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CERTIFICATE

This is to certify that the Dissertation entitled **"FORMULATION AND EVALUATION OF P^H TRIGGERED IN SITU GELLING SYSTEM OF LEVOFLOXACIN"** submitted by **Ms.E.Mohanambal** in partial fulfillment of the requirement for the degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by her, under the guidance and supervision of **Mr.K.Arun, Assistant reader**, during the academic year 2009 – 2010 in the Department of Pharmaceutics, Madurai Medical College, Madurai-20.

I wish him success in all his endeavors.

Place: Madurai

Date: **(A.Abdul Hasan Sathali)**

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Place: Madurai

Date: **(K.Arun)**

CHAPTER I

INTRODUCTION

Designing formulations and delivery systems for topically applied ophthalmic drugs is challenging. The search for novel ways of delivering therapeutically active agents has been the focus of attention in the past 2 to 3 decades. For any noninvasive route of administration, the need to prolong the duration of residence of the dosage form is evident. This is especially true in the area of ophthalmic drug delivery.

An effective ocular drug delivery device must be easy to use, comfortable to the patient and must improve the therapeutic performance of the drug over conventional dosage forms.[1] Some ocular diseases may be treated by systemic dosage forms, either by oral ingestion or parenteral injections, however the systemic routes presents the great disadvantage of exposing all organs of the body to the action of the drug, thus leading to the unwanted side effects.[2]

Topical application of drugs to the eye is the most popular and well accepted route of administration for the treatment of various eye disorders.[3] Except skin, the eye is the most easily accessible site for topical administration of a medication.[4] Various systems have been designed to maximize ocular absorption of ophthalmic drugs. There are two main strategies for improvements, increasing the corneal permeability and prolonging the contact time on the ocular surface. Formulations were maximizing the absorption through prolongation of the drug residence time in the conjunctival sac.[5]

Drugs are commonly applied to the eye for a localized action, on the surface, or in the interior of the eye. Poor bioavailability of drugs from ocular dosage forms is mainly due to the precorneal loss factors which include tear dynamics, non-productive

absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane.

Due to these physiological and anatomical constraints only a small fraction of the drug is ocularly absorbed. The effective dose of medication administered ophthalmically may be altered by varying the strength, volume, or frequency of administration of the medication or the retention time of medication in contact with the surface of the eye. So far, attempts have been made to improve ocular drug bioavailability by extending drug residence time in the conjunctival sac and improving drug penetration across the cornea, the major pathway of drug entry into the internal eye.

TOPICAL OCULAR DRUG DELIVERY AND THE CONSTRAINTS TO OCULAR THERAPY

For ailments of the eye, topical administration is usually preferred over systemic administration for obvious reasons:

- The systemic toxicity of many ophthalmic drugs,
- The rapid onset of action, and
- The smaller dose required compared to the systemic route.

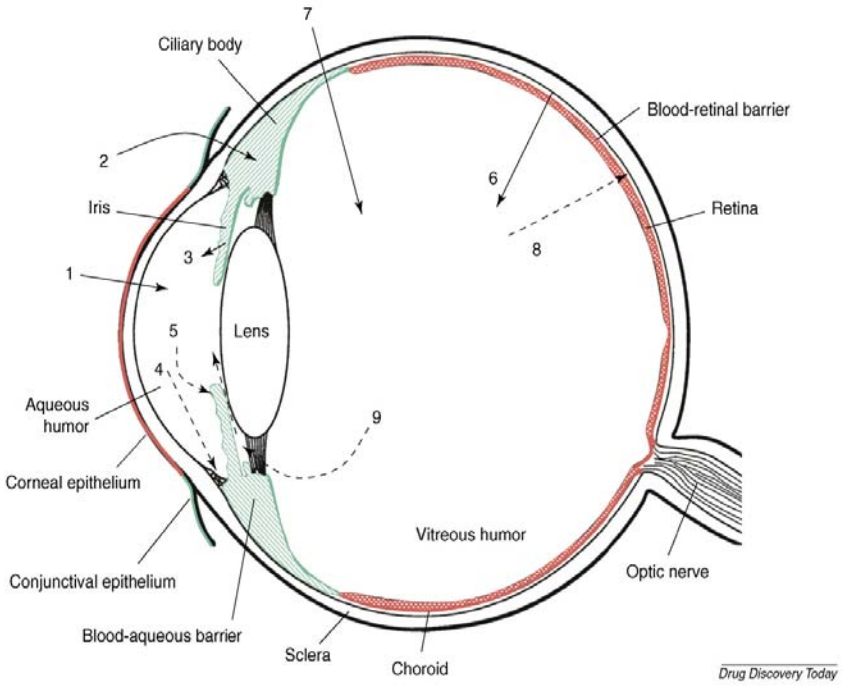
The topically applied ocular drugs have to reach inner parts of the eye to elicit responses. The trans-corneal penetration is believed to be the major route for drug absorption. Most ocular drugs seem to penetrate the cornea by diffusion. The paracellular (i.e.,through intercellular space) and the transcellular pathways (i.e., through intracellular space) are the two mechanisms for drug transport across the cornea.

The cornea is divided into five layers (the epithelium, Bowman's membrane, the substantia propria or the stroma, Descemet's membrane, and the endothelium). The epithelium and the endothelium are rich in lipids; on the other hand, the stroma has high

water content. The epithelium is reported to be the rate-limiting barrier to transcorneal transport. Its barrier function depends favorably on the lipophilicity of molecules and excludes the macromolecules (r>10 A°). The corneal epithelium is a layered structure of 50–100 mm thickness. These cells have skirting intercellular junctions, termed ‘‘tight junctions,’’ forming a strong barrier.[4]

Ocular pharmacokinetics: barriers in drug delivery

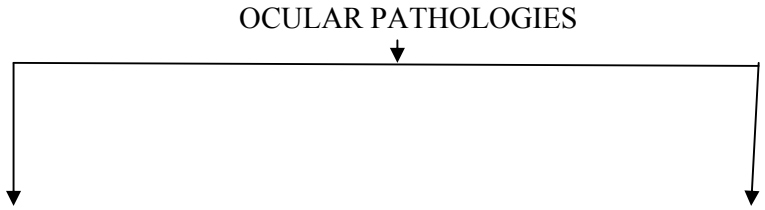
The ocular tissues can be reached either by local or systemic drug administration. The tissue barriers limit the access of drugs to their targets (Figure). The blood–aqueous barrier, composed of the uveal capillary endothelia and ciliary epithelia, limits the access of compounds from the systemic circulation to the anterior chamber, whereas the blood–retina barrier limits the drug diffusion from the systemic blood to the retina and vice versa.



The barrier has two components: outer and inner blood–retina barriers that are formed by the retinal pigment epithelium (RPE) and the tight retinal capillary walls, respectively.[6]

PATHOLOGIES OF OCULAR SYSTEM

The pathologies of ocular system can be divided into topically treated pathologies and intraocular pathologies



Topically Treated Pathologies	Intraocular pathologies
(i) Inflammations	(i) Glaucoma Filtering Surgery failure
(ii) Keratoconjuctivities sicca	(ii) Proliferative vitreoretino pathy
(iii) Glaucoma	(iii) Cytomegalovirus retinitis
	(iv) Endopththalmitis
	(v) Posterior uveitis
	(vi) Posterior capsule opacification
	(vii) Retinal Degenerative disease

These are all the various major pathologies involved in the ocular system.[2]

REASONS FOR POOR BIOAVAILABILITY OF OCULAR PREARATIONS

- Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics and nasolacrimal drainage.[7]

- Most of the topically applied drugs are washed off from the eye by various mechanisms include lacrimation, tear dilution and tear turn over resulting in low ocular bioavailability of drugs.[8]

- The residence time of most conventional ocular solutions ranges between 5 and 25 minutes. Only 1-10% of topically applied drug is absorbed.[9]

- The corneal epithelium is the main barrier of drug absorption into the eye.

- Only few percent of the applied dose is delivered into ocular tissues, the major part of the dose absorbed systemically.

- The systemic absorption of ocularly applied drug is nearly complete.

- This has caused systemic side effects varying from mild to life threatening events.[5]

- Systemic absorption can be minimized by,

A) Reducing the instilled volume, B) Controlling drug release,

C) Prodrug derivatization, D) Adding vasoconstrictive agents

These are all the reasons for poor bioavailability.[9]

VARIOUS APPROACHES INVOLVED IN OCULAR DRUG DELIVERY SYSTEM

Ophthalmic Solutions

Ophthalmic solutions are the most common dosage form for delivering drugs to the eye. These are used for soluble drugs, require frequent instillation of highly concentrated solutions.[10] More than 90% of the marketed ophthalmic formulations are in the form of eye drops.[8]

Advantages

a) Cost effective

b) Greater simplicity of formulation development and production

c) Good acceptance by patients[10]

Disadvantages

a) Poor ocular drug bioavailability

b) Pulse drug entry after topical administration

c) Systemic exposure because of nasolacrimal duct drainage

d) Frequent instillation of eye drops results in local side effects such as,

- Head ache due to ciliary muscle spasm
- Decreased vision in poor illumination due to miosis & accommodative myopia.[11]

OPHTHALMIC SUSPENSIONS

Suspensions are dispersions of finely divided, relatively insoluble, drug substances in aqueous vehicle containing suitable suspending and dispersing agent.

Advantages

a) The contact time and duration of action exceed that of a solution

b) Good acceptance by the patients

Disadvantages

a) Brisk shaking is always required to distribute the suspended particles before each use

b) Suspension favours the phenomenon of polymorphism, due to crystal growth chances for increased or decreased bioavailability

c) Blurred vision

OPHTHALMIC OINTMENTS

Ophthalmic ointments are primarily anhydrous and contain mineral oil and white petroleum as the base. Dosage availability of ophthalmic ointments is probably greater than the solutions.

Advantages

a) Longer contact time and greater total drug bioavailability

b) Popular as a pediatric dosage form and for post operative use

Disadvantages

a) Blurred vision due to greasy nature

b) Due to interference with the vision their use is usually limited to bed time instillation

c) Moisture sensitive drugs cannot be formulated as ointment[10]

OPHTHALMIC GELS

Ophthalmic gel formation is an extreme case of viscosity enhancement through the use of viscosity enhancers. Despite extremely high viscosity gels achieve only limited improvement in bioavailability.

Advantages

a) Increased bioavailability than solutions.

b) Dosing frequency is less to once a day at most.

Disadvantages

a) Blurred eye vision and matted eye lids

b) Reduced patient acceptability[7]

OCULAR INSERTS

Ocular inserts have been developed in which the drug is delivered on the basis of diffusional mechanism. Such a solid dosage form delivers an ophthalmic drug at a near constant know rate, minimizing side effects by avoiding excessive absorption peaks.

Advantages

a) Ocusert gives sustained or controlled drug release

Disadvantages

a) Self administration is so difficult

b) Foreign body sensation

c) Inadvertent loss from the eye

d) Rupture of its membrane causing excessive bolus drug release.[12]

PENETRATON ENHANCERS

In the early stages of ophthalmic drug delivery research, chelating agents, preservatives, surfactants and bile salts were studied as possible penetration enhancers.

Advantages

a) Enhanced corneal drug penetration

Disadvantages

a) ocular irritation and toxicity

PRODRUGS

The principle of prodrug is to enhance corneal drug permeability through modification of the hydrophilic of the drug.

Advantages

a) Enhanced corneal drug penetration

Disadvantages

a) Difficult to design and develop[7]

LIPOSOMES

The use of liposomes as a topically administered ocular drug delivery system began in the early stages of research in to ophthalmic drug delivery. The results were favourable for lipophilic drugs, but disappointing for hydrophilic drugs.

Advantages

a) droppable, biocompatible, biodegradable

b) potential for bioavailability improvement

c) toxicity reduction

d) sustained release and site specific delivery[13]

Disadvantages

a) low drug loading

b) Inadequate aqueous stability

c) Manufacturing difficulties for sterile preparations.[7]

IN SITU-FORMING GELS

In situ forming hydrogels are liquids upon instillation and undergo a phase transition to form a viscoelastic gel in response to environmental changes like temperature, pH and electrolyte composition.[14]

Advantages

a) In situ forming hydrogels are attractive as conventional ocular solutions because of facile dosing as a liquid

b) Ensures complete and rapid ocular coverage

c) They also allow for accurate and reproducible quantities to be administered to the eye in contrast to pre-gelled formulations[2]

d) Prolonged contact time and increased bioavailability

e) Improved level of patient acceptance

f) Once or twice day dosing is the great advantage[14]

CHAPTER III

LITERATURE REVIEW

1. **Srividya et al.,** studied a sol-to-gel ophthalmic delivery system of ofloxacin based on PH-triggered in situ gelation using poly(acrylic acid) (Carbopol940) in combination with HPMC (Methocei E50LV) and showed the system was therapeutically efficacious,stable,non-irritant and provided sustained release over 8-hour period.

2. **Naseem A. et al.,** reported sol-to-gel system of ciprofloxacin hydrochloride using HPMC (K15M) and carbopol 940 and showed the system was active against selected microorganism compared with marketed for more than 2 years.

3. **Zhidong Liu et al.,** evaluated the in situ gel forming system based on ion activated gelation by using HPMC(E50LV) and alginate and showed alginate/HPMC mixture can be used as an in situ gelling vehicle to enhance ocular bioavailability and patient compliance.

4. **Raida S.et al.,** designed a controlled release sol-to-gel ophthalmic delivery system for ciprofloxacin with HPMC and methyl cellulose combined with carbopol to increase the viscosity of the gels and the formulation showed increase in viscosity,gelling capacity and efficiency against gram positive and gram negative organism including E.coli,Staphylococcus and Pseudomonas aeruginosa and also showed its prolonged antimicrobial activity.

5. **Mai Mansour et al.,** prepared in situ forming hydrogel using Poloxamer407(P407) and Poloxamer 188(P188) HPMC or HEC and evaluated for in vitro drug release ,sol-to-gel transition temperature,rheological behaviour and showed optimum release and improved ocular bioavailability when compared to marketed conventional eye drops.

6. **Amel El-Kamel et al.,** prepared a environmentally responsive sol-to-gel formulation by using gelrite and showed pseudoplastic behaviour with thixotropic characteristics and the viscosity of the prepared system increased as the polymer concentration increases. Formulations containing gelrite and 1% drug showed significantly improved bioavailability compared with commercial aqueous preparation.

7. **Ega Chanra Mohan et al.,** prepared formulations of an ophthalmic drug delivery system of an antibacterial agent, ciprofloxacin based on concepts of PH-triggered in situ gelation by using polyacrylic acid (Carbopol 940) in combination with HPMC ,thermo reversible gelation by using pluronic F-127 (14%) in combination with HPMC and ion activated system by using gellan gum and showed the developed system was therapeutically efficacious, stable, non irritant and sustained release.

8. **Mitan R. et al.,** prepared and developed a system of ophthalmic drug delivery system of model drug tropicamide based on the concept of pH-triggered in situ gelation. Polyacrylic acid(Carbopol 940) was used in combination with HPMC K15M and all formulation sterilized in an autoclave at 121◦C and 15 psi for 20

minutes. The system showed higher in vitro gelling capacity, exhibit pseudoplastic behaviour in both solution and gel and in vitro drug release of more than 8-hours.

9. **Sindhu Abraham et al.,** reported an ophthalmic delivery system of an antibacterial agent ofloxacin based on the ion activated in situ gelation.sodium alginate in combination with HPC was used. The release studies indicated that the alginate/HPC solution retained the drug better than the alginate or HPC solution alone and showed the developed system were therapeutically efficacious, sterile, stable and provided sustained drug release.

