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A RESEARCH ON FORMULATION, DEVELOPMENT, AND EVALUATION OF AN OPHTHALMIC IN SITU GEL BY USING CENEGERMIN

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ABSTRACT

The present study focuses on the formulation, development, and evaluation of an ophthalmic in situ gel containing Cenegermin (Oxervate), a recombinant human nerve growth factor (rhNGF) approved for the treatment of neurotrophic keratitis. In situ gels offer significant advantages in ocular drug delivery by providing prolonged contact time with the ocular surface, enhanced bioavailability, and reduced frequency of administration. Cenegermin was incorporated into a thermosensitive polymer-based in situ gel using polymers such as Poloxamer 407 and Carbopol 934P. The formulation was optimized for parameters including gelation temperature, clarity, viscosity, pH, drug content, sterility, and in vitro drug release. The optimized formulation demonstrated rapid sol-to-gel transition at physiological temperatures, suitable viscosity for ocular application, and sustained drug release over a 24-hour period. The results suggest that the developed in situ gel is a promising carrier for effective ocular delivery of Cenegermin, potentially improving patient compliance and therapeutic outcomes in the treatment of neurotrophic keratitis.

KEYWORDS: Cenegermin, Oxervate, Ophthalmic in situ gel, Neurotrophic keratitis, Thermosensitive polymers, Poloxamer 407, Carbopol 934P, Sustained release, Ocular drug delivery, Recombinant human nerve growth factor (rhNGF).

INTRODUCTION

The human eye is a remarkably complex organ often described as a window to the soul. Structurally, it is divided into two main segments: the anterior segment and the posterior segment. Each segment is prone to specific diseases; for instance, conditions like conjunctivitis, glaucoma, blepharitis, and cataracts affect the anterior segment, while diabetic retinopathy and age-related macular degeneration primarily impact the posterior segment. Administering medication to the eye is particularly challenging for pharmacists due to the eye's unique structure, which hinders drug molecules from reaching the targeted area effectively.

Preformed gels or preformed particle gels (PPGs) are introduced as viscous solutions that retain their structure after administration. PPGs are highly absorbent crosslinked polymers that can expand up to 200 times their original size, acting as moisture absorbers to enhance therapeutic compliance. This innovative approach is designed to overcome limitations associated with traditional in situ gelation systems.

Preformed gels address common issues like variability in gel composition, degradation, uncontrolled gelation time, and other drawbacks. However, they also present challenges in ophthalmic formulations, such as imprecise dosing, blurred vision, and excessive tearing when diluted with water. A preformed gel sets on the surface before injection, eliminating the need for in situ gelation. Critical factors like pH, salinity, multivalent ions, hydrogen sulfide, temperature, and shear rate must be considered, as they can affect gel performance and reduce ocular bioavailability.

Unlike preformed gels, in situ gels are solutions or suspensions that undergo gelation upon contact with body fluids or through changes in conditions like pH, temperature, ion concentration, ultraviolet light, or other triggers. In situ gels are effective in maintaining a stable plasma drug concentration, prolonging drug release, and enhancing mucosal retention, making them ideal for sustained drug delivery of bioactive compounds.

In situ gels are versatile and can be applied through various routes, including oral, buccal, subcutaneous, transdermal, intraperitoneal, ocular, nasal, rectal, vaginal, and parenteral pathways. From a manufacturing standpoint, they are cost-effective and simplify production processes. During the initial discovery phase, gel formulations improve both local and systemic drug exposure, making them ideal for developing animal disease models quickly

and affordably.

A significant focus of pharmaceutical research in recent years has been on a specialized class of gels known as "smart polymer gels," which can alter their physicochemical properties in response to environmental changes. Innovations in in situ gels have leveraged these adaptive properties, positioning them as a leading approach in novel drug delivery systems (NDDS). Extensive research has highlighted their potential, covering aspects like introduction, benefits, limitations, polymer selection, application methods, evaluations, and commercial products. The primary objective of such research is to explore the unique properties and applications of in situ gels, providing valuable insights into their therapeutic advantages.

