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Formulation and Characterization of Gemifloxacin Mesylate Ophthalmic *In-Situ* Gel

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Abstract

Objective: Gemifloxacin Mesylate is an fluoroquinolone antibacterial agent with a broad spectrum of activity. It is a fourth generation fluoroquinolone, has high potency against Gram-positive, Gram-negative bacteria and its bactericidal activity is through inhibition of bacterial topoisomerase II and IV enzymes which are critical in the maintenance, synthesis and replication of DNA. Gemifloxacin mesylate showed good *in vivo* activity in a model of infective keratitis due to *St.aureus* ,in comparison to all third generation fluoroquinolones. **Method:** Gemifloxacin Mesylate *in situ* gels were formulated using sodium alginate as main polymers and HPMC K4 as co-polymer. Sodium alginate and HPMCK4 were used in different concentration (0.5, 0.1, 1.5 and 2%) whereas HPMCK4 was in (0.6, 0.8, 1 and 1.2 %) respectively and benzalkonium chloride as preservative. All the prepared *in-situ* gel formulations were evaluated for gelation, pH, drug content, viscosity, *in-vitro* release study, and the optimized formulation was tested for accelerated stability studies. **Results:** FT-IR and DSC studies revealed that drug was found to be compatible with formulation excipients. All the formulations were developed using combination of sodium alginate with HPMC K4 and the developed formulations showed satisfactory results for *in-vitro* gelling capacity, rheology and other physical properties. **Conclusion:** The *in-situ* gel is a promising approach for ocular infection. This gel combines the advantage of a solution and its administration convenience with favourable residence time and sustained release.

Keywords
Gemifloxacin Mesylate, *in-situ* gel, HPMCK4, sodium alginate.

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INTRODUTION:

- The landscape of ophthalmic drug delivery is highly competitive and rapidly evolving. New classes of pharmaceuticals and biologics are fuelling the demand for novel drug delivery systems.
- The main aim of pharmacotherapeutics is the attainment of effective drug concentration at the intended site of action for a sufficient period of time to elicit a response. A major problem of ophthalmic drug delivery is not the lack of efficient drugs but the attainment of their optimal concentration at the site of action.[1]
- Ophthalmic drug delivery is one of the most interesting and challenging endeavours facing the pharmaceutical scientist. The anatomy, physiology and biochemistry formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage.

- The traditional ophthalmic dosage forms (solutions, suspensions and ointments) have been described commonly used and accepted forms. [2]
- It is a common knowledge that, the ocular bioavailability of drugs applied topically as eye drops is very poor. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of eye and by other concomitant factors

For examples

- Drainage of instilled solution
- Lacrimation and tear turnover
- Metabolism
- Tear evaporation
- Non-productive absorption/ adsorption
- Limited corneal area and poor corneal permeability
- Binding by the lacrimal proteins
- Several new preparations have been developed for ophthalmic use which includes liposomes, polymer matrices, mucoadhesive polymers, absorbable gelatin sponge and inserts, both erodible as well as non-erodible (for example medicated contact lenses, collagen shields, the minidisk etc.)[3].
- Successful results were obtained with inserts and collagen shields, although these preparations present some disadvantages, such as non-compliance especially by elderly patients. From the point of view of patient acceptability, a liquid dosage form is preferable. This problem can be overcome by using *in situ* forming gel as ophthalmic drug delivery systems[4].

IN-SITU FORMING GELS FOR OPHTHALMIC DRUG DELIVERY – A NOVEL CONCEPT

Recently, controlled and sustained drug delivery has become the standard in modern pharmaceutical design and intensive research has been undertaken in achieving much better drug product effectiveness, reliability and safety. In this regard, many polymers are very useful which undergo reversible sol to gel phase transition in response to physiological stimuli[5].

In situ gels are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favourable residence time. The sol-gel transition occurs as a result of a chemical/ physical change induced by physiological environment. This type of gel combines the advantage of a solution being patient convenient with the favourable residence time of a gel for enhancing the ocular bioavailability[6,7].