10. **Jagadish balasubramaniam et al.,** developed an ophthalmic delivery system of the NSAID indomethacin based on ion activated in situ gelation.The developed formulations were therapeutically efficacious and provided in vitro release of drug over 8-hour period in vitro.

11. **Doijad R.C, et al.,** reported an ophthalmic drug delivery system of an antibacterial agent gatifloxacin, based on the concept of ion activated system. Sodium alginate was used in combination with HPMC (E50LV) and showed therapeutically efficacious, stable, non-irritant formulations and provided sustained release of the drug over an 8-hour period.

12. **Sivanaga S. et al.,** studied pilocarpine loaded ocular hydrogels and demonstrated that in situ forming hydrogels posses the viscoelastic, and sustained delivery properties required for an efficient ocular drug delivery system.

13. **Indu pal kaur et al.,** reported that incorporation of acetazolamide into in situ forming ophthalmic drug delivery system showed increase in residence time.

14. **Miyazaki S.et al.,** studied about the vehicles for ocular delivery and showed sustained release of all in situ gels over 6hrs period of time.

15. **Gang wei,et al.,** demonstrated three fold increase in corneal residence time of in situ gels.

16. **vandamme S.et al.,** studied ophthalmic vehicles for ocular delivery and showed prolonged drug residence time for ophthalmic route.

17. **Yasmin Sultana,M.et al.,** developed and characterized a series of carbopol and methyl cellulose solutions as the in situ gelling vehicle and reported the mixture of carbopol(0.3%) and methyl cellulose(1.5%) showed a significant enhancement in gel strength and showed the mixture can be used as an in situ gelling vehicle to enhance the ocular bioavailability.

18. **Chunjie Wu et al.,** developed a pH-triggered in situ gelling vehicle for ophthalmic delivery of puerarin. They demonstrated that in vitro release studies of combined polymer system of carbopol 980NF/HPMC E4M performed better in retaining capacity than conventional eye drops.

19. **Eun-young Kim.et al.,** studied the effect of poloxamer and showed the poloxamer gel could be applicable for the development of effective ophthalmic delivery.

20. **Zhidong liu et al.,** demonstrated ion-activated in situ ophthalmic gel of gatifloxacin by microdialysis. The developed formulation has a higher bioavailability and longer residence time than ophthalmic solutions.

21. **Sathish kumar P.Jain,et al.,** reported ophthalmic delivery system for ciprofloxacin hydrochloride based on the concept of pH-triggered in situ gelation by using poly(acrylic acid)(Carbopol 980NF) as a phase transition polymer and HPMC(k100LV) as release retardant and reported the formulation was stable and non irritant to rabbit eyes and showed sustained in vitro release.

22. **Sibel Bozdag et al.,** demonstrated viscous solutions of cysteamine hydrochloride using HPMC and reported increased pH and increased viscosity and showed drug release for 8 hrs. No irritation was observed when the viscous solutions were tested on rabbit eye.

23. **Wen-Di Ma,et al.,** developed in situ gelling system of pluronic F127-g-poly(acrylic acid) copolymer as a ophthalmic vehicle and showed significant increase in drug resident time and better rheological behaviour.

24. **Maria Cristina Bonferoni,et al.,** reported the ion-exchange based ophthalmic delivery system of carrageenan and showed increase in in vitro release.

25. **Odile Sechoy,et al.,** studied alginic acid ophthalmic solutions for prolonged drug release of carteolol and reported that alginic acid vehicle is an excellent drug

carrier and could be used for the development of a long-acting ophthalmic formulation of carteolol.

26. **Shivanand Swamy P.et al.,** demonstrated novel in situ gum based ophthalmic delivery system of linezolid and showed extended release of the formulation and non irritant to rabbit eyes.

27. **Katarina Edsman et al,** studied carbopol 974P and 1342NF and showed better ocular residence time due to elastic property of the gel and rheological studies were satisfactory.

28. **Helene Hagerstrom,et al.,** reported the two ion sensitive polymers of carbopol 934 and Gelrite (deacetylated gellan gum) and showed the rheological behaviour of both the polymer and the polymers were found to be have better rheological property.

29. **John Carlfors,et al.,** studied Gelrite in situ gels for in vivo study to determine precorneal residence time .They showed high rate of the sol/gel transition results in long contact time.

30. **Hong-Ru Lin,et al.,** demonstrated a series of carbopol and pluronic based solutions as the in situ gelling vehicle for ophthalmic delivery and the mixture of 0.3% carbopol and 14.0% pluronic solution showed significant enhancement in gel strength and free flowing at pH 4.0 and 25◦C.The results showed that the carbopol/pluronic solutions had better ability to retain drug than the carbopol or

pluronic solutions alone and showed the mixture can be used as an in situ gelling vehicle to enhance the ocular bioavailability.

31. **Katarina Edsman,et al.,** developed the sol-to-gel transition of an in situ gel of Poloxamer 407 and showed the increase in corneal contact time when there is increase in Poloxamer concentration and the system was found to be temperature dependant.

32. **R.C.Doijad,et al.,** studied ophthalmic delivery system of an antibacterial agent,gatifloxacin, based on the ion activated system using sodium alginate and HPMC E50LV .The developed formulations were therapeutically efficacious,stable, non-irritant and provided sustained release of the drug over an 8-h period of time.

33. **J.K.Pandit,et al** studied the different types of in situ system of indomethacin and showed the formulated system was concentration dependant and the drug release was extended upto8-h and followed zero order kinetics.

34. **Hongyi Qia,et al.,** developed a thermosensitive in situ gelling and mucoadhesive ophthalmic drug delivery system containing puerarin based on poloxamer analogs (21% (w/v) poloxamer 407/5% (w/v) poloxamer 188) and carbopol (0.1% (w/v) or 0.2% (w/v) carbopol 1342P NF).The in vitro release studies demonstrated diffusion-controlled release of puerarin from the combined solutions over a period of 8 hrs and showed ocular bioavailability can be increased.

35. **Wu C,et al.,** developed a pH-triggered in situ gelling vehicle for ophthalmic delivery of puerarin in which Carbopol 980NF was used as the gelling agent in combination with HPMC (Methocel E4M) which acted as a viscosity-enhancing agent. The combined solution could flow freely under non- physiological condition and showed the character of pseudoplastic fluid under both conditions. Both in vitro release studies and in vivo pharmacokinetics studies indicated that the combined polymer systems performed better. Results demonstrate that the Carbopol 980NF/HPMC E4M can be a viable alternative to conventional puerarin eye drops to enhance ocular bioavailability and patient compliance.

CHAPTER II

IN SITU GELS - A REVIEW

A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action.[15] Various systems have been designed during the past two decades to maximize ocular absorption of ophthalmic drug.

The various approaches attempted in the early stages can be divided in to two main categories. Bioavailability improvement and controlled release drug delivery.[7]

A more desirable dosage form would be one that can be delivered in a drop form, creates little to no refractive index problem for vision.[16] Current research efforts are focused towards the design and evaluation of ocular delivery systems that are easy to administer, require decreased administration frequency and provide controlled and possibly sustained release, to increase therapeutic efficacy and patient compliance.[11]

Major progress has been made by ophthalmic gel technology in the developent of droppable gels or Insitu forming gels.[14] In the present era, sol-gel technolgy has the most promising applications in drug delivery systems as well as in industries[17] Hydrogels are expected to provide prolonged corneal contact time, when used in ocular drug delivery system, and also provide reduced precorneal drug loss, convenience in administration as compared to eye drops, suspensions or ointments.[11]

HYDROGELS

The most common way to improve drug retention on the corneal surface is by using polymers to increase solution viscosity. Hydrogels are cross linked network of hydrophilic polymers that have the ability to absorb large amounts of water and swell,

while maintaining their three dimensional structure. Molecules of different sizes can diffuse in and out of the hydrogel network, which allows their possible use as a drug depot for controlled release applications.

Hydrogels show minimum tendency to absorb protein from body fluids due to their low interfacial tension. They closely resemble living tissue due to their high water content and soft/rubbery characteristics. The viscoelastic properties of hydrogels should allow for sufficient mechanical strength to resist clearance due to blinking, resulting in prolonged ocular residence time.[14]

Hydrogels: A brief overview

Hydrogels are a three-dimensional network of hydrophilic polymers held together by association bonds such as covalent bonds and weaker cohesive forces such as hydrogen and ionic bonds and intermolecular hydrophobic association.

These networks are able to retain a large quantity of water within their structure without dissolving. Due to their superior chemical and physical properties, hydrogels have received much attention for preparing drug delivery systems.

The elastic nature of the hydrated hydrogels when used as implants has been found to minimize irritation to surrounding tissue.

The low interfacial tension between the hydrogel surface and the aqueous solution has been found to minimize protein adsorption and cell adhesion.

General Classification of Hydrogels:

Source:

1) Natural 2) Synthetic

Component:

- Homopolymer
- Copolymer
- Multipolymer

Preparation method:

- Simultaneous polymerization
- Crosslink of polymer

Electric charge:

- Nonion
- Anion
- Cation
- Zwitter ion

Physical structure:

- Amorphous
- Semicrystaline
- Hydrogen bonded
- Crosslink Covalent bond
- Intermolecular force

Functions:

- Biodegradable
- Stimuli responsive
- Superabsorbent

Fundamentals

Wichterle and Lim introduced a type of hydrophobic gel for biological uses in the early 1960s.A huge sum of efforts and studies has been devoted to advancing and extending the potentials attributed to hydrogels. Ever-growing hydrogel technology has led to dramatic advances in pharmaceutical and biomedical era.

Due to the contribution of these groups and domains in the network, the polymer is thus hydrated to different degrees (sometimes, more than 90%wt.), depending on the nature of the aqueous environment and polymer composition.

Properties:

1. Water Content and Swelling Ratio

The polymer chains of hydrogels interact with the solvent molecule and tend to expand to the fully solvated state. On the other hand, the crosslink structure works as the re-tractive force to pull back the polymer chain inside.

This retractive force is described by the Flory rubber elasticity theory.To describe the swelling behavior of hydrogels, their swelling ratio or water content is currently used in most cases. The water content of a hydrogel is expressed in terms of percentage of water by weight:

$$\text{Water content} = \frac{\text{Weight of water} \times 100}{\text{Weight of water + weight of dry gel}}$$

For instance, most of hydrogel contact lenses have water content between 38 and 75%. When the water content of the hydrogel is over 90%, the hydrogel is called super adsorbent hydrogel. Another index which characterizes hydrogels is the swelling ratio,

expressed by the ratio of the weight of swollen sample over that of the dry sample: Swelling ratio: weight of swollen gel / weight of dry gel

Peppas suggested that the swelling characteristic of hydrogels is a key for the use of hydrogels in biomedical and pharmaceutical applications, since the equilibrium swelling ratio influences the solute diffusion coefficient, surface wettability and mobility, and optical and mechanical properties of hydrogels.

Permeability

The permeability of target molecules is of utmost importance for medical application of hydrogels. For instance, oxygen permeation for contact lens, nutrient and immunological biosubstance transport for immunoisolation, and releasing drugs and proteins for drug delivery systems are core characteristics for each application.[18]

MECHANISIMS OF DRUG RELEASE FROM HYDROGELS

It involves a combination of diffusion and erosion of the gel surface. Due to hydrophilic nature of the gels, tears readily diffuse into the gel interior their by rapidly leaching out water soluble drugs.

Release mechanism from hydrogel matrices

Since the most common mechanism of drug release from hydrogels is passive diffusion, molecules of different sizes and characteristics would freely diffuse into/out of hydrogel matrix during the loading and storage periods. The hydrophilic nature of a hydrogel makes it highly different from non-hydrophilic polymer matrices with respect to the release behavior of the incorporated agents.

Mechanisms of drug release from hydrogels can be categorized as:

i) Diffusion-controlled

ii) Swelling-controlled

iii) Chemically-controlled

According to Fick's first law of diffusion (with constant or variable diffusion coefficients), the diffusion-controlled behavior is the most dominantly applicable mechanism to describe the drug release from hydrogels.

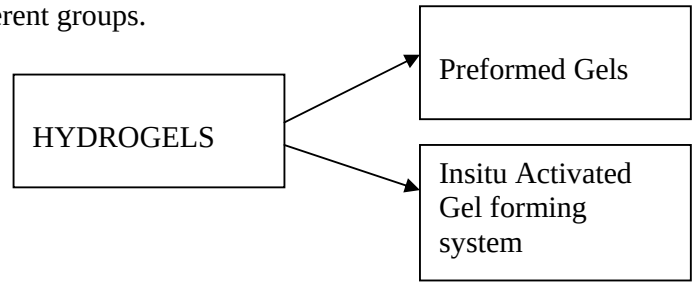
The drug diffusion out of a hydrogel matrix is primarily dependent on the mesh sizes within the matrix of the gel, which, in turn, is affected by several parameters, including, mainly, the degree of crosslinking, chemical structure of the composing monomers, and, when applicable, type as well as intensity of the external stimuli.

DESIRED PROPETIES OF POLYMERS USED IN OPHTHALMIC HYDROGELS AS DRUG DELIVERY SYSTEMS

- Chemical compatibility with the other excipients and the drug
- Stability after prolonged storage and if possible heat stability permitting sterilization by autoclaving
- Absence of ocular irritation or toxicity
- Little or no problem for vision
- Promotion of precorneal retention due to viscosity or bio adhesive properties.
- Physicochemical properties (pH, osmolality) compatible with the ocular use.

CLASSIFICATION OF HDROGELS USED IN OPHTHALMOLOGY

Polymeric hydrogels used in ophthalmology are generally classified into two different groups.



Both systems has different method of preparation and different in their activity.

PREFORMED HYDROGELS

These are systems which are administered as viscous preparations which do not under go any other transformation on the eye such as an eventual dilution by lacrimation or gradual elimination by lacrimal drainage, these hydrogels are called preformed gels and they are structured before ocular application.

METHODS OF LOADING OF HYDROGELS AS DRUG CARIERS

In this method the hydrogel monomer is polymerized in the presence of the drug by addition of chemicals (initiator) and cross linker, so that the drug remains trapped in the polymeric matrix.

Disadvantage

In some polymerization conditions may have deleterious effects on the drug and some chemicals are toxic.

VARIOUS TYPES OF PREFORMED HYDROGELS

Cellulose derivatives

Semi synthetic water soluble cellulosic polymers have been widely used in ophthalmic preparations such as eye drops, artificial tears and contact lens solutions as viscosity increasing agents.

The main cellulosic compounds used in ocular preparations include

- Hydroxy ethyl cellulose
- Hydroxy propyl cellulose
- Hydroxy propy methyl cellulose
- Sodium carboxy methyl cellulose

The common properties of above polymers are,

- Wide range of viscosity
- Good ocular tolerance

POLY ACRYLIC ACID

Polyacrylic acids also called carbomers or carboxy vinyl polymers, are acrylic acid based polymers which are available in different molecular weights and different structures.

SODIUM HYALURONATE

Hyaluronic acid is high molecular weight linear unbranched polysaccharide consists of repeating disaccharide units of glucuronid acid and N-acetyl glucosamine.

POLYVINYL ALCOHOLS

Polyvinyl alcohols are synthetic long chain polymer obtained by condensation of vinyl acetate and partial or full hydrolysis of the resulting polyvinyl acetate.

