley A., 2009. In situ hydrogels. In: Encyclopaedia of controlled drug delivery systems, page no. 609-612.
7. Cao F., Zhang X., Ping Q., 2010. New model for ophthalmic deliveryof azithromycin by Poloxamer/carbopol-based in situ gelling system, drug delivery, 17(7), page no. 500-507.
8. Mythri.G, K. Kavitha, M. Rupesh kumar, Sd. Jagadeesh Singh, Novel Mucoadhesive polymer – A review, journal of applied pharmaceutical science.
9. Liu Y., Liu J., Zhang X., Zhang R., Huang Y., Wu C.,2010. In situ gelling gel rite/alginate formulations as vehicle for ophthalmic drug delivery. AAPS Pharm. Sci Tech, 11(2), page no.610-620.
10. John C. Lang., 1995. Ocular drug delivery conventional ocular formulations. Advanced drug delivery reviews, page no. 39-44.
11. Manjanna K,. Kumar P., Shivkumar B., 2010. Natural polysaccharide hydrogels as novel excipients for modified drug delivery systems. Int. J. of Chem. Tech. Research.2, page no.509-526.
12. AroraKunal,SinghSumitaandChaurasiaLovely.,2019.OphthalmicDrugDeliverySystemAConciseReviewonitsConventionalandNovelApproaches,*CurrentResearchinPharmaceuticalSciences*,2018;08(04). Page no. 263-269.
13. Gupta H., Jain S., Mathur R., Mishra P., Mishra A., 2007. Sustained ocular drug delivery from temperature and pH triggered Novel in situ gel system, page no. 507-514.

14. Calfors J., Peterson R., Jornving K., 1998. Rheological evaluation of gel rite in situ gels for ophthalmic use. Eur. J. Of Pharm. Sci, page no. 11-120.

15. Ishibashi T., Norihiko Yokoil, Bron J., Tiffany J., Komurol A., Kinoshita S., 2003. Retention of reversibly thermo gelling Timolol on the human ocular surface studied by video meniscometry. Current eye research,2: page no.117-123.
16. ChaudharyS.,chaudhary. , A bookof*Humananatomyandphysiology*,S.Vikasandcompany(medicalpublishers)pageno.226-228.
17. Ishibashi T., Norihiko, Yokoil, Bron J., Tiffany J., Kinoshita A., Kinoshita S., 2003. Retention of reversibly thermo gelling Timolol on the human ocular surface studied by video meniscometry. Current eye research,: page no.117-123.
18. DhanapalRamaiyanandRatnaJ.Vijaya.,2012.Oculardrugdeliverysystem–isview,*InternationalJournalofInnovativeDrugDiscoveryRamaiyanDhanapal*,Volume 2, Issue1/2012/4.page no.15.
19. GadadAnandPanchakshri,WadklarPadmajaDharmanand,DandghiPanchakshri,PatilArchana.,2016.ThermosensitiveInsituGelforOcularDeliveryofLomefloxacin,*IndianJournalofPharmaceuticalEducationandResearch*,|Volume 50|Issue2(Suppl.)Apr-Jun,2016.
20. GandhiJaimini,ShahPranav.,2018.AReviewonCurrentPerspectivesandRecentAdvancesinOcularDrugDeliverySystem,*InternationalJournalofChemTechResearch*,2018,11(06), page no.314-326.
21. GargRam,KumarVikas,SharmaVandana.,2019.EmergingTrendsinOcularDrugDeliverySpecial ReferencetoInSituOphthalmicGel,*PharmaceuticalandBiosciencesJournal, Volume 7* (3), page no.08-17.
22. Jyothirmai,ManasaM.,SravaniD.,GT.DeviSundari.2017. Formulationandevaluationofocularinsituhydrogelsof Acyclovirhydrochloride,*IJRPC*,KSL,ISSN: 2017,7(2), page no.162-170.
23. KunoNoriyukiandFujiiShinobu.,2011.RecentAdvancesinOcularDrugDeliverySystemsPolymers2011, polymers, *www.mdpi.com/journal/polymers*,3,page no.193-221.
