

FORMULATION AND EVALUATION OF AN *IN SITU* GEL FOR OCULAR DRUG DELIVERY OF ANTICONJUNCTIVAL DRUG

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The aim of the present study was to formulate and optimize Norfloxacin *in situ* gels for the treatment of conjunctivitis. Norfloxacin ophthalmic solution has been shown to be effective in ocular infections and may be used in patients with chronic conjunctivitis or ocular irritation. Norfloxacin *in situ* gel was prepared using various concentrations of polymers, such as Carbopol-940 (0.1, 0.2, 0.3 0.4 and 0.5% w/v), HPMC-E50LV (1.5% w/v), HPMC E4M (0.6% w/v) and HPMC K4M (0.5% w/v), as a pH triggered gelling system, with the objectives of increasing contact time, achieving controlled release, reducing the frequency of administration and obtaining greater therapeutic efficacy of the drug. The prepared *in situ* gels were then evaluated for their visual appearance, clarity, pH, drug content, *in situ* gelation. Also, rheological studies, sterility testing, texture analysis and *in vitro* drug release studies were carried out. It was evident from these studies that the polymeric *in situ* gels formed were transparent and clear, and possessed a satisfactory gelling capacity. The drug contents of all optimized formulations were found to range between 98.30-99.97%. The formulations of our *in situ* gels possibly possessed characteristics of a pseudoplastic behavior. The developed formulations were light yellow in colour, therapeutically efficacious, stable, non-irritant and provided sustained release of the drug up to eight hours' time.

Keywords: Norfloxacin, *in situ* gel, ophthalmic gel

INTRODUCTION

Ocular drug delivery is one of the most interesting and challenging fields for the pharmaceutical scientists. As an isolated organ, the eye is very difficult to study from a drug point of view; drugs are commonly applied to the ocular system for a local action on the surface or interior of the eye. The ocular system is most interesting due to its drug disposition characteristics. Ocular drug delivery has remained as one of the most challenging tasks for pharmaceutical scientists.¹ The unique structure of the eye restricts the entry of drug molecules at the required site of action. Drug delivery to the eye can be targeted at anterior or posterior eye segments. Conventional systems like eye drops, suspensions and ointments mainly target the anterior segment eye diseases.² Although more than 90% of the marketed ophthalmic formulations are in the form of eye drops, these formulations cannot be considered optimal in the treatment of vision threatening ocular diseases. Most of the topically applied drugs are washed off from the eye by various mechanisms (lacrimal drainage, tear dilution and tear turnover),

resulting in low ocular bioavailability of the drugs. Moreover, human cornea, comprising epithelium, substantia propria and endothelium, also restricts the ocular entry of drug molecules. The poor ocular drug bioavailability is thus due to ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, and nasolacrimal drainage.³ As a result of these factors, less than 5% of the administered drug enters the eye.⁴ Also, conventional drug delivery systems, like solutions, suspensions and ointments, are no longer sufficient to fulfil the present day requirements of providing a constant rate delivery for prolonged time. One of the main reasons for that is the poor residence time of the drug at the site of action, which results in poor bioavailability.⁵ *In situ* gel forming systems are liquid aqueous solutions before administration, which are transformed to gel under physiological conditions. These are delivery systems that can be instilled as eye drops and undergo an immediate gelation when in contact with the eye.⁶

In the present study, Norfloxacin ophthalmic gel was prepared using polymers Carbapol-940, HPMC-E50LV, HPMC E4M and HPMC K4M as a pH triggered gelling system to enhance contact time and controlled release, to reduce the frequency of administration and increase the therapeutic efficacy of the drug.

EXPERIMENTAL

Materials

Norfloxacin was obtained as a gift sample from Dr. Reddy's Laboratory Ltd., Hyderabad (India). All other chemicals were purchased from Loba Chemicals Pvt. Ltd., Mumbai (India) and were of analytical grade.

Methods

Formulation of *in situ* gel

Optimized method for ophthalmic *in situ* hydrogel preparation

The detailed procedure for preparing the Norfloxacin *in situ* gel forming system as a pH triggered system is outlined below. Formulation ingredients with their quantities were as given in Table 1. The buffer salts were dissolved in 75 mL of purified water, hydroxypropyl methylcellulose (HPMC-E50LV/HPMC-E4M/HPMCK4M) was added and allowed to hydrate, Carbapol 940 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer, Norfloxacin was dissolved in a small quantity of water, and benzalkonium chloride (BKC) was added to this solution, the drug solution was added to the polymer solution. Purified water was then added to make up the volume to 100 mL, and then the solution was filtered through 0-2 mm filter paper. (When the drug solution

and polymer solution were mixed, immediate precipitation of Carbapol occurred due to the decrease in pH brought about by Carbapol. Therefore, the drug was incorporated in a sufficient quantity of 0.1M NaOH and then added to the polymer solution to get a clear solution of drug and polymer).