INSITU FORMING HYDROGELS

Insitu forming hydrogels are liquid upon instillation and undergo phase transition in the cul-de-sac to form viscoelastic gel and this provides a response to environmental changes.

METHODS OF LOADING OF HYDROGELS AS DRUG CARIERS

In this method, a polymer is allowed to swell in a suitable drug solution or he drug is added to a preformed hydrogel and diffuses through the polymeric network.

Advantages of in situ forming hydrogels

- Prolonging the corneal contact time of the drug and decreasing its drainage rate, thus increasing ocular bioavailability.
- It lowers the frictional resistance between the cornea and eyelids during blinking, there by exerting a lubricating effect.

Three methods have been employed to cause phase transition.

(i) Gelling triggered by change in pH

(ii) Gelling triggered by change in temperature

(iii) Gelling triggered by change in ionic strength[2]

GELLING TRIGGERED BY CHANGE IN pH

The concept of using hydrogels based on pH sensitive polymers for the ocular route began in the 1980s. These hydrogels are based on latex and carbomer.

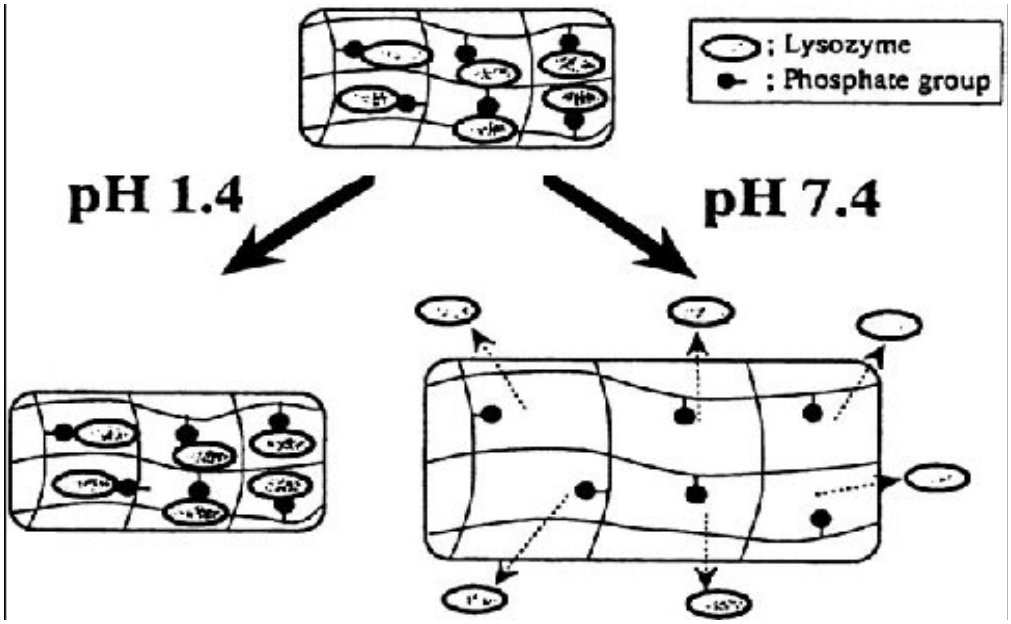
LATEXES

The term latex defines a low viscosity, stable polymeric dispersion of particles generally in an aqueous solvent.

Pseudo latexes are pH sensitive aqueous colloidal dispersion which undergo spontaneous coagulation and gellation after instillation in the conjunctival cul-de-sac because of pH increase.A change in pH is the most useful signal in the human body, because variation in pH occurs naturally in some areas of the body. In general, such pH-responsive hydrogels can be prepared by using polymers with ionizable groups.

Drug release systems controlled by pH-responsive swelling can be developed, which exhibit zero-order or near-zero-order release of the incorporated drug.

The special hydrogels were sensitive to both temperature and pH, and respond to the pH change to a much greater extent than normal hydrogels with carboxyl groups. Only a negligible amount of indomethacin as a model drug was released at pH 1.4, while more than 90% of the total drug in the gels was released at pH 7.4.



The swelling ratio of the hydrogels with the phosphate groups increased steeply at pH 5 and 10 because the phosphate group acts as an acidic charged divalent group. Furthermore, the hydrogels with the phosphate groups also showed swelling changes in response to temperature and solvent composition.[19]

PREREQUISITES NECESSARY FOR OPTIMAL FORMULATION OF OPHTHALMIC PSEDUO LATEX

- Solubility of the polymer in organic solvents and insolubility in water
- Existence on the macromolecule of ionizable groups, which can react with the electrolytes of the lacrimal fluid.
- Use of high molecular weight polymer

- Compatibility of the different components of the colloidal dispersion with precorneal tissues.[2]

CELLULOSE ACETATE PHTHALATE LATEX (CAP-Latex)

The ability of CAP latex to be free running solution at pH4.2 and a gel at 7.2, and finally, the latex stable at low pH.

CARBOMER

Cross linked Poly(acrylic acid) of high molecular weight, commercially available as carbopol, is widely used in ophthalmology to enhance precorneal retention to the eye. Four mechanisms of interaction between mucin and poly(acrylic acid) have been described: electrostatic interaction, hydrogen bonding, hydrophobic interaction, and inter-diffusion.

These mechanisms can be explained by the similar features of the mucus network and the cross-linked poly (acrylic acid): macromolecular expanded network, negative charges, and significant hydration in aqueous media and significant number of carboxyl groups. As the concentration of Carbopol increases in the vehicle, its acidic nature may cause stimulation to the eye tissues. In order to reduce the total polymer content and improve the gelling properties, an ocular drug delivery system based on a combination of Carbopol and methylcellulose has been developed.

Carbopol is a poly(acrylic acid)[PAA] polymer, which shows a sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5. Methylcellulose, a viscosity enhancing polymer, exhibits a sol to gel transition in aqueous solution in the range of 50–55 °C. The rheological properties of this system were investigated and sol to gel transition occurred primarily by an increase in pH due to the

presence of Carbopol; the temperature-mediated effect occurred only at very low shear rates.

The development of a similar delivery system by the combinations of Carbopol and hydroxypropylmethylcellulose, For both systems it was found that a reduction in the Carbopol concentration without compromising the in situ gelling properties as well as overall rheological behaviors can be achieved by adding a suitable viscosity enhancing polymer.[14]

GELLING TRIGGERED BY CHANGE IN TEMPERATURE

In this system, the drugs were released rapidly from the hydrogels due to the squeeze effect by their collapse as soon as the hydrogels were transferred from an aqueous solution below LCST (Lower critical solution temperature) to the release media above LCST.

As the temperature-responsive hydrogels were swollen at a low temperature, the drug could be released from the hydrogels. However, when the temperature increased beyond their LCST, dense skin formation during the deswelling process stopped the release of the drug. [19,20,21]

Temperature induced gelation

These hydrogels are liquid at room temperature (20–25 °C) and undergo gelation when in contact with body fluids (35– 37 °C), due to an increase in temperature. Different thermal setting gels have been described in this review, including for example Poloxamers, cellulose derivatives, and xyloglucan.

Poloxamers (Pluronic®)

The Poloxamers consist of more than 30 different non-ionic surface active agents. These polymers are ABA-type triblock copolymers composed of polyethylene oxide (PEO) (A) and polypropylene oxide (PPO) units (B).

The Poloxamer series covers a range of liquids, pastes, and solids, with molecular weights and ethylene oxide–propylene oxide weight ratios varying from 1100 to 14,000 and 1:9 to 8:2, respectively. Poloxamers, commercially available as Pluronic®, are the most commonly used thermal setting polymers in ophthalmology.

They are formed by central hydrophobic part (polyoxypropylene) surrounded by hydrophilic part (ethylene oxide). Depending on the ratio and the distribution along the chain of the hydrophobic and hydrophilic subunits, several molecular weights are available, leading to different gelation properties. Pluronic F-127, which gives colorless and transparent gels, is the most commonly used polymer in pharmaceutical technology.

Poloxamers have been widely investigated as ocular drug delivery systems At room temperature (b25 °C), the solution behaves as a mobile viscous liquid, which is transformed into a semisolid transparent gel at body temperature (37 °C).

Cellulose derivatives

Thermo reversible gels can be prepared with naturally occurring polymers. Most natural polymer aqueous solutions form a gel phase when their temperature is lowered. Classic examples of natural polymers exhibiting a sol–gel transition include gelatin and carrageenan. At elevated temperatures, these polymers adopt a random coil conformation in solution. Upon cooling, a continuous network is formed by partial helix formation.

Some cellulose derivatives are an exception to this gelation mechanism. At low concentrations (1–10 wt. %), liquid at low temperature, but gel upon heating. Methylcellulose and hydroxypropyl methylcellulose (HPMC) are typical examples of such polymers. Methylcellulose solutions transform into opaque gels between 40 and 50 °C, whereas HPMC shows phase transition between 75 and 90 °C.

These phase transition temperatures can be lowered by chemical or physical modifications. For example, NaCl decreases the transition temperature of methylcellulose solutions to 32–34 °C. Similarly, by reducing the hydroxypropyl molar substitution of HPMC, its transition temperature can be lowered to ~40 °C. Gelation of methylcellulose or HPMC solutions is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution.

Xyloglucan

Xyloglucan is the principal hemicellulose of primary cell walls of dicots and in about half of the monocots. It is structurally related to cellulose as it shares the same backbone of ß (1 .4)-linked glucose residues. The main repeating unit contains four glucose units. Three out of four glucose units are substituted with a (1 .6) xylose residues. Some xylose units are further substituted by galactose through a ß (1 .2) bond. In addition to these sugar units, the galactose residues can be further substituted with a (1 .2) fucose.

The XG side chains give raise to radically different physical properties of the polymer compared to cellulose; xyloglucan is highly water soluble and cannot form ordered crystalline microfibrils as cellulose.

Xyloglucan, a polysaccharide derived from tamarind seed, forms thermoresponsive gels in water, under certain conditions. Xyloglucan is composed of a (1-4)-ß-Dglucan backbone chain (GLU) which presents (1-6)-a-D-xylose branches (XYL) partially substituted by (1-2)-ß-D-galactoxylose (GAL).

The transition temperature is inversely related to polymer concentration and the galactose removal ratio. For example, the sol–gel transition of xyloglucan was shown to decrease from 40 to 5 °C when the galactose removal ratio increased from 35 to 58%.

GELLING TRIGGERED BY CHANGE IN IONIC STRENGTH

In this method, gelling of the solution instilled is triggered by change in the ionic strength.

Gelrite:

Gellan gum (Gelrite) is a linear, anionic heteropolysaccharide secreted by the microbe Sphingomonas elodea (formerly known as Pseudomonas elodea).The polysaccharide can be produced by aerobic fermentation and then isolated from the fermentation broth by alcohol precipitation.

The polymer backbone consists of glucose, glucuronic acid, and rhamnose in the molar ratio 2:1:1. These are linked together to give a tetrasaccharide repeat unit. The native polysaccharide is partially esterified with L-glycerate and acetate, but the commercial product Gelrite has been completely de-esterified by alkali treatment. Gelrite® (deacetylated gellan gum) is one of the most interesting in situ gelling polymers that has been tested since it seems to perform very well in humans.

Gelrite forms double helices at room temperature. This solution has a viscosity close to that of water and the helices are only weakly associated with each other (by van der Waals attraction). When gel-promoting cations are present, some of the helices associate into cation-mediated aggregates, which cross-link the polymer.

Gelrite has also provided corneal residence times superior to those of other hydrogel preparations based on polymers such as cellulosic derivatives or xanthan gum. The rheological properties of gellan gum such as thixotropy, pseudoplasticity, and thermoplasticity are further advantages for its use in ophthalmology: the fluidity of the solution can be increased simply by shaking or slightly warming the preparation.

Alginates

Being a family of unbranched binary copolymers, alginates consist of (1 .4) linked ß-D-mannuronic acid (M) and a-Lguluronic acid (G) residues of widely varying composition and sequence. By partial acid hydrolysis, alginate was separated into three fractions. Two of these contained almost homopolymeric molecules of G and M, respectively, while a third fraction consisted of nearly equal proportions of both monomers and was shown to contain a large number of MG dimer residues.

The alginate forms 3-dimensional ionotropic hydrogel matrices, generally by the preferential interaction of calcium ions with the G moieties resulting in the formation of inhomogeneous gel. Calcium-crosslinked alginate gels have shown good mechanical properties even when prepared from relatively low solution concentrations of the polymer, ~0.5% w/v, and they can physically entrap a whole array of molecules, and sustain their release.

Advantages of in-situ gelling system:

In situ forming hydrogels are attractive as conventional ocular solutions because of facile dosing as a liquid. Ensures complete and rapid ocular coverage. They also allow for accurate and reproducible quantities to be administered to the eye in contrast to pre-gelled formulations.[2] Prolonged contact time and increased bioavailability. Improved level of patient acceptance. Once or twice day dosing is the great advantage[14]

CHAPTER IV

AIM OF WORK

The ocular drug delivery has remained as one of the most challenging task for pharmaceutical scientists.

The unique structure of the eye restricts the entry of drug molecules at the required site of action. Conventional systems like eye drops, suspensions and ointments cannot be considered optimal in the treatment of vision threatening ocular diseases.[8]

The bioavailability is very poor when given in the form of ophthalmic solutions due to efficient productive mechanisms of the eye. There are more than 90% of the formulations available as ocular solutions. Frequent instillations of eye drops are necessary to maintain a therapeutic drug level in the tear film or at site of action. But, the frequent use of highly concentrated solutions may induce toxic side effects and cellular damage at the ocular surfac[3]

The typical pulse entry type drug release observed with conventional preparations can be replaced by a more controlled, sustained and continuous drug delivery using a controlled release ocular drug delivery system.

These systems can achieve therapeutic action with a smaller dose and a fewer systemic and ocular side effects, such systems include implants, ocuserts, collagen shields, nanoparticles, microspheres and liposomes, but the limitations of the above systems are

- Poor patient compliance and difficulty of insertion in ocular inserts.
- Tissue irritation and damage caused by penetration enhancers and collagen shields.

- Toxicity caused by insertion of foreign substances like albumin and poly butyl cyanoacrylates as in case of nanoparticles and microsheres.
- Cell toxicity and ocular irritation caused by stearylamine positive liposomes.[14]

To overcome the above mentioned problems, an alternative approach of in situ gelling systems or phase transition systems which are instilled in a liquid form and shift to a gel or solid phase in cul-de-sac by change in pH, temperature and electrolytes present is selected as a novel system.[3]

The aim of the current work is to formulate and evaluate the pH-triggered in situ gelling system to deliver sustained drug release.Due to the gelling capacity of the pH-triggered in situ gelling system after instillation in to the eye by increase in pH of tear fluid, the drainage of formulation is minimal compared to conventional ophthalmic eye drops.

The in situ gelling system provides longer contact time and sustained delivery of the drug, hence decrease in frequency of administration and improved patient compliance are the major advantages. The ocular conjunctivitis caused by various micro organism causes permanent conjunctival damages, corneal ulceration, systemic infections. The role of topical quinolones reserved principally for severe bacterial conjunctivitis.[22]

The second generation flouroquinolone, levofloxacin 0.5% solution is 2 times potent than the ofloxacin ophthalmic solution and hence selected as a drug in the formulation of pH-triggered in situ gelling system.

The formulation of pH-triggered in situ gelling system would be a alternative to conventional marketed eye drops by providing sustained drug release and improved patient compliance due to less frequency in administration.

