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Review Article

A REVIEW ON LEVOFLOXACIN *IN SITU*-GEL FORMULATION

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ABSTRACT

Several *in situ* gelling system have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. These systems consist of polymers that exhibit sol to gel phase tansititions due to change in specific physico chemical parameter (pH, temperature) in their environment, the cul-de-sacin this case. Depending on the method employed to cause sol-to-gel phase transition on the eye surface the following three types of systems are recognized, pH Triggered system Eg: Carbopol, Cellulose acetatephthalate latex, temperature dependant system Eg: pluronics, tetronics, methyl cellulose, ion activated system Eg: Gelrite, Sodium alginate etc. The principal advantage of *in situ* gels is that they can be easily administered with accurate and reproducible dose compared to that of preformed gels and have an advantage that they can be easily instilled in liquid form, and are capable of prolonging the residence time Levofloxacin hemi hydrate is broad spectrum antibacterial drug, which acts by inhibiting bacterial DNAgyrase enzyme which is required for DNA replication, gelrite is an anionic deacetylatedexo cellular polysaccharide secreted by *Pseudomonas elodea*, with a tetrasaccharide repeating unit of one -L-rhamnose,one-D-glucuronic acid and two -D-glucose residues. It has the property of cation-induced and temperature dependent gelation. Exploitation of polymeric Insitu gels for controlled release of various drugs provides a number of advantages over conventional dosage forms. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems. *In situ* activated gel-forming systems seem to be favored as they can be administered in drop form and produce appreciably less inconvenience with vision.

Keywords: Carbopol, Levofloxacin, *In situ* gels.

INTRODUCTION

Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response, because high tear fluid turns over and dynamics cause rapid precorneal elimination of the drug. A high frequency of eye drop instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation is an attempt to overcome bioavailability problem is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolachrymal duct. Various ophthalmic vehicles such as inserts, ointments, suspensions, and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These ocular drug delivery system show ever have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts.

Several *in situ* gelling system have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. These systems consist of polymers that exhibit sol to gel phase transitions due to change in specific physicochemical parameter (pH, temperature) in their environment, the cul-de-sacin this case. Depending on the method employed to cause sol-to-gel phase transition on the eye surface the following three types of systems are recognized, pH.

Triggered system e.g., carbopol, cellulose acetate phthalate latex, temperature dependent system e.g., pluronics, tetronics, methyl cellulose, ion activated system e.g., gelrite, sodium alginate etc.

The principal advantage of *in situ* gels is that they can be easily administered with accurate and reproducible dose compared with that of performed gels and have an advantage that they can be easily instilled in liquid form, and are capable of prolonging the residence time.

Levofloxacin hemihydrate is broad spectrum antibacterial drug, which acts by inhibiting bacterial DNA gyrase enzyme which is required for DNA replication, gelrite is an anionic deacetylatedexo cellular polysaccharide secreted by *Pseudomonas elodea*, with a tetrasaccharide repeating unit of one-L-rhamnose, one-D-glucuronic acid and two-D-glucose residues. It has the property of cation-induced and temperature dependent gelation.

ANATOMY OF HUMAN EYE

The eye is a spherical structure with of three layers; the outer part sclera, the middle parts choroid layer, ciliary body and iris and the inner section nervous tissue layer retina. The sclera is tough fibrous coating that protecting the inner tissues of eye which is white except for the transparent area at the front, and the cornea allows light to enter the eye. The choroid layer, situated located in the sclera, contains many blood vessels that modified at front of the eye as pigmented iris the colored part of the eye (blue, green, brown, hazel, or grey).

THE STRUCTURE OF THE CORNEA

The clear transparent bulge cornea situated at the front of the eye that conveys images to the back of the nervous system. The adult cornea has a radius of approximately 7-8 mm that covers about one-sixth of the total surface area of the eye ball that is a **v**ascular tissue to which provides nutrient and oxygen are supplied via lachrymal fluid and aqueous humor as well as from blood. The cornea is made of five layers as epithelium, bowman's layer, stroma, Descemet's membrane and endothelium that is main pathway of the drug permeation to eye. The epithelium made up of 5-6 layers of cells. The corneal thickness is 0.5-0.7 mm in the central region. The main barrier of drug absorption into the eye is the corneal epithelium, in comparison to many other epithelial tissues (intestinal, nasal, bronchial, and tracheal) that is relatively impermeable. The epithelium is squamous stratified, (5-6 layer of cells) with thickness of around 50-100 μm and turnover of about one cell layer every day. The basal cells are packed with a tight junction, to forming not only an effective barrier to dust particle and most microorganisms, and also for drug absorption.