Evaluation of ophthalmic *in situ* gel

Visual clarity and appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

Determination of pH

pH is one of the most important parameters involved in ophthalmic formulations. The two areas of critical importance are the effect of pH on solubility and stability. The pH of an ophthalmic formulation should be such as to ensure formulation stability and at the same time to cause no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have a pH ranging between 5 and 7.4. The developed formulations were evaluated for pH by using a digital pH meter.

Determination of drug content

The drug content was determined by diluting 1 mL of the formulation to 50 mL with freshly prepared simulated tear fluid having pH 7.4. An aliquot of 5 mL was withdrawn and further diluted to 50 mL with simulated tear fluid. Norfloxacin concentration was then determined at 272 nm using a UV-Visible spectrophotometer.

Table 1
Composition of *in situ* gelling formulation

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Norfloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Carbapol 940	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
HPMC E 50 LV	1.5	1.5	1.5	1.5
HPMC E 4 M	0.6	0.6	0.6	0.6
HPMC K 4 M	0.5	0.5	0.5	0.5
Citric acid	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407
Disodium hydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125
Sodium hydroxide	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tween 80	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Benzalkonium chloride	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Distilled water	100	100	100	100	100	100	100	100	100	100	100	100

Gelling capacity test (*sol-to-gel transition/in vitro gelation study*)

All prepared formulations were evaluated for gelling capacity, time and viscosity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by

placing a drop of the system in a vial containing 2 mL of freshly prepared simulated tear fluid and equilibrated at 37 °C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

Viscosity and rheological measurements

The rheological properties of solutions and gels were measured using a Brookfield DV III programmable viscometer. The developed formulation was poured into the small adaptor of the Brookfield DV III programmable viscometer using spindle no.62 and the angular velocity (shear rate) was increased gradually from 10 to 100 rpm. The hierarchy of the angular velocity was reversed. The average of two readings was used to calculate the viscosity. The formulation was then poured into an ointment jar and the pH was raised to 7.4 by adding simulated lachrymal fluid.

In vitro diffusion study

An *in vitro* release study of the *in situ* gel solution was carried out in simulated tear fluid at pH 7.4 using a Franz diffusion cell. The formulation was placed in the donor compartment and the freshly prepared simulated tear fluid in the receptor compartment. Between the donor and receptor compartments, a dialysis membrane (previously soaked overnight in the dissolution medium) was placed (0.22 μm pore size). The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at $37\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$. 1 mL of sample was withdrawn at a pre-determined time interval of 1 h to 8 h and the same volume of fresh medium was replaced. The withdrawn samples were diluted to 10 mL in a volumetric flask with the respective solvent and analyzed by a UV spectrophotometer at the respective nm using a reagent blank. The drug content was calculated using the equation generated from the standard calibration curve. The % cumulative drug release (%CDR) was calculated.

RESULTS AND DISCUSSION**Optimized method for *in situ* hydrogel formulation**

The use of Carbapol 940 in *in situ* gel-forming systems is substantiated by the property of its aqueous solutions to transform into stiff gels when the pH is raised. Hydroxypropyl methylcellulose (HPMC E5OLV, HPMC E4M, and HPMC K4M) was used to increase the viscosity of the formulation. The citrophosphate buffer was used as a vehicle in the pH triggered gelling system.

Evaluation parameters***Clarity and visual appearance***

The clarity of all the formulations was found to be satisfactory, as shown in Table 2. Moist heat sterilization by autoclaving had no effect on the clarity and other physicochemical properties of the formulations. The haziness that was observed after autoclaving (due to precipitation of HPMC at elevated temperature) was found to disappear,

the original clarity was regained after overnight standing.

pH

The pH of the formulations was found to be satisfactory and was in the range of 5.4-7.2, as shown in Table 2. The formulations were liquid at room temperature and at the pH formulated. Terminal sterilization by autoclaving had no effect on the pH.

Drug content determination

The drug content of the ophthalmic formulations of Norfloxacin *in situ* gel was found satisfactory (ranging between 98.30-99.70), indicating uniform distribution of the drug. Table 2 shows the percent drug content of F1-F12.

Gelling capacity test (sol-to-gel transition temperature and gelling time/in vitro gelation)

The formulation should have an optimum viscosity, which would allow easy instillation into the eye as a liquid (drops), but would also allow the formulation to undergo rapid sol-to-gel transition. Additionally, to facilitate sustained release of the drug to the ocular tissue, the gel formed *in situ* should preserve its integrity without dissolving or eroding for a prolonged period of time.