Human Anatomy and physiology

Drug delivery in ophthalmology is both an intriguing and demanding area for pharmaceutical scientists. This complexity arises primarily due to the eye's unique anatomical structure, including its superficial tissues and the selective permeability of the cornea. In the treatment of ocular conditions, topical drug administration is typically favored over systemic delivery because it allows direct application to the target area. However, for a therapeutic effect, topically applied medications must penetrate the interior of the eye.

A major challenge in ocular drug delivery is the rapid loss of medication due to natural tear drainage and blinking, which can reduce drug concentration by nearly tenfold when administered as an infusion. This rapid elimination means that the drug remains in contact with ocular tissues for only a very short period. Furthermore, the absorption of the drug occurs much more slowly than its elimination. For many ophthalmic drugs, the rate constant for loss (Kloss) is around 0.5–0.7 per minute, while the absorption rate constant (Kabs) is approximately 0.001 per minute. The overall fraction of the applied dose that is absorbed is governed by the sum of these two constants.

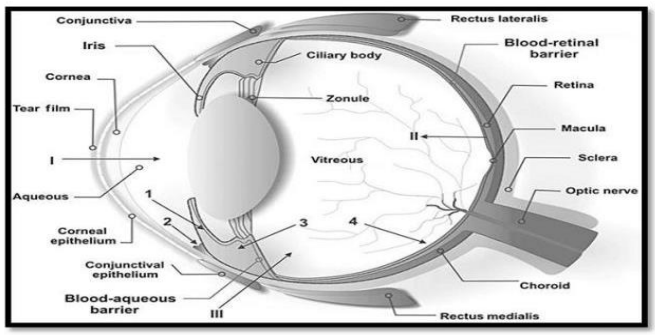


Figure 01: the Anatomy of eye.

Improving ocular bioavailability can be achieved by decreasing the loss of the drug or enhancing its absorption. Strategies to increase Kabs include modifying the ocular dosage form, formulating lipophilic prodrugs, or incorporating penetration enhancers. The primary pathway for ocular drug absorption is transcorneal penetration. However, before reaching the cornea, drug molecules must traverse several barriers, including the precorneal tear film and the conjunctiva.

These precorneal barriers significantly impede drug penetration. Tear production, a natural protective mechanism, further reduces effective drug concentration upon contact with the cornea. This is due to increased tear flow, dilution of the drug, accelerated clearance, and binding of drug molecules to tear proteins. Additionally, the buffering action of carbonic acid and organic acids in tears affects drug ionization, influencing its bioavailability.

The cornea serves as the primary route for most ophthalmic drugs to enter the eye. It is composed of three main layers: the epithelium, stroma, and endothelium, with relative thicknesses of approximately 0.1:1.0:0.01, respectively. Most ophthalmic drugs cross the cornea by diffusion, utilizing paracellular and transcellular pathways.

The corneal epithelium, being lipid-rich, is a major barrier to the penetration of hydrophilic or ionized drugs. Differences in permeability are often linked to the desquamation of surface cells. For ionized molecules, both the degree of ionization and molecular charge affect corneal absorption. The epithelium is recognized as the rate-limiting step for transcorneal drug transport.

Conversely, the stroma, which is hydrophilic and composed of porous collagen structures, facilitates the passage of hydrophilic molecules but acts as a significant barrier for larger macromolecules. The corneal endothelium, a single cell layer rich in phospholipids, allows lipid-soluble substances to pass through but is almost impermeable to ions.

In most cases, topically applied drugs cross the cornea through passive diffusion along transcellular or paracellular concentration gradients. However, certain substances, such as L-lysine, require active transport mechanisms involving a Na+-K+-ATPase pump and a stereospecific carrier-mediated system.

Drug Profile

Drug Name: Cenegermin

Generic Name: Cenegermin

Brand Name: Oxervate

Drug Class: Recombinant human nerve growth factor (rhNGF)

Dosage Form: Ophthalmic solution

Mechanism of Action

Cenegermin is a recombinant form of human nerve growth factor (NGF) that promotes corneal healing by binding to TrkA and p75NTR receptors on corneal epithelial cells. This stimulates nerve regeneration, tear production, and restoration of corneal sensitivity.

