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Research Article

Formulation and Evaluation of Norfloxacin Ocular *In-situ* Gel

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Abstract

The study aims to prepare and evaluate the in situ Norfloxacin gel for the treatment of Conjunctivitis. The pronounced fluctuations and repeated administration of conventional eye drops are the main disadvantages of ophthalmic drug delivery. The ocular *in situ* gelling system is designed to achieve a prolonged therapeutic effect by improving residence time at the application site. Infrared spectroscopy studies of Norfloxacin, Sodium alginate, Gellan gum, and HPMC K4M alone and their physical mixture revealed that Norfloxacin is compatible with all the polymers used. The Ophthalmic *in situ* gelling system of Norfloxacin was successfully formulated using different gelling agents viz. Sodium alginate, Gellan gum, and HPMC K4M are viscosity-enhancing agents. Prepared *in situ* gels were subjected to evaluation such as visual appearance, pH, and drug content. All formulations were found transparent and clear, the pH of the formulations was between 7.1 to 7.4, and drug content was found within 95-98% in all *in situ* gelling systems. All the formulations showed instantaneous gelation when contacted with simulated tear fluid. The viscosity of all formulations decreased as the shear rate increased, indicating the pseudoplastic fluid's character. *In vitro* release of Norfloxacin from the prepared formulations was studied for 6 hours. Results reveal that all formulations exhibited sustained release of the drug from the gelrite polymeric network over 6 hrs. The stability studies confirmed that *in situ* gelling formulations of Norfloxacin remained more stable at ambient temperature and humidity. The present work was a satisfactory preliminary study in developing *in situ* gelling systems of Norfloxacin.

Keywords: Conjunctivitis, Norfloxacin, *in situ* gel, Sodium alginate, sustained release.

INTRODUCTION

In recent years, much focus has been placed on developing novel drug delivery methods. Controlled drug delivery can increase the therapeutic efficacy and safety of drugs supplied using conventional methods by allowing for more accurate spatial and temporal placement within the body. Targeted Delivery, Controlled Delivery, and Modulated Delivery are the three primary drug delivery methods [1].

To prevent the unintended danger of eye injury due to a high blood concentration of the drug, the eye as a drug delivery route is often reserved for local therapy in contrast to systemic therapy. The unique structure, physiology, and biochemistry of the eye render this organ resistant to outside chemicals, making it a perpetual challenge for formulators to breach the eye's protective barriers without causing irreversible tissue damage. Most ocular treatments, such as eye drops and suspensions, involve the topical administration of opthalmically active drugs to tissues surrounding the ocular cavity [2]. Most of the drug in these dose forms is diluted in the tear film upon instillation of the eye drop solution into the cul-de-sac and swiftly drained from the precorneal cavity by continual tear flow and lacrimal-nasal drainage. Consequently, the targeted tissues absorb a negligible amount of the injected dose. For the installation to have an appropriate therapeutic impact, concentrated solutions and frequent dosage are required. One of the new classes of drug delivery systems, polymeric film ocular drug delivery systems/ocular inserts, which are receiving worldwide acclaim, release medications at a preprogrammed rate for a longer duration by extending the precorneal residence time [3].

The disadvantages of conventional drug administration methods such as suspension, ointment, and solution are increased pre-corneal drainage, impaired vision, limited bioavailability, and short residence time [4]. The absorption of drugs in the eye is severely limited by protective mechanisms that ensure proper eye function and by concomitant factors such as drainage of instilled solutions, lachrymation and tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, limited corneal area and poor corneal permeability, and binding by lacrimal proteins. The primary objective of ocular therapies is to surpass the eye's structural barriers and defensive mechanisms to induce the appropriate pharmacological response [5].

The goal of designing a therapeutic system is to attain the optimal concentration and duration of the drug at the active site. A therapeutic agent's ocular disposition and elimination are determined by the agent's physicochemical qualities and pertinent ocular anatomy and physiology. Therefore, a successful drug delivery system design necessitates an integrated understanding of the drug molecule and the ocular route of administration [6]. To produce ocular delivery systems with high therapeutic efficacy [7], it is necessary to develop better, more sensitive diagnostic procedures and novel therapeutic agents. This is because current systems have limitations that make them less effective.