CHAPTER V

PLAN OF WORK

1. Determination of λmax of levofloxacin hemihydrate
2. Calibration curve for the drug in simulated tear fluid pH 7.4
3. Formulation of levofloxacin in situ gelling system by using different concentrations of Carbopol 940 and HPMC (k4m, E50LV, E15LV) by pH-triggered in situ gelling system
4. Determination of gelling capacity
5. Sterilization by autoclave at 121◦C and 15 p.s.i for 15 minutes.
6. In –vitro release characteristics of pH-triggered in situ gelling system by using simulated tear fluid pH7.4
7. Rheological evaluation of pH-triggered in situ gelling system using different shear stress starting from 0.01,0.1,0.5,1.0,5.0,10,20,50,75 to 100 rpm.
8. Sterility test for selected formulation.
9. Evaluation of formulation for anti-microbial activity for selected formulation by using different strains of micro-organisms.
10. Comparisons of In –vitro release pattern of pH-triggered in situ gelling system with marketed formulation of eye drops.
11. Accelerated stability studies of pH-triggered in situ gelling system.
12. Ocular irritancy test of selected formulation of in situ gelling system on rabbit eye by using modified Draize protocol.

CHAPTER VI

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Levofloxacin hemihydrate -Shasun laboratories
2. Poly acrylic acid(Carbopol 940) - Shasun laboratories
3. Hydroxy propyl methyl cellulose(k4m) - Shasun laboratories
4. Hydroxy propyl methyl cellulose(E50LV) - Shasun laboratories
5. Hydroxy propyl methyl cellulose(E50LV) - Shasun laboratories
6. Tween 20 - S.D.Fine chem Ltd
7. Benzalkonium chloride - Nice chemicals
8. Di-sodium hydrogen phosphate - Vin biotech systems
9. Citric acid - Nice chemicals
10. Sodium chloride - Loba chemie
11. Hydrochloric acid - Nice chemicals
12. Sodium hydrogen carbonate - Merck
13. Calcium chloride - Nice chemicals
14. Sodium hydroxide - Nice chemicals

EQUIPMENTS USED

1. Mechanical stirrer	- Bombay India ltd.
2. Brookfield viscometer	- A.R. and Companies
3. Electronic Balance	- A&D Company, Japan
4. Magnetic Stirrer	- MC Dalal & co
5. UV Visible Spectrophotometer	- UV Pharma spec 1700, Shimadzu
6. FTIR Spectrophotometer	- FTIR,Shimadzu
9. Environmental chamber	- Inlab equipments (Madras pvt ltd)

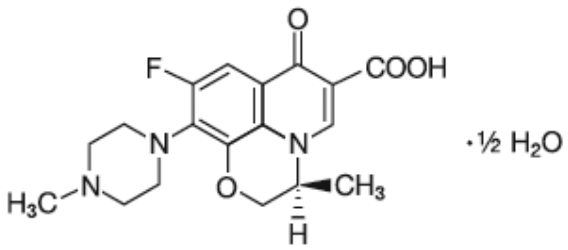
CHAPTER – VII

DRUG PROFILE

Levofloxacin:

Levofloacin, is a synthetic broad-spectrum antibacterial agent and a chiral fluorinated carboxyquinolone, is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin. The chemical name is (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemi hydrate.[23,24]

Structural form:



Empirical formula:

$C_{18}H_{20}FN_3O_4 \cdot ½\ H_2O$

Systematic IUPAC Name:

Molecular Weight:

370.38

Description:

Levofloxacin is a light yellowish-white to yellow-white crystal or crystalline powder.

CHEMICAL PROPERTIES:

Solubility:

The data demonstrate that from pH 0.6 to 5.8, the solubility of levofloxacin is essentially constant (approximately 100 mg/mL). Levofloxacin is considered soluble to freely soluble in this pH range. Above pH 5.8, the solubility increases rapidly to its maximum at pH 6.7 (272 mg/mL) and is considered freely soluble in this range. Above pH 6.7, the solubility decreases and reaches a minimum value (about 50 mg/mL) at a pH of approximately 6.9.

Melting point: 218^{o}c

PKa:

Partition co-efficient:

CLINICAL PHARMACOLOGY

Mechanism of action

Levofloxacin is the L-isomer of the racemate, ofloxacin, a quinolone antimicrobial agent The antibacterial activity of ofloxacin resides primarily in the L-isomer. The mechanism of action of levofloxacin and other fluoroquinolone antimicrobials involves inhibition of bacterial topoisomerase IV and DNA gyrase (both of which are type II topoisomerases), enzymes required for DNA replication, transcription, repair and recombination.

Anti-bacterial spectram

The materials have broad spectrum antibacterial and antifungal powerfulfeatures, the majority of Enterobacteriaceae bacteria such Klebsiellapneumoniae,Proteus is, typhoid Salmonella spp, Shigella species, some E. coli, and has strong antibacterial activity, part of

staphylococcus, streptococcus pneumonia,influenzabacillus,Pseudomonas aeruginosa, gonorrhea, and chlamydia have good antibacterial effect.

Dosage and Administration

Levofloxacin is applied topically to the eye as an ophthalmic solution.The recommended dosage of levofloxacin for the treatment of bacterial conjunctivitis is 1 or 2 drops of 0.5% solution in the affected eyes every 2-hours upto 8 times for 2 days,then 1 or 2 drops every 4-hours upto 4 times for next 5 days.

Common adverse effects

Transient decrease in vision,transient ocular blurring,ocular pain or discomfort,foreign body sensation,headache,fever,pharyngitis,and photophobia occur in 1-3 % of patients. Allergic reactions, lid edema, ocular dryness, and ocular itching occur in less than 1% of patients.

Contraindications

Known hypersensitivity to levofloxacin, other quinolones, or any ingredients in the formulation.

Drug Interactions

The systemic absorption may occur following topical application of levofloxacin to the eye. The reported interactions with theophylline, caffeine, anti coagulants and cyclosporine.

Indication

Conjunctivitis & otitis media.

Storage

Levofloxacin should be stored between 15-30 C (59-86 F) [25,26,27,28,29]

CHAPTER - VIII

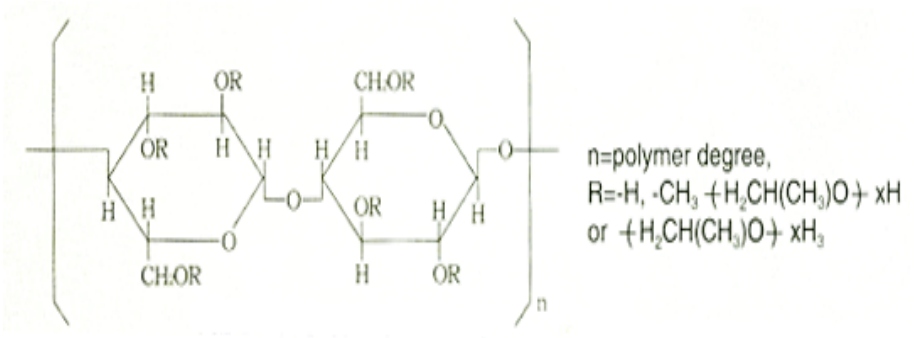
EXCIPIENTS PROFILE

1) HYDROXY PROPYL METHYL CELLULOSE (K4M):

Synonym:

Hypromellose, Methocel

Structure:



Structural Formula

Where R is H, CH3, or CH3CH(OH)CH2

Empirical formula:

Partly O-methylated and O-(2-hydroxy propylated) Cellulose.

Molecular weight:

10 000 – 1 500 00

Description:

An odorless and tasteless, white or creamy-white fibrous or granular powder.

Solubility:

Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95 %) and ether, but soluble in mixtures of ethanol and dichloromethane and mixtures of water and alcohol.

Methoxyl content:

19-24%

Hydroxy propyl content:

7-12%

Functional Category:

Coating agent, Film- former, stabilizing agent, Tablet binder, viscosity increasing agent.

Typical Properties

Acidity/alkalinity: pH -5.5–8.0 for a 1% w/w aqueous solution.

Ash:

1.5–3.0%, depending upon the grade and viscosity.

Autoignition temperature:

3608C

Density (bulk):

0.341 g/cm3

Density (tapped):

0.557 g/cm3

Density (true):

1.326 g/cm3

Melting point:

Browns at 190–2008C; chars at 225–2308C.

Glass transition temperature

170–1808C.

Moisture content

Hypromellose absorbs moisture from the atmosphere; the amount of water absorbed depends upon the initial moisture content and the temperature and relative humidity of the surrounding air

Viscosity:

Methocel K4M Premium - 4000 mPas

Applications in Pharmaceutical Formulation or Technology

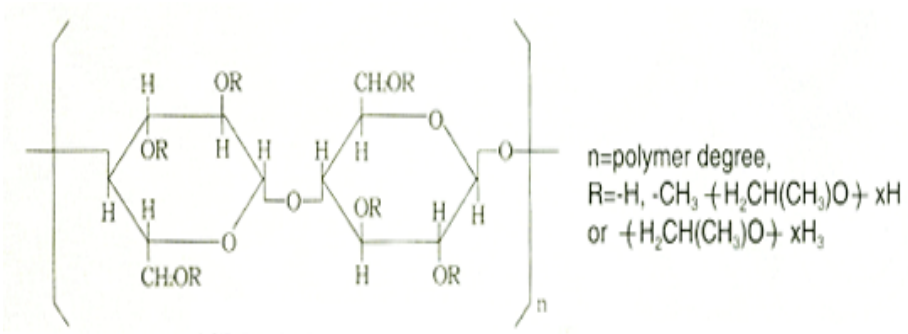
Hypromellose is widely used in oral, ophthalmic and topical pharmaceutical formulations.In oral products, hypromellose is primarily used as a tablet binder,(1) in film-coating,(2–7) and as a matrix for use in extended-release tablet formulations.

2) HYDROXY PROPYL METHYL CELLULOSE (E50LV):

Synonym:

Hypromellose, Methocel

Structure:



Structural Formula

Where R is H, CH3, or CH3CH(OH)CH2

Empirical formula:

Partly O-methylated and O-(2-hydroxy propylated) Cellulose.

Molecular weight:

10 000 – 1 500 00

Description:

An odorless and tasteless, white or creamy-white fibrous or granular powder.

Solubility:

Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95 %) and ether, but soluble in mixtures of ethanol and dichloromethane and mixtures of water and alcohol.

Methoxyl content:

19-24%

Hydroxy propyl content:

7-12%

Functional Category:

Coating agent, Film- former, stabilizing agent, Tablet binder & viscosity increasing agent.

Typical Properties

Acidity/alkalinity: pH - 5.5–8.0 for a 1% w/w aqueous solution.

Ash:

1.5–3.0%, depending upon the grade and viscosity.

Autoignition temperature:

3608C

Density (bulk):

0.341 g/cm3

Density (tapped):

0.557 g/cm3

Density (true):

1.326 g/cm3

Melting point:

Browns at 190–2008C; chars at 225–2308C.

Glass transition temperature

170–1808C.

Moisture content

Hypromellose absorbs moisture from the atmosphere; the amount of water absorbed depends upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Viscosity:

Methocel E50LV Premium	-	50 m Pas

Applications in Pharmaceutical Formulation or Technology

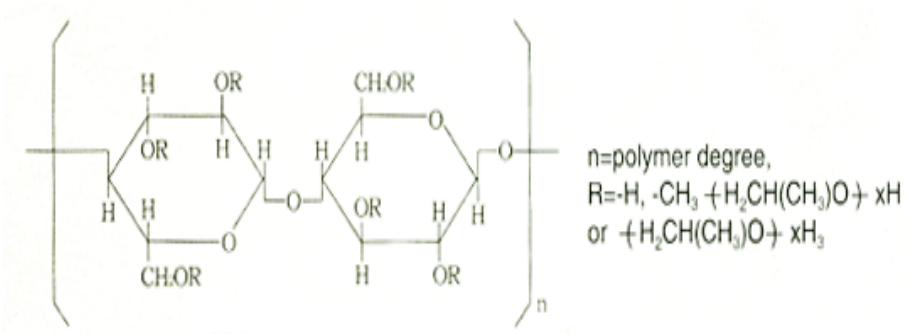
Hypromellose is widely used in oral, ophthalmic and topical pharmaceutical formulations.In oral products, hypromellose is primarily used as a tablet binder,(1) in film-coating,(2–7) and as a matrix for use in extended-release tablet formulations.

3) HYDROXY PROPYL METHYL CELLULOSE(E15LV):

Synonym:

Hypromellose, Methocel

Structure:



Structural Formula

Where R is H, CH3, or CH3CH(OH)CH2

Empirical formula:

Partly O-methylated and O-(2-hydroxy propylated) Cellulose.

Molecular weight:

10 000 – 1 500 00

Description:

An odorless and tasteless, white or creamy-white fibrous or granular powder.

Solubility:

Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95 %) and ether, but soluble in mixtures of ethanol and dichloromethane and mixtures of water and alcohol.

Methoxyl content:

19-24%

Hydroxy propyl content:

7-12%

Functional Category:

Coating agent, Film- former, stabilizing agent, Tablet binder, viscosity increasing agent.

Typical Properties

Acidity/alkalinity: pH - 5.5–8.0 for a 1% w/w aqueous solution.

Ash:

1.5–3.0%, depending upon the grade and viscosity.

Autoignition temperature:

3608C

Density (bulk):

0.341 g/cm3

Density (tapped):

0.557 g/cm3

Density (true):

1.326 g/cm3

Melting point:

Browns at 190–2008C; chars at 225–2308C.

Glass transition temperature

170–1808C.

Moisture content

Hypromellose absorbs moisture from the atmosphere; the amount of water absorbed depends upon the initial moisture content and the temperature and relative humidity of the surrounding air

Viscosity:

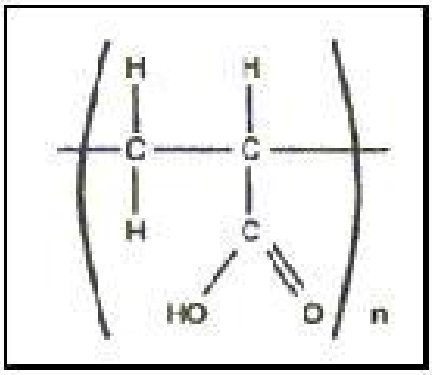
Methocel E15LV Premium - 15 m Pas

Applications in Pharmaceutical Formulation or Technology

Hypromellose is widely used in oral, ophthalmic and topical pharmaceutical formulations.In oral products, hypromellose is primarily used as a tablet binder,(1) in film-coating,(2–7) and as a matrix for use in extended-release tablet formulations.

4) CARBOMER (CARBOPOL 940)

Structure:



Synonyms

Acritamer; acrylic acid polymer; Carbopol; carboxy polymethylene,polyacrylic acid; carboxyvinyl polymer; Pemulen;Ultrez. Chemical Name and CAS Registry Number Carbomer

Empirical Formula and Molecular Weight

Carbomers are synthetic high-molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56% and 68% of carboxylic acid (COOH) groups 104 400 g/mol for Carbopol 940 have been reported.

Structural Formula

Carbomer polymers are formed from repeating units of acrylic acid. The monomer unit is shown above. The polymer chains are crosslinked with allyl sucrose or allyl pentaerythritol.

Functional Category

Bioadhesive; emulsifying agent; release-modifying agent; suspending agent; tablet binder; viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology

Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents.Formulations include creams, gels, and ointments for use in ophthalmic,(5–7) rectal,(8–10) and topical preparations.