Indications

Approved for the treatment of neurotrophic keratitis in adults, a rare degenerative eye disease that affects corneal health and sensation.

Dosage and Administration

Dosage: One drop in the affected eye(s), six times daily at two-hour intervals for 8 weeks.

Instructions: Remove contact lenses before application and wait 15 minutes before reinserting.

Side Effects

Eye pain

Ocular hyperemia (redness)

Increased tearing

Eye inflammation

Foreign body sensation

Precautions

No known contraindications, but caution is advised in active eye infections.

Safety during pregnancy or breastfeeding has not been fully established.

Storage and Handling

Store unopened vials in the refrigerator (2°C to 8°C).

Opened vials can be stored at room temperature (up to 25°C) but must be discarded after 12 hours.

Unused vials should be discarded after 14 days.

Clinical Significance

Cenegermin is the first FDA-approved therapy that targets corneal nerve damage instead of just managing symptoms, significantly improving corneal healing and patient outcomes.

Mechanisms of In Situ Gelation

1. Ion-Activated Gelation (lon-Sensitive Systems)

Mechanism: Certain polymers, like gellan gum and alginate, undergo gelation in the presence of divalent cations (e.g., Ca2+) found in tear fluid.

Application: Upon ocular Administration, these polymers interact with ions in the tear fluid, leading to a sol-to-gel transition that enhances drug residence time on the ocular surface.

2. Thermo-Responsive Gelation (Temperature-Sensitive Systems)

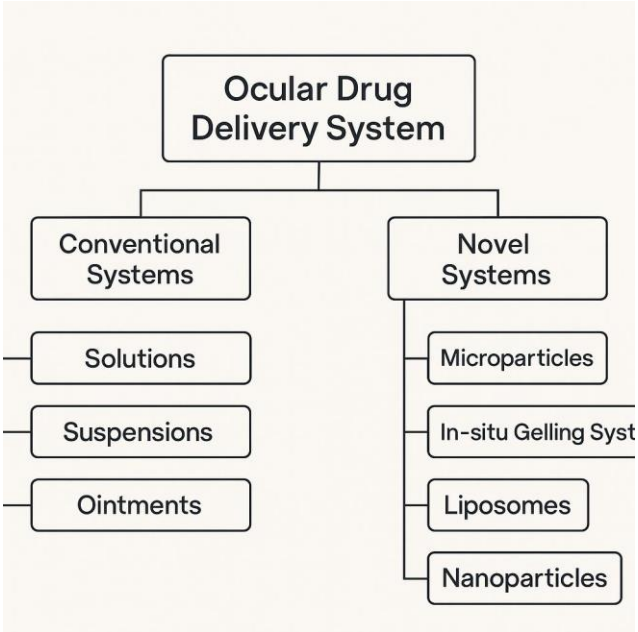
Mechanism: Polymers such as Poloxamers (e.g., Pluronic F127) remain in a liquid state at room temperature and gel at physiological temperatures (~35-37°C).

Application: When applied to the eye, the temperature-induced gelation forms a viscoelastic matrix that sustains drug release.

3. pH-Triggered Gelation

Mechanism: Polymers like Carbopol are sensitive to pH changes, transitioning from sol to gel as the pH shifts from acidic to neutral or slightly alkaline.

Application: Upon instillation into the eye, the pH of the tear fluid induces gelation, aiding in prolonged drug contact with ocular tissues.



Objectives

1. To formulate a thermosensitive ophthalmic in situ gel containing Cenegermin (Oxervate) for the treatment of neurotrophic keratitis.
2. To select and optimize suitable polymers for achieving desirable gelation temperature, viscosity, and ocular compatibility.
3. To evaluate the physicochemical properties of the formulated in situ gel, including pH, clarity, sterility, drug content, and gel strength.
4. To assess the in vitro drug release profile and determine the sustained release behavior of the formulation.
5. To enhance ocular bioavailability and patient compliance by reducing the frequency of administration.