The transcellular or paracellular pathway is the main pathway to penetrate drug across the corneal epithelium. The lipophilic drugs choose the transcellular route whereas the hydrophilic one chooses paracellular pathway for penetration (passive or altered diffusion through intercellular spaces of the cells). The Bowman's membrane is an acellular homogeneous sheet with 8-14 μm thick situated between the basement membrane of the epithelium and the stroma. The stroma, or substaniapropria, composed of around 90% of the corneal thickness that contains about 85% water and about 200-250 collagenous lamellae. The lamellae provide physical strength while permitting optical transparency of the membrane. The hydrophilic solutes diffuse through the stroma's open structure. The Descemet's membrane is secreted by the endothelium and lies between the stroma and the endothelium.

Conjunctiva

The conjunctiva protects the eye and also involved in the formation and maintenance of the precorneal tear film. The conjunctiva is a thin transparent membrane lies in the inner surface of the eyelids and that is reflected onto the globe. The conjunctiva is made of an epithelium, a highly vascularized substantia propria, and a submucosa. The bulbar epithelium contains 5-7 cell layers. The structure resembles a pallisade and not a pavemente corneal epithelium cells are connected by tight junctions, which render the conjunctiva relatively impermeable. The molecules up to 20,000 Da can cross the conjuctiva, while the cornea is restrict to molecules larger than 5000 Da. The human conjunctiva is about 2 and 30 times more absorption of drugs than the cornea and also proposed that loss of drug by this route is a major path for drug clearance. The highest density of conjunctiva is due the presence of 1.5 million globlet cell varying with age depended among the intersujects variability and age. The vernal conjunctivitis and atopic kerato conjunctivitis occurs due to the great variation in goblet cell density results only in a small difference in tear mucin concentration.

Nasolachrymal drainage system

Nasolachrymal drainage system consists of three parts; the secretory system, the distributive system and the excretory system. The secretory portion is composed of the lacrimal gland that secreted tears are spread over the ocular surface by the eyelids during blinking. The secretory system is stimulated by blinking and temperature change due to the tear evaporation and reflux secretors that have an efferent parasympathetic nerve supply and secrete in response to physical and emotional state e.g. crying. The distributive system consists of the eyelids and the tear meniscus around the lid edges of the open eye, which spread tears over the ocular surface by blinking, thus preventing dry areas from developing. The excretory part of the nasolachrymal drainage system consists of the lachrymal puncta, the superior, inferior and common canaliculi; the lachrymal sac, and then asochrymal duct. In humans, the two puncta are the openings of the lachrymal canaliculi and are situated on an elevated area known as the lachrymal papilla. It is thought that tears are largely absorbed by the mucous membrane that lines the ducts and the lachrymal sac; only a small amount reaches the nasal passage.

Tear film

A thin fluid layer is covered the exposed part of the eye called as precorneal tear film. The film thickness is about 3-10 Am depending on the measurement method with the resident volume approximately 10 μl. The osmolality of the tear fluid is approx. 310-350 mOsm/kg in normal eyes and is maintained by the monovalent and divalent inorganic ions present in fluid such as Na+, K+, Cl−, HCO_3−, and proteins. The mean pH of normal tears is about 7.4. Diurnal patterns of pH changes the pH of tear, which a general shift from acid to alkaline during the day. The buffer capacity of the tears fluid is determined by bicarbonate ions, proteins, and mucins.

Mechanism of drug release

The mechanism of controlled drug release into the eye is as follows:

A. Diffusion
B. Osmosis
C. Bio-erosion.

Diffusion

In the diffusion mechanism, the drug is released continuously at a controlled rate through the membrane into the tear fluid. If the insert is formed of a solid non-erodible body with pores and dispersed drug. The release of drug can take place via diffusion through the pores. Controlled release can be further regulated by gradual dissolution of solid dispersed drug within this matrix as a result of inward diffusion of aqueous solutions.

In a soluble device, true dissolution occurs mainly through polymer swelling. In swelling-controlled devices, the active agent is homogeneously dispersed in a glassy polymer. Since glassy polymers are essentially drug-impermeable, no diffusion through the dry matrix occurs. When the insert is placed in the eye, water from the tear fluid begins to penetrate the matrix, then swelling and consequently polymer chain relaxation and drug diffusion take place. The dissolution of the matrix, which follows the swelling process, depends on polymer structure: Linear amorphous polymers dissolve much faster than cross-linked or partially crystalline polymers.

Osmosis

In the osmosis mechanism, the insert comprises a transverse impermeable elastic membrane dividing the interior of the insert into a first compartment and a second compartment; the first compartment is bounded by a semi-permeable membrane and the impermeable elastic membrane, and the second compartment is bounded by an impermeable material and the elastic membrane. There is a drug release aperture in the impermeable wall of the insert. The first compartment contains a solute, which cannot pass through the semi-permeable membrane and the second compartment provides a reservoir for the drug, which again is in liquid or gel form. When the insert is placed in the aqueous environment of the eye, water diffuses into the first compartment and stretches the elastic membrane to expand the first compartment and contract the second compartment so that the drug is forced through the drug release aperture.