The many attempts to improve the bioavailability and duration of therapeutic action of ophthalmic drugs can be

categorized into two distinct groups. The first is based on sustained drug delivery systems, which deliver ophthalmic drugs in a controlled and continuous manner. The second objective is to maximize corneal drug absorption while decreasing precorneal drug loss [8]. In recent years, substantial emphasis has been paid to developing *in situ* gel systems due to the several advantages this polymeric system has, including ease of administration and reduced frequency of administration, enhanced patient compliance, and comfort. *In situ* gel formation is caused by various stimuli, including pH change, temperature modulation, and solvent exchange [9].

MATERIAL AND METHODS

Norfloxacin (NFX) was obtained as a free sample from IPCA Laboratories Ltd, Indore. Gellan Gum, HPMC, Sodium Alginate, Sodium chloride, Sodium bi carbonate, Potassium chloride, Di Sod Hydrogen Phosphate, and Potassium dihydrogen orthophosphate were acquired from S.D. Fine-Chem Ltd., India.

Calibration curve of Norfloxacin

Accurately weighed, 100 mg of NFX was dissolved in PBS pH 7.4, and volume was made up to 100 ml, resulting in a stock solution of 1000µg/ml. Then, 10 ml of this stock solution was diluted to 100ml with PBS pH 7.4 to get a stock solution of 100µg/ml. The stock solution was taken in aliquots of 0.2 ml, 0.4 ml, 0.6 ml up to 2.0 ml into a series of 10 ml volumetric flasks, and volume was made up to the mark with PBS pH 7.4. The solutions were filtered through Whatman filter paper no. 1, and the filtrate was analyzed at λmax 273 nm using a UV visible spectrophotometer. PBS pH 7.4 was used as the blank solution. The standard curve was plotted between absorbance and concentration [10].

Drug - Excipients Compatibility Studies

The infrared spectrum of any drug or medicine provides information about the groups present in that drug. Using the KBr disc method, the IR absorption spectra of pure drug and physical admixtures of drug with various additives were obtained in the region of 4000-400 cm^{-1}. FTIR analysis was used to determine drug-excipient compatibility. The spectrum of physical mixtures obtained was examined for prominent peaks and documented [11].

Preparation of *in situ* gelling system of Norfloxacin

Aqueous solutions of *in situ* gel systems were formulated by dissolving polymers (gellan gum, sodium alginate, HPMC) in warm (70°C) phosphate buffer (pH 7.4) by constant stirring. The polymeric solution was subsequently cooled to room temperature (25 ± 1°C) to which specified quantities of Norfloxacin (0.3%W/V) was added and stirred until completely dissolved (Table 1). Terminal sterilization of the ophthalmic gels was carried out by heating in an autoclave and kept in a refrigerator until further investigation [12].

Table 1: Composition of Norfloxacin *in situ* gel systems

S. No.	Ingredients	Formulation code			
		N1	N2	N3	N4
1.	Norfloxacin	0.3g	0.3g	0.3g	0.3g
2.	Gellan gum	0.5g	0.7g	0.5g	0.7g
3.	Sodium alginate	0.3g	0.3g	0.3g	0.3g
4.	HPMC K4M	0.4g	0.4g	0.5g	0.5g
5.	PBS pH 7.4	100ml	100ml	100ml	100ml

Evaluation of Prepared formulation

The prepared gels were evaluated for drug content, pH, viscosity, *In vitro* release characteristics, and the selected gel formulation was subjected to stability studies.

Appearance and pH

Clarity testing was done on all developed formulations by visually examining the samples to determine the presence of any transparent or colored particle materials or turbidity. The pH of the various gels was evaluated by calibrated pH meter at 25±0.5°C as per the standard protocol [12].

Drug content

The drug content was determined using freshly prepared simulated tear fluid pH 7.4 (STF). STF was prepared by dissolving 0.670 g of sodium chloride, 0.200 g of sodium bicarbonate, 0.008 g of calcium chloride dihydrate in purified water and finally make up to 100 ml volume with purified water. 1 ml of the formulation was diluted to 50 ml using STF. 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 273nm by using UV-Vis spectrophotometer [13].

In vitro gelation

The gelation of gels was evaluated using a polypropylene vial containing simulated tear fluid as the gelation solution and equilibrated in a water bath at 37±0.5°C. Using a micropipette, 100 µl of each mixture was accurately transferred into a separate vial, followed by the addition of 2ml of simulated tear fluid. Gelling capacity was determined by visually seeing the formation and measuring the time required for gelation and the time needed to dissolve the gel [14].