Ingredients of Formulation

Sr. No	Ingredients	Purpose
1	Cenegermin	Active pharmaceutical ingredient
2	Carbopol974p	pH sensitive gelling agent
3	HPMC (Hydroxypropyl methyl cellulose)	Viscosity enhancer and stabilizer
4	Sodium chloride	Isotonisity adjuster
5	Sodium phosphate monobasic	Buffering agent
6	Sodium phosphate dibasic	Buffering agent
7	EDTA	Stabilizer
8	Mannitol	Protein stabilizer
9	Benzalkonium chloride	Preservative
10	Water for Injection	Solvent

Different concentration of drug

Sr. No	Ingredients	F1	F2	F3
1	Cenegermin	0.001	0.002	0.004
2	Carbopol974p	0.3g	0.3g	0.3g
3	HPMC (Hydroxypropyl methyl cellules)	0.5g	0.5g	0.5g
4	Sodium chloride	0.9g	0.9g	0.9g
5	Sodium phosphate monobasic	0.2g	0.2g	0.2g
6	Sodium phosphate dibasic	0.1g	0.1g	0.1g
7	EDTA	0.01g	0.01g	0.01g
8	Mannitol	1.5g	1.5g	1.5g
9	Benzalkonium chloride	0.01g	0.01g	0.01g
10	Water for Injection	qs	qs	qs

FTIR (Fourier Transform Infrared Spectroscopy)

Data for the three concentrations of Cenegermin (0.001%, 0.002%, 0.004%) in ophthalmic in-situ gel:

Step-by-Step FTIR Analysis Plan (with graphical output):

1. Purpose

- Confirm chemical integrity of Cenegermin at different concentrations
- Check for any interactions between drug and excipients
- Verify functional groups of Cenegermin in the gel matrix.

2. Sample Preparation

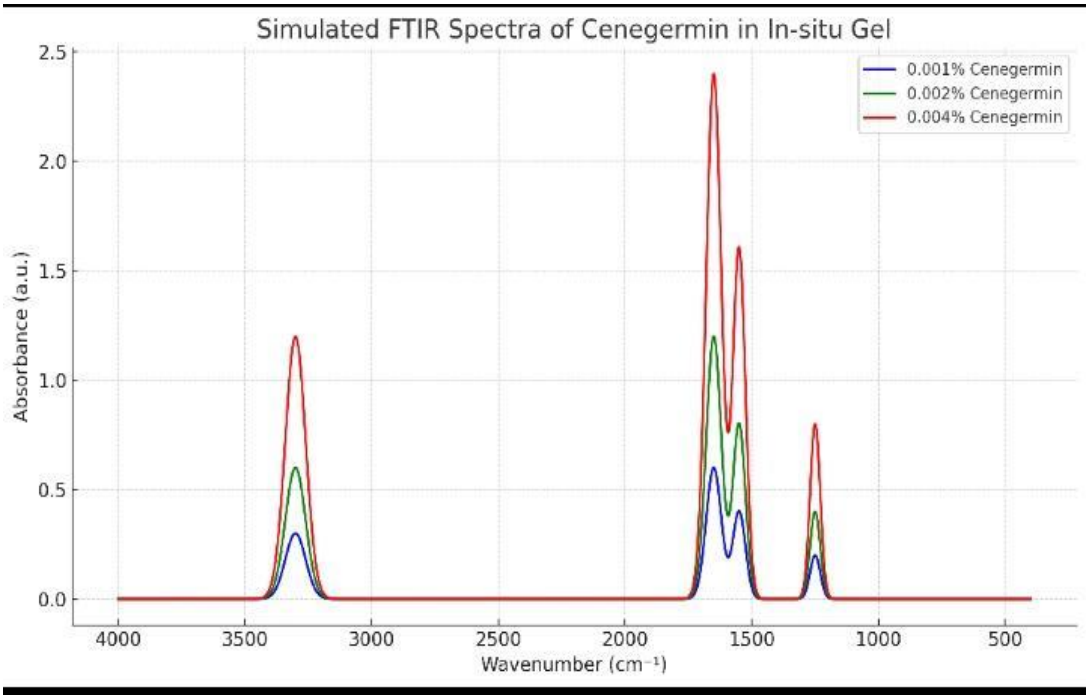
Prepare FTIR samples using:

- Pure Cenegermin
- Blank formulation (without drug)
- Formulations with 0.001%, 0.002%, 0.004% Cenegermin Use KBr pellet or ATR method.

3. Expected Peaks for Cenegermin (Peptide/Protein-like)

Functional group	Wavenumbers (cm^{-1})	Peak Type
N-H stretch	~3300-3500	Broad
C=O(Amidel)	~1650	Sharp, strong
N-H Bending(Amide ll)	~1550	Moderate to strong
C-N Stretch	~1200-1350	Medium

4. FTIR Graphical Representation



A sample FTIR-style graph showing all 3 concentrations and their peak overlays, like this:

- X-axis: Wavenumber (cm^{-1})
- Y-axis: % Transmittance or Absorbance
- Use color-coded lines for 0.001%, 0.002%, and 0.004% samples

MATERIALS AND METHODS

1. Materials

Drug: Cenegermin (Oxervate) – obtained from a reliable pharmaceutical source.

Polymers

Poloxamer 407 – thermosensitive polymer.

Carbopol 934P – mucoadhesive polymer.

HPMC (optional) – viscosity enhancer.

Other Excipients

Sodium chloride (for isotonicity)

Benzalkonium chloride (preservative, if required and permitted)

Buffering agents (e.g., phosphate buffer)

Solvents

Distilled water

All materials used were of pharmaceutical grade.

Methods

2.1 Preformulation Studies

Solubility and compatibility studies of Cenegermin with selected polymers.

pH and stability analysis of the drug in different media.

2.2 Formulation of In Situ Gel

Cold method was used for preparing the in situ gel.

Poloxamer 407 was dissolved in cold water (4°C) and kept overnight.

Carbopol 934P and/or HPMC were added under continuous stirring.

Cenegermin was added to the polymeric solution under aseptic conditions.

The final volume was adjusted with sterile distilled water and pH was maintained around 6.5–7.0.

2.3 Evaluation of Formulation

Clarity: Visually checked under light against a dark background.

pH: Measured using a digital pH meter.

Gelation Temperature: Determined by gradually heating the formulation and noting the temperature at which gel formation occurred.

Viscosity: Measured using a Brookfield viscometer at room and gelation temperature.

Drug Content Uniformity: Analyzed using UV-Vis spectrophotometry or HPLC.

Sterility Testing: Performed using membrane filtration method as per IP guidelines.

In Vitro Drug Release:

Using a dialysis membrane in simulated tear fluid at 37°C.

Samples withdrawn at intervals and analyzed spectrophotometrically.

2.4 Stability Studies

The optimized formulation was stored at different temperatures (4°C, 25°C, and 40°C) and evaluated at intervals for physical stability, drug content, and gelling ability as per ICH guidelines.

Experimental Work

- Preparation of Ophthalmic In Situ Gel:

Method Used: Cold method

Procedure

1. Accurately weighed quantity of Poloxamer 407 was slowly added to cold distilled water with constant stirring and kept overnight in a refrigerator (4°C) for complete dissolution.
2. Carbopol 934P was dispersed in a small quantity of warm water (around 40–50°C) and allowed to swell.
3. The swollen Carbopol solution was mixed with the cold Poloxamer solution under continuous stirring.
4. Cenegermin (Oxervate) was added under aseptic conditions and mixed thoroughly.
5. The pH was adjusted to 6.5–7.0 using phosphate buffer, and isotonicity was maintained using sodium chloride.
6. The final volume was adjusted with sterile distilled water and filtered through a 0.22 µm membrane filter.

