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Preparation and Characterization of Natamycin Loaded Bioadhesive *in Situ* Ophthalmic Gel for Enhanced Bioavailability

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Abstract: Natamycin, an antifungal agent that has been approved in the management of exterior fungal infections of the oculus such as fungal keratitis, blepharitis, and conjunctivitis. The ophthalmic preparation needs frequent installation into the eye due to the quick precornel drug loss which may lead to poor bioavailability. The present investigation aimed at formulation development and characterization of *in situ* ophthalmic gel of natamycin by using a blend polymer of sodium alginate, ethylcellulose, and Xanthan gum for better residence time to improve the bioavailability of the drug. The six different formulations (F1 to F6) of natamycin *in situ* gel were prepared. All the formulations were evaluated for clarity, visual appearance, pH, gelling capacity, drug content, drug release, release kinetic, ocular irritancy, and *in vitro* stability. The results were found to have complied with the pharmacopoeial specification. The *in vitro* drug releases of F3 formulation established maximum drug release for 8 h as compared to other formulations in sustained manner. Further, the F3 formulation was found to be stable, safe and innocuous. The studies suggested that prepared *in situ* ophthalmic gel of natamycin will be a valuable alternative to conventional eye drops to counter the precorneal loss.

Keywords: Natamycin, bioadhesive *in situ* gel, ophthalmic preparation, bioavailability

1. Introduction

There are a lot of challenges in ophthalmic drug delivery for pharmaceutical scientists. The outer layer of the eye obstructs the entry of foreign particles. The chief problem in ophthalmic preparation is to maintain optimal drug concentration at the site of action. The eye drop is the most commonly used for eye preparation for the healing of eye diseases. Further eye drops are associated with various disadvantages such as swift removal of drugs due to tear turnover, repeatedly instillation of eye drops, metabolism of drugs by enzymatic drainage from nasolacrimal, conjunctival absorption, fuzzy vision, and absence of controlled release [1-3].

Apart from these disadvantages, noteworthy developments have been made in ocular drug delivery. Several new techniques of ophthalmic drug delivery systems have been introduced. This new drug delivery system can improve therapeutic efficacy and also retain inside the eyes. This system can retain the drug for a longer time in the eye cavity [4-6].

The drug can retain in the corneal surface by adding viscosity enhancing agents such as methylcellulose. But these dosage forms give only marginally more sustained drug-eye contact than eye drop solutions and do not yield a constant

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drug bioavailability as originally hoped. Similarly, the ocusert system has been introduced, and it decreases the dosing. The ocusert has better therapeutic properties due to enhance bioavailability. The *in situ* gelling systems are a solution when administered into eye, but changes to the gel phase upon exposure to physiological conditions. The gel-forming enhances the pre-corneal residence time of the drug and increases its bioavailability. It led to a decrease in the drug release and maintained therapeutic efficacy for a longer time [6-8].

Fungal Keratitis is an inflammation of the cornea caused by a fungal infection in which the patient has a decreased vision, ocular pain, red-eye, and often a cloud / opaque cornea. It is usually characterized by a corneal epithelial defect and inflammation of the corneal stroma. The topical administration is the most common and accepted the administration route, but it results in very poor drug bioavailability. To maintain therapeutic levels of a drug, frequent applications, and high concentrations are necessary, which may induce local and systemic side effects and thus reduces the patient's compliance. The successful treatment of eye diseases requires an effective concentration of drugs at the eye for a sufficient period of time [9-12]. Natamycin is an antifungal medication. Natamycin ophthalmic (for the eyes) is used to treat the eyes' fungal infections [12-15]. The study aimed to develop *in situ* gel of Natamycin by using a blend polymer of sodium alginate, ethylcellulose, and HPMC.

2. Materials and Method

2.1 Preparation of Formulation of *in Situ* Ocular Gel of Natamycin (pH Triggered System and Ion Activated System)

The *in situ* ocular gel of Natamycin was prepared by mixing with different concentrations of polymers. The compositions of the ingredient of *in situ* ocular gel are shown in Table 1. The polymeric solution was prepared by dispersing the required quantity of sodium alginate as the main polymer and Xanthan gum, Ethyl cellulose as copolymers in water using a magnetic stirrer until the polymers completely dissolve. An aqueous solution of natamycin was added into the polymeric solution with continuous stirring. Buffering and osmolality agents were added to the resulting solution along with benzalkonium chloride. The solution's pH was adjusted to 6.5, using 0.1 N NaoH/0.1 N HCl [13-15].

