**FORMULATION AND EVALUATION OF NOVEL IN SITU GEL OF**

**ANTIBACTERIAL DRUG**

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**Abstract**

The present work describes the formulation and evaluation activated of an ophthalmic delivery system of an anti-bacterial agent- Norfloxacin. Sodium alginate was used as the gelling agent for Ion activated in situ gel, Polyacrylic acid (Carbopol- 934) was used as the gelling agent for pH-triggered in situ gel and Poloxamer was used as the gelling agent for temperature activated in situ gel in combination with HPMC K4 M which acted as a viscosity enhancing agent. The evaluated for determination of visual appearance, clarity, pH and Drug Content, In vitro gelation studies, Rheological studies, In vitro drug release study, antimicrobial efficacy Studies, Ocular irritation studies, Sterility testing, Stability studies. Cumulative percent release of 86.89 %, 63.23 % and 76.37 %, was observed for optimized formulations

**Keywords:** In situ-forming system, ophthalmic hydrogel, Norfloxacin, Sodium alginate, Carbopol 934, Poloxamer

# INTRODUCTION

Extensive research has been carried in designing of polymeric drug delivery systems. The development of *in situ* gel systems has received considerable attention over the past few years.9 This interest has been sparked by the advantages shown by *in situ* forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort.10 Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered.11 *In situ* gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulations. Various natural and synthetic polymers are used for formulation development of *in situ* forming drug delivery systems.

**Mechanism:**

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.

# Ophthalmic drug formulations

Ophthalmic drugs are formulated to bring the active drugs in contact with the eye surface to allow for absorption. Extension of corneal contact time may result in increased drug penetration & higher intraocular drug delivery. In addition to the active drug, ophthalmic formulations should contain other ingredients to control various characteristics of the formulation, such as the buffering and pH, osmolality & tonicity, viscosity & antimicrobial preservatives. Although these ingredients are listed inactive, they can affect permeability of drug across the ocular surface barriers & alter the therapeutic effectiveness of the drug. ***In Situ* gel system**

The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery or as tear substitutes. They do not allow accurate and reproducible administration of quantities of drugs and, after administration; they often produce blurred vision, crusting of eyelids, and lachrymation. A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the later. Thus, in situ hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with eye. The liquid to semisolid phase change can be triggered by increased temperature, increased pH and ionic strength of the tear film. Based on different stimuli, in situ forming hydrogels can be classified as follow:

1. Ion-sensitive hydro gels
2. pH-sensitive hydro gels
3. Temperature-sensitive hydrogels

# Ionically induced gelation

Gellan gum is an anionic exocellular polysaccharide by the bacterium pseudomonas elodea, having the characteristic property of cation- induced gelation. The acetylated form is commercially available as gelrite (Kelco division of Merck and Co, USA). The sol-gel transition process is induced by the presence of monovalent or divalent ions such as Na+ and Ca+. Some other parameters influence the phase transition. e.g.: The concentration of polysaccharide, the temp of the preparation, and the nature and the concentration of cations. It was determined that divalent ions such as magnesium or calcium were superior to monovalent cations in promoting the gelation of the polysaccharide. **pH induced gelation**

Pseudolatexes can be defined as artificial latexes prepared by the dispersion of a preexisting polymer in aqueous medium in situ gelling pseudo latexes for ophthalmic use can be described as aqueous colloidal dispersions of polymer, which become viscous gels after instillation in the conjunctival cul-de-sac due to modification of the pH. Pseudo latexes are obtained by dispersion of an organic solution of a preformed polymer in an aqueous medium, leading to an o/w emulsion. Two principal methods are commonly used to prepare ophthalmic pseudo latexes, the solvent evaporation process and the salting out process. Both methods allow the production of a lyophilized and easily re dispersible power. Thus, pseudo latexes have the advantage of the latex as well as the stability of active compounds such as pilocarpine, which is sensitive to aqueous media. In addition, such systems represent an interesting technological alternative that avoids the use of organic solvents, which can cause problems such as toxicity. **Thermo reversible hydro gels**

These hydro gels are liquid at room temperature (20-250 C) and undergo gelation when in contact with body fluids (35-370 C), due to an increase in temperature. Different thermal settings gels have been described in this Review. For example acrylic acid copolymers and N- isopropylacrlamide derivatives ophthalmic administration such as tolerance have limited the choice of such polymers. Poloxamers, commercially available as pluronic (BASF–Wyandotte, USA), are the most commonly used thermal setting polymers in ophthalmology. They are formed by a central hydrophobic part (poly oxy propylene) surrounded by hydrophilic part (ethylene oxide). Depending on the ratio and distribution along the chain of the hydrophobic and hydrophilic sub units, several molecules weights are available, leading to different gelation properties. Pluronic F-127, which gives colorless and transparent gels, is the most commonly prepared by solubilization of the polymer in cold water (5-100 C) followed by gelation up on warming to ambient temperature.

