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Review Article

FORMULATION, DEVELOPMENT AND EVALUATION OF OPTHALMIC IN SITU FORMULATION OF NORFLOXACIN

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ABSTRACT

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergo a sol–gel transition in the cul-de-sac. Norfloxacin ophthalmic solution has been shown to be effective 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 Gellan gum and HPMC by ion activated gelling system with objectives of increasing contact time, achieving controlled release, reduction in frequency of administration and greater therapeutic efficacy of drug. The prepared in-situ gels were then evaluated for their visual appearance, clarity, pH, drug content analysis, in-vitro gelation (Gelling capacity), rheological studies, sterility testing and *in-vitro* drug release studies. It is evident from these studies that, formed polymeric in-situ gels had transparent, clear possessing satisfactory gelling capacity. The developed formulation was transparent in colour, therapeutically efficacious, stable, non-irritant with sustained release of drug.

Keyword: Norfloxacin, In situ gel, Gellan gum, Ocular delivery, sol-gel transition.

INTRODUCTION [1-5]

The eye is a complex sensory organ design differently from other organs from its anatomy andphysiology point of view. Unique anatomical and physiological features of eye make it impermeable to foreign particles. There are various dosage forms for ocular drug delivery butdue to strong protective mechanism and barriers exerted by the eyes the ocular drug absorptionand penetration especially into the posterior part of the eyes in desired therapeutic concentrations is not achieved successfully.

Ocular drugs are mostly applied locally to the surface of the eye as eye drops for treatment ofeither the external ocular infections such as conjunctivitis, blepharitis, keratitis sicca, or intraocular diseases such as glaucoma, proliferative vitreoretinopathy, endophthalmitis, recurrent uveitis, acute retinal necrosis and cytomegalovirus retinitis etc. However, due to efficient protective mechanisms of the eye (e.g. lachrymal secretion, blinking reflex) and systemic absorption in the conjunctiva, major part of the drug is rapidly eliminated from the ocular surface and only a small fraction of drug is absorbed into the eye, which results in poorbioavailability of the drugs. This needs frequent dosing of eye drops, which causes pulse kinetics of the drugs in the eye.

In situ gelling systems can fulfill these criteria successfully as they can be retained at the ocularsurface for longer duration and thus can increase the residence time of the drug at the site of action, resulting in enhanced drug bioavailability and lesser patient incompliance as comparedto conventional ocular drug delivery system. A wide variety of drug molecules and materials of therapeutic advantages such as antibiotics (Ofloxacin, Ciprofloxacin, Gatifloxacin), beta blockers (Timolol, Carteolol), NSAIDs (Ketorolac Tromethamine, Indomethacin), Pilocarpine hydrochloride, Puerarin, Acyclovir has been delivered through in situ gelling systems, which shows the importance of in situ gelling formulations as the future drug delivery systems.

Classification of in-situ gelling polymer

A) According to their origin in situ gelling systems can be divided into two types:

1. Natural: examples include chitosan, alginic acid, xyloglucan, gellan gum, sodium hyaluronate, pectin.

2. Synthetic/semi synthetic: e.g. hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), cellulose acetate phthalate (CAP), Carbopol, Pluronics, poly (lactide-co-glycolide) (PLGA).

B) According to physiological mechanisms gelation of polymers causing by:

1. pH Change
2. Temperature Change

3. Ion Activation

MATERIAL

Table: 1- List of material and supplier

Sr.No.	Chemicals	supplier
1.	Norfloxacin	Shah Enterprises, Mumbai.
2.	Gellan gum	Suvidhinath Laboratory, Vadodara, Gujarat.
3.	HPMC	Dr. V.V. P. F's College of pharmacy, Vilad ghat, Ahmednagar.
4.	Benzalkonium chloride	Dr.V.V.P.F's College ofpharmacy, vilad ghat, Ahmednagar.
5.	Distilled water	Dr.V.V.P.F's College of Pharmacy, vilad ghat, Ahmednagar.

