Formulation and Evaluation of Natamycin Solid Dispersion Incorporated Ophthalmic Films

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ABSTRACT

Background: The aim of this study was to enhance the solubility of Natamycin, an anti-fungal agent used in fungal keratitis, through solid dispersion incorporated ophthalmic films with the purpose to avoid frequent dosing, enhance the period of retention, and sustaining release for better patient compliance. Materials and Methods: Firstly, solid dispersions were prepared by the solvent evaporation method and then, the best solid dispersion formulation was incorporated into films which were prepared by the solvent casting method using retarding polymers Ethyl cellulose, PVA and Chitosan. PEG-400 was used as a plasticizer. The films were evaluated for their thickness, surface pH, drug content, weight uniformity, folding endurance, tensile strength, antimicrobial activity, in-vitro and ex-vivo permeation studies, and in-vitro antifungal studies. Results and Discussion: The films exhibited good mechanical properties with promising results and were in an acceptable range. The optimized formulation NF6 shows more drug release in in-vitro studies as compared to ex-vivo. This is due to the varying pore size of the cornea and disturbance of other tissues. An ocular irritation test revealed that the prepared formulation was non-irritant. Conclusion: Thus, the formulated ophthalmic film seems to be a promising formulation for the safe and effective delivery of Natamycin through the ocular route in the treatment of fungal keratitis.

Key words: Ophthalmic films, Natamycin, Ethyl cellulose, PVA, Chitosan.

INTRODUCTION

The Ocular Drug Delivery System was an advancement that came into existence in the past twenty years and combats over the utilization of the suspensions, eye drops, ointments, and other ocular drug delivery systems. Ocular route serves as a real benefit over the conventional dosage forms since the drainage of the drug from nasolacrimal path; non-corneal absorption gets and flushes out from the pre-corneal path without reaching the targeted site. The cornea is the main part through which light gets reflected onto the retina, making it a good site for rapid absorption and high bioavailability. Fungal keratitis, which can lead to blindness, is the leading cause of ocular morbidity. Fungi are responsible for over 62 percent of microbial keratitis in warm, tropical climates. Plant trauma is one of the predisposing causes of keratitis. Candida species, Fusarium species, and Aspergillus species are the most commonly detected fungal causes of keratitis. Antiviral, antifungal, and antibacterial treatments are given depending on the spread of infection. Polyene antibiotics (Amphotericin B, Natamycin (pimaricin), Nystain, and others) and Azoles (Imidazole: Clotrimazole, Ketoconazole, Miconazole, and others) are common tropical antifungals used for eye infections. Pathogenic fungi can be found in filamentous moulds or intracellular yeast, and they can infect animals or people. Chitin and polysaccharides, which function as a barrier to drug penetration, are used to make the fungal cell wall stiff. Fungi static is the responsibility of the
Natamycin is a BCS class-II drug which is now marketed as a 5 percent w/v ophthalmic suspension. When used in the frontal portion of the eye, conventional formulations permeate through the cornea to the inner tissues of the eyes in a relatively small amount (5%). Thus, to enhance bioavailability, the retention time of the drug on the administered site should be extended. To enhance the bioavailability of Natamycin, various systems have been developed by researchers, which include, niosomes, solid lipid nanoparticles, mixed micelles, nanosuspension, etc. Ophthalmic films are polymer-based films that can be kept in the outer region of the eye for longer periods of time. These are made up of several grades of polymer that dissolve when exposed to physical stressors. They have numerous advantages over other conventional forms since they are biodegradable in nature, retain for a longer period of time at the site of action, and are less expensive, that leads to enhanced bioavailability and patient compliance.

As a result, the primary goal of this study was to develop and evaluate a Natamycin ophthalmic film using a solid dispersion approach to improve solubility, increase drug retention time on the site, and improve bioavailability. As a retarding polymer, PVA, Chitosan, and ethyl cellulose were utilized.

**MATERIALS AND METHODS**

Natamycin IP was procured from Chihong biotechnology, China. Ethyl cellulose (M/s Colorcon Pvt. Ltd. Goa), Polyvinyl alcohol (Fisher scientific Mumbai), Chitosan (Sigma Aldrich, Mumbai) PEG 400 (MERCK Pvt. Ltd Mumbai), All other chemicals were procured from SD fine chemicals Mumbai.