Table 1 - Pharmaceutical composition of various natamycin loaded *in situ* ocular gels (formulation code: F1-F6)

Ingredient	F1	F2	F3	F4	F5	F6
Natamycin (mg)	400	400	400	400	400	400
Sodium alginate (mg)	600	600	600	600	600	600
Xanthan gum (mg)	600	500	400	300	200	100
Ethyl cellulose (mg)	100	200	300	400	500	600
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02
Sodium Chloride (mg)	600	600	600	600	600	600
Distilled water	qs	qs	qs	qs	qs	qs

2.2 Characterization

(a) Clarity and Visual Appearance

The clarity and visual appearance of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

(b) pH Determination

The pH of each of the prepared ophthalmic formulations was determined by using pH meter (equip-tronics). The pH meter was calibrated before each use with standard pH 4, 7, and 9.2 buffer solutions.

(c) Gelling Capacity

The prepared formulation's gelling capacity was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. Further, the gelling time of in situ ocular gel was noted [16].

(d) Drug Content

The drug content estimation was carried out by diluting 1 ml of prepared formulation in 100 ml of distilled water and analyzed using a UV-visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan) at 304 nm [17].

(e) In Vitro Dissolution Study

The *in vitro* release of Natamycin from the prepared formulations was studied using a modified diffusion testing apparatus. The freshly prepared simulated tear fluid (pH 7.4) 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 an inner diameter of 3.4 cm. Two millilitres of the formulation were accurately pipette into the glass cylinder known as the donor chamber. The cylinder was suspended in a beaker (Acceptor chamber) containing 100 ml of diffusion medium so that the membrane just touches the surface of the medium. The acceptor chamber was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using a magnetic stirrer. About 1 ml of the sample was withdrawn at a time interval of 1 hour and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and analyzed at 304 nm using UV spectrophotometer [18-22].

(f) Kinetic Modeling

The formulations were exposed to determine the kinetics of drug release. The *in vitro* drug release data were analyzed by fitting them into different kinetic models, namely zero-order, first-order, Higuchi, and Korsmeyer-Peppas, to investigate the release mechanism of natamycin from the formulation [23,24].

(g) Ocular Irritancy

Ocular irritation study was performed on the optimized formulation in four albino rabbits (male). The Institutional Animal Ethical Committee approved the experimental protocol of Research. The registration no. is MIP/IAEC/1555/P'Col/2020/06 (1555/PO/a/11/CPCSEA), as per the guidance of the "Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)" and institutional regulations and national criteria for animal experiments. Each rabbit weighing about 2 to 3 kg, and 0.1 ml of the optimized sterile natamycin formulation was instilled into cul-de-sac twice a day for 14 days. The eyes of each rabbit were examined at a particular time interval after the instillation of the optimized formulation (F3). The rabbits were monitored periodically for redness, swelling, watering of the eye [25,26].

(h) Stability Study of Prepared Ocular Gel

Stability studies were carried out as per International Conference on Harmonization (ICH) guidelines. The stability studies of in situ gel of Natamycin was stored at 25°C ± 2°C / 60% RH ± 5% and 40°C ± 2°C / 75% RH ± 5 % for 3 months. The gel was analysed for physiochemical parameters like color change, clarity, visual appearance, gelling capacity, drug content, and pH for three months [24,27].

3. Results and Discussion

3.1 Visual Appearance

During the preparation of the *in-situ* ocular gel of natamycin drug, the visual appearance of formulation on varying the concentration of polymer in the drug has been observed. The visual appearance of Natamycin ocular gel is displayed in Table 2. The visual appearance of various formulations was transparent. It depicted that the uniform distribution of the drug in the formulation.