Table 2 shows the gelling capacity of all formulations, which is depicted as + (gel forms in 60 seconds and dissolves rapidly), ++ (gel forms within 60 seconds and remains stable for 3 hours) and +++ (gel forms within 60 seconds and remains for 6 hours). The gelling capacity increases with increasing concentration of gelling agent both at higher and lower concentration. Table 2 shows the gelling capacity of formulations F1 to F12. All the formulations, except F1, F5, F6 and F9, showed instantaneous gelation when contacted with artificial simulated tear fluid (STF). However, the nature of the gel formed depended on the concentration of the polymers used. The formation of instantaneous gels can be attributed to the buffering capacity of the simulated tear fluid. Formulation F5 showed gel formation within 60 seconds, which dissolved rapidly. Formulations F1, F6, and F9 showed immediate gelation within 60 seconds and remained stable for a few hours, whereas formulations F2, F3, F4, F7, F8, F10, F11 and F12 showed immediate gelation within 60 seconds and remained stable for an extended period.

Table 2
Physicochemical properties of prepared *in situ* gel

Formulation code	Drug content, %	pH	Gelling capacity at 25 °C	Gelling capacity at 37 °C
F1	99	6.9	-	++
F2	98.3	6.91	-	+++
F3	99.2	7.10	-	+++
F4	99	7.13	-	+++
F5	99	5.9	-	+
F6	98.7	6.2	-	++
F7	98.5	5.47	-	+++
F8	98	6.0	-	+++
F9	99.97	5.9	-	++
F10	99.6	6.0	-	+++
F11	99.65	6.02	-	+++
F12	99.5	6.1	-	+++

+ gelation within 50-60 seconds, dissolves rapidly
 ++ gelation within 60 seconds and remains stable for 3 hours
 +++ gelation within 60 seconds and remains stable for 6 hours

Viscosity and rheological measurements

Viscoelastic fluid with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rates are preferred. In order to evaluate the rheological behavior, the viscosity of the formulation before and after addition of simulated lacrimal fluid was evaluated by a Brookfield DV III Programmable rheometer, using increased shear stress and varying the angular velocities or shear rate. All the selected formulations were shear thinning, exhibiting pseudo plastic behavior. All the formulations were liquid at room temperature and underwent rapid gelation upon raising the pH to 7.4, as shown in Table 2. The results obtained from the

rheological study of the prepared *in situ* gelling system (F1-F12) revealed that the viscosity decreased as the angular velocity or shear rate increased. The viscosity of formulations F1-F12 ranged from 12-467 cps at room temperature 25 °C. The viscosity of the formulations ranged from 91-2504 cps at 37 °C in gel. The rheological profile of the prepared *in situ* gelling systems of Norfloxacin before and after gelation is shown in Figures 1, 2, 3 and 4. To assess the rheological behavior, the viscosity of the formulation (F1-F12), before and after addition of simulated tear fluid, was evaluated using a Brookfield viscometer (Spindle no. 62), varying the angular velocities.