The sol-gel transition can be induced by a shift in the pH as for cellulose acetate phthalate, a shift in temperature as for the thermo gelling Poloxamer 407 or by presence of cations as for deacetylated HPMCK4 and alginates.

Thus, the *in-situ* gelling systems for ophthalmic use can be classified as pH sensitive, temperature sensitive and ion-activated systems. The rate of gel formation in situ, is important since when dropped in the eye, before a strong gel is formed, a solution or a weak gel is prone to elimination by the fluid mechanics of the eye[8].

The ion activated *in situ* gelling system can be formulated using sodium alginate, the sodium salt of alginic acid, as a natural hydrophilic polysaccharide containing two types of monomers, β-D-mannuronic acid (M) and x-L-guluronic acid (G) which forms a gel in the cul-de-sac due to the presence of divalent calcium ions in the lacrimal fluid[9]. Thus, with the use of these *in situ* gelling systems, residence time of the drug in the eye is increased. Continuous delivery of drugs in a controlled manner to the anterior chamber of the eye will eliminate the requirement for frequent drug administration, causing better patient compliance and will result in extended duration of action, hence lower amount of total dose required, which in turn will minimize the local and/or systemic side effects[10].

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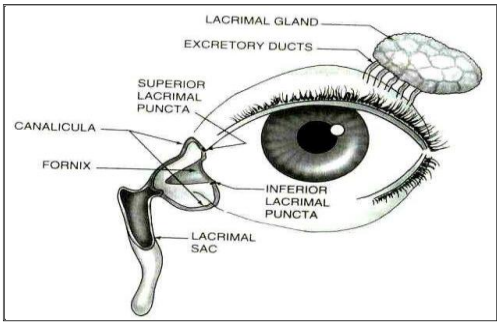
MECHANISM OF OCULAR DRUG ABSORPTION

Drugs administered by instillation must penetrate the eye and do so primarily through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera and appear to be particularly important for drugs that are poorly absorbed across the cornea[11,12].



Schematic illustration of the Ocular Disposition of Topically Applied Formulations Corneal permeation:

The penetration of drugs across the corneal membrane occurs from the precorneal space. Thus, the mixing and kinetic behaviour of drug disposition in tears have a direct bearing on efficiency of drug absorption into the inner eye. The productive absorption of most ophthalmic drugs results from diffusional process across the corneal membrane. The efficiency of absorption process is a function of rate and extent at which the transport processes occur. The flux of any drug molecule across a biological membrane depends on the physicochemical properties of the permeating molecule and its interaction with the membrane. The extent to which the transport or absorption process occurs is also a function of physiological mechanism of pre- corneal fluid drainage or turnover.

Ocular Bioavailabilty:

Bioavailability of drugs administered to the eye is an important consideration. There are physiologic factors, which can affect a drug's ocular bioavailability including protein binding, drug metabolism and lacrimal drainage. Protein bound drugs are incapable of penetrating the corneal epithelium due to the size of the protein-drug complex. Because of the brief time in which an ophthalmic solution may remain present in the eye (due to lacrimal drainage), protein binding of a drug substance could quickly negate its therapeutic value by rendering it unavailable for absorption.

As in the case with other biological fluids, tears contain enzymes (such as lysozyme) capable of the metabolic degradation of drug substance. In addition to physiologic factors affecting ocular bioavailability, other factors as the physicochemical characteristics of the drug substance, and product formulation are important. Because the cornea is a membrane-barrier containing both hydrophilic and lipophilci layers, it is permeated most effectively by drug substances having both hydrophilic and lipophilic characteristics.

