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Formulation and Development of Ophthalmic in situ gel Forming System of Antifungal Drug Clotrimazole

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ABSTRACT: The purpose of the present study was to formulate and evaluate an ion-activated in situ gelling system of antifungal drug clotrimazole for administration into the eye. The suspension of the drug was prepared using gellan gum as an in-situ gelling agent and HPMC as a sustained release polymer. The formulation was evaluated for visual appearance, PH, drug content, particle size, gelling capacity,viscosity,sterility test, antifungal activity, and stability studies.
The suspension was turbid and showed a particle size of 8.8-12.30 µm and pH of 7.4 suitable for ophthalmic use. The suspensionshowed immediate gelation in the STF and a Sustained releasewas obtained as 97.30 % over 8 hrs. The formulation was sterile, showed effective antifungal activity against candida, and was stable over a period of one month at accelerated conditions.
Keywords:in situ gels, in vitro drug release, sustained drug release.

I. INTRODUCTION

In the field of pharmaceutical research, ocular drug delivery is intriguing and demanding. Conventional ocular administration systems such as eye drops have poor bioavailability and therapeutic response.Because of the high tear fluid turnover and nasolacrimal drainage, both of which induce side effects, frequent administration and the use of concentrated solutionsare used to achieve the desired therapeutic effect.If the drug's precorneal residence time could be increased, ocular therapy would be significantly improved[1].

Several new preparations like inserts, aqueous gels, ointments &collagen shields have been developed not only to prolong the contact time of the vehicle on the ocular surface but also to slow down the drug elimination. These suffer from a few drawbacks like blurring of vision, loss of device, and patient compliance. From the point of view of patient acceptability, a liquid dosage form is preferable[2].

By using in situ gel-forming ophthalmic drug delivery system this problem can be overcome through prepared polymeric formulations that exhibit reversible phase transitions (sol-gel-sol) and pseudo-plastic behavior to minimize interference with blinking. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye, which upon exposure to physiological conditions shifts to the gel phase, thus increasing the pre-corneal residence of the delivery system and enhancing ocularbioavailability[3].

Clotrimazole is a broad-spectrum antifungal agent having very poor water solubility. It is mainly fungistatic but also acts as fungicidal against some organisms in a dose-dependent manner. It is chemically 2-(2,4-difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol belonging to the azole category. It inhibits fungal growth by inhibition of ergosterol biosynthesis, an essential constituent of fungal cell membranes. There are different Clotrimazole formulations available in the market like gels, eye drops, creams, etc.

The sol to gel phase change on the ocular surfacecan be achieved bypH-activated systems, temperature-dependent systems, and ion-activated systems. The latter can be formulated using Sodium alginate and gellan gum.Gellan gum is a high molecular mass; linear anionic heteropolysaccharide produced aerobically from the bacterium Auromonas(Pseudomonas) elodea, renamed Sphingomonaspaucimobilis.It has the capacity to form gels in presence of cations[4,5].

The purpose of the present study was to develop an ion-activated in situ gelling system comprising of drug Clotrimazole against ophthalmic Candida infection.

II. MATERIALS AND METHODS

2.1 Materials

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Clotrimazole was obtained as a gift sample from NuLife Pharmaceuticals Pvt. Ltd. Pune India. gellan gum and HPMC K4M were obtained from Sisco LabsPvt.Ltd. and H.D. Fine Chemicals respectively. All other reagents were of analytical grade.

2.2 Methods

Pre-formulation

Clotrimazole-excipient compatibility studies: Clotrimazole and Excipients in combination were kept in the oven at 40^0C for 30 days and analyzed through Fourier transform infrared spectrophotometer(Shimadzu, miracle-10) in the region of 4000-400cm-1 to confirm drug compatibility with various excipients used in the preparation of in-situ gel[7].

The polymeric solution was prepared by dispersing gellan gum into deionized water and heating up to 80°c for 20 minutes. This polymeric solution was cooled to room temperature. To this solution under continuous stirring copolymer, HPMC K-4 M was added to form a clear solution followed by the addition of drug clotrimazole and stirred using a magnetic stirrer to form a homogeneous suspension. Finally, buffering agent, osmolality agent(Nacl) along withpreservative(Benzalkonium chloride)were added. Ophthalmic suspension formed. 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[8-10].