**Advantages of *in-situ* forming gel:**

Generally more comfortable than insoluble or soluble insertion.

✓ Less blurred vision as compared to ointment.

# PREFORMULATION STUDY Determination of melting point

Melting point of norfloxacin was determined by capillary method. Norfloxacin was filled in capillary and tied with a thermometer.

# Solubility

Solubility is an important consideration in ophthalmic formulations as clarity of the solution is an essential requirement. The solubility of norfloxacin was tested in various solvents such as Distilled water, Ethyl alcohol, Acetic acid, Chloroform and Acetone.

# IR Spectroscopy and Compatibility studies

The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug, using potassium bromide (KBr)

discs

# Spectrophotometric method for estimation of norfloxacin

The calibration curve for estimation of norfloxacin for determination drug content and cumulative percent release (CPR) were prepared in 1% v/v acetic acid and artificial tear solution respectively.

* Increased bioavailability due to – Increased precorneal residence time, Decreased nasolacrimal drainage of the drug
* Chances of undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal duct is reduced
* Drug effect is prolonged hence frequent instillation of drug is not required **Preparation of stock solution**

10 mg of norfloxacin was weighed accurately and transferred to 100 ml volumetric flask. Dissolve the drug in 1% v/v acetic acid and artificial tear solution and the volume was made up to 100 ml with respective solution to get the final concentration of 100 μg/ml. **Preparation of calibration curve** The above stock solutions were scanned for the maximum absorbance using

Shimadzu 1700 UV-Visible spectrophotometer. The λmax for norfloxacin was found to be 273 nm in 1% v/v acetic acid and artificial tear solution.

# Calibration curve in 1% v/v acetic acid

The above stock solution (100 μg/ml) prepared in 1% v/v acetic acid was further diluted to get concentration in the range of 1-5 μg/ml for 1% v/v acetic acid. From the stock solution aliquots of 1-5 ml were withdrawn and further diluted to 100 ml with 1% v/v acetic acid to obtain a concentration range of 1-5 μg/ml. Absorbance of solution was measured at

273 nm using Shimadzu 1700 UV- Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated. **Calibration curve in artificial tear solution**

The above stock solution (100 μg/ml) prepared in artificial tear solution was further diluted to get concentration in the range of 1-5 μg/ml for artificial tear solution. Absorbance of solution was measured at 273 nm using Shimadzu 1700 UV-Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated.

# Preparation of in situ gelling system

**Preliminary Trials for sodium alginate based Ion activated in situ gel system**

**For Sodium alginate:** In batches P1 to P8 (table 5.4), the concentration of sodium alginate were 0.2 to 1.5% and all the formulations were checked for their pH, viscosity, drug content and gelling capacity.

# Formulation of factorial batches of Ion activated Norfloxacin *in-situ* ophthalmic gel

The table 5.6 shows the composition of all the formulations. Sodium alginate and HPMC K4 M were dissolved in a beaker containing purified water, and this solution was heated about 85°C for 15 min, then beaker was cooled with stirring. Norfloxacin (0.3% W/V) was dissolved in 0.1 N NaoH to get clear solution. After cooling benzalkonium chloride and drug solution were added to the polymer solution and volume was made up to 100 ml with distilled water and this solution was filtered through 0.2 mm filter paper. The formulations were sterilized by terminal autoclaving at 121°C for 20 min at 15 psi. All glassware used during the preparation of the *in situ* forming gels was sterilized by autoclaving and the entire procedure was carried out in a laminar flow hood. STF was prepared using NaCl

0.67 g, NaHCO3 0.20 g, CaCl2 · 2H2O 0.008 g and water up to 100.0 g.

# Evaluation of formulation

**Determination of visual appearance, clarity, pH and Drug Content:**

The appearance and clarity were determined visually. The pH of the formulations was measured by using pH meter. The drug content was determined by diluting 1 ml of the formulation to 50 ml freshly prepared simulated tear fluid (pH 7.4). The formed gel was completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel got completely dispersed to give a clear solution. The volume was adjusted to 100 ml with simulated tear fluid. The solution was filtered through a

0.45-mm filter membrane and

Norfloxacin concentration was then determined at 272 nm by using UV- Vis spectrophotometer. The results were the means of three runs. **Rheological studies:** The rheological properties of solutions and gels were measured using a

Brookfield synchroelectric viscometer.