**Methods**

**Solubility Analysis**

Natamycin solubility study was carried out in a metabolic shaker for 24 hr at 37±2°C. The solvents used were methanol, phosphate buffer pH 7.5 and a mixture of ethanol: 6.8 pH buffer (1:2). The drug content assay was performed by UV spectrophotometer (Shimadzu UV 1900 Pharmaspec, Japan) $\lambda_{\text{max}}$ 303nm after serial dilution within the Beer’s range (2-10µg/ml).

**Preparation of Solid Dispersions**

The solvent evaporation method was used to prepare the solid dispersion product. The drug and polymer complex were prepared by varying the drug: polymer ratio and concentration of polymers (HPMC K100M, HPMC K4M and PVA) as shown in Table 1. Natamycin and polymers were dissolved in ethanol: acidic buffer of pH 6.8 (1:2 ratio) with the aid of a magnetic stirrer for 2 hr at room temperature. The clear solvents were evaporated on water bath at 60°C for 3 hr. The dried mass was pulverized and passed through 60 mesh and stored in an air tight container.

**Evaluation of Solid Dispersions**

**Drug Content and In-vitro Dissolution Study**

With continuous stirring on a magnetic stirrer, the weighed quantity of solid dispersion was dissolved in 7.4 pH phosphate buffer, and dilutions were made according to the beer’s range. A UV spectrophotometer set at 303nm was used to determine the drug content. In 900 ml of 7.4 pH phosphate buffer, an in-vitro dissolution analysis of solid dispersed product was conducted using a USP type II apparatus maintained at 37±0.5°C temperature with continuous stirring at 50 rpm. At predetermined intervals, 5 ml of the samples were collected from the media and replaced with fresh dissolution medium in the same amount. The withdrawn samples were filtered via Whatman filter paper and quantified at 303 nm using a UV Spectrophotometer.

**Formulation of Natamycin Solid Dispersions Incorporated Ophthalmic Films**

Six Natamycin ophthalmic films were prepared by using the solvent casting method. Chitosan, ethyl cellulose, and PVA polymers were used as retarding polymers along with casting solvents acetic acid + distilled water, chloroform, and distilled water respectively. PEG 400 was used as a plasticizer. The detailed compositions of

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug: polymer ratio</th>
<th>Natamycin (mg)</th>
<th>HPMC K100M (mg)</th>
<th>HPMC K4M (mg)</th>
<th>PVA (mg)</th>
<th>Ethanol:acidic buffer (1:2) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1</td>
<td>1:1</td>
<td>500</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>SD2</td>
<td>1:2</td>
<td>500</td>
<td>1000</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>SD3</td>
<td>1:3</td>
<td>500</td>
<td>1500</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>SD4</td>
<td>1:1</td>
<td>500</td>
<td>-</td>
<td>500</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>SD5</td>
<td>1:2</td>
<td>500</td>
<td>-</td>
<td>1000</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>SD6</td>
<td>1:3</td>
<td>500</td>
<td>-</td>
<td>1500</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>SD7</td>
<td>1:1</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>500</td>
<td>20</td>
</tr>
<tr>
<td>SD8</td>
<td>1:2</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>1000</td>
<td>20</td>
</tr>
<tr>
<td>SD9</td>
<td>1:3</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>1500</td>
<td>20</td>
</tr>
</tbody>
</table>
the Natamycin solid dispersion incorporated films are given in Table 2. The casting solutions were prepared by dissolving the specified concentration of polymers in suitable solvents using a magnetic stirrer for 30 min to get a uniform dispersion. Natamycin dispersions equivalent to 1.44gms of drug and plasticizer (30%) were added during constant stirring (600 rpm) in the polymeric solution. It was then set aside for ½ hr to get rid of the bubbles. The mixture was poured into a pre-lubrication petridish with glycerin. The petridish was enclosed in an inverted funnel and left untouched overnight for regulated evaporation. The films were carefully removed and cut into 4x2mm pieces before being wrapped in butter paper and stored at room temperature. The pictorial representation of the method of preparation of Natamycin ophthalmic films is shown in Figure 1.