Table: 2- List of Instruments

Sr. No.	Instrument	Manufacturer
1.	Electronic balance	Shimadzu
2.	UV spectrophotometer	Jasco (V 630)
3.	Magnetic Stirrer	Remi Instruments Ltd.
4.	FT-IR spectrophotometer	Jasco (IR 4500)

METHOD

Preparation of ophthalmic formulations of norfloxacin: Gellan was dissolved in hot phosphate buffer pH 7.4 (prepared from potassium dihydrogen ortho phosphate and sodium hydroxide in fresh water for injection at 70 °C under laminar flow), by continuous stirring at 40 °C. The quantity of Norfloxacin required to give a final drug concentration of 0.3% (m/V) was added to the polymeric solutionand stirred until dissolved. The formulations were filled in 10 ml amber colored glass vials, capped with rubber bungs and sealed with aluminum caps. In their final pack, the formulationswere terminally sterilized by autoclaving at 121 °C and 15 Pa for 20 minutes. Sterilized formulations were stored in a refrigerator (4–8 °C). [(6)]

Table: 3- Formulation of In-situ ophthalmic preparation

Sr. No.	Ingredients	Concentration %		
		F1	F2	F3
1.	Norfloxacin	0.1	0.1	0.1
2.	Gellan gum	0.1	0.2	0.3
3.	HPMC	0.1	0.1	0.1
4.	Benzalkonium chloride	0.001	0.001	0.001
5.	Deionized water	Up to 10ml	Up to 10ml	Up to 10ml

Preformulation studies:[7-9]

1. **Organoleptic characteristics of drug and excipients:** The physical characteristics of drug and excipients are carried out by observing physical nature of drug and excipients.
2. **Solubility studies:** Solubility of drug is defined as the amount of solute (drug) that dissolves into a given solvent (solution) to obtain saturated solution of drug at constant temperature and constant pressure. Solubility is an integral parameter of reformulations which has been studied extensively. This focuses on the drug-solvent mechanism which can occur during the delivery of drugs. Such knowledge is important for the formulator, as it gives the information about the selection of best solvent medium for drug substance, recognize and overcome the challenges that occur in the formulation process of pharmaceutical solutions. Solubility of Norfloxacin was tested in various solvents viz; distilled water, methanol, acetone, glacial acetic acid.
3. **Melting point determination:** Melting point determination of Norfloxacin was carried out by using melting point apparatus. Readings were recorded in triplicates.
4. **FT-IR spectroscopy analysis:** Infrared spectra of pure water free Norfloxacin sample is recorded by using FT-IR spectrophotometer (Jasco IR 4500) by suitably diluting with potassium bromide (KBr) at ambient temperature Spectrum were recorded for purity analysis of Norfloxacin at scanning range 400 to 4000 c

Evaluation of in-situ ophthalmic formulation [7-9]

1. **PH:** pH of the formulation was measured by using digital pH meter.
2. **Appearance:** Formulation observed physically.
3. **Clarity:** Checked by observing formulation against clarity test apparatus.
4. **Gelling capacity of the in-situ gel formulation:** Gelling capacity of formulations was evaluated in order

to identify the formulations suitable for use as in-situ gelling systems. Gelation studies were carried out in specially fabricated gelation cells. The cells were cylindrical reservoirs capable of holding 3 mL of solution (simulated tear fluid, STF). Within the cells at the bottom, a 250 L transparent plastic cup was located to hold the gel sample in place after its formation. The studies were carried out using STF of composition 1 (sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g and purified water sufficient to make 100 g) , which simulated the divalent cation content. The antibiotic formulation (100 L) was carefully placed into the cavity of the cup using a micropipette and 2 mL of gelation solution (STF) was added slowly in to it. Gelation was assessed by visual examination.