24. KurniawansyahInsanSunan,SopyanIyan,WathoniNasrul,FillahDastyLatifa,PradityaRahadianti Umi.,2018.Applicationandcharacterizationofinsitugel,*internationaljournalofappliedpharmaceutics*,Volume 10,Issue6,2018, page no34-37.
25. MakwanaS.B,PatelV.A,ParmarS.J.,2015.Developmentandcharacterizationofinsitugelforophthalmicformulationcontainingciprofloxacinhydrochloride,*pharmascience*,6(2016), page no.1-6.

26. NagalakshmiS.,AnbarasanB.,RameshS.,ShanmuganathanS. andThankaJ.,2015.AnOverview-StimuliSensitiveHydrogelsinOcularDrugDeliverySystem,*Journalofpharmaceuticalsciencean dresearch*,Volume 7(10),2015, page no.818-822.
27. OhanesianRogerV.,ShahsuvaryanMarianneL.,2005.*Themanualbookofaneyedisease*,2005, page no.41.
28. Shastri D., Prajapati S., Patel L., 2010. Thermo reversible Mucoadhesive ophthalmic in situ hydrogel, design and optimization using a combination of polymers. Acta Pharm,60 ; page no.350-361.

29. SawarkarS.,RavikumarP.andPashteS.,2016.Insituophthalmicgelformingsolutionofmoxifloixacinhydrochlorideforsustainoculardelivery,*IJPSR*, 2016; Volume7(3): page no.1192-1205.
30. Gupta S., Vyas S., 2010. Carbopol/ Chitosan based Ph triggered in situ gelling system foe ocular delivery of Timolol Maleate Sci. Phar,78: page no. 959-976.
31. ShastriDivyeshH.,PrajapatiShaileshT.,PatelLaxmanbhaiD.,2010.Thermo reversible Mucoadhesive ophthalmicinsituhydrogel: Designandoptimizationusingacombinationofpolymers,ActaPharm.60(2010), page no.349–36.
32. Mahanvi T., Aminabhavi M., Agnihotri S., B. Naidu V., 2004. Rheological properties and drug releases characteristics of pH responsive hydrogels. Wiley periodicals, Inc.J Appl.polym. Sci,94, page no. 2057-2064.
33. SharmaAnjali,DassRameshwar,DhimanShivani,SinghRanjit.,2018.Hydrogelsinophthalmicdrugdeliverysystem-Aminireview,*AsianPacificJournalofHealthSciences*,|Volume 5|Issue2|April-June|2018, page no.96-104.
34. VenkataRatnamG.,MadhaviS.,RajeshP.,2011.Oculardrugdelivery:anupdatereview,*IJPBS*,|Volume1|Issue4|OCT-DEC|2011, page no.437-446.
35. Abraham S., Furtado S., Bharath S., Basavaraj B., Deveswaran R., and Madhavan V., 2009. Sustained ophthalmic delivery of ofloxacin an ion-activated in situ gelling system,22: page no. 175-179.
36. Mario La Rosa, Elena Lionetti, Michele Reibaldi, Andrea Russo, Antonio Longo, Salvatore Leonardi, Stefania Tomarchio, Teresio Avitabile, and Alfredo Reibaldi allergic conjunctivitis: a comprehensive review of the literature.
37. WadhwaKaran,SharmaChandan,GoswamiManish,ThakurNishant.,2018.In-situoculargel-an overall approachtowardsoculardrugdelivery.
38. Lekhraj Verma L., Sakir M., Singh N., Mehra R., Mehan S., 2010. Development of phase change solutions for ophthalmic drug delivery based on ion activated and pH induced polymers. Int. J. of Pharma Research, page no. 136-144.
39. Bhagat BV, Hapse SA, Mane AR, Pagar HJ, Wagh VS. Development of ophthalmic in situ gelling formulation of ciprofloxacin hydrochloride using gellan gum. Research Journal of Pharmacy and Technology. 2011; 4(11):1742-1745.