Table 2: Composition of Natamycin Solid Dispersion Incorporated Ophthalmic Films.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation Code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NF1</td>
</tr>
<tr>
<td>Natamycin dispersion</td>
<td>5.76</td>
</tr>
<tr>
<td>equivalent to 1.44 gms</td>
<td></td>
</tr>
<tr>
<td>PVA (gms)</td>
<td>0.2</td>
</tr>
<tr>
<td>Ethyl Cellulose (gms)</td>
<td>_</td>
</tr>
<tr>
<td>Chitosan (gms)</td>
<td>_</td>
</tr>
<tr>
<td>Chloroform (ml)</td>
<td>_</td>
</tr>
<tr>
<td>Distilled Water (ml)</td>
<td>15</td>
</tr>
<tr>
<td>Dist water + Acetic acid (ml)</td>
<td>_</td>
</tr>
<tr>
<td>PEG 400 (% W/V)</td>
<td>30%</td>
</tr>
</tbody>
</table>

Evaluation of Prepared Films

Physical Appearance and Surface Texture

The prepared formulations were inspected visually for their appearance, texture, and clarity.

Surface pH

To swell the film, it was kept in contact with 0.5ml of neutral water. The electrode was made to come into contact with the ocular film, and the pH was recorded in triplicates after one minute of equilibration with a pH meter (Elite Scientific Corporation, Haryana, India).

Thickness of Film

Using a vernier caliper, the thickness of the film was measured. The film was folded a certain number of times and the measured value was divided by the number of folds. This demonstrates the film’s thickness uniformity as well as the polymer’s distribution homogeneity. All measurements were carried out three times.

Uniformity of Weight

Five films were chosen at random from each batch of the formulation, and their weight was assessed using a digital balance. The average weight was reported in triplicates.

Folding Endurance

The film was folded repeatedly in the same spot until it broke at that point. The number of folds required to break the film was calculated. This was analyzed three times and the average was considered.

Drug Content Uniformity

All ophthalmic film formulations of 4x2mm were dissolved in 7.4 pH phosphate buffer, stirred for 1hr on a magnetic stirrer, filtered and filtrate was analyzed for drug content at 303nm by UV-spectrophotometer. For all the formulations, triplicate study was performed.

Tensile Strength

The 3x4cm ophthalmic film was clamped between two clamps spaced 3cm apart. The tensile strength of the film was determined by adding weights to the pan until the film broke. The total weight was recorded, and the tensile strength was calculated in triplicate. Using, following formula.

\[
\text{Tensile strength (g/(cm)}^2 = \left(\frac{\text{Load at failure (g)} \times 100}{\left(\text{Cross sectional area ((cm)}^2\right)}\right)
\]

Percent Moisture Absorption

It is one of the ways of checking the physical stability of the ophthalmic film under high humidity conditions.
The prepared ophthalmic films were weighed and stored for three days in the desiccator. As a desiccant, aluminium chloride solution is employed (79.5 percent humidity). All ophthalmic films were weighed, and moisture absorption percentages were estimated using the formula, in triplicate.

\[
\text{Percent moisture absorption} = \frac{(\text{Final weight} - \text{Initial weight})}{(\text{Final weight})} \times 100
\]

**Percent Moisture Loss**

Anhydrous calcium chloride is present in the dissector. The films were weighed and stored for three days. The moisture loss was determined using the following equation after the films were re-weighed.

\[
\text{Percent moisture loss} = \frac{(\text{Initial weight} - \text{final weight})}{(\text{Initial weight})} \times 100
\]

**In-vitro Diffusion Study**

The Franz diffusion cell was used to determine the in-vitro drug release profile of ophthalmic films. The donor compartment contained the 8mm films, while the receptor compartment held the simulated tear solution. The dialysis membrane (molecular weight cutoff of 12,000-14,000 Dalton) was fixed as a semi-permeable membrane between the donor and receptor compartments, with the rpm set to 50 and the temperature set to 37±1°C. Samples were withdrawn at a specified interval of up to 8hr. Sink condition was maintained and samples were analyzed at 303 nm.