It is advantageous for corneal penetration to adjust the pH of solution to increase the proportion of unionized drug in the instilled dose. Drugs which are highly water soluble do not readily permeate the cornea. Suspensions of drugs and ophthalmic ointments mix with lacrimal fluids less readily than do solutions, and thus, remain in the cul-de-sac for longer period of time, enhancing the bioavailability of the drug substance. Ophthalmic solutions of increased viscosity also remain in cul-de-sac longer than solutions with lower viscosity[13]. pothesis that race is a major risk factor for glaucoma is based on the data indicating a higher prevalence of the disease among

MATERIAL AND METHOD:

Sr. No.	Drug/Excipients
1.	Gemifloxacin mesylate
2.	Sodium alginate
3.	HPMCk4
4.	Sodium chloride
5.	Benzalkonium Chloride

DRUG AND PROFILES:

Gemifloxacin Mesylate [14,15]

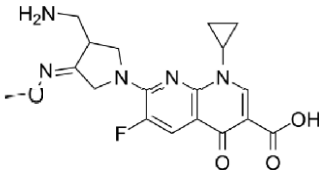
Gemifloxacin Mesylate is a salt from of gemifloxacin, is a new group of fluoroquinolone antibacterial agent with a broad spectrum of activity. It is a frouth generation fluoroquinolone, has highly potency against gram positive and gram negative bacteria and its bactericidal activity is through inhibition of bacterial topoisomerase II and IV enzymes

Chemical name: 7-[(4*Z*)-3-(aminomethyl)-4-methoxyiminopyrrolidin-1-yl]- 1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid;methanesulfonic acid

Molecular formula: $C_{19}H_{24}FN_5O_7S$

Molecular weight: 485.49g/mol

Structure Formula:



Brand name: FACTIVE
Solubility: Freely soluble in water

Mechanism of action:

Gemifloxacin is Quinolone/ fluroquinolone antibiotic. Gemifloxacin in bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called gyrase, which allows the untwisting required to replicate one DNA double helix into two

Pharmacokinetic properties:

- **Absorption:** Rapidly absorbed from the gastrointestinal tract. The absolute bioavailability averages approximately 71%
- **Protein binding:** 60-70%
- **Metabolism:** Limited metabolism by the liver to minor metabolites
- **Excretion:** Feces (61%) urine (36%)
- **Half –life:** 7-8 hours

Medical uses:

- It is prescription medicine used to treat the symptoms of **Pneumonia** and ***Actue Exacerbation of Chronic Bronchitis***.

Adverse effects:

- Tendon problems, arthritis or other joint
- Epilepsy or other Seizure disorder
- Heart problems
- High blood pressure
- Dizziness
- Burning pain
- Memory problem
- Hallucination

***Preparation of* in-situ *gelling solution*[16]:**

The polymeric solutions were prepared by dispersing sodium alginate and HPMC K4 in various concentrations, soaked separately overnight in deionised water at 4^0 C until the polymers completely dissolve. The drug solution was prepared by dissolving desired amount of Gemifloxacin mesylate, benzalkonium chloride, sodium chloride in 5ml of deinosied water. Both polymeric and drug solutions were combined and stirred using magnetic stirrer until the mixture is formed at constant 50rpm.

Composition of Gemifloxacin mesylate* in-situ *Gel.

Name of the Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8
Drug	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium Alginate	0.5	1	1.5	2	-	-	-	-
HPMC K4	-	-	-	-	0.4	0.6	0.8	1
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.
Benzalkonium Chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Deionized water	100	100	100	100	100	100	100	100

EVALUATION PARAMETERS

***Determination of melting point*[17]:**

Melting point of Gemifloxacin Mesylate was determined by both capillary method and with digital melting point apparatus.

✓ ***Capillary method: -***

In this method the capillary was sealed with gentle heating from one end. Then the small quantity of pure drug Gemifloxacin mesylate was filled into the sealed capillary. Capillary was inserted into to the tube which was dipped in mineral oil phase. Gently the oil bath was heated, as soon as the powder had started melting the heating was stopped and the temperature was noted down.

Drug Excipients Compatibility Studies:

A proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Compatibility must be established between the active ingredients and other excipients to produce a stable, efficacious, attractive, and safe product. If the excipients are new and if no previous literature regarding the use of those particular excipients with an active ingredient is available then compatibility studies are of the paramount importance. Hence, before producing the actual formulation, compatibility of Gemifloxacin mesylate with different polymers and other excipients was tested using FT-IR and DSC studies.