Emulsifying agent

0.1–0.5

Gelling agent

0.5–2.0

Suspending agent

0.5–1.0

Tablet binder

5.0–10.0

Description

Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odor.

Pharmacopeial Specifications

Carbomer 940 (0.5 w/v) — 40 000–60 000(a)

Typical Properties

Acidity/alkalinity

pH = 2.7–3.5 for a 0.5% w/v aqueous dispersion;

pH = 2.5–3.0 for a 1% w/v aqueous dispersion.

Density (bulk)

1,.76–2.08 g/cm3

Density (tapped):

1.4 g/cm3

Glass transition temperature:

100–1058C

Melting point

Decomposition occurs within 30 minutes at 2608C.

Moisture content

Normal water content is up to 2% w/w.However, carbomers are hygroscopic and a typical equilibrium moisture content at 258C and 50% relative humidityis 8–10% w/w. The moisture content of a carbomer does notaffect its thickening efficiency, but an increase in the moisture content makes the carbomer more difficult to handle because it is less readily dispersed.

Particle size distribution

Primary particles average about 0.2 mm in diameter. The flocculated powder particles average 2–7 mm in diameter and cannot be broken down into the primary particles. Recently, a granular carbomer having a particle size in the range 180–425 mm has been introduced. Its bulk and tap densities are also higher than those of other carbomers.

Solubility

Soluble in water and, after neutralization, in ethanol (95%) and glycerin. Although they are described as 'soluble', carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels.

Viscosity (dynamic)

Carbomers disperse in water to form acidic colloidal dispersions of low viscosity that, when neutralized,produce highly viscous gels. Carbomer powders should first be dispersed into vigorously stirred water, taking care to avoid the formation of indispersible lumps, then neutralized by the addition of a base.

Stability and Storage Conditions

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 1048C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 2608C. Dry powder forms of carbomer do not support the growth of molds and fungi.

CHAPTER IX

EXPERIMENTAL DETAILS

CALIBRATION CURVE FOR LEVOFLOXACIN HEMIHYDRATE

PREPARATION OF CALIBRATION MEDIUM

Simulated tear fluid or artificial tear fluid

Sodium chloride – 0.670g,sodium bi carbonate - 0.200g,calcium chloride - 0.008g is placed in 100ml volumetric flask and dissolved and make up to volume with distilled water.[31]

Preparation of standard curve for levofloxacin hemihydrate

The standard stock solution of levofloxacin hemihydrate is prepared by dissolving a known amount of drug in sodium hydroxide solution and dilution with simulated tear fluid. From the above stock solution, different concentrations of 5,10,15,20.....50µg /ml is prepared in simulated tear fluid. The resulting solution is scanned in UV Spectrophotometer to find λmax and the absorbance is measured at λmax (288nm)[32]. The standard curve is plotted by taking concentration in X-axis and absorbance in Y-axis. The standard curve is used to estimate drug content and percentage drug release.

FORMULATION OF pH TRIGGERED IN SITU GELLING SYSTEM OF LEVOFLOXACIN

Optimum concentration of polymers

Carbopol is a polyacrylic acid (PAA) polymer, which shows sol to gel transition in aqueous solution as the pH is raised above its pka of about 5.5.The concentration of PAA required to form stiff gels results in highly acidic solutions which are not easily neutrlised by buffering action of tear fluid.A reduction in PAA concentration without compromising gelling capacity and rheological property achieved by addition of viscosity enhancing polymer HPMC.

It is noted that carbopol concentration 0.2% (W/V) had free flowing properties and these composition could not form gel at physiological condition. On the other hand carbopol concentration above 0.5%(W/V) formed stiff gel even at pH 4.0.As the concentration of carbopol solution increased, the solution become highly acidic ,which may stimulate the eye tissues.[33]

Based on the work of Helene hagerstrom, the carbopol concentration was fixed from 0.25 %(W/V) to 0.5 %(W/V). The HPMC of different grades in concentration fixed form 0.5 %(W/V) to 1.5 %(W/V) and the formulations made.[34]

Various in situ gelling system of levofloxacin hemihydrate is prepared by utilizing the phase transition properties of hydroxy propyl methyl cellulose E15LV, E50LV, K4M grade and carbopol 940 in different ratios by using pH triggered in situ gelling system which is shown in table no. 2

SELECTION OF VEHICLE

The vehicle is selected based on the solubility and stability of the drug. The solubility of levofloxacin is starting from the pH 2.0 to pH 6.5 and maximum at pH6.2.The marketed formulation showed the pH 6.2.For these reasons the citrophosphate buffer pH 6.0 is selected as vehicle for levofloxacin.

PREPARATION OF pH-TRIGGERED IN SITU GELLING SYSTEM

Aqueous solutions of varying concentrations of Carbopol 940 and Hydroxy propyl methyl cellulose of different grades are prepared.

The buffer salts are dissolved in specified quantity of purified water. To this methocel (E15LV, E50LV, K4M) is added and allowed to hydrate. Carbopol 940 is sprinkled over this solution and allowed to hydrate overnight.

The solution is stirred with an overhead stirrer, Tween 20 added whilst stirring. Levofloxacin is dissolved in sodium hydroxide solution and pH is adjusted. Benzalkonium chloride (BKC) is then added and the solution is filtered.

The drug solution is added to the Carbopol – HPMC solution under constant stirring until a uniform solution is obtained. Purified water is then added to make up the volume to 100ml.

The formulations are filled in 5-ml capacity vials, closed with gray butyl rubber closures and sealed with aluminium caps for further evaluations. All the formulations are sterilized using autoclave at 121◦C and 15 p.s.i for 20 minutes.[35]

EVALUATION OF FORMULATED IN SITU GELLING SYSTEM

The formulated levofloxacin in situ gels are evaluated for clarity, pH measurement, gelling capacity, drug content measurement, rheological study, sterility test, in vitro release study, anti microbial activity, eye irritation testing and stability testing.

Determination of visual appearance, clarity and color

The visual appearance, clarity and color of the in situ gel formulation is noted.[36]

PH measurement

The pH of all the prepared in situ gelling system is measured by using pH meter.[37,65]

Drug content analysis

Drug content of in situ gel is determined by taking 2ml of in situ gel containing a known amount of drug in a 100ml volumetric flask and diluted with simulated tear fluid of ph 7.4 to get the concentration of 10µg/ml. The standard solution of concentration 10µg/ml is also prepared. Then the absorbance is measured at λmax (288nm) using simulated tear fluid as blank by UV-spectrophotometer to calculate the percentage drug content.[38,39,41]

Gelling capacity

The prepared in situ gelling system is evaluated for gelling capacity in order to identify the composition suitable for use as in situ system./The in situ gelling system is mixed with simulated tear fluid in the proportion of 25:7(application volume 25µl.normal volume of tear fluid in the eye is 7µl).The gelation is assessed by visual examination of

gel formation and noting the time for gelation and the time taken for the formed gel to dissolve.[39,40,41]

In vitro release studies

The drug release from the prepared formulation is studied by using a modified method reported earlier. The test solution was placed in a circular plastic cup of 2.5cm internal diameter and1.2cm depth. This was in turn placed on an inverted USP basket kept inside a 250-ml beaker. Dissolution medium of 200ml of simulated tear fluid is added and stirred with a magnetic bead. Temperature of 37±1◦C is maintained throughout the study. Samples of 1ml are withdrawn at regular intervals and make up to 10ml with simulated tear fluid and replaced with an equal volume of fresh medium. The drug release study of in situ gelling system is compared with that of marketed conventional formulation of eye drops.

The absorbance of the samples is measured atλmax (288nm)by UV-spectrophotometer using blank to calculate amount of drug release from in situ gel. The percentage of drug release is plotted against time to find the drug release pattern of all in situ gel preparation.[42,43,44,45,68,70,71]

Rheological studies

The relationship between contact time and the rheology is easily understood for viscosity enhanced ophthalmic solutions. It is noted from various literature that, the formulations before gelling should have a viscosity of 5 to 1000 m pa and after gelling in the eye, will have a viscosity from about 50-50,000m pa. Viscosity determinations of the prepared formulations are carried out by Brookfield synchroelectric viscometer(LVDV

Pro II),spindle S18(small sample adaptor) and the angular velocity increased from 0.01,0.1,0.5,1.0,5.0,10,20,50,75 to 100 and measurements are noted.[46,47, 48,49,51,52,53.54,66,67]

Antimicrobial activity

Antimicrobial efficiency studies are carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This is determined by agar diffusion test employing "cup plate technique". Sterile solution of marketed levofloxacin eye drops is used as a standard.The standard solution and the developed formulations(test solution)are poured into separate cups bored into sterile Muller Hinton Agar(MHA) previously seeded with organisms(Staphylococcus aureus and Pseudomonas aeruginosa). After allowing diffusion of solutions for two hours, the plates are incubated for 24 h at 37◦C.The zone of inhibition(ZOI)measured around each cup is compared with that of the standard.[36,41,55,56,57,69]

Sterility testing

Sterility testing is carried out by incubating formulations for not less than 14 days at 30 to 35◦ c in case of fluid thioglycolate medium for bacteria and at 20 to 25◦c in case of soyabean-casein digest medium for fungi and observations made.[38,41]

Ocular irritancy studies

Ocular irritation studies are performed on male albino rabbits weighing 1-2kg.The modified Draize technique is designed for the ocular irritation potential of the ophthalmic product. According to Draize test ,the amount of substance applied to the eye is normally 100 µl placed to the lower cul-de-sac and observed at the time interval of

1hr,24hrs,48hrs,72hrs,and 1 week after administration. The rabbits observed periodically for redness, swelling and watering of the eyes.[37,39,46,58,59,60,61,64,72,73,74.]

Accelerated stability studies

The sol-to-gel systems are placed in amber colored vials and sealed with aluminium foil for a short term accelerated stability study at 40◦±2◦c and 75±5% RH as per International Conference on Harmonization states guidelines. Samples are analysed every 15 days for appearance, pH, gelling studies and drug content.[37,41,49,50]

FT-IR studies

The possibility of drug-excipient interactions are further investigated by FTIR. The FTIR graph of pure drug and combination of drug with excipient are recorded .The analysis is performed using KBR pellets.[57,62,63.]

CHAPTER IX

RESULTS AND DISCUSSION

CALIBRATION CURVE OF LEVOFLOXACIN HEMIHYDRATE

Calibration curve of levofloxacin hemihydrate was done in simulated tear fluid pH-7.4.Levofloxacin hemihydrate shows λmax of 288nm in simulated tear fluid pH7.4.The correlation coefficient was 0.99929866.Hence, Levofloxacin hemihydrate obeys the beer's law within the concentration range of 5 to 50µg/ml.Calibration plots of levofloxacin hemihydrate in simulated tear fluid was showed in table 1 and fig.1.The maximum absorbance showed in fig.2.

FORMULATION OF pH-TRIGGERED LEVOFLOXACIN HEMIHYDRATE IN SITU GELLING SYSTEM

Eighteen formulations of levofloxacin hemihydrate in situ gelling systems were prepared by using various concentrations of carbopol 940 along with different grades of hydroxyl propyl methyl cellulose in different ratios by using pH triggered in situ gelling method. The drug concentration kept as constant for each formulation (25mg/5ml).

The two main prerequisite of an in situ gelling system are viscosity and gelling capacity. The formulation should have an optimum viscosity so that will allow easy instillation into the eye as liquid and undergo a rapid sol-to-gel transition (triggered by rise in pH from 6.0 to 7.4).

EVALUATION OF FORMULATION

Appearance and clarity

The appearance of the formulation was light yellow and the system was clear except for formulations F-3 and F-6. Terminal sterilization by autoclaving had no effect on the formulations. The haziness observed during autoclaving due to precipitation of HPMC at elevated temperature was found to disappear and the clarity was regained after overnight standing. The observations are showed in table no.3.

pH measurements

The pH of all the formulations were noted and it was from the pH 6.0 to 6.4 and found to be satisfied and showed in table no.3

Drug content

The drug content of all the formulations noted and found to be within the normal range. The values are showed in table no.4

Gelling capacity

The viscosity and gelling capacity plays important role for in situ gelling system. The formulation should have an optimum viscosity for easy instillation into the eye as a liquid which undergo sol-to-gel transition. The gelling capacity of various formulation given in table no.4

Formulations F-1,F-2,F-3 prepared from Carbopol940(0.5%) and HPMC K4M,F-7,formulations F-8,F-9 prepared from Carbopol 940(0.5%) and HPMC

E50LV,formulation F-15 prepared from Carbopol 940(0.5%) and HPMC E15LV showed better gelling capacity. The other formulations were not having desirable gelling capacity.

In vitro release studies

The cumulative percentage drug release of formulations F-1 to F 18 given in table no.5. The order of release of drug from the formulations as follows,

F-13> F-12>F-4> F-14> F-5> F-7 >F-6> F-8 >F-1> F-2> F-9> F-3

The above formulations showed drug release for 8-hrs period of time and remaining formulations released drug before 8-hrs and the cumulative percentage drug release of F-13:100.57%, F-12:99.89%, F-4:99.42%, F-14:97.03%, F-5:93.94%, F-7*9.97%, F-6:89.51%, F-8:83.36%, F-1:80.88%, F-2:77.75%, F-9:75.06% and F-3:64.78% respectively and considered to be suitable for novel pH-triggered in situ gelling system of levofloxacin. The in vitro release observations were in accordance with the Carbopol/HPMC system reported by Kumar and Himmestein (1995).

The formulation prepared from HPMC K4M [F-3(0.9%W/V)] and carbopol 940(0.5%W/V) showed maximum controlled drug release of 64.78% followed by formulation prepared from HPMC E50LV [F-9(0.9%W/V)] and carbopol 940(0.5%W/V)of drug release, followed by formulation prepared from HPMC K4M [F-2(0.9%W/V)] and carbopol 940(0.5%W/V) 77.75% at 8-hrs.

The cumulative percentage drug release of marketed formulation of levofloxacin eye drops was compared with that of the selected formulations prepared from different grades of HPMC (K4M, E50LV, E15LV) with Carbopol and the observations showed in table.5G and fig.no.9.

The formulations were selected based on sustained in drug release at 8-hrs period of time and the graph is shown in figures.3-8.

Even formulation F-3 achieved maximum sustained release achieved, due to turbidity the formulation is not considered as best formulation.

From the results it was concluded that the polymer concentrations of both Carbopol 940 and different grades of HPMC with higher viscosity plays important role in the release of drug from the formulations. When the polymer concentration increases drug release decreases, and when polymer concentration decreases drug release from the formulation increases.

The in vitro release of in situ gelling system containing low concentrations of polymer showed increase in drug release and high concentrations of polymer decrease in drug release during initial sampling.

The initial fast release from the formulation could be explained based on the fact that these systems formulated in aqueous vehicle .The matrix formed on gellation was already hydrated and hence hydration and water permeation could no longer limit the drug release.

The eye drops formed opaque matrix immediately upon addition to the dissolution medium, due to increase in pH of the simulated tear fluid. Hence the release of drug from this matrix was possibly by diffusion and /or erosion of the matrix.

The combination of these processes seemed to result in the overall diffusion controlled release kinetics. The in vitro release conditions may be vary from those likely to be encountered when instillation into the eye. There were, the results showed that the formed gel had the ability to retain levofloxacin for the duration of the study (8hrs).

The cellulose derivatives like HPMC (K4M, E50LV, E15LV) with higher molecular weight dissolve in water and yield much more viscous solution in combination with carbopol 940.Thus increase in viscosity might have contributed to decrease in rate of drug release from the formulation.