**In-vitro Transcorneal Permeation Studies**

A local sloutry house provided the goat eye. The core layer was excised and cleaned with saline, which was kept at 4°C until it was used. The cornea was rinsed in cold normal saline to remove the protein. The cornea was positioned between the donor and receptor compartments so that the outer epithelial faced the donor compartment. For 8 hr, the receptor compartment was filled with simulated tear fluid that was kept at 37°C with continual stirring at 50rpm using a Teflon coated magnetic bead. The cornea in the donor compartment was covered with a 4x2mm ophthalmic film, and the donor compartment was sealed with a glass cover slip. At specified time interval 1ml of fluid was withdrawn (from receptor compartment) and replaced with fresh simulated tear fluid to maintain the sink condition. Withdrawn samples were analyzed at 303nm for drug content.

**Ocular Toxicity Study**

The Draize test was adapted to assess ocular toxicity in accordance with OECD recommendations. The Institutional Animal Ethical Committee (IAEC) provided ethical clearance for the management of experimental animals (albino rabbits of either sex) (Resolution No. KLECOP/CPCSEA/Res.No-221/Po/Re/S/2000/CPCSEA. Reg. 27-24-12-2018). The animals were divided into 3 groups, each group containing 6 rabbits marked as test, control, and placebo groups, respectively. Throughout the experiment, they were kept in an animal housing at room temperature and supplied with standard feed and water. The placebo group received a placebo film, whereas the control group received nothing. The test group received sterile best formulation. The ophthalmic film was placed in the cul-de-sac of the rabbit (test group) once a day for 7 days and corneal irritancy was tested at specific time intervals of 24 hr, 48 hr, 72hr. After 1 week, all the animals were observed for redness, swelling, discharge, hemorrhaging, cloudiness, ulceration and blindness.

**Antimicrobial Activity**

A *Candida albican* media containing Sabouraud dextrose agar was used to test antimicrobial activity *in vitro*. For inoculation, the streak method was utilized, and the samples were incubated at 35±4°C for 24 hr. The autoclave was used to sterilize 60ml of Sabouraud dextrose agar, which was then placed into the previously sterilized petridish and solidified at room temperature. The fungal suspension was made using sterile saline water, and 1ml of it was placed into a 9cm diameter petridish and incubated at 35±4°C temperature. Wells (8mm) were made using cork bores. In three petridishes, 4×2mm (30mg) optimized ophthalmic film, 0.6ml of marketed suspension (5%), and 4×2mm placebo ophthalmic films were introduced respectively, in the aseptic condition. All petridishes were incubated at 35±4°C for 24h. Zone of inhibition was measured after 24hr.

**Kinetic Modeling of the Drug Release**

The cumulative amount of drug permeated per square centimeter of all the ophthalmic film was fitted into zero order, first order, Higuchi kinetic model, and Korsmeyer peppas models, to calculate the release constant and regression coefficients (R^2) using PCP DISSO V3 software.

**Stability Studies**

Stability testing provides information on the shelf-life of drugs or dosage forms and their recommended storage conditions. The stability study of NF6 formulation was carried out at normal condition 25±2°C and RH 60±5% and accelerated condition at 40 ±2°C and 75
RH ±5% in stability chamber for a period of 90 days. Films were wrapped in a butter paper followed by aluminium foil and placed in an aluminium pouch. They were evaluated for drug content, tensile strength and *in-vitro* diffusion studies after storage of 15 days, 30 days, 60 days and 90 days.

**RESULTS AND DISCUSSION**

**Solubility Studies**

The maximum solubility of Natamycin was 5mg/ml in a mixture of ethanol and acidic buffer (pH 6.8) in 1:2 ratio. It is practically insoluble in water and 0.05mg/ml was soluble in methanol.

**Evaluation of Solid Dispersions**

The solid dispersion was prepared by utilizing three polymers: HPMC K100M, HPMC K4M, and PVA in 1:1, 1:2, and 1:3 ratios, respectively. The drug release was investigated for each polymer ratio. In comparison to different polymers and their ratios, it was discovered that drug: HPMC K4M in a 1:3 (SD-6) ratio had the maximum drug release. The HPMC K4M solid dispersed product was further utilized for film formulation. Table 3 shows the drug content and percent drug release of all solid dispersions.