Table 2 - Physicochemical characterization of prepared natamycin loaded *in situ* ocular gel formulations

Formulations	Visual appearance	Clarity	pH*	Drug content (%)*	Gelling capacity
F1	Transparent	Clear	6.46 ± 0.12	97.11 ± 1.52	++
F2	Transparent	Clear	6.82 ± 0.06	96.43 ± 1.33	++
F3	Transparent	Clear	6.18 ± 0.49	97.52 ± 0.49	++
F4	Transparent	Clear	6.39 ± 0.24	95.63 ± 1.22	++
F5	Transparent	Clear	6.27 ± 0.09	96.51 ± 1.65	++
F6	Transparent	Clear	6.54 ± 0.11	96.28 ± 1.41	++

**Values are mean ± S.D*

"++" signs mean, the formulation present in semi solid state (gel form)

3.2 Clarity

During the preparation of *in-situ* ocular gel of Natamycin drug, clarity of formulation on varying the polymer concentration in the drug has been observed. The clarity of Natamycin ocular gel is displayed in Table 2. The clarity of various formulations was transparent. It depicted the uniform distribution of the drug in the formulation.

3.3 pH Determination

The pH of the various formulations observed and is displayed in Table 2. The pH of the formulation was found to be in the range of 6.18–6.82, which is good for the eye. All the formulations of ocular gel were shown pH nearer to the eyes.

3.4 Drug Content

This was measured for formulations F1 to F6 in triplicate, and results are illustrated in Table 2. The drug content capacity for F1 to F6 ranged between 95.63% to 97.52%. The F6 revealed the lowest drug content capacity while F3 displayed the highest drug content capacity, and it was the highest drug content capacity compared to other formulations.

3.5 Gelling Capacity

The gelling capacity was measured for formulations F1 to F6 in triplicate, and results are illustrated in Table 2. The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed.

3.6 Assessment of *in Vitro* Drug Release

The *in vitro* releases of the drug from *in situ* ocular gel are illustrated in Fig. 1. It represents the percentage of drug release from the formulation at a different rate. The formulation F3 has maximum drug release as compared to other formulations. The percentage release of various formulations was 97.4±0.35%, 89.3±0.92%, 95.1±0.36%, and 87.6±0.52% for F1, F2, F5, and F6, respectively at 6 hr. Similarly, the F3 and F4 showed drug release 97.5±0.85% and 92.5±0.13% at 8 hr. The F3 demonstrated sustained drug release compared to other formulations. This could be the reason for higher concentration of sodium alginate and ethyl cellulose among the developed formulations. The polymer and sodium alginate undergoes rapid gel formation due to formation of complex of calcium alginate by interacting with divalent cation (Ca^{2+}) present in lachrymal fluid (pH 7.4) [28,29]. Alginate can be ionically cross-linked in the presence of divalent cations. Thus the *in vitro* dissolution test designated the sustained release nature of *in situ* gel of natamycin.

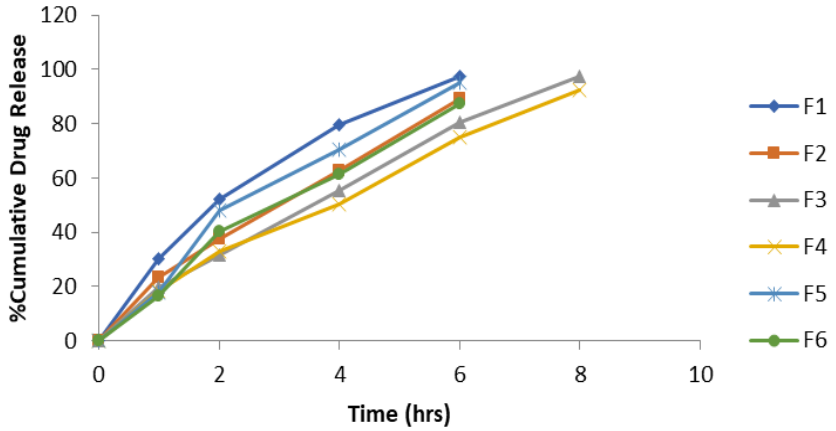

Fig. 1 - *In-vitro* drug release study of various formulations of natamycin loaded *in situ* ocular gels (Formulation code: F1-F6)

3.7 Kinetic Modeling

The formulations of Natamycin *in situ* gel were subjected to four model fitting analysis. The findings demonstrated that the formulations of all the formulations follow the zero-order kinetics as the co-efficient of regression (R^2) was nearer to unity as compared to the regression value of first-order and Higuchi model. Among all the formulations, it was observed that R^2 value of formulation F3 was nearer to one than formulations. Hence it can be concluded that the major mechanism of drug release follows zero-order kinetics and thus showing that the drug release rate was

independent of the residual concentration of the drug. The zero-order describes the drug release rate is independent of its concentration. Based on this parameter, F3 was selected for further study.