Rheological studies

At 34±1°C, the viscosity of in situ gels was measured using a Brookfield Viscometer. The developed formulation was put into the viscometer's small adapter, and the angular velocity raised gradually from 10 to 100 rpm. The angular velocity hierarchy was reversed. The viscosity was calculated using the average of the two measurements. The composition was then placed into an ointment jar, and simulated tear fluid was added to raise the pH to 7.4 [15].

In vitro Drug Release Studies

In vitro release tests for formulations N1 to N4 were conducted using a modified USP dissolution testing device. The temperature of the dissolving medium (STF) was maintained at 37±1°C. Shafts were permitted to rotate at a consistent rate (50 rpm). Throughout 8 hrs, aliquots were removed and replaced with an equal volume of the receptor media at specified intervals. At 273 nm, using a UV-visible spectrophotometer, the drug concentration in the withdrawn samples was measured [16].

Stability Studies

Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. The stability study of the formulated gel (N1) was carried out under different environmental conditions of 2-8°C (45% RH), 25-30°C (60% RH), and 45-50°C (75% RH) for a period of 3 months. The gel was characterized for the drug content during the stability study period [17].

RESULTS AND DISCUSSION

Determination of wavelength maxima (λ_{max}) and Calibration Curve of Norfloxacin

The UV spectrum of the drug was obtained by scanning drug solutions (10µg/ml) and showed maximum absorption at 273nm. The calibration curve was prepared in PBS pH 7.4 at

273nm and linearly regressed. The correlation coefficient for standard curves was found to be near one, indicating an excellent co-linear correlation between concentrations 2-20 µg/ml (Figure 1). Hence, the drug follows Beer-Lambert Law in the above range that gives a straight line with an equation: y = 0.084x- 0.003 and r^2 = 0.999.

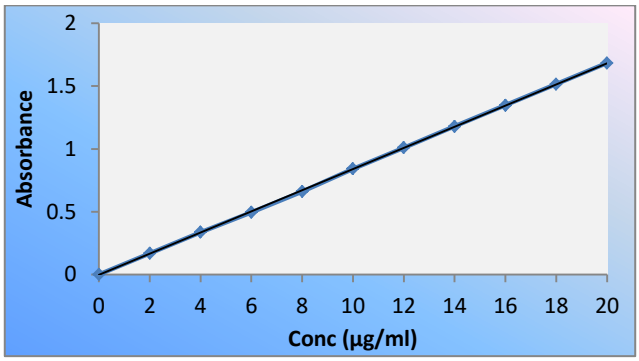

Figure 1: Calibration curve of NFX in PBS pH 7.4 at 273nm

Drug-Excipients Compatibility Study

FTIR analysis was performed to confirm the drug and excipient interaction. The scan was evaluated for the presence of principal drug peaks, shifting and masking of drug peaks, and the appearance of new peaks due to excipient interaction. There are no extra peaks seen other than the normal peak in the spectra of the mixture of the drug and excipients, so there is no interaction with the drug and excipient, and they are compatible with each other.

Evaluation of NFX *in situ* gel

Appearance and pH

In situ gels (N1-N4) were prepared and assessed for different parameters. The visual observation of N1-N4 signifies that the formulations varied from clear solution to turbid with transparent (T) or less transparent (L) in nature (Table 2). The pH of formulations ranged between 7.1–7.4 and was not influenced by the polymers studied in the current investigation.

Table 2: Physicochemical characteristics of *in situ* gel formulations

S. No.	Formulation code	Parameter	
		Transparency	pH
1.	**N1**	T	7.2 ±0.2
2.	**N2**	L	7.4 ±0.08
3.	**N3**	T	7.2 ±0.2
4.	**N4**	T	7.1 ±0.3

Drug content

The drug content was determined using freshly prepared simulated tear fluid pH 7.4 (STF). The drug content of the *in situ* gels were determined and found to be more than >95% (Table 3). 1 ml of the formulation was diluted to 50 ml using STF. The formed gel was utterly crushed and filtered through a 0.45-mm filter membrane, and Norfloxacin concentration was then determined at 273nm by using a UV-Vis spectrophotometer.

Table 3: % Drug content of *in situ* gel formulations

S. No.	Formulation code	% Drug Content
1.	**N1**	97.48±1.22%
2.	**N2**	96.24±2.38%
3.	**N3**	95.98±2.42%
4.	**N4**	96.71±3.2%

In vitro gelation

The visual observation of time taken for gel formation, gel remains for the time period and gel dissolves was done for N1-N4 *in situ* gels. Formulations demonstrated immediate gelation and also was stable for an extended period (Table 4).