**Evaluation of Natamycin Ophthalmic Films**

**Physical Appearance, Surface Texture and pH of Ophthalmic Films**

All the formulations prepared (NF1-NF6) were clear and elegant in appearance and the films had a smooth surface without any cracks and imperfections. The film’s acidity and alkalinity are determined by the pH of the film’s surface. The pH of the ocular fluid ranges from 6.8 to 7.5. The film’s varied alkalinity may cause irritation or harm to the eye. The surface pH of the formulation NF1-NF6 was in the acceptable range of 6.8-7.2 pH indicating films have less potential to irritate eyes and are highly acceptable by patients. All prepared films possess a surface pH which was compatible with simulated tear fluid of pH 7.4. (Table 4).

**Thickness, Weight Variation and Folding Endurance**

The thickness of the formulations NF1-NF6 was in the range of 0.0245 ± 0.38mm -0.07031 ± 0.75mm. These findings revealed that the polymer and drug were distributed uniformly. The average weight of all formulations NF1-NF6 was in the range of 126 ± 1.252mg to 129 ± 2.02 mg which was within the acceptable limit. The folding endurance reflects the mechanical strength of the film and for (NF1-NF6) formulations it was in the range of 260-453 (Table 4).

**Drug Content and Tensile Strength**

The goal of determining drug content is to ensure that the drug is distributed evenly throughout the film. Formulations NF1-NF6 showed drug content in the range of 95.30 % to 98.28%. Tensile strength of the formulations NF1-NF6 were in the range of 29±0.812-45±0.8779 and was in an acceptable range (Table 5). It was observed that, as the concentration of the plasticizer increased, gradually there was an increase in the folding endurance and tensile strength, the films of all the formulations NF1 to NF6 had good mechanical strength, flexibility and elasticity at the particular concentration of polymer.

| **Table 3: Evaluation of Natamycin Solid Dispersed Products.** |
|-------------|-------------|----------------|
| Formulation code | % Drug content | % Drug release at 60 mins |
| SD1 | 95.67±1.8 | 74.93±3.4 |
| SD2 | 96.23±2.1 | 83.84±2.7 |
| SD3 | 94.38±0.9 | 93.52±2.8 |
| SD4 | 92.64±2.2 | 82.14±3.5 |
| SD5 | 95.87±1.5 | 90.67±3.2 |
| SD6 | 98.22±2.4 | 95.11±4.1 |
| SD7 | 96.09±1.6 | 61.32±2.6 |
| SD8 | 93.76±1.8 | 57.45±3.2 |
| SD9 | 98.22±0.8 | 69.03±1.2 |

| **Table 4: Evaluation of Natamycin Solid Dispersion Incorporated Ophthalmic Films.** |
|-------------|-------------|-------------|----------------|-------------|-------------|----------------|
| Formulation Code | Surface Texture | Surface pH | Thickness(mm) | Weight (mg) | Folding Endurance |
| NF1 | Smooth | 7.1±0.241 | 0.024±0.38 | 126±1.252 | 260±0.451 |
| NF2 | Smooth | 6.8±0.152 | 0.070±0.75 | 128±2.516 | 453±1.732 |
| NF3 | Smooth | 7.3±0.115 | 0.063±0.10 | 126±2.081 | 350±0.577 |
| NF4 | Smooth | 7.0±0.192 | 0.043±0.25 | 127±1.244 | 345±1.254 |
| NF5 | Smooth | 7.2±0.210 | 0.070±0.75 | 129±2.025 | 280±1.356 |
| NF6 | Smooth | 7.2±0.120 | 0.035±0.42 | 127±1.527 | 311±2.645 |
Moisture Absorption and Moisture Loss

The moisture absorption and moisture loss of all formulations were depicted in Table 5 and were found in the range of 2.752±0.348 to 3.96±0.422 and 2.387±0.768 to 3.821±0.421 respectively, and were in an acceptable range.