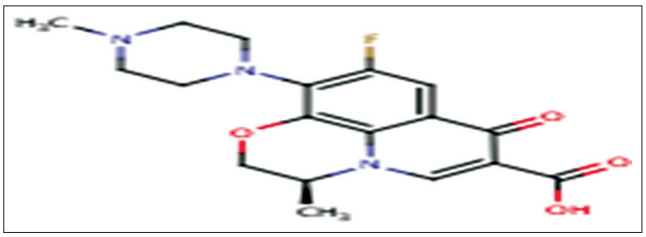
Bioerosion

In the bioerosion mechanism, the configuration of the body of the insert is constituted from a matrix of bioerodible material in which the drug is dispersed. Contact of the insert with tear fluid results in controlled sustained release of the drug by bioerosion of the matrix. The drug may be dispersed uniformly throughout the matrix, but it is believed a more controlled release is obtained if the drug is superficially concentrated in the matrix.

In truly erodible or E-type devices, the rate of drug release is controlled by a chemical or enzymatic hydrolytic reaction that leads to polymer solubilization, or degradation to smaller, water-soluble molecules. These polymers, as specified by Heller, may undergo bulk or surface hydrolysis. Erodible inserts undergoing surface hydrolysis can display zero order release kinetics; provided that the devices maintain a constant surface geometry and that the drug is poorly water-soluble.

Drug profile		
1	Name	Levofloxacin
2	Lambda max	293 nm
3	Solubility	Water, methanol
4	Ph	6.7-7.2
5	Bioavailability	80-90%
6	Half-life	12 hrs
7	Protein binding	30-50%
8	Molecular weight	361.367

Molecular structure



Levofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe or life-threatening bacterial infections or bacterial infections that have failed to respond to other antibiotic classes. It is sold under various brand names, such as levaquin and tavanic, the most common. In the form of ophthalmic solutions it is known as oftaquix, quixin and iquix.

Levofloxacin is associated with a number of serious and life-threatening adverse reactions as well as spontaneous tendon ruptures and irreversible peripheral neuropathy. Such reactions may manifest long after therapy had been completed and in severe cases may result in life-long disabilities. Hepatoxicity has also been reported with the use of levofloxacin.

Levofloxacin is used to treat a number of infections, including: Respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, endocarditis, meningitis, pelvic inflammatory disease, and traveler's diarrhea.

Serious adverse events occur more commonly with fluoroquinolones than with any other antibiotic drug classes. In most adverse reactions are mild to moderate; however, on occasion, serious adverse effects occur. There have been a number of regulatory actions taken as a result of such adverse reactions, which included published warnings, additional warnings and safety information added to the package inserts, which includes Black Box Warnings together with the issuance of "Dear Doctor Letters" concerning the recent addition of the Black Box Warnings.

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Children and the elderly are at a much greater risk of experiencing such adverse reactions. Such reactions may manifest during, as well as long after fluoroquinolone therapy had been discontinued.

MECHANISM OF ACTION

Levofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a Type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division.

The fluoroquinolones interfere with DNA replication by inhibiting an enzyme complex called DNA gyrase. This can also affect mammalian cell replication. In particular, some congeners of this drug family display high activity not only against bacterial topoisomerases but also against eukaryotic topoisomerases, and are toxic to culture mammalian cells and *in vivo* tumor models. Although the quinolone is highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. Quinolone-induced DNA damage was first reported in 1986.

Recent studies have demonstrated a correlation between mammalian cell cytotoxicity of the quinolones and the induction of micronuclei. As such some fluoroquinolones may cause injury to the chromosome of eukaryotic cells.

There continues to be a debate as to whether or not this DNA damage is to be considered one of the mechanisms of action concerning the severe and non-abating adverse reactions experienced by some patients following fluoroquinolone therapy.

PREFORMULATION STUDIES

Instrument for the standard curve

Ultraviolet (UV)-Spectophotometer set at lamda max. 293 nm and sample were recorded in 10 mm quartz cell.

Preparation of standard solution

Solution was prepared by dissolving 150 µg drug in 150 ml water (levofloxacin) in a volumetric flask of concentration 150 µg/ml.

Determination of maximum absorption

- From the standard solution (150.0 µg/ml) approximately, 3.0 ml was taken and scanned from 200 to 400 nm with UV-Visible spectrophotometer.
- The water was used as blank.
- Levofloxacin presented maximum absorption at 293 nm.

Calibration curve

- The calibration curve was constructed by analyzing 10 different concentrations of standard solution, prepared on the same day
- The range of solutions varied from 2.0 to 20.0 µg/ml
- All determinations were conducted in triplicate.