III. PREPARATION OF IN SITU GEL

Table 1. Composition of clotrimazole in situ gel formulation

MATERIALS	PHARMACEUTICAL USE
Clotrimazole	Drug
Gellan gum	In situ gel-forming polymer
HPMC K4 M	SR polymer& Viscosity enhancing agent
Nacl	Osmolality agent
Benzalkonium Chloride	Preservative
Distilled water	Vehicle

IV. EVALUATION

4.1 Determination of visual appearance: The visual evaluation was used to determine the appearance and clarity under light alternatively against white and black backgrounds[6].

4.2 pH:A digital pH meter was used to determine the pH of prepared ophthalmic formulation.

4.3 Drug content: It was determined by taking a sample of 2ml and diluted with simulated tear fluid (STF) of pH 7.4 to get the concentration of 10mg/ml. Then absorbance was measured at lambda max (261nm) using a UV spectrophotometer [3].

4.4 Particle size: Particle size was determined by using Malvern Zeta sizers NanoZS90(Malvern Instrument, UK) Based on laser diffraction with a beam length of 2.40mm, range lens of 300 RF mm, and 14.4% obscuration
[13-14].

4.5 Gelling capacity:0.05 ml of the formulation was added in a vial containing 2 ml of freshly prepared STF and visually observed for gelling and the time taken for it was noted[11].

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Composition of simulated tear fluid:
Sodium chloride : 0.670gm
Sodium bicarbonate : 0.200gm
Calcium chloride dehydrate : 0.08gm
Deionized water: 100ml

4.6 Viscosity: Brookfield synchro electric (DV II+PRO) viscometer was used to measure the viscosity of the gel at 37°C and a T bar (96) spindle was used. The velocity of the spindle was increased from 1 to 4 RPM and the viscosity of the formulation was measured [12].

4.7 Texture analysis: The hardness of in situ gel formulation was determined by placing it in the lower cone of the texture analyser (Brookfield Ametek CT3100). Texture Pro CT software was used to examine hardness after the base was locked in place and the male cone was made to align perfectly with the lower female cone.

4.8 In vitro release studies: Bi chambered donor receiver compartment type (Franz diffusion cell) was used for in vitro release studies.The freshly prepared mixture of STF (pH 7.4) and PEG 400 10% was used as a diffusion medium.One end of the diffusion tube was covered by a cellophane membranewhich was previously soaked overnight in a diffusion medium. The receptor chamber was maintained at a temp of 37 ± 2°C with a stirring rate of 50 rpm using a magnetic stirrer.Then1 ml formulation was spread on the cellophane membrane and the membrane was placed such that it just touches the diffusion medium (STF+PEG400) present in the receptor chamber. 1ml samples were withdrawn at the interval of 1,2,4,6,& 8hrs from the diffusion medium and diluted to 10 ml analyzed at 261 nm by a UV spectrophotometer using STF as blank[15-16].

4.9 Sterility Testing: The direct inoculation method was chosen for sterility testing.A sterile pipette, a sterile syringe, or a needle were used to remove 2 ml of liquid from the test containerSeparately.The test liquid was transferred aseptically to fluid thioglycolate medium (20 ml) and soybean-casein digest media (20 ml).The liquid was mixed with the media.The inoculation media were kept at 30°C to 35°C in the case of fluid thioglycollate medium and 20°C to 25°C in the case of soybean-casein digest medium for at least 14 days[18-19].

4.10 Antifungal Efficacy Studies: Antifungal drug efficacy of the formulation was performed using the cup plate method. The fungiCandida albicans (NCIM No. 3674) was received from the National Collection of Industrial Microorganism,Pune. The fungal culture was revived using the streak plate technique. For the revive process, the fungi were streakedon the Sabourad Dextrose Agar medium containing 2% of agar and to grow at 30°C in the incubator. The grownculture was then inoculated in the sterilized Soybean casein broth and kept in the mechanical shaker at 30°Cfor a day. The grown culture was then poured into the five sterilized Petri plates in aseptic condition and mixedwith the 20 ml sterilized SDA media in each 4 Petri plates. After solidification of the media, each Petriplate was bored using a cup borer to form the wells. 2 wells were bored in each Petriplate. Formulations were added to the well. A zone of inhibition was recorded after 24 hrs[20-21].

4.11 Accelerated stability studies: The ophthalmic formulations in amber-colored vials were employed for short-term accelerated stability experiments by storing them at 40°±2°C and 75±5% RH according to ICH recommendations[17].