**In-vitro Permeation Studies**

*In-vitro* permeation study for NF1, NF2, NF3, NF4, NF5 and NF6 showed the release of 79.48 %, 90.63 %, 85.147 %, 85.72 %, 82.81 % and 80.21 % respectively. Based on the *in-vitro* study NF1, NF3 and NF5 showed faster and complete release in 4 to 5 hr and thus were rejected. The use of retarding polymers in the formulation (PVA, Ethyl cellulose and Chitosan) successfully contributed to the sustained release of the drug and *in-vitro* permeability of NF2, NF4 and NF6 for 8 hr was found to be in the range of 90.63 %, 85.14 % and 80.21 % respectively. On the basis of the overall evaluation parameter, NF6 was determined to be the best of the three formulations.

The decreased drug release at higher concentrations of PVA (NF1 and NF2) and chitosan (NF5 and NF6) may be due to their tendency to form an intense and denser molecular network. Ethyl cellulose (EC) is the most utilized polymer in the preparation of sustained drug delivery systems because it’s biocompatible, versatile and lower cost.\(^\text{28}\) As the EC concentration ratio increased from 0.25gm 0.30gm, (NF3 and NF4) the *in vitro* drug release decreased, refer to Figure 2. The reason for this could be longer diffusion pathway of the drug in the higher ratio, so the release was retarded. This agrees with Yadav *et al.*\(^\text{29}\)

**Ex-vivo Permeation Studies**

*Ex-vivo* permeation study was conducted using the cornea of a goat and at the end of 8hrs percentage cumulative drug permeation of optimized formulation NF6 was found to be 76.30 %. The permeation of NF6 by *in-vitro* was 80.21% and by *ex-vivo* was 76.30%.

The drug permeation was high in the *in-vitro* studies as compared to *ex-vivo*. This is due to the fact that the, real cornea is a semipermeable membrane with varying pore size and also some other fatty tissues hinder the permeation of drugs through the cornea. Also, the cornea is made up of the epithelium (lipophilic), stroma (hydrophilic) and endothelium (less lipophilic than epithelium) which act as a lipophilic-hydrophilic barrier for corneal penetration, while the dialysis membrane acts as a mechanical barrier.\(^\text{31}\) The *in vitro* release profile of all formulations (NF1-NF6) is depicted in Figure 2 and the comparative *in vitro-ex vivo* release of the optimized formulation is shown in Figure 3.

**Antimicrobial Activity Using Cup-let Method**

The zone of inhibition observed (24hr) for optimized NF-6 film was 9.5mm and for the marketed formulation was 10mm and for placebo it was 3mm as shown in Figure 4. The results of the optimised formulation were satisfactory. Chitosan is an antibacterial agent itself, according to a placebo report. After 10-15 days of incubation at 35°C±4°C, the ophthalmic film was released in a controlled manner. According to the

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**Table 5: Evaluation of Solid Dispersion Incorporated Ophthalmic Films.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Tensile strength (g/cm(^2)) ± SD</th>
<th>Drug content (%) ± SD</th>
<th>Moisture Absorption (%) ± SD</th>
<th>Moisture Loss (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>35.0±0.76</td>
<td>96.82±0.28</td>
<td>2.75±0.34</td>
<td>3.82±0.42</td>
</tr>
<tr>
<td>NF2</td>
<td>45.0±0.87</td>
<td>97.27±0.41</td>
<td>2.92±0.44</td>
<td>3.70±0.39</td>
</tr>
<tr>
<td>NF3</td>
<td>42.5±0.62</td>
<td>95.30±0.38</td>
<td>3.26±0.38</td>
<td>3.11±0.05</td>
</tr>
<tr>
<td>NF4</td>
<td>32.0±0.81</td>
<td>98.28±0.47</td>
<td>3.86±0.24</td>
<td>3.67±0.24</td>
</tr>
<tr>
<td>NF5</td>
<td>30.4±0.12</td>
<td>95.74±0.41</td>
<td>3.96±0.42</td>
<td>3.55±0.27</td>
</tr>
<tr>
<td>NF6</td>
<td>31.2±1.22</td>
<td>96.20±0.46</td>
<td>3.58±0.39</td>
<td>2.38±0.76</td>
</tr>
</tbody>
</table>

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**Figure 2: In-vitro release profile of all Natamycin ophthalmic films.**

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28. Ethyl cellulose (EC) is the most utilized polymer in the preparation of sustained drug delivery systems because it’s biocompatible, versatile and lower cost.