Table 4: Gelling Capacity of *in situ* gel formulations

S. No.	Formulation code	Gelling Capacity
1.	**N1**	+++
2.	**N2**	++
3.	**N3**	+++
4.	**N4**	++

++ gelation immediate and remains for few hours, +++ shows gelation immediate and remains for extended period

Rheological studies

The viscosity is a critical factor in determining the ocular residence time of the instilled formulation. The viscosity of N1-N4 are listed in Table 5. It was demonstrated that *in situ* gels displayed pseudo-plastic flow or shear-thinning rheological behavior as shown by a drop in viscosity with higher angular velocity. Polymers (gellan gum, sodium alginate, and HPMC) have the inherent capacity to produce a gel. This peculiar phenomenon endorses the *in situ* gelling characteristics of the N1-N4 gels.

Table 5: Viscosity of *in situ* gel formulations (±SD, n=3)

S. No.	Formulation code	Viscosity (cps)
1.	**N1**	2175±112
2.	**N2**	3050±208
3.	**N3**	1140±126
4.	**N4**	3700±234

In vitro Drug Release Studies

The drug release from *in situ* gels is imperative for absorption and to elicit a therapeutic response. A comparison of the cumulative amount (%) of NFX released from N1-N4 is presented in figure 2. It is apparent from figure 4 that the drug release profiles of N1-N4 *in situ* gels were relatively distinct. However, the percentage of NFX release increases as a function of time. Specifically, the drug release rate decreased marginally as follows; N3>N4>N2>N1. These minor variations in drug release could be correlated to the concentration of polymers used. N1 showed an extended release profile and was selected for further studies.

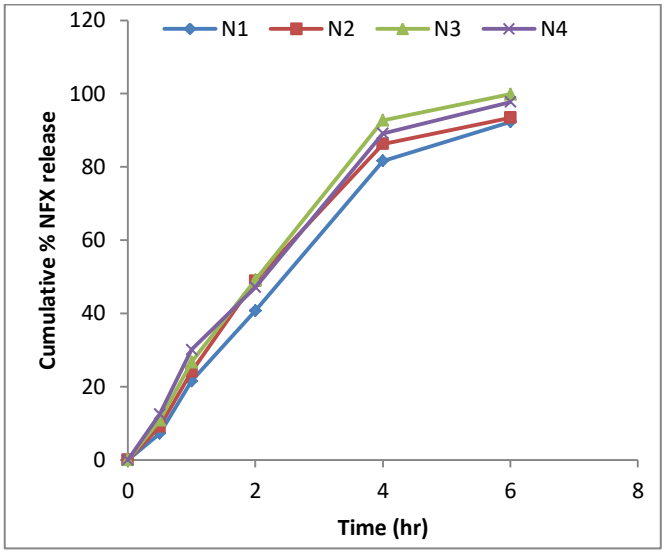

Figure 2: % drug release of NFX from *in situ* gel formulations

Stability study of best formulation (N1)

Since the release pattern of drug from formulation N1 was found to be good compared to all formulations, further stability studies were carried out on it. Stability studies as per ICH guidelines performed showed that the optimized formulation was stable (Table 6).

Table 6: Stability studies at different conditions (N1)

Storage Conditions	Formulation (N1)	Observations on storage for Drug content (%)			
		Initial	1 month	2 months	3 months
4-5°C	% Drug Content	98.15±1.6	98.15±3.6	97.49±3.3	97.08±1.4
25±2°C and 60±5%	% Drug Content	98.15±1.6	98.15±1.5	98.03±1.6	97.87±3.3
37±0.5°C and 75±5%	% Drug Content	98.15±1.6	98.12±2.7	98.01±3.1	96.53±1.7

Values are mean± SD (n=3)

CONCLUSIONS

Since the novel ocular in-situ gel of Norfloxacin was found to be non-irritant and to provide sustained release with improved ocular residence time by reducing dosage frequency, the above research work led to the conclusion that the in-situ gel system is a viable alternative to conventional eyedrops due to its ability to enhance bioavailability via its longer pre-corneal residence time, ability to maintain drug stability, and in the event of administration enhances patient compliance. It is, therefore, concluded that personalized delivery of ophthalmic medicines represents the future of ophthalmic drug delivery.

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CONFLICTS OF INTEREST

Author reported no conflicts of interest.

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