5. **Rheological studies:** Viscosity values were measured using programmable digital viscometer for solutions and gels. Guard leg was mounted on the viscometer. Helipath spindle was used for measurement of low viscosity of solution. (Range 3.0-12.0). Helipath spindle was inserted in the test material until fluid level was at the immersion groove on the spindle. The spindle was attached to the lower shaft of the viscometer. The shaft was lifted slightly; holding it firmly with one hand while screwing the spindle. The spindle code 00 was used for measurement of the viscosity of solution and spindle code S 92 was used for measurement of the viscosity of gel. The motor was turn on and spindle was rotated. The value of viscosity was recorded from display window.

In vitro release studies: The Franz diffusion cell was used for in vitro study is placed on magnetic stirrer. The receiving fluid, STF (Simulated tear fluid) was placed in to the diffusion cell. The sterile cellophane membrane was placed on the diffusion cell along with test solution (1 ml) after formation of gel with STF. Temperature of 37 ± 1 °C was maintained throughout the study. Samples (1 mL) were withdrawn at regular time intervals and replaced with an equal volume of pre warmed medium. The sample was withdrawn after 1 hour regularly for 8 hours.

RESULTS AND DISCUSSION

Pre-formulation studies:

1. **Organoleptic characteristics of drug and excipients:**

Table: 4- Organoleptic characters of drug and excipients.

Sr.No	Drug and excipients	Organoleptic characters		
		Colour	Odour	Taste
1.	Norfloxacin	White to pale yellow	Odourless	Bitter

2.	Gellan gum	White	Odourless	Tasteless
3.	HPMC	Creamy white	Odourless	Tasteless
4.	Benzalkonium chloride	colourless	Characteristic odour	Tasteless

2. **Solubility study:** solubility of the drug is carried out in various organic solvents such as, water, glacial acetic acid, ethanol, and methanol. norfloxacin is freely soluble in glacial acetic acid and very slightly soluble in ethanol, methanol and water.
3. **Melting point determination:** melting point determined by melting point apparatus, melting point of Norfloxacin is 221ºC.
4. **FT-IR spectroscopy:**