Fourier Transform Infra-Red Spectroscopy[18]:

In the preparation of liquid formulation, drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug. FT-IR spectroscopy is one of the most powerful analytical techniques, when it comes to the determination of presence of various functional groups involved in making up the molecule. It provides very well acceptable spectral data regarding any

change in functional group characterization of a drug molecule occurring while in the processing of a formulation.

Procedure: Integrity of the pure drug and physical mixture of pure drug with excipients was checked by taking an IR spectrum. The spectra were obtained using Shimadzu FTIR 8700 spectrophotometer.

In this study, palletization of potassium bromide (KBr pellets) was employed. Before forming the pellet of potassium bromide, it was completely dried at 100°C for one hour and after drying it was thoroughly mixed with the sample in the ratio 1 part of drug and 100 parts of KBr. The mixture was compressed to form a disc using the dies. This disc was placed in the sample chamber and a spectrum was obtained which was further subjected to interpretation.

Differential Scanning Calorimetry (DSC) Analysis[19]:

DSC (Perkin-Elmer thermal analysis) studies were carried out in order to characterize the physical state of drugs. Sample of pure drug and physical mixture were placed in the aluminum pans and thematically sealed. The heating rate was 10°C per min using nitrogen as pure gas. The DSC instrument was calibrated for temperature using indium. In addition, the enthalpy calibration indium was sealed in aluminum pan with sealed empty pans as reference.

Analytical Method: -

a. Preparation of reagents[20]:

I. Potassium dihydrogen orthophosphate 0.2M: 27.218 grams of potassium dihydrogen phosphate was dissolved in little quantity of distilled water and volume adjusted to produce 1000 ml.

II. Sodium hydroxide 0.2 M: 8 grams of NaOH was dissolved in distilled water and volume adjusted to produce 1000 ml.

III. Preparation of phosphate buffer pH 6.6 solution: Place 50 ml of 0.2M KH_2PO_4 in a200 ml volumetric flask, add the specified volume of 0.2 M NAOH and volume adjusted to produce 1000 ml.

b. Preparation of standard stock solution of Gemifloxacin mesylate

Standard stock solution-I of Gemifloxacin mesylate was prepared by dissolving accurately weighed 100 mg of Gemifloxacin mesylate in little quantity of 6.6 phosphate buffer. The volume was then made up to 100 ml by phosphate buffer pH to obtain the solution 1000 µg/ml.

C. Determination of analytical wavelength of Gemifloxacin mesylate

From the stock solution-I 3ml was pipetted out into 100 ml volumetric flask. The volume was made up to 100 ml with phosphate buffer pH 6.6. The resulting solution containing 30µg/ml was scanned between 200-400 nm. The wavelength of drug max was selected.

Calibration curve of Gemifloxacin Mesylate in phosphate buffer pH 6.6: From the standard stock solution-II, a series of dilutions were prepared using phosphate buffer pH 6.6 to get concentration of 3-15µg/ml. The absorbance of these solutions was measured using UV-spectrophotometer (Shimadzu 1800. Japan). Standard curve was obtained by plotting absorbance vs. drug concentration[4].

Physical appearance and Clarity:[21,22]

Physical appearance and clarity test was observed by visual inspection under a good light, viewed against a black and white background, with the contents set in motion with a swirling action. Also, it was observed for formation of turbidity, or any unwanted particles dispersed in the solution.

Measurement of pH:

Each formulated batch, pH was measured using pH meter which was previously calibrated using standard buffers of pH 4 and pH 7 as per the established procedure[53].

Determination of the gelling capacity:

The gelling capacity was determined by placing a drop of the *in-situ* gel in a test tube containing 2 ml of freshly prepared simulated tear fluid (pH 7.4) equilibrated at 35 ± 1^0 C, the time taken for its gelling formation then dissolution of the gel was visually observed, and the gelling capacity was evaluated as follows[23]:

(-) No gelation
(+) The gel formed after few minutes and dissolved rapidly
(++) Immediate gelation and remains for few hours
(+++) Immediate stiff gelation which remains for extended period of time

Viscosity measurement of* in-situ *gels:[24]

The viscosity measurements were carried out using Brookfield viscometer (Model no LVDV 2P230). The *in-situ* gel formulations were placed in the sampler tube. The samples were analyzed at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each measurement. The angular velocity of the spindle was increased 1 to 4 and the viscosity of the formulation was measured.