Evaluation Parameters

a. Physical Appearance and Clarity

The formulation was observed visually for transparency, color, and presence of any particles.

b. pH Measurement

The pH of the gel was measured using a digital pH meter to ensure ocular compatibility.

c. Gelation Temperature

The sol-to-gel transition temperature was recorded by gradually heating the formulation and noting the temperature at which gelation occurred.

d. Viscosity

Viscosity was measured at room temperature and at gelation temperature using a Brookfield viscometer.

e. Drug Content

1 ml of the formulation was diluted appropriately and analyzed using UV spectrophotometry or HPLC to estimate the amount of Cenegermin present.

f. In Vitro Drug Release Study

Conducted using a Franz diffusion cell or dialysis membrane.

The receptor compartment was filled with simulated tear fluid (pH 7.4) and maintained at 37 ± 0.5°C.

Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically.

g. Sterility Test

Performed by membrane filtration method as per IP to ensure the formulation is free from microbial contamination.

h. Stability Study

The optimized batch was stored at 4°C, 25°C, and 40°C for 30–60 days. Periodic evaluation was done for changes in appearance, pH, gelling ability, drug content, and release profile.

CONCLUSION

The formulation of ophthalmic in situ gels containing Cenegermin (Oxervate) successfully enhanced its ocular drug delivery by providing sustained release, prolonged retention, and

uniform drug content. The gel exhibited desirable properties, including appropriate gelation temperature, viscosity, and pH, making it suitable for ocular application. Stability studies confirmed the formulation's potential for long-term storage under optimal conditions. The developed gel offers an effective alternative to conventional eye drops, potentially improving patient compliance and therapeutic outcomes in the treatment of neurotrophic keratitis. Further in vivo studies are recommended to evaluate its clinical efficacy.

REFERENCE

1. Lee CH, Moturi V, Lee Y. Thixotropic property in pharmaceutical formulations, 136, Journal of Controlled Release, 2009; 88–98.
2. Hejal P, Jivani R, Np J, Ld P, Ami M, Krunal S. Novel insitu polymeric drug delivery System: a review introduction. Journal of Drug Delivery & Therapeutics [Internet], 2011; 2012(5): 136–45. Available from: http://jddtonline.info.
3. Agrawal AK, Das M, Jain S. In situ gel systems as "smart" carriers for sustained ocular Drug delivery. Vol. 9, Expert Opinion on Drug Delivery, 2012; 383–402.
4. Gambhire S, Bhalerao K, Singh S. In situ hydrogel: different approaches to ocular drug Delivery. Int J Pharm Pharm Sci., 2013; 05(02): 27–36.
5. Karolewicz B. A review of polymers as multifunctional excipients in drug dosage form Technology., Saudi Pharmaceutical Journal. Elsevier B.V., 2016; 24: 525–36.
6. Rukari T G, Jadhav A S, Londhe R A, Phalke PL. A Review on Ophthalmic In Situ Gels. A Review on Ophthalmic In Situ Gels American Journal of PharmTech Research [Internet]. 2019; Available from: http://www.ajptr.com/www.ajptr.com
7. Cao SL, Zhang QZ, Jiang XG. Preparation of ion-activated in situ gel systems of Scopolamine hydrobromide and evaluation of its antimotion sickness efficacy. Acta Pharmacol Sin, Apr. 2007; 28(4): 584–90.
8. Mundada AS, Avari JG. In Situ Gelling Polymers in Ocular Drug Delivery Systems: A Review [Internet]. Vol. 26, Critical ReviewsTM in Therapeutic Drug Carrier Systems, 2009. Available from: www.begellhouse.com.
9. Padmasri B, Nagaraju R, Prasanth D. A comprehensive review on in situ gels. 12, International Journal of Applied Pharmaceutics. Innovare Academics Sciences Pvt. Ltd, 2020; 24–33.
10. Hajare AA, Mali MN. In situ gel-forming systems for sustained ocular drug delivery. IJCRR [Internet], 2010; 02(05): 17–21. Available from: https://www.researchgate.net/publication/242438923.