40. Jayant D, Shah B, Anil B. Design and development of pHmonitored in situ gel of lomefloxacin. Journal of Pharmaceutical Science and Bioscientific Research. 2013;3(2):10-15.
41. Harsha Vardhani Kondepati, Girish Pai Kulyadi, Vamshi Krishna Tippavajhala. A Review on In Situ gel forming ophthalmic drug delivery systems. Research J. Pharm. and Tech. 2018; 11(1): 380- 386.
42. Choudhary NG, Syed AM, Kale VV, Avari JG. Oral sustained release in situ gel forming polymeric drug delivery systems. Research Journal of Pharmacy and Technology. 2010; 3 (3): 682- 687.
43. Swapnil D, Sonawane, Swaroop L. Design and evaluation of ion induced in situ gel formulation for levofloxacin hemihydrateocular delivery. International Journal of Pharmaceutical Science Invention. 2014;3(3):38-43
44. Prerana V, Asmita S, Sudha R. Microspheric in-situ gel for ocular drug delivery system of bromfenac sodium. International Journal of Pharmaceutical Sciences and Research. 2014;5(3):179-185.

45. Nisha G Shetty and Charyulu RN. Phase transition ocular drug delivery system for antazoline phosphate. International Journal of Drug Formulation and Research. 2012;3(1);27-39.
46. Baladaniya M, Vadgama N, Patel P. Gastro retentive in situ floating gel formulation – an overview. Research Journal of Pharmaceutical Dosage Forms andTechnology.2014;6(2):140-145. 9. Tanvi PP, Moin KM, Vishnu MP. Sustained ophthalmic delivery of ciprofloxacin hydrochloride from an ion-activated in situ gelling system. Der Pharmacia Lettre. 2011;3(4):404-410.
47. NS Malekar, SB Gondkar, RB Saudagar. In- situ nasal gel: a review. Research Journal of Pharmaceutical Dosage Forms and Technology. 2015; 7(4): 285-293.
48. Prasanth VV, Della GT, Shashi R. Formulation and evaluation of in situ ocular gel of levofloxacin. Journal of Drug Delivery and Therapeutics. 2017; 7(4):68-73.
49. Mahdi ZH, Maraie NK, Amer ZA. Application of liquisolid technology to enhance the dissolution of cefixime from its oral capsules. International Journal of Applied Pharmaceutics. 2018; 10(5): 214-219.
50. Gupta SK, Singhvi IJ. In situ gelling system and other possible innovative approach for ocular disease: a review. Research Journal of Pharmacy and Technology. 2011; 4(6): 872-882.
51. Sandeep DS, Narayana CR, Anoop NV. Smart in situ gels for glaucoma- an overview. International Journal of Pharmaceutical Sciences Review and Research. 2018;50(4):94-100.
52. Mahdi ZH, Maraie NK. Overview on nanoemulsion as a recently developed approach in drug nanoformulation. Research Journal of Pharmacy and Technology. 2019; 12(11): 1-7. 16. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Results in Pharma Sciences. 2016;6(2):1-6.
53. Nazia K, Mohammed A, Ameeduzzafar, Syed SI, Asgar A. Development and evaluation of a novel in situ gel of sparfloxacin for sustained ocular drug delivery: in vitro and ex vivo characterization. Pharmaceutical Development and Technology.2019;9(3):1-8.
54. Reeshanteni B, Abdullah K, Rajermani T. Formulation of in-situ gelling system for ophthalmic delivery of erythromycin. International Journal of Students Research in Technology and Management. 2017;5(1):1-8.
55. Mundhada DR, Chandewar AV. An overview on in-situ gel. Research Journal of Pharmaceutical Dosage Forms and Technology. 2015; 7(4): 261-265.
56. Sonjoy M, Manjunath KM, Thimmasetty J, Prabhushankar GL, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. International Journal of Pharmaceutical Investigation. 2012; 2(2): 78-82.
57. Mudit D, Parthasarthi KK, Narayana C. Enhancing solubility and dissolution of naproxen by spray drying technique. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 4(1): 715-725.