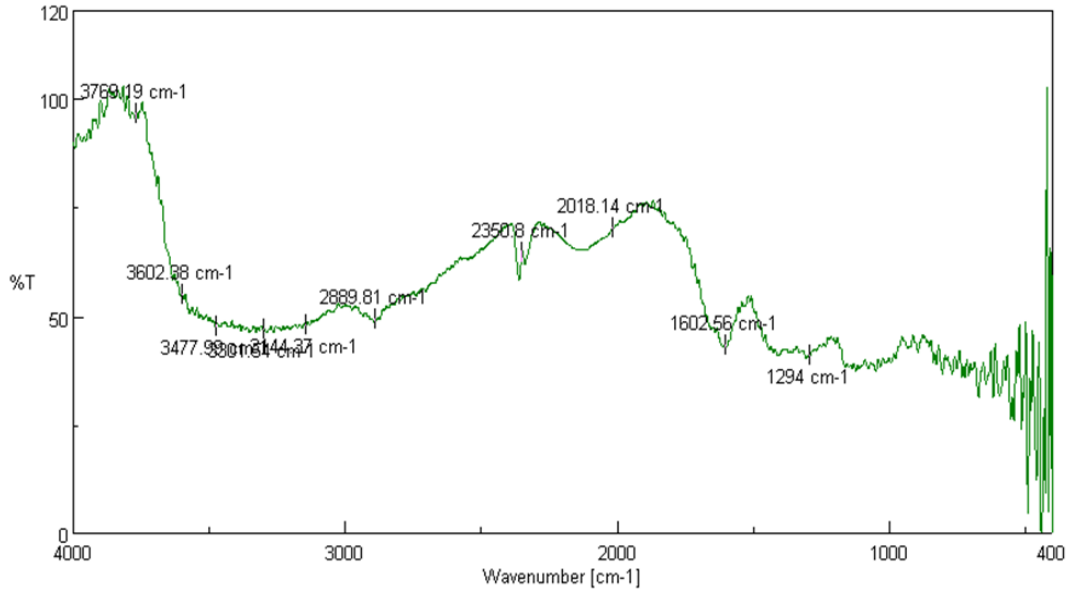

Fig: 1- IR of Norfloxacin

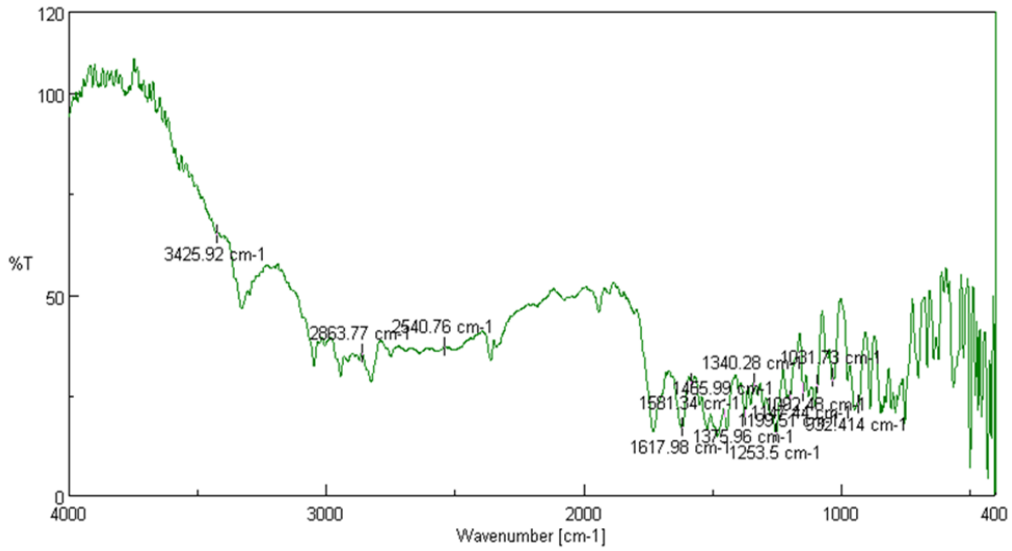

Fig: 2- IR of Gellan gum

Evaluation of in-situ ophthalmic formulation:

1. Appearance, clarity, pH:

The appearances of all formulations were transparent and clear. Terminal sterilization by autoclaving had no effect on the formulations. The haziness observed during autoclaving due to precipitation of HPMC at elevated temperature was found to disappear and the clarity was regained after overnight standing. The pH of all the formulations was found to be within the range of 6.8 to 7.4, which is desirable for the ophthalmic formulations

Table: 5- Appearance, clarity, pH

Formulation code	Visual appearance	Clarity	pH
F1	Transparent	Clear	6.8
F2	Transparent	Clear	7.2
F3	Transparent	Clear	7.4

2. Gelling capacity:

The viscosity and gelling capacity plays important role for in situ gelling system. The formulation should have an optimum viscosity for easy instillation into the eye as a liquid which undergo sol-to-gel transition. Prepared in situ gelling systems were evaluated for the in vitro gelation capacity. All the formulations gave satisfactory results.

Table: 6- Gelling capacity

Formulation	Gelling capacity

F1	+
F2	++
F3	+++

+ Gelation immediate (60-90 sec.), remains stable for 1 hrs. , ++ Gelation immediate (60-90 sec), remains stable for 2 to 3 hrs. , +++ Gelation immediate (60-90 sec.), which remains for extended periods 7 to 8 hrs.

3. Rheological studies:

The rheological study of the formulations exhibited decrease in viscosity on increase in shear rate because of the pseudo plastic behavior of the formulations. Moreover, the pseudo plastic property of these formulations may be in favor of sustaining the release of drug in the conjunctival sac of the eye.