***In vitro* Drug Release Studies:**

The *in vitro* release studies were carried out on formulation codes F1 to F9 using a modified USP dissolution testing apparatus. **Sterility Testing**

The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically were maintained the study.

# RESULT AND DISCUSSION

**Determination of melting point:** Melting point of Norfloxacin was found to be in the range of 220- 225ºC as reported in literature, thus indicating

**Identification of drug by IR Spectroscopy** transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30˚C to 35˚C in the case of fluid thioglycolate medium and 20˚C to 25˚C in the case of soya bean casein digest media. Both positive and

negative controls

purity of the drug sample. Any impurity, if present, will cause variation in the melting point of a given drug substance.

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| E:\microbiology\article reserch 3 july\S1.JPG  **Figure : FT IR spectra of Norfloxacin**  |

**Solubility:** Norfloxacin was found soluble in acetic acid.

# Selection of Vehicle

Buffers play a pivotal role in formulating ophthalmic drops. They contribute significantly to chemical stability and clinical response and also influence the comfort and safety of the product; hence the importance of selecting a suitable buffer ensures product stability and desired drug solubility. The studies in various buffer solutions indicated the drug was soluble in acetate buffers of pH 6 & 6.5 at the dosage level desired (0.3% w/v). The solutions were stable to elevated temperatures and autoclaving. However, their instability to light as evidenced by discoloration of the exposed solutions necessitated their packing in amber via

**Spectrophotometric method for estimation of norfloxacin:**

**Calibration curve in 1% V/V acetic acid (For Drug Content)** The curve was found to be linear in the range 1-5 μg/ml at λmax 273 nm.



# Formulation of Optimized Batch

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| **Table: Formulation of Optimized Batch**  |
| **Ingredient**  | **Quantity (% w/v)**  |
| Norfloxacin  | 0.3  |
| Sodium alginate  | 0.94  |
| HPMC K4 M  | 0.51  |
| Edetate disodium  | 0.01  |
| Benzalkonium chloride  | 0.01  |
| Citric Acid I.P.  | 0.407  |
| Disodium hydrogen phosphate I.P  | 1.125  |
| Purified Water I.P.  | 100ml  |

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| --- | --- |
| **Table : Evaluation of selected optimized batch**  |  |
| **Values**  | **Viscosity (cps)**  | **Gelling Capacity**  | **Drug release(%)**  |
| **Predicted value**  | 623.93  | 2.6  | 89.73  |
| **Actual value**  | 618  | 2  | 86.89  |

# CONCLUSION

Infrared spectroscopy studies of Norfloxacin, Sodium alginate, Carbopol 934, Pluronic F 127 and HPMC K4M alone and their physical mixture revealed that, Norfloxacin is compatible with all the polymers used. Ophthalmic *in situ* gelling system of Norfloxacin was successfully formulated using three different gelling agents viz. Sodium alginate, Carbopol 934 and Pluronic F 127 as ion-sensitive, pH-sensitive and temperature sensitive respectively along with HPMC K4M as viscosity enhancing agent. 32 full factorial design was applied to all the three method of *in situ* gel to select optimized formulations**.**

The clarity of the prepared formulations was found satisfactory. The pH of all formulations was found to be satisfactory in the range of 6 - 7.4. The drug content of the prepared formulation was within the acceptable range, and ensures dose uniformity. All the optimized formulations showed instantaneous gelation when contacted with simulated tear fluid (STF).

Cumulative percent release of 72.25 %, 62.10 % and 76.37 %, was observed for formulation F6, F18 and F23 respectively. Cumulative percent release

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of 86.89 %, 63.23

% and 76.37 %, was observed for optimized formulations NF1, NF2 and NF3 respectively. Optimized formulations showed sustained drug release for a period of 8 hour. It was observed that two optimized formulations NF1 and NF2 followed the zero order drug release, suggesting drug release in a controlled manner. Optimized formulation with pluronic F 127, NF3 showed higuchi release order suggesting drug release in a sustained manner.

The formulation passed the antimicrobial efficacy studies. The results of the ocular irritation studies indicate that all the three formulations were non-irritant and excellent ocular tolerance was noticed. Results of sterility test confirmed that all the formulations were sterile. From the stability studies it was confirmed that *in situ* gelling formulations of Norfloxacin remained more stable at ambient temperature (25°C) and humidity. The maximum instability of *in situ* gelling formulations was observed at 40°C and 4°C (significant decrease in drug content and *in vitro* drug release).

preliminary study in developing *in situ* gelling system of Norfloxacin. The *in vitro – in vivo* correlation need to be established to guarantee the bioavailability of prepared formulatio 10-30.

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1. INTRODUCTION

143