58. Gupta SK, Singhvi IJ. Sustained ophthalmic delivery of moxifloxacin hydrochloride from a pH triggered in situ gelling system. Research Journal of Pharmacy and Technology. 2012; 5(12):1538-1542.
59. Gulecha BS, Shahi S, Lahoti SR. Floating in situ gelling drug delivery system of verapamil hydrochloride. American Journal of Pharm Tech Research. 2012; 2(4):954-969.

60. Nagesh C, Manish P, Chandrashekhara S, Rahul S. A novel in situ gel for sustained ophthalmic delivery of ciprofloxacin hydrochloride and dexamethasone- design and characterization. Der Pharmacia Lettre. 2012; 4(3): 821-827.
61. Vazir AA, Shiv Kumar HG, Paranjothy KLK, Mohd K. Ophthalmic drug delivery of diclofenac potassium from different polymer formulations: in situ sol gels. Research Journal of Pharmaceutical Dosage Forms and Technology. 2009; 1(2): 158- 161.
62. Sasaki H, Yamamura K, Nishida K, Nakamurat J, Ichikawa M. Delivery of drugs to the eye by topical application. Progress in Retinal and Eye Research, 15 (2), 1996, 553-620. 2. Macha S, Mitra AK. Ophthalmic drug delivery systems; second edition revised and expanded. Chapter 1, Overview of Ocular Drug Delivery. p 1-3. 3. Mundada AS, Avari JG, Mehta SP, Pandit SS, Patil AT. Recent advances in ophthalmic drug delivery system. Pharm Rev., 6(1) 2008, 481-489. 15 Ramaiyan Dhanapal. / Vol 2/ Issue 1/ 2012/ 4-15.
63. Wagh VD, Inamdar B, Samanta MK. Polymers used in ocular dosage form & drug delivery system. Asian J Pharm., 2(1), 2008, 12-7.
64. Khurana AK, Khurana I. Anatomy & physiology of Eye; 2nd ed. CBS publishers & Dist. 2007. 6. Khurana AK. Comprehensive ophthalmology; 4th ed. Age International (P) Ltd Pub. 2007.
65. Snell RS, Michel A. Clinical Anatomy of the eye; 2nd ed. Cemp. Blackwell science. 8. http://www.ivy-rose.co.uk/HumanBody/Eye/Anatomy_Eye.php. (Access on date 08/08/2009; 4.30 pm.)
66. Hosoyaa K, Vincent HL, Kim KJ. Roles of the conjunctiva in ocular drug delivery: a review of conjunctival transport mechanisms and their regulation. Eur J Pharm Biopharm, 60, 2005, 227–40.
67. Cross JT. Fluoroquinolones Seminars in Pediatric Infectious Diseases, 12, 2001, 211-23.
68. Urtti A. Challenges and obstacles of ocular pharmacokinetics and drug delivery. Adv Drug Deliv Rev, 58, 2006, 1131–35.
69. Jtirvinena K, Tomi J, Urttia SA. Ocular absorption following topical delivery. Adv Drug Deliv Rev, 16, 1995, 3-19.
70. Nanjawade BK, Manvi FV, Manjappa AS. In situ-forming hydrogels for sustained ophthalmic drug delivery. J Control Release, 122, 2007, 119–34.
71. Meqi SA, Deshpande SG. Ocular drug delivery: Controlled and novel drug delivery. New delhi:CBS Publishers; 2002, p 82- 84.
72. Eva M, Amo D, Urtti A. Current and future ophthalmic drug delivery systems. A shift to the posterior segment. Drug Discov Today, 2004;13, 2004, 135-143.
73. Blondeau JM. Fluoroquinolones: Mechanism of Action, Classification, and Development of Resistance. Surv Ophthalmol., 49, 2004, S73-S78.
74. Martinez M¸ McDermott P, Walker R. Pharmacology of the fluoroquinolones: A perspective for the use in domestic animals. The Veterinary Journal, 172, 2006, 10– 28.