4. In vitro drug release studies:

The release profile of the formulations shown in figure below. The results indicated that the formulation F-3 showed better sustaining effect amongst all formulations. This may be due to the presence of higher concentration of Gellan gum along with HPMC in the formulation F-3. Results indicated that, the drug release was significantly prolonged by using the in situ gelling system due to the addition of the polymers Gellan gum and HPMC.

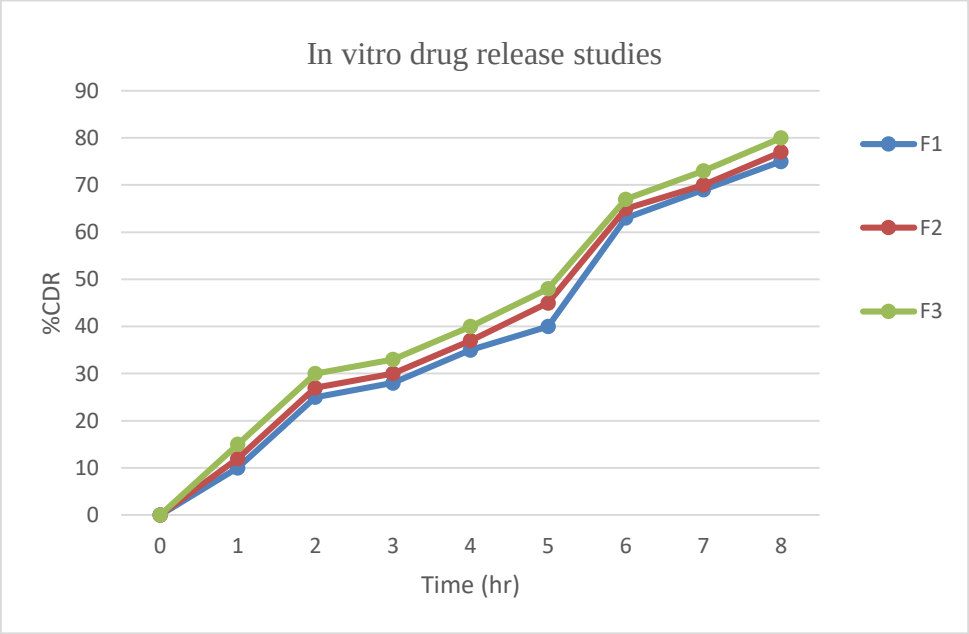

Fig: 3- In vitro drug release studies

Table: 7- In-vitro Drug release studies

Time	**% Cumulative Drug Release**		
	F1	**F2**	**F3**
0	0	0	0
1	10	12	15
2	25	27	30
3	28	30	33
4	35	37	40
5	40	45	48
6	63	65	67
7	69	70	73
8	75	77	80

CONCLUSION

Norfloxacin, which is broad spectrum antibacterial agent used in treatment of ocular infections, was successfully formulated as an in-situ gelling system by using gellan gum as gelling agent and HPMC as viscosity enhancing agent. The formulated system was provided release of the drug over 8-hour period in vitro. Out of 3 different batches prepared only one batch was shoeing optimum results. Optimized formulation F3 were liquid in consistency before in contact with ion(salt) and underwent rapid gelation upon contact with ions(salt) the formulation were found to be clear, translucent having good in situ gelling capacity. The pH of the formulations was found to be within the range of 6.8 to 7.4, which is desirable for the ophthalmic formulations

Hence, from the above results this can be concluded that in situ ophthalmic eye drop of norfloxacin is the better for treatment of ocular infections. It is the best mode of retaining the drug into the site of action and got better bioavailability of drug in formulation. Hence, this can be viewed as a viable alternative to conventional eye drops by virtue of its ability to enhance pre-corneal residence time and thereby ocular bioavailability. The ease of administration coupled with its ability to provide sustained release could probably result in less frequent administration, thus enhancing patient compliance.

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