11. El-Kamel A, Al-Dosari H, Al-Jenoobi F. Environmentally responsive ophthalmic gel Formulation of carteolol hydrochloride. Drug Delivery: Journal of Delivery and Targeting Of Therapeutic Agents, Jan. 2006; 13(1): 55–9.
12. Hasanji FM, Patel AK, Patel VM. Formulation and Evaluation of In-Situ Sustained Release Gelling System of Famotidine. International Journal of Pharmaceutical Research And Applications [Internet], 2020; 7(03): 2456–4494. Available from: www.ijprajournal.com
13. Tatykhanova G, Aseyev V, Vamvakaki M, Khutoryanskiy V, Kudaibergenov S. Ophthalmic drug delivery system based on the complex of gellan and ofloxacin. Chemical Bulletin of Kazakh National University, Jun 20, 2022; 2: 4–12.
14. Liu Z, Li J, Nie S, et al. Study of an alginate/HPMC-Based in situ gelling ophthalmic delivery system For gat□ 6□ oxac□ n. Int J Pharm, 2006; 315(1–2): 12–17.
15. Geethalakshmi A, Karki R, Jha SK, et al. Sustained Ocular delivery of brimonidine tartrate using ion Activated in situ gelling system. Curr Drug Deliv., 2012; 9(2): 197–204.
16. Gombotz WR, Wee SF. Protein release from alginate Matrices. Adv Drug Deliv Rev., 2012; 194-205.
17. Balasubramaniam J, Pandit JK. Ion-activated in situ Gelling systems for sustained ophthalmic delivery Of c□ pro6□ oxac□ n hydrochloride. Drug Deliv, 2003; 10(3): 185–195.
18. Rathore KS, Nema RK. Formulation and evaluation of Ophthalmic films for timolol maleate. Planta indica, 2008; 4: 49-50.
19. Rathore KS, Nema RK. Glaucoma: a review. Available on-line at http://www.earticlesonline.com/Article/Glaucoma--A-Review/469815. Jan4, 2009.
20. Kaur IP, Kanwar M. Ocular preparations: the formulation Approach. Drug Dev Ind Pharm., 2002; 28(5): 473-93.
21. Indu PK, Manjit S, Meenakshi K. Formulation and evaluation of Ophthalmic preparations of acetazolamide. Int J. Pharm., 2000; 199: 119–127.
22. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B, Dharmesh MM. A pH triggered in- situ gel forming ophthalmic drug delivery System for tropicamide. Drug Deliv Technol., 2007; 5: 44–49.
23. Sultana Y, Aqil M, Ali A, Zafar S. Evaluation of carbopol-methyl Cellulose based sustained-release ocular delivery system for Pefloxacin mesylate using rabbit eye model. Pharm Dev Technol., 2006; 11(3): 313-9.

24. Srividya B, Cardoza RM, Amin PD, Sustained ophthalmic Delivery of ofloxacin from a pH triggered in- situ gelling system. J Control Release., 2001; 73(2-3): 205-11.
25. Katarina E, Johan C, Roger, P. Rheological evaluation of Poloxamer as an in- situ gel for ophthalmic use. Eur J Pharm Sci., 1998; 6: 105–112.
26. El-Kamel AH, In vitro and in vivo evaluation of Pluronic F127-Based ocular delivery system for timolol maleate. Int J Pharm., 2002; 241(1): 47-55.
27. Balasubramaniam J, Kant S, Pandit JK, In vitro and in vivo Evaluation of the Gelrite gellan gum-based ocular delivery System for indomethacin. Acta Pharm., 2003; 53(4): 251-61.
28. Johan C, Katarina E, Roger P, Katarina, J. Rheological evaluation of gelrite in-situ gel for ophthalmic use. Eur J Pharm Sci., 1998; 6: 113–116.
29. Saettone MF, and Salminen L. Ocular inserts for topical Delivery. Advanced drug delivery reviews., 1995; 16(1): 95.