29. Yadav *et al.*

31. Chitosan is an antibacterial agent itself, according to a placebo report.
findings, there was no microbial development in the inhibited area. *In-vitro* anti-microbial tests showed that the zone of inhibition was sustained for 24hr, and the same zone size was maintained for up to 15 days. The eye irritation score from individual rabbits was added to get the total irritation score that was subsequently divided by the total number of rabbits used for the ocular irritancy test to obtain the final eye irritation score. The calculated eye irritation score was 0.23 in control while for NF6 it was 0.47, which demonstrates good ocular tolerance. All the rabbits were kept under observation for 24hr. There was no irritation, inflammation, and redness found in the placebo with respect to optimized formulation NF6 and control eye. The ocular irritation test revealed that prepared formulation was non-irritant for ocular administration (Figure 5).

**Kinetic Modeling of the Drug Release**

To obtain the release constant and regression coefficients ($R^2$), the release data was fitted into various kinetic models. The drug release profile for all formulations NF1 to NF6 revealed Higuchi kinetics among the models studied. The diffusion mechanism was responsible for the release profiles. All of the formulations (NF1-NF6) had a diffusion exponent ($n$) of less than 0.5, indicating a Fickian mechanism of drug release.

**Short-term Stability Studies**

The stability study of the optimized formulations for 90 days revealed that the formulations were stable at room temperature as well as in the accelerated stability conditions as there was no significant difference in drug content, tensile strength, and *in-vitro* diffusion studies after storage for 90 days, which confirms the potentiality of the films for longer storage.

**CONCLUSION**

This study evaluated the suitability and feasibility of delivering Natamycin in the form of an ophthalmic film through the ocular route with the goal of avoiding the loss of the drug, an increase in the retention time of the drug and preventing the frequent dosing, along with enhancement in the rate of drug release, diffusion, and better patient compliance. Ophthalmic films were successfully prepared by using polymers (PVA, Ethyl cellulose, and Chitosan) by the solvent casting method. It was observed that as the concentration of the plasticizer increased, gradually there was an increase in the folding endurance and tensile strength. The films of all the formulations NF1 to NF6 had with good mechanical strength, no deviation in weight, and good folding endurance. The optimized formulation NF6 shows more drug release in *in-vitro* studies as compared to *ex-vivo*. This is due to the varying pore size of the cornea.
and disturbance of other tissues. An ocular irritation test revealed that the prepared formulation was non-irritant. Thus, the formulated ophthalmic film seems to be a promising formulation for the safe and effective delivery of Natamycin through the ocular route in treatment of fungal keratitis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HPMC: Hydroxypropyl methylcellulose; PVA: Poly (vinyl alcohol); PEG: Poly(ethylene glycol); SD: Solid dispersion; EC: Ethyl cellulose.

REFERENCES

SUMMARY

- Natamycin belongs to BCS Class II and currently available Natamycin formulation is conventional suspension with doses of 5mg, and has several disadvantages, such as drug loss during the administration and frequent dosing. This leads to greater variability in response.
- In this present study, an attempt was made to formulate sustained release formulation of Natamycin ophthalmic films to avoid the loss of dose and frequent dosing, increase retention time and patient compliance.
- The Natamycin solid dispersions were and based on percent drug release profile SD-6 formulation is selected to incorporate in to ophthalmic films which were prepared by solvent casting method with PFG 400 as plasticizer.
- Further, the formulations were subjected to various evaluation parameters viz. surface pH, drug content, weight uniformity, thickness, folding endurance, tensile strength, in-vitro and ex-vivo permeation studies.
- The films of all the formulations NF1 to NF6 were with good mechanical strength, no deviation in weight, and good folding endurance. The optimized formulation NF6 shows more drug release in the in-vitro studies as compared to ex-vivo.
- Stability study of the optimized formulations for 90 days revealed that the formulations were stable at room temperature as well as in the accelerated stability conditions which confirms the potential of the films for longer storage.
- Thus, it was concluded that the formulated ophthalmic films seem to be a promising formulation for the safe and effective delivery of Natamycin through ocular route in treatment of ocular fungal keratitis.