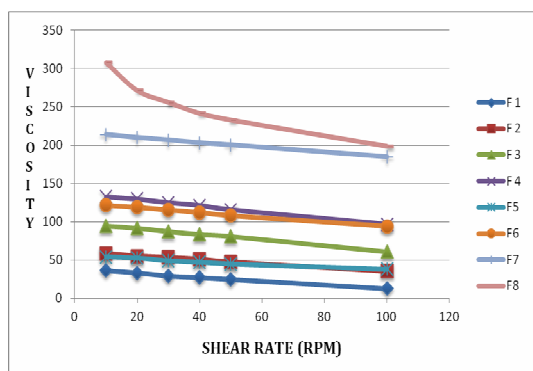


Figure 1: Rheological evaluation of formulations F1-F8 before gelation

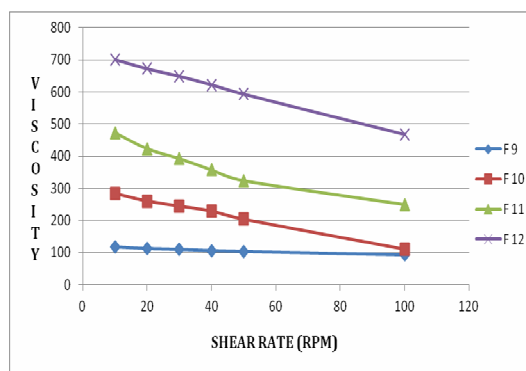


Figure 2: Rheological evaluation of formulations F9-F12 before gelation

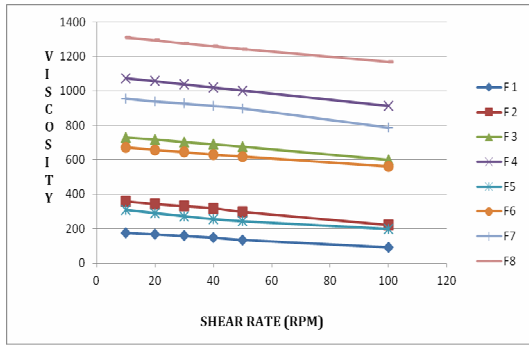


Figure 3: Rheological evaluation of formulations F1-F8 after gelation

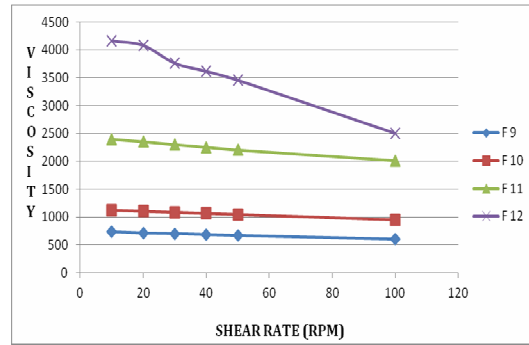


Figure 4: Rheological evaluation of formulations F9-F12 after gelation

In vitro drug release/diffusion test

The *in situ* gelling formulations of Norfloxacin, F1-F12, were subjected to *in vitro* release studies, which were carried out using simulated tear fluid (STF) of pH 7.4 as dissolution medium. *In vitro* release data indicated that formulation F8 showed a better sustained effect than the other formulations did. The prolonged release in the later stage could be attributed to the slow diffusion of the drug through the polymer matrix. The initial burst release of the drug could be explained by the fact that the *in situ* gelling system was formulated in water and hence the polymer was completely hydrated. When they came in contact with STF, gelation occurred and a rehydrated matrix was formed in which hydration and water penetration no longer limited drug release, leading to an apparent diffusion-controlled release.

The *in vitro* drug release conditions may be very different from those likely to be encountered in the eye. However, the results clearly show that the gels have the ability to retain the drug for a prolonged period of time (8 hours) and that

premature drug release will not occur. In the cul-de-sac, the gels will probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movement. It is also observed that the dissolution in the cul-de-sac will proceed more slowly than that seen in the *in vitro* experiments, as the normal resident volume of the lachrymal fluid in the human eye is 7.5-10 μ L. On visual inspection at periodic intervals during the *in vitro* drug release experiments, the gels showed gradual swelling after 6 hours, which resulted in an increase in the volume of most gels. No discernible relationship between the extent of swelling and gel composition could be established. Also, no apparent changes or disruptions in the integrity of the gels were noticed during the course of experiment. The only evidence to suggest a gradual dissolution of the polymers comprising the gels was that the filtration of the aliquot of release medium became increasingly difficult after each successive withdrawal. This shows the *in vitro* release of the drug from the *in situ* formulation follows the diffusion mechanism.

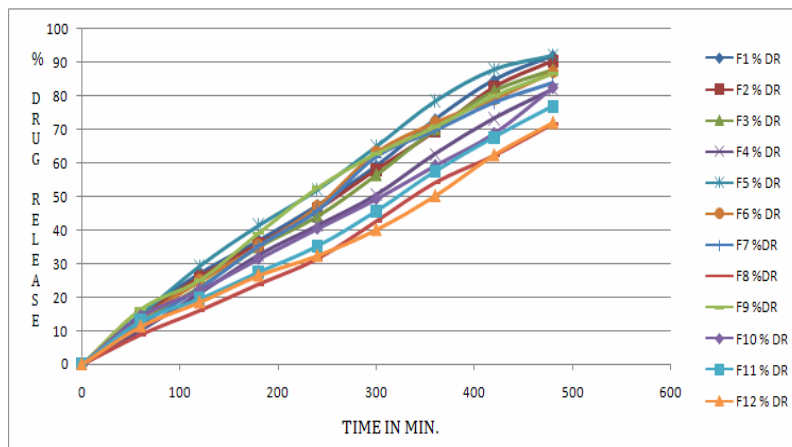


Figure 5: Percentage drug release of *in situ* gel (F1-F12)

CONCLUSION

An ophthalmic *in situ* gelling system of Norfloxacin hydrochloride was successfully formulated using three different gelling agents HPMC E50 LV, HPMC E4M, and HPLC K4M. The clarity of the prepared formulations was found satisfactory. The pH of all formulations was found to be satisfactory ranging between pH 6-7.4. The drug content of the prepared formulation was within acceptable limits and ensured dose uniformity. Formulation F9 showed the maximum drug content. All the formulations, except F1, F5 and F9, showed instantaneous gelation when contacted with simulated tear fluid. Formulations F4, F8 and F12 showed sustained drug release for a period of 8 hours. Formulation F8 showed the most sustained drug release.

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