The formulations prepared from each grade of HPMC (F-2, F-9, F-15) selected for rheological evaluation.

RHEOLOGICAL STUDIES

The viscosity of the selected formulations based on in vitro release showed in table no.6.The viscosity of all these formulations decreased as the shear rate increased, which showed the character of pseudoplastic fluid.

Formulation F-2 showed better pseudoplastic behavior compared to all three formulations. The formulation F-2 selected as best formulation out of all 18 formulations based on clarity, pH, gelling capacity, *in vitro* release and viscosity. The pseudoplastic behavior of formulations showed in figure no.6 and fig.10

The formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity (Pseudoplastic rheology). The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the precorneal tear film. At formulation pH (pH 6.0), the formulations were in a liquid state and exhibited low viscosity. At pH of tear fluid (pH 7.4) the solution transformed to gels with high viscosity.

During blinking the shearing force on the preparation is large. The ocular shear rate is ranging from 0.03 s during inter-blinking periods to 4250 – 28500 s diring blinking, viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rate which is called as pseudoplastic fluid, is often preferred.

If the viscosity at high shear rate is too high, It result in irritation. On the other hand ,if viscosity is too low ,it will give rise to increased drainage.

The pseudoplastic property of these formulations is in favour of sustaining drainage of drug from the conjunctival sac of the eye,also without blinking difficulty for undergoing shear thinning. The remaining evaluations are performed only for the selected formulation F-2.

ANTIMICROBIAL ACTIVITY

The zone of inhibition (ZOI) measured around each cup was compared with that of control (Standard and marketed formulation).The results were showed in table.7 and in fig.11.The in vitro efficacy study of selected in situ gelling formulation of levofloxacin retained its antimicrobial efficacy when formulated as an in situ gelling system compared with that of marketed formulation of levofloxacin eye drops and the drug was active against the selected strains of microorganism.

The diameter of zone of inhibition produced by formulation F-2 against both test organisms was equal to that produced by marketed eye drops. The anti-microbial activity of levofloxacin in situ gel formulation is probably due to a fairly rapid initial release of drug into the viscous solution formed by dissolution of gel, followed by formation of a drug reservoir that permits the drug to be released to the target site relatively slowly.

FT-IR STUDIES

FT-IR spectrum of pure drug and mixture of drug and polymers and given in fig no. From this study it was observed that there were no significant changes between the spectrum of pure drug and the mixture. It showed that there were no specific interactions between the drug and excipients used in the formulations.

STERILITY TEST

The test for sterility is an important aspect for ophthalmic preparations. The test for sterility is intended for detecting the presence of viable bacteria, fungi and yeast in sterilized preparations and observations showed in table no.8

The formulation F-2 passed the test for sterility as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 14 days at 30-35◦c in case of fluid thioglycolate medium and at 20-25◦c in the case of soyabean casein digest medium.

OCULAR IRRITANCY TEST

The observations of ocular irritancy were showed in table no.9. The ocular irritancy test was carried out in male albino rabbits by using modified Draize test. For this purpose F-2 formulation was tested by comparison with marketed formulation of levofloxacin eye drops. Ocular tolerability results showed no evidence of redness, swelling or lacrymation in rabbit eyes. The score for ocular presentations were determined as zero at all observations.

STABILITY STUDIES

All the formulations did not showed any change in appearance, clarity, pH and color of the preparations. It was also noted that no change in gelation properties of the prepared in situ gelling system.

The drug content of the formulations was tested regular interval and there was no significant change in the drug content. From the stability studies it was observed that there was no significant change in any of the parameters evaluated. It was found that the formulated in situ gelling system is stable. The observations showed in table no.10.

CHAPTER XI

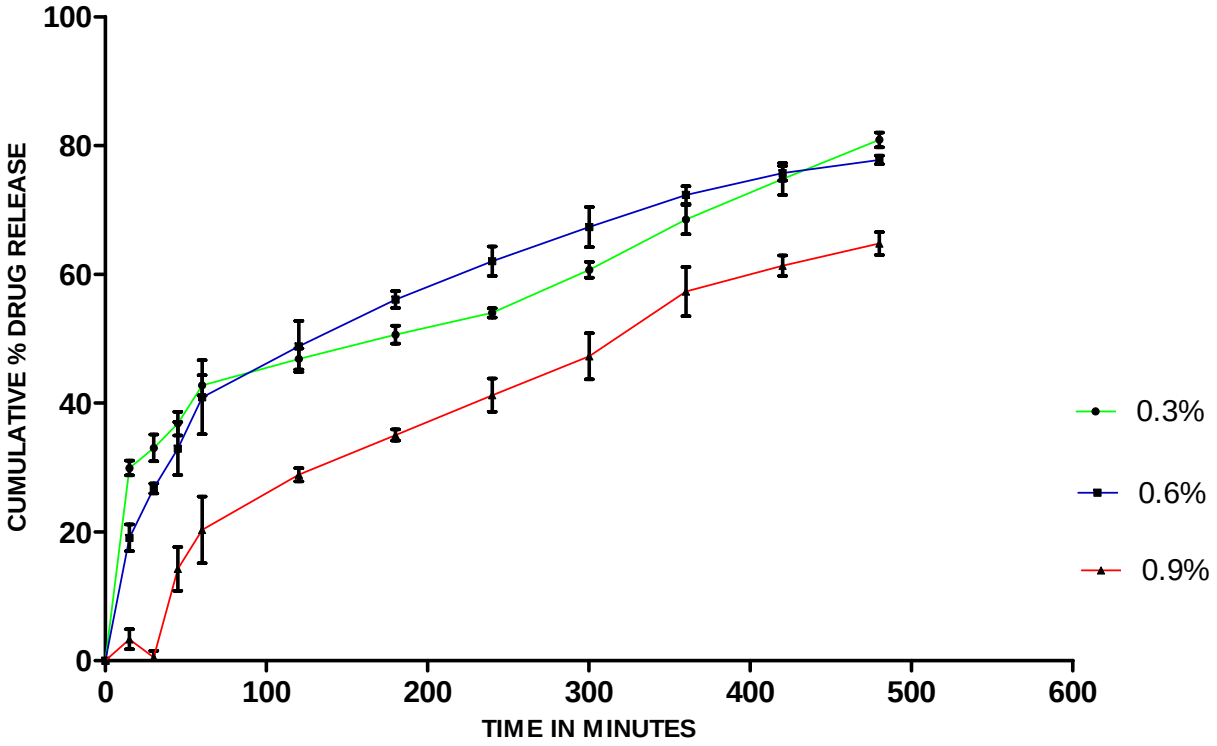
SUMMARY AND CONCLUSION

- The purpose of this research was to develop a novel ophthalmic in situ drug delivery system of levofloxacin hemihydrate to improve its poor ocular bioavailability.
- The ocular in situ gel of levofloxacin hemihydrate was prepared by using carbopol 940 and HPMC (K4M, E50LV and E15LV) by pH-triggered in situ gelling technique.
- The broad spectrum antibacterial agent used in the treatment of ocular infections like conjunctivitis was successfully formulated in situ gelling system using 0.5%W/V of levofloxacin.
- The formulated in situ gelling system were characterized for appearance, color, pH, gelling capacity, rheological character, in vitro release in simulated tear fluid.
- All the formulations were clear except, F-3 andF-6 and pH of the formulations was from 6-6.4.
- The better gelling capacity was obtained by optimized concentrations of carbopol 940 and HPMC when these two vehicles were combined, the gel strength and gelling capacity under physiological conditions were appropriate.
- The formulations showed better gelling capacity which can be easily instill as a drop.

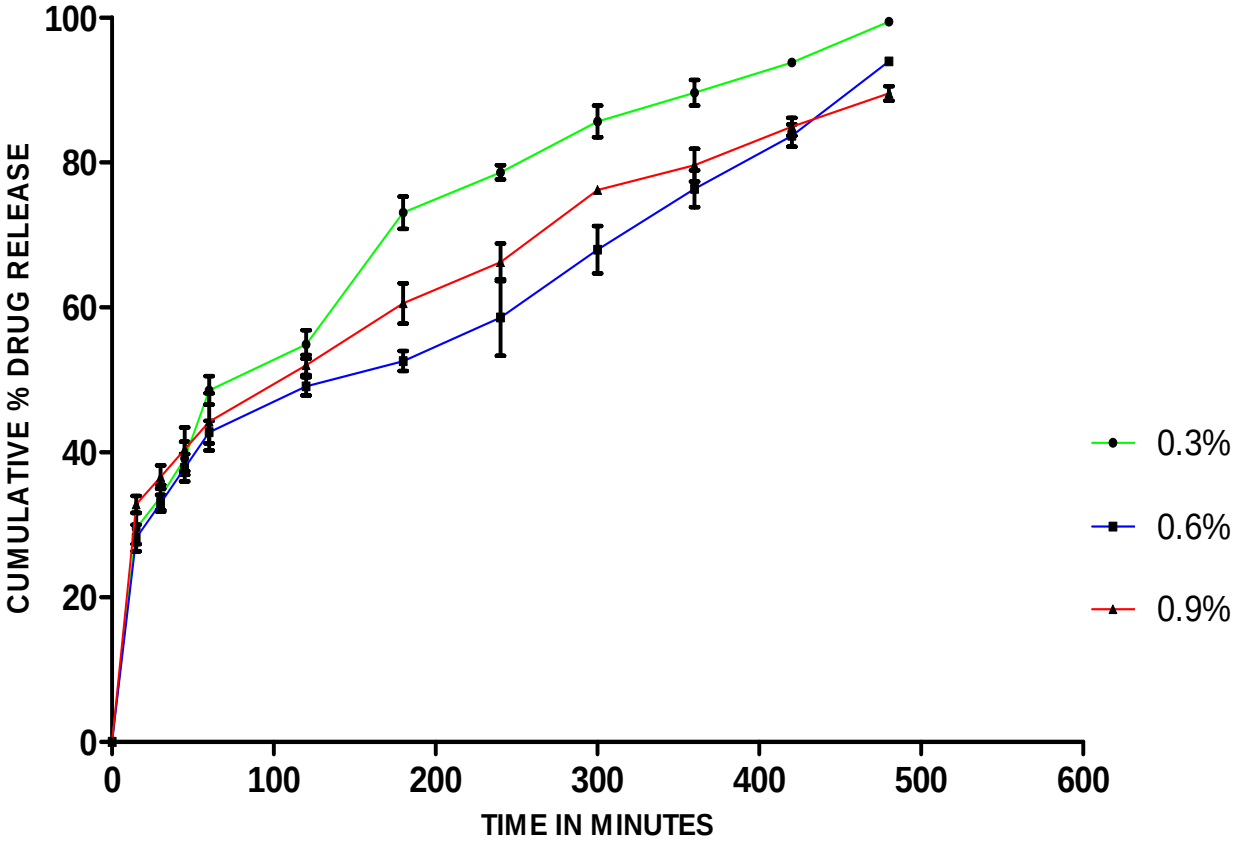
- The cumulative percentage of drug release of F-3 was 64.78% and F-2 was 77.75%.The drug release of F-3 retarded much but the formulation was turbid, Hence the F-2,F-9,F-15 selected for evaluation of Rheological character.
- The results of viscosity revealed that F-2 showed better pseudo plastic behavior in lowest concentration of HPMC k4m(0.6%W/V) than other formulations
- The in vitro release studies revealed that the increase in polymer concentration retards the drug release and the decrease of decrease in polymer concentration increases the drug release.
- In order to achieve high stability all the formulations sterilized by autoclave at 121c and 15 p.s.i for 20 minutes. The test for sterility showed no turbidity of the inoculated medium suggests that the pH activated in situ gelling system of levofloxacin was sterile.
- The test for anti-microbial efficacy proved the formulation to be therapeutically efficacious. The comparison of anti-microbial activity of in situ gelling system with marketed formulation showed equal efficacy in zone of inhibition (ZOI).
- The addition of benzalkonium chloride and autoclave sterilization of selected formulation F-2 had no influence on pH and viscosity.
- The absence of irritant activity by animal study shows that the system is promising for ophthalmic use.
- Stability studies indicated that the drug retention capacity of in situ gelling system was not changed significantly.
- The FTIR results proved that no interactions between the drug and polymers of in situ gelling system.

- The methodology adopted for the in situ gelling system is cost effective
- The pH-triggered in situ gelling system afforded sustained drug release over 8-h period of time.
- The in situ gel forming system could have good patient compliance because it's easy instillation.
- These hydrogel in situ formulations are administered into the eye as a solution, rapidly forming a hydrogel that is able to withstand the shear forces in the cul-de-sac.
- The pH-triggered in situ gel of levofloxacin has sustained drug release than conventional ophthalmic solutions of levofloxacin, and which is a viable alternative to conventional eye drops by its ability to retard drug release.
- The prolonged precorneal residence time of in situ gelling system to get higher bioavailability and reduced systemic side effects caused by prevention of drainage from the nasolacrymal duct makes the formulation advantages over conventional eye drops.
- The pH triggered in situ gel of levofloxacin formulation was effective in treatment of conjunctivitis and the drug was released from the formulation in a constant manner for the desired period of time.
- The in situ gelling formulation evaluated here has potential in ophthalmic use, for reason that it is more readily administered and hence the pH-triggered in situ gelling considered to be promising for prolonging the ocular residence time without causing irritation to eyes and a viable alternative to marketed eye drops.