3.8 Ocular Irritation

An ocular irritation study was performed using healthy albino rabbits after getting prior permission from the institutional animal ethics committee. There was no sign of redness, continuous blinking, swelling, or watering of eyes. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctiva were visible. The result of ocular irritation studies indicates that formulations containing all ingredients are non-irritant to the rabbit eye.

3.9 Stability Study of the Ocular Gel

With the recent trend towards globalization of manufacturing operation, it is imperative that the final product be sufficiently rugged for marketing worldwide under various climatic conditions, including tropical, subtropical and temperate. Stability studies were carried out as per ICH guidelines. Stability studies for F3 gel were performed for three months, at room temperature (25 ± 2ºC) and at 40ºC / RH 75%. The F3 gel was evaluated for physical properties to determine the drug compatibility with polymer. The prepared F3 gel was subjected to drug release after every one-month interval (Table 3 and Table 4). The data depicts that the F3 gel stored at room temperature and accelerated temperature were found to be comparatively stable and no colour changes was observed during storage stability studies.

Table 3 - Physicochemical characterization of optimized best formulation (F3) of natamycin loaded *in situ* ocular gel at the accelerated storage condition

Parameters	Initial (0 day)	30 days	60 days	90 days
Clarity	Clear	Clear	Clear	Clear
Visual appearance	Transparent	Transparent	Transparent	Transparent
pH	6.21 ± 0.17	6.36 ± 0.56	6.32 ± 0.21	6.45 ± 0.85
Drug content (%)	98.28 ± 0.31	98.63 ± 0.73	98.14 ± 0.48	98.57 ± 0.52
Gelling capacity	++	++	++	++

Table 4 - Physicochemical characterization of optimized best formulation (F3) of natamycin loaded *in situ* ocular gel at the room temperature

Parameters	Initial (0 day)	30 days	60 days	90 days
Clarity	Clear	Clear	Clear	Clear
Visual appearance	Transparent	Transparent	Transparent	Transparent
pH	6.53 ± 0.52	6.71 ± 0.63	6.29 ± 0.32	6.91 ± 0.72
Drug content (%)	97.82 ± 0.61	97.43 ± 0.48	97.31 ± 0.73	97.11 ± 0.35
Gelling capacity	++	++	++	++

**Values are mean ± S.D*
"++" signs mean, the formulation present in semi solid state (gel form)

4. Conclusion

Six different F1 to F6 i*n situ* ocular gels of Natamycin were formulated by using a varied ratio of polymers xanthan gum, Ethylcellulose, and sodium alginate. The formulation F3 demonstrated the best findings compared to other formulation. The F3 formulation was found to be non-irritant and safe to use. The stability study findings indicate that the F3 gel stored at room temperature and accelerated temperature were found to be comparatively stable. The F3 can be used as a substitute to conventional eye drops in terms of ease of administration and have sustained drug release, which may ultimately improve patient compliance. Natamycin loaded bioadhesive *in situ* ophthalmic gel form bioadhesive and permeation- enhancing *in situ gel via* enhanced *permeability* and retention (EPR) effect and flexibility at neovascularization site. Bioadhesive *in situ* ophthalmic gel appears to be a valuable approach for improved drug solubility and permeability for the management of disease of eye. Major progress might be made *in situ* gel forming systems to prolong the precorneal residence time of a drug and increase ocular bioavailability especially in eyes. it will reduce the burden of invasive therapies to patients and will optimize the localization of drugs to cul-de-sac of the eye. Safe and effective treatment of eye diseases is the anticipated outcome.

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