METHODOLOGY

1. Preparation of *in situ* gel
   - Polymer solution was prepared by dispersing gelrite deionized water by heating up to 90°C for 20 minutes followed by cooling to room temperature
   - Drug solution was prepared by dissolving levofloxacin hemihydrates in mixture of propylene glycol and water (1:0.08)
   - Drug solution was mixed with a polymer solution using a magnetic stirrer
   - Benzalkonium chloride was added which acts as a preservative
   - The prepared *in situ* gels were filled in glass vials closed with rubber closures and sealed with aluminum caps
   - And sterilized by autoclave at 121°C for 20 minutes.
2. Formulations table of *in situ* gel preparation.

EVALUATION PARAMETERS

Appearance and homogeneity

Formulations were examined visually for color and clarity against white background and for the presence of particulate matter any if present.

pH and gelation studies

pH was determined by using pH meter. Gelling capacity of formulations was evaluated in order to identify the formulations suitable for use as *in situ* gelling systems. Gelling was determined by mixing the formulation with simulated tear fluid in the proportion 25:7 and examined visually.

Note: **++** gelation immediate and remains for few hours; **+++** shows gelation immediate and remains for extended period.

Viscosity measurement

The viscosity of formulated *in situ* gel was determined. The determination aswas carried out on brook-field viscometer using spindle S-04 and the determination was carried out and reading is recorded. The results were shown.

Spreading coefficient

Spreading coefficient was determined by apparatus. A ground glass slide was fixed on the wooden block. An excess of emulgel (about 2 g) under study was placed on this ground slide. Gel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 1 g was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of gel between the two slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. The time (in seconds) required by the top slide to separate from ground slide was noted. A shorter interval indicates better spreading coefficient.

It is calculated by using the formula:

$$S=M.L/T$$

Where, M=Weight tied to upper slide
L=Length of glass slides
T=Time taken to separate the slides.

Composition of simulated tear fluid

1. Sodium chloride: 0.670 g
2. Sodium bicarbonate: 0.200 g
3. Calcium chloride dihydrate: 0.08 g
4. Deionized water: 100 g.

***In vitro* drug release**

- *In vitro* release, studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell)
- *In vitro* release of levofloxacin, hemihydrates were carried out in formulations with different concentrations of gelrite using cellophane membrane
- The diffusion medium 100 ml of simulated tear fluid stirred at 50 rpm at 370°C±0.50°C
- One end of the diffusion tube was covered with a cellophane membranes
- The 1 ml formulation was spread on the cellophane membrane, and membrane was placed such that it just touches the diffusion medium (simulated tear fluid [STF]) present in the receptor compartment
- The drug samples were withdrawn at the interval of one hour for the period of 8 hrs from diffusion medium and analyzed by a UV-spectrophotometer at 287.5 nm using simulated tear fluid as blank.

Drug content

- The vials containing formulation were properly shaken for 2-3 minutes
- One ml of the formulation was transferred into 100 ml volumetric flask with 1 ml calibrated graduated pipette
- 50 ml of simulated tear fluid with pH 7.4 was added gel was completely crushed with the help of glass road, followed by vigorous shaking until the formed gel gets completely dispersed to give a clear solution
- Final volume was adjusted to 100 ml with slandered solution, aliquot of 1 ml was taken and further diluted to 10 ml with STF
- Obtained solution was filtered through 0.45 micron filter membrane, and the drug concentration was determined by UV-visible spectrophotometer at 287.5 nm.

Stability study

Stability study was performed on F2 formulation. The preparations were packed in collapsible aluminum tubes (5 g) and subjected to stability studies at 40°C/75% RH, for a period of 3 months. Samples were withdrawn at interval of 45-days and were evaluated for physical appearance, rheological properties, and drug content. All the test results were found to be in limits. Hence, the formulations were stable understated storage condition.

CONCLUSION

The primary requirement of a successful controlled release product focuses on increasing patient compliance, which the *in situ* gels offer. Exploitation of polymeric *in situ* gels for controlled release of various drugs provides a number of advantages over conventional dosage forms. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug delivery systems. *In situ* activated gel-forming systems seem to be favored as they can be administered in drop form and produce appreciably less inconvenience with vision. Moreover, they provide better sustained release properties than drops. The prepared *in situ* gel formulations were subjected to stability studies as per ICH guidelines for the period of 3 month. The formulation was found to be satisfactory results. The physical parameter pH, appearance, homogeneity, viscosity, spreadibility and the % drug content were found to be satisfactory. The % drug content was found to be 72.0. The pH was 7.25, color was off-white semisolid gel, viscosity was 32,727 cps, and spreadibility was 9.12. This type of dosage forms are used now a day in combat glaucoma, dry eye syndrome, Sjogren's syndrome, age-related macular degeneration, trachoma etc. It was conclude that levofloxacin hemihydrates *in situ* gel can be formulated for the treatment of various eye diseases.

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