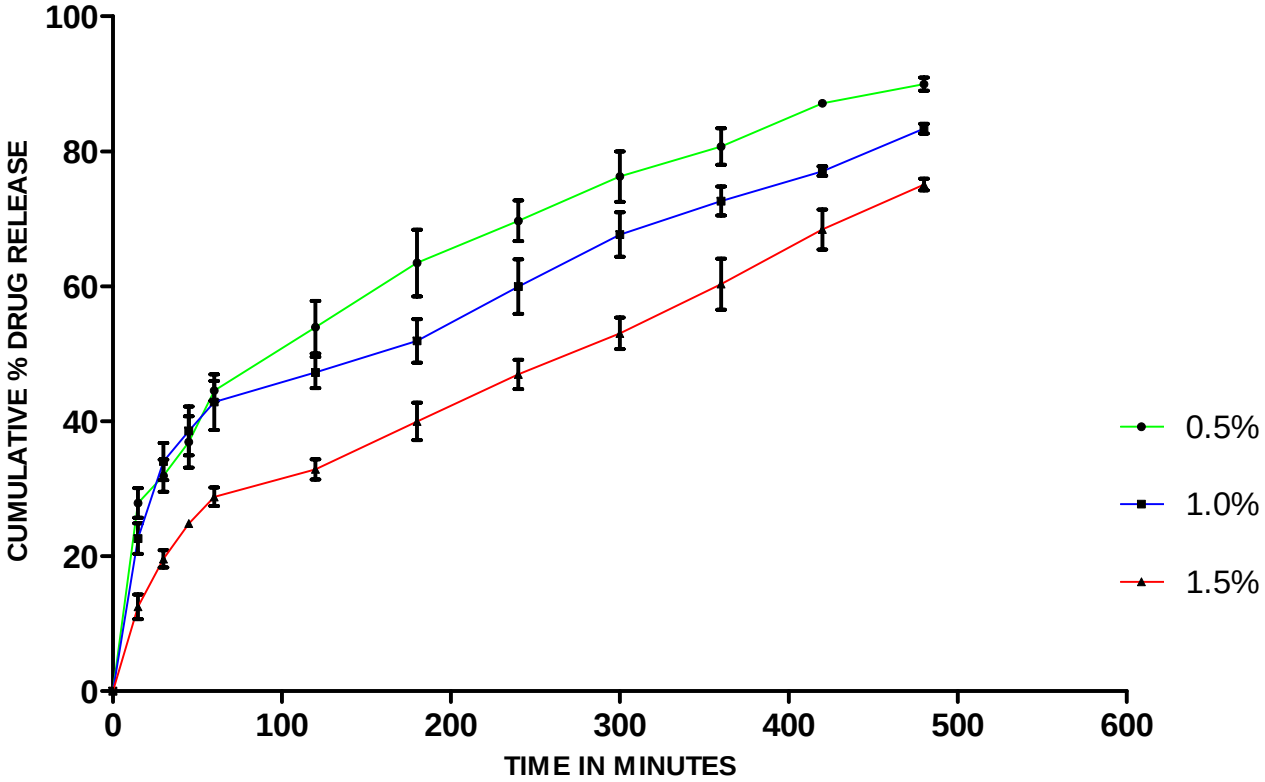
Comparison of invitro release profile of in situ gelling system containing various concentration of HPMC K4M in carbopol 0.5%

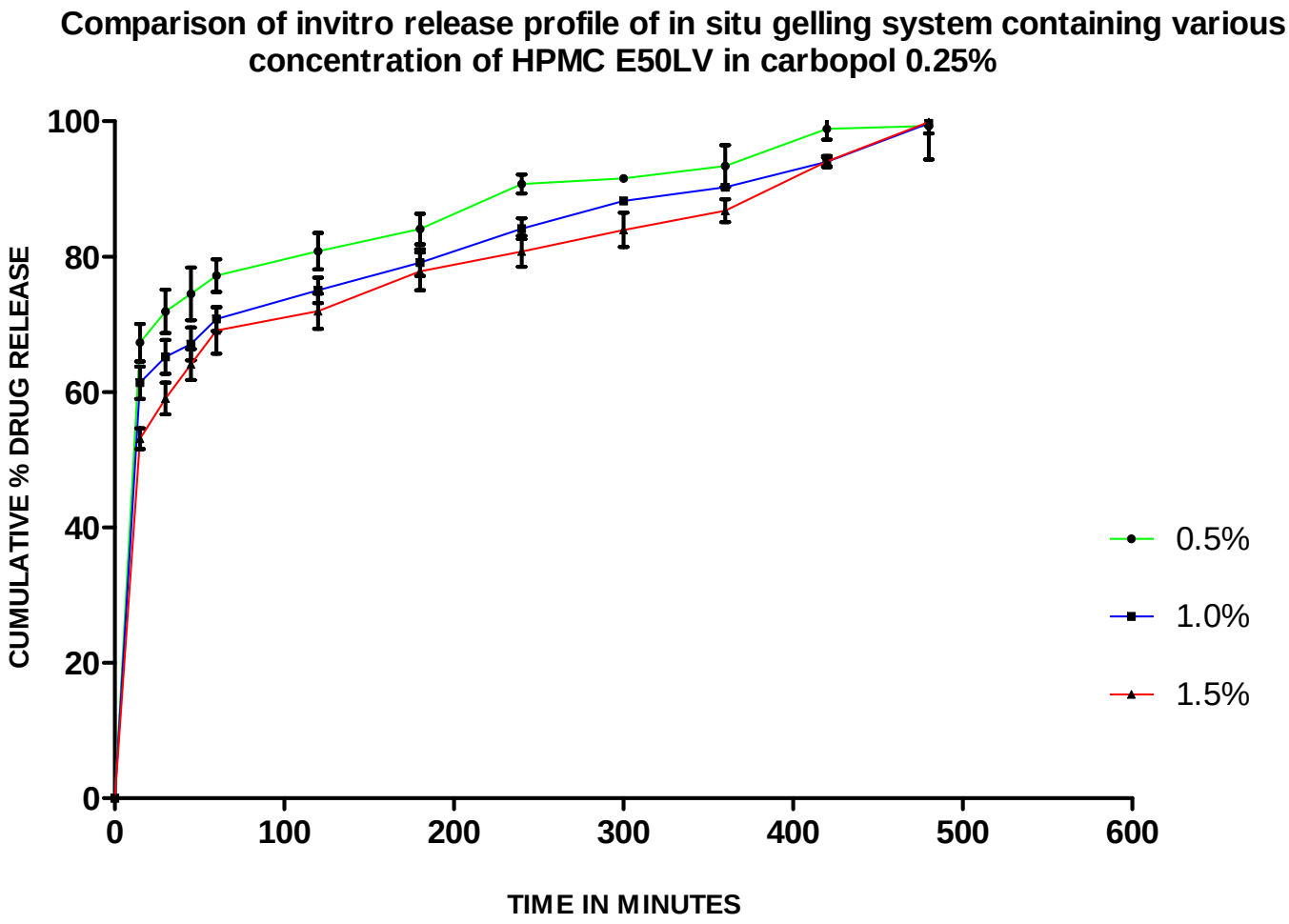


Comparison of invitro release profile of in situ gelling system containing various concentration of HPMC K4M in carbopol 0.25%

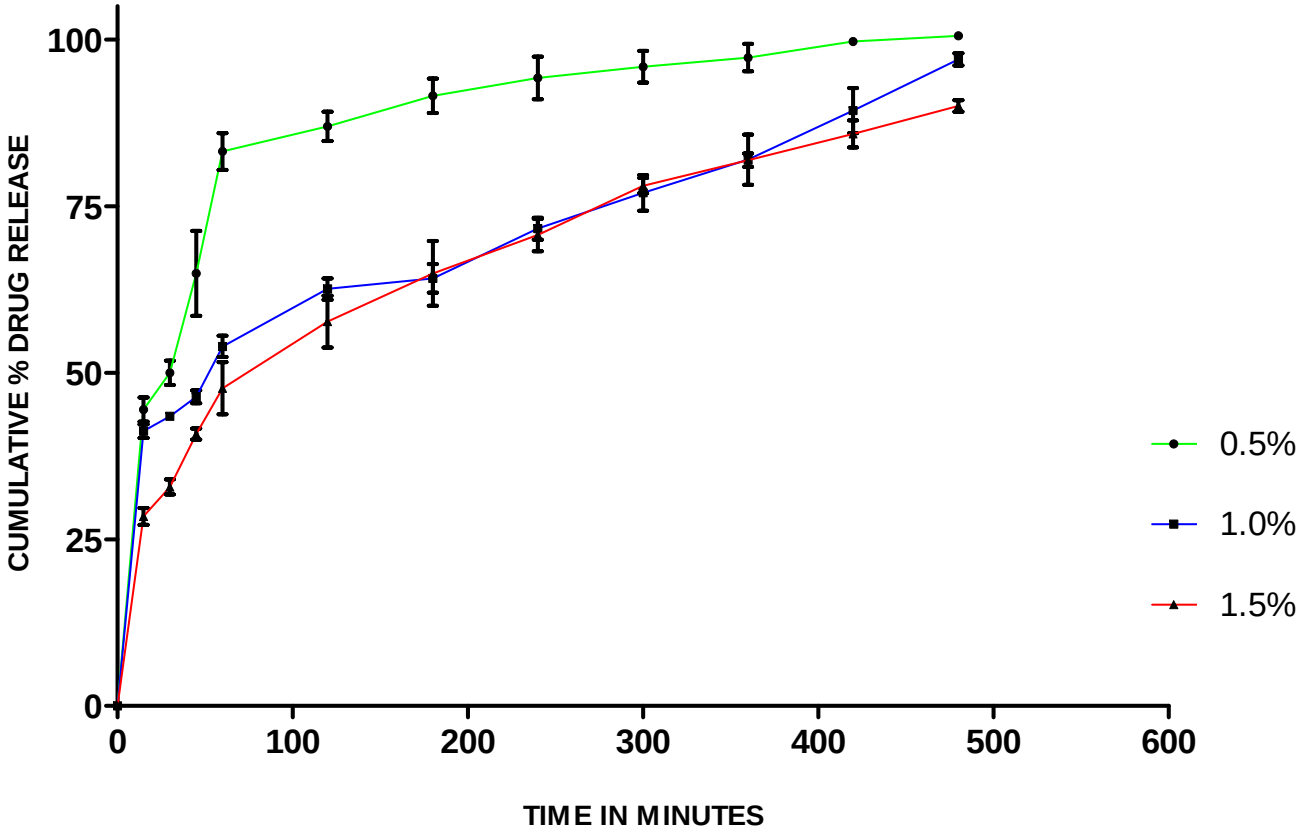


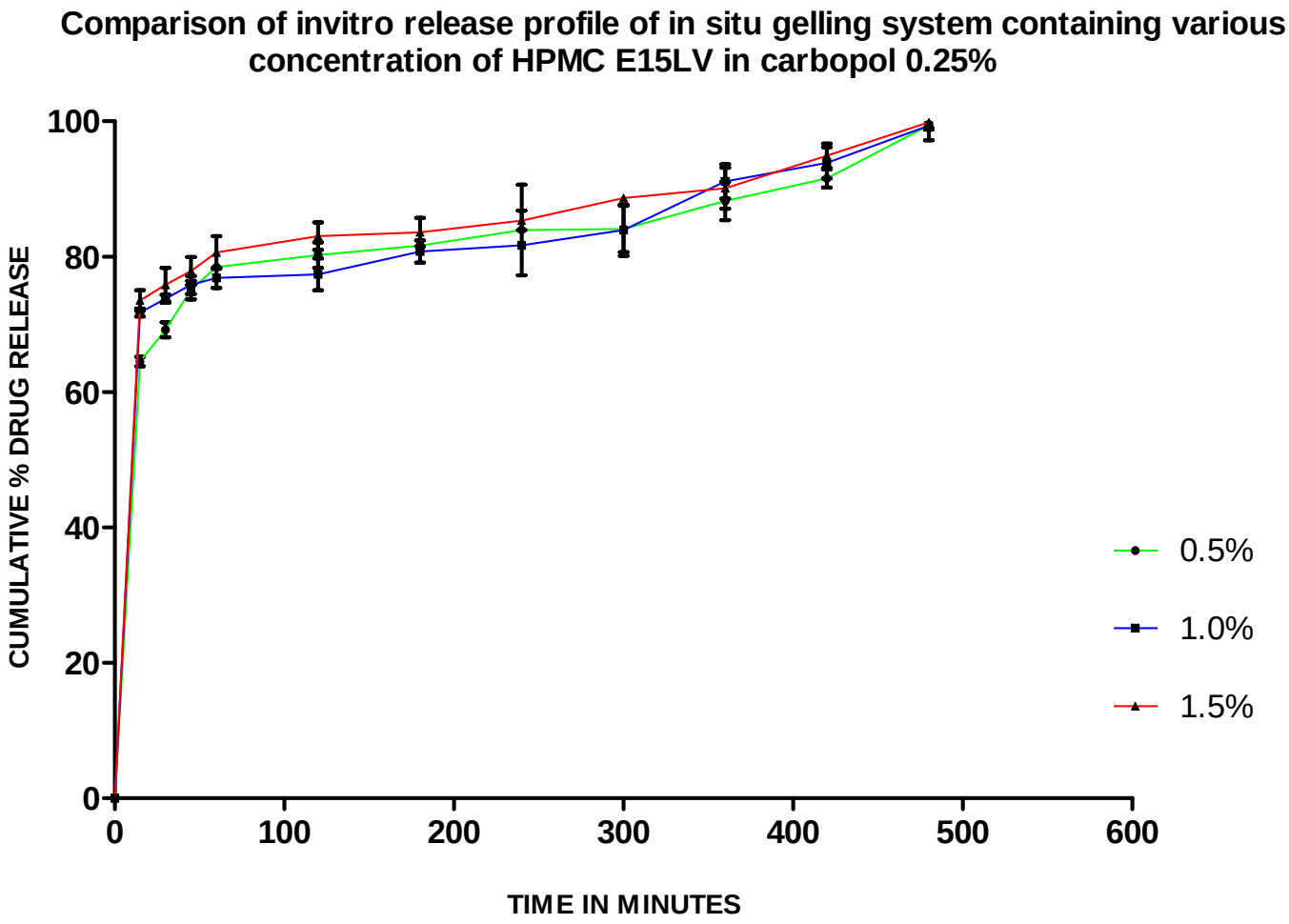
Comparison of invitro release profile of in situ gelling system containing various concentration of HPMC E50LV in carbopol 0.5%



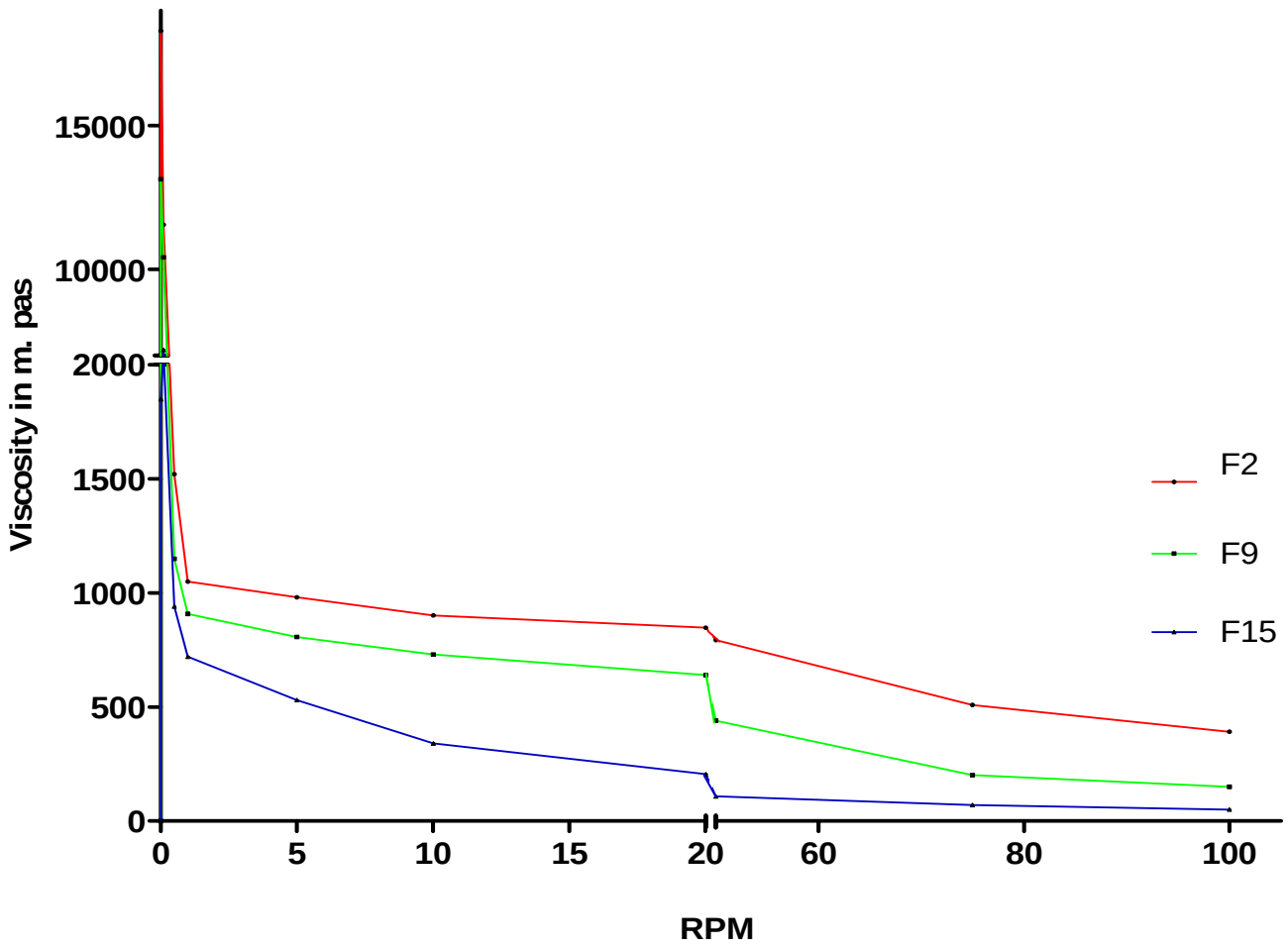

Comparison of invitro release profile of in situ gelling system containing various concentration of HPMC E50LV in carbopol 0.25%
CUMULATIVE % DRUG RELEASE
100
80
60
40
20
0
0
100
200
300
400
500
600
TIME IN MINUTES
0.5%
1.0%
1.5%