Determination of drug content:

The drug content estimation was carried out by dissolving 1 ml of prepared formulation and volume was made up to 100ml with phosphate buffer pH 6.6and drug content was determined after suitable

dilution using UV-visible spectrophotometer at 271 nm.

***In-vitro* drug release: -**

The *in vitro* release of Gemifloxacin Mesylate from the prepared formulations was studied using a modified diffusion testing apparatus. The freshly prepared simulated tear fluid (pH 6.6) was used as a diffusion medium. Semi permeable membrane, previously soaked in the diffusion medium for overnight, was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter of 3.4 cm. Two ml of formulation was placed in to donor chamber. The cylinder was suspended in a beaker (receptor chamber) containing 100 ml of diffusion medium so that the membrane just touches the surface of the medium. Receptor chamber was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using magnetic stirrer. About 5 ml of sample was withdrawn at different time interval and replaced with an equal volume of fresh diffusion medium. The aliquots were suitably diluted with the diffusion medium and analyzed at 271 nm using UV spectrophotometer. In a similar manner.

Stability Test[25]: -

The stability of optimized formulation [S4] was studied at 40 ± 2°C with 75 ± 5% RH. *In-situ* gel was stored in well tight closed container and maintained at oven temperature 40 ± 2°Cwith 75 ± 5% RH for 3 months as per ICH guidelines. Changes in the appearance and drug content of the stored gel were investigated after storage. The data presented are the mean of three determinations.

RESULT AND DISCUSSION:

The preparation of *in-situ* gelling system for ocular diseases was carried out by using different polymeric systems like sodium alginate as main polymer and HPMC-K4M as co-polymers. Prepared *in-situ* gel formulations were subjected to general appearance, pH, gel strength, drug content, *in-vitro* diffusion studies and stability studies.

Preformulation study:

Melting point: -

The melting point of Gemifloxacin Mesylate was found to **206^{0C}** which is within reported range of 204-208^{0C} It complies with official standard. Thus, indicating the purity of sample.

Compatibility study:

FT-IR studies: -

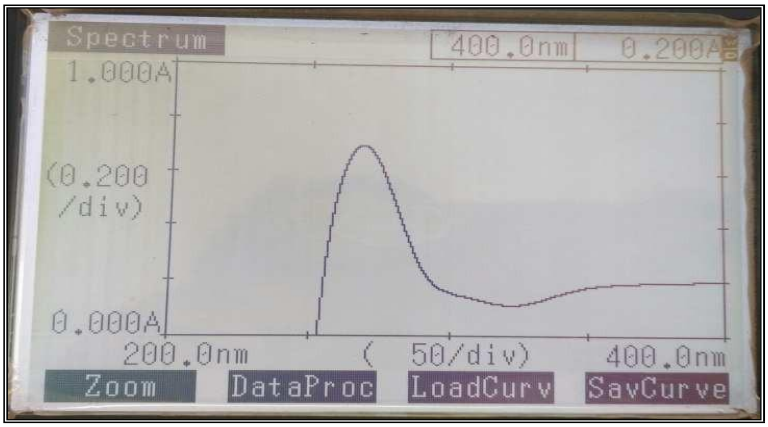
Major Peaks of Gemifloxacin Mesylate and physical mixture of drug and polymers

Name of compound	C-H stretching	C=O stretching	C=C Stretching	C-F Bending
Gemifloxacin Mesylate	2915.18	1705.14	1506.75	1037.79
Physical mixture of drug and sodium Alginate	2937.68	1716.70	1503.40	1040.37
Physical mixture of drug and HPMC K4M	2922.35	1714.03	1502.85	1037.37

Determination of wavelength

The solution of Gemifloxacin Mesyalte in 6.6 pH buffer was scanned using UV-spectrophotometer over the range between 200 - 400 nm using 6.6-pH buffer as blank. The absorption spectra of Gemifloxacin Mesylate showed absorption peak at 271 nm, which represent the maximum absorption (λ max) of the drug.

Analytical wavelength of Gemifloxacin Mesylate



STANDARD CALIBRATION CURVE OF GEMIFOLXACIN MESYLATE:

shows the absorbance value of standard solution of Gemifloxacin Mesylate in pH 6.6 buffer. standard calibration curve for Gemifloxacin Mesylate in pH 6.6 buffers. The curve was found to be linear in the range of 3 to 15 µg/ml with regression value of **0.997** at 271 nm

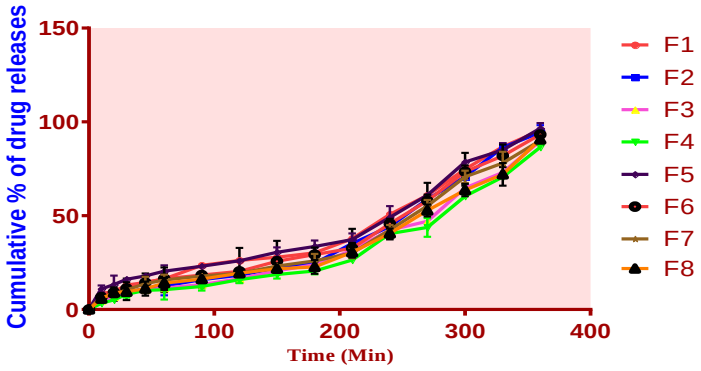
Calibration data of Gemifloxacin Mesylate 271 nm

Sl. No.	Concentration (µg/ml)	Absorbance (at 265 nm)
1	0	0± 0.00
2	3	0.122± 0.21
3	6	0.264± 0.34
4	9	0.399± 0.12
5	12	0.526± 0.08
6	15	0.657± 0.16

Evaluation Parameters of Gemifloxacin mesylate *in-situ* Gel

Formulation code	Gel strength	Surface pH	Viscosity (Cp)	Drug content (%)
F1	+	6.22	76	81.25± 2.42
F2	++	6.16	79	83.23± 1.37
F3	++	6.34	88	90.22± 1.12
F4	+++	6.88	94	94.35± 1.68
F5	+	6.34	79	80.35± 1.78
F6	+++	6.78	83	86.16± 0.66
F7	++	6.65	87	86.73± 1.05
F8	+++	6.60	90	92.2± 1.32

***In-vitro* drug release profile of Gemifloxacin Mesylate *In-situ* gel**



Stability study of Gemifloxacin Mesylate based *in-situ* gel (F4)

Sl No.	Time period for sampling	Drug content (%)
1.	Initial	94.35± 1.68
2.	After 1 month	94.05± 1.22
3.	After 2 months	93.78± 0.64
4.	After 3 months	93.45± 1.02

CONCLUSION:

The present work was taken up to use of the gel forming solution in order to develop a *in-situ* gel of Gemifloxacin Mesylate for treatment of ocular infection which can be expected to prove beneficial for overcoming the limitations of oral and parenteral administration routes. Some of the important conclusions that have been drawn from 'Results and Discussions' are as follows:

- ✓ IR and DSC spectra revealed that there were no possible interactions between the drug and polymer.
- ✓ The method employed for the preparation of *in-situ* gel was simple and reproducible.

- ✓ The concentration of sodium alginate and HPMCK4 chosen for the formulations underwent gelation in simulated salivary fluid easily and was found to have good gelling capacity at higher concentration in the presence of HPMC K4.
- ✓ All the formulations were found to have desired amount of drug content, indicating that the method adopted for making of the formulation was suitable.
- ✓ The pH of the formulations varied from 6.1 – 6.6 which is considered safe for ocular delivery.
- ✓ Increase in the polymer concentration increases the viscosity and thereby increases strength of the formed gel.
- ✓ The *in-vitro* release followed a controlled release pattern. Formulation exhibited matrix diffusion release kinetics. This is one of the most important observations recorded.
- ✓ Stability studies indicated that the developed formulations have stable at room 40°C for a period of 3months.

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