Comparison of invitro release profile of in situ gelling system containing various concentration of HPMC E15LV in carbopol 0.5%

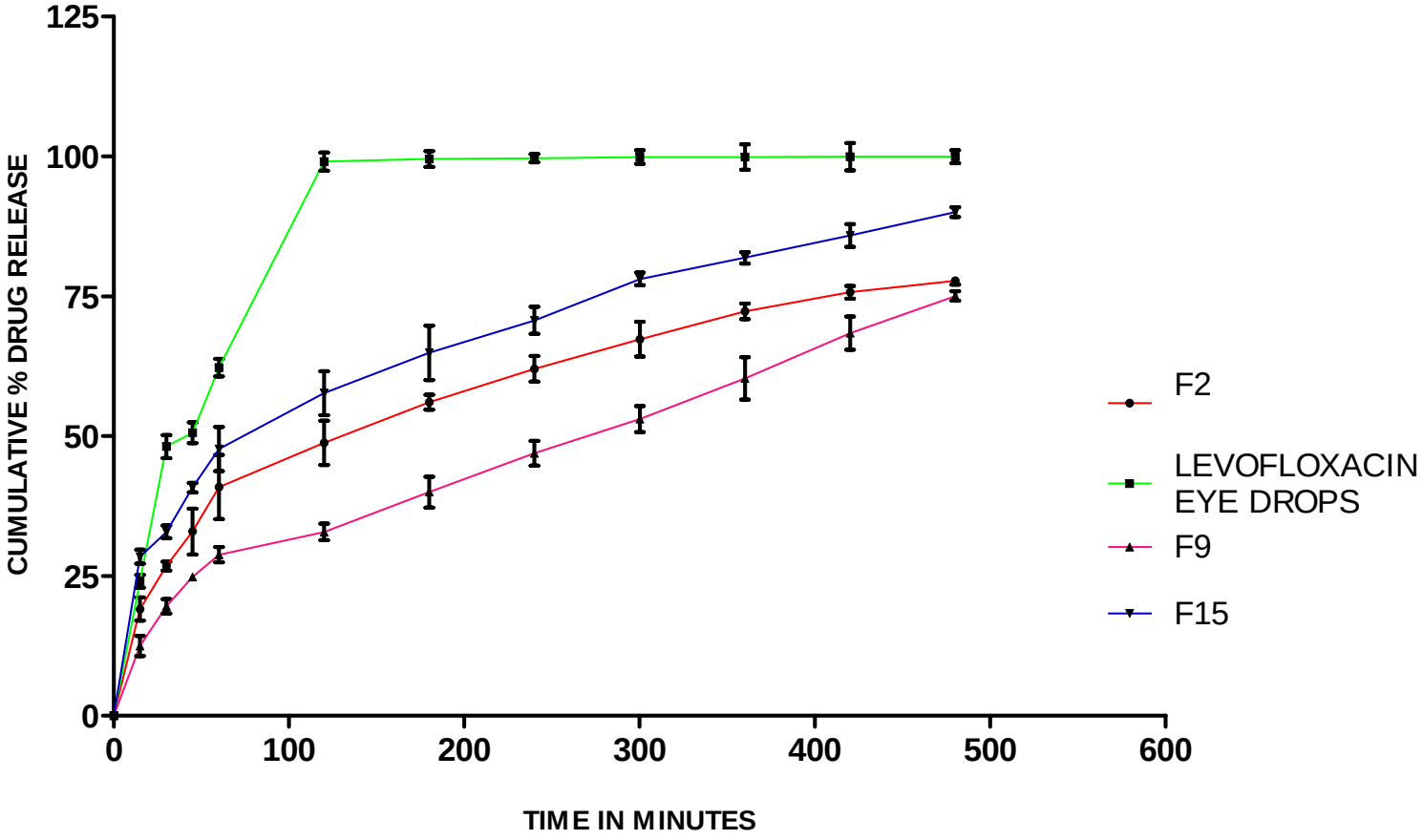


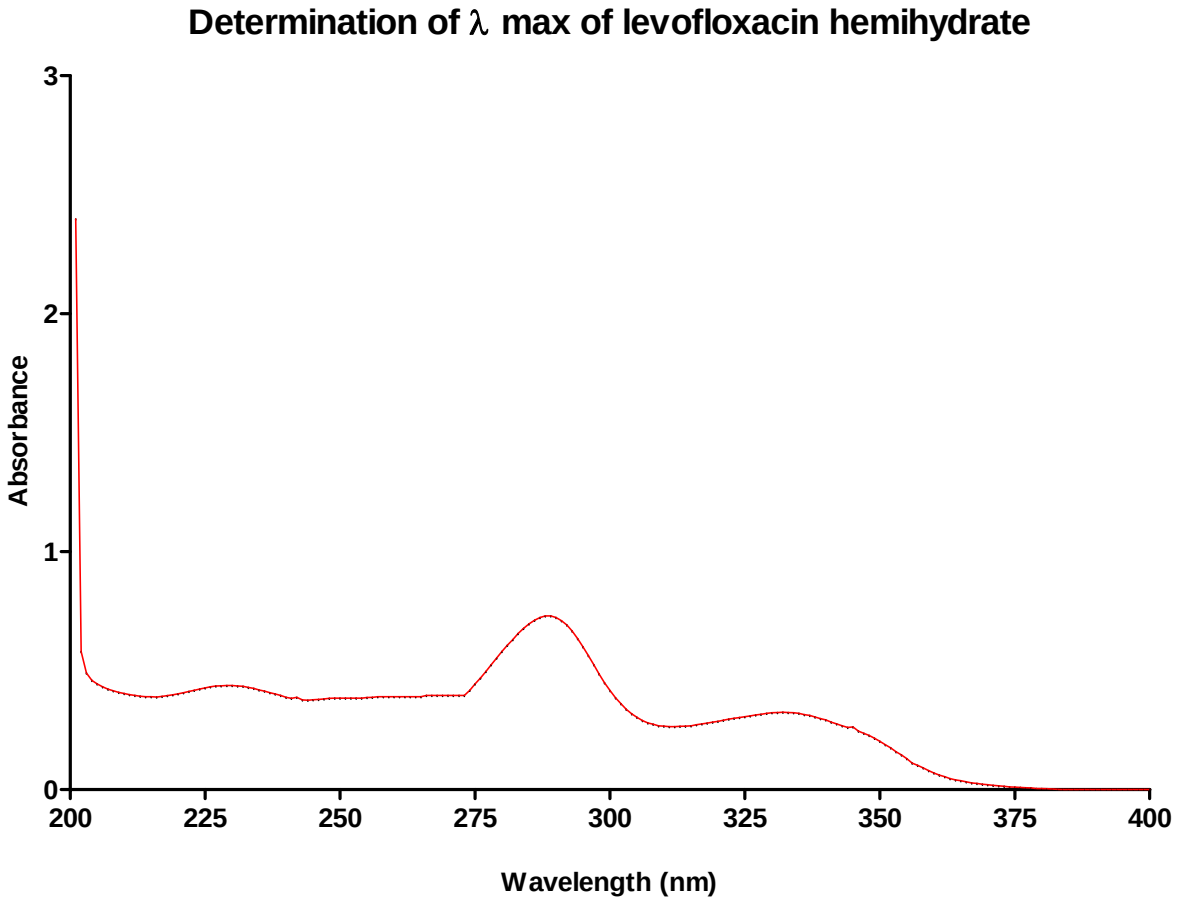

Comparison of invitro release profile of in situ gelling system containing various concentration of HPMC E15LV in carbopol 0.25%
CUMULATIVE % DRUG RELEASE
100
80
60
40
20
0
0
100
200
300
400
500
600
TIME IN MINUTES
0.5%
1.0%
1.5%

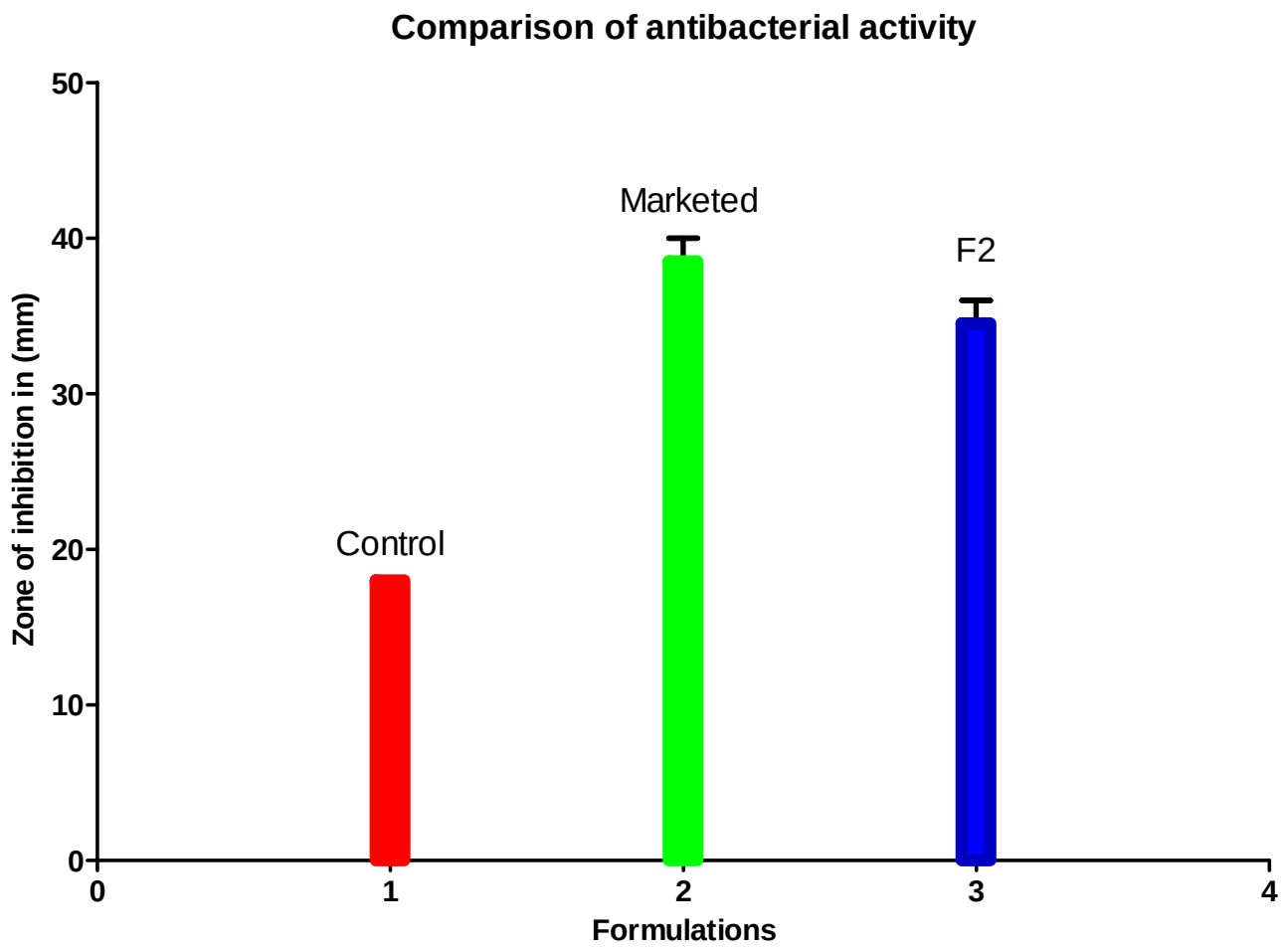
RHEOLOGICAL EVALUATION OF FORMULATIONS



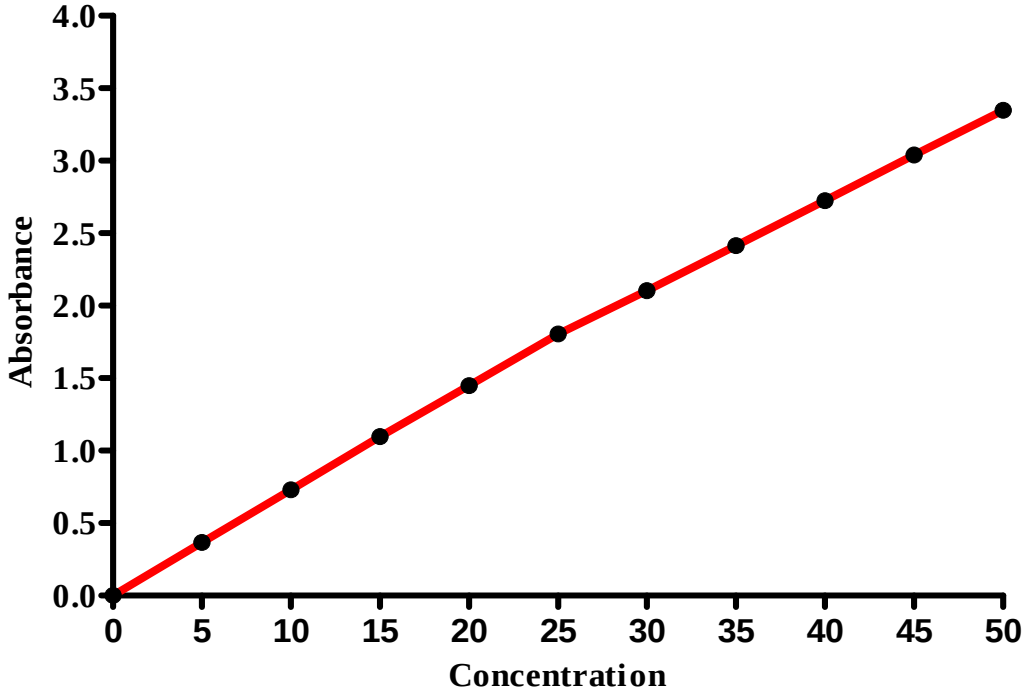
Comparison of in vitro release of selected in situ gel formulations (F2, F9, F15) and marketed levofloxacin eye drops




Determination of λ max of levofloxacin hemihydrate
Absorbance
3
2
1
0
200
225
250
275
300
325
350
375
400
Wavelength (nm)


Comparison of antibacterial activity
Zone of inhibition in (mm)
50
40
30
20
10
0
Control
Marketed
F2
0
1
2
3
4
Formulations

Calibration curve for levofloxacin hemihydrate in simulated tear fluid pH-7.4



CHAPTER-XII

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