V. RESULTS AND DISCUSSION:

Formulation of Clotrimazole in situ gelling systems was prepared by using fixed concentrations ofgellan gum (0.5%w/v) along with hydroxy propyl methyl cellulose K4 M (0.5%w/v) and the drugconcentration of (1%w/v) clotrimazole.

5.1 Visual appearance: The formulation was observed under light alternatively against white and black backgrounds and it was found to be turbid suspension.

5.2 Drug excipients compatibility studies: The result of the FTIR Interaction Study for the drug excipient compatibility studies is shown in table no. 2. The peaks obtained from pure clotrimazole are the same as the peaks obtained from the clotrimazole and excipients (gellan gum and HPMC K4M) in combination. The CH stretch of the pure clotrimazole i.e., at 3167 cm^{-1} has no change in the clotrimazole combination withgellan gum and HPMC K4M Also, the C=N stretch was found at 1570 cm^{-1} in clotrimazole in combination with the same excipients which indicate no change in the C=N stretch of the pure clotrimazole. This confirms that there is no interaction between the drug and polymers and polymers are not making changes in the drug's chemical structure and functional group necessary for the drug's activity. So, we can say that there was no physical as well as

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chemical interaction between the drug and excipients and the excipients are compatible with the drug.

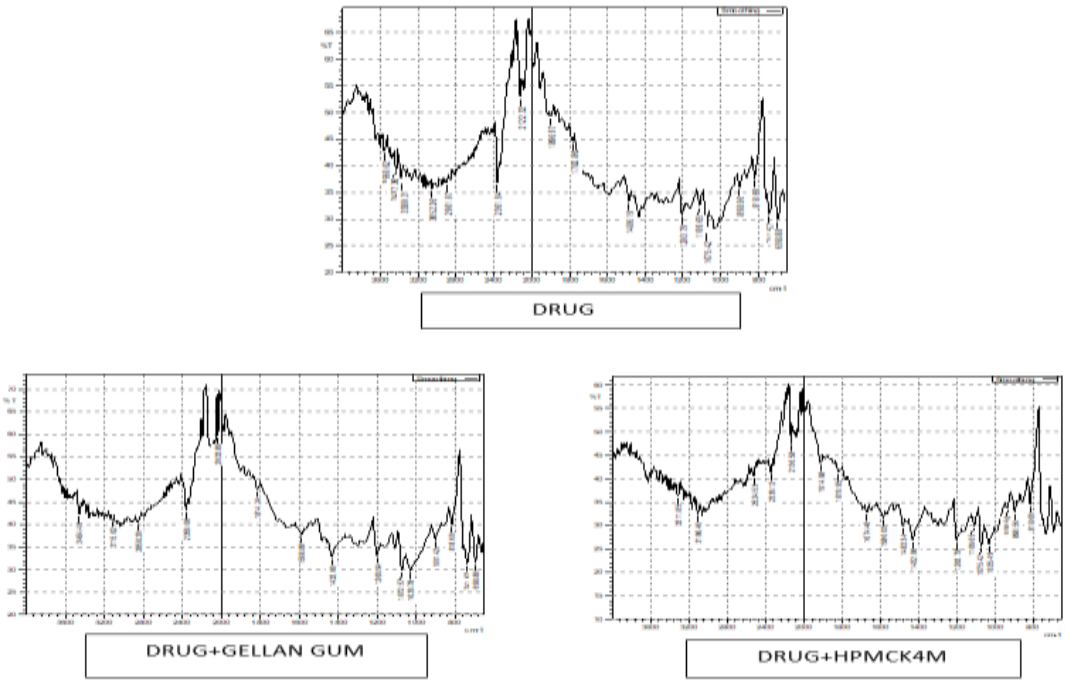

Figure1. IR of Drug excipient compatibility

Table 2. Excipients And The Clotrimazole Compatibility Results

Sr. no	Clotrimazole with	IR observation	Physical appearance
1	HPMC K4M	No peaks shifting	No change
2	Gellan gum	No peaks shifting	No change
3	HPMC K4M and Gellan gum	No peaks shifting	No change

5.3 pH: The pH is one of the most significant factors concerned in ophthalmic preparation. During the formulation of in situ gels, care has been taken that there should not be any irritation while administration. The pH of the prepared formulations was found to be 7.4. This pH is compatible with the eye.

5.4 Drug content: The drug content of the clotrimazole in situ gel formulation was foundto be 98.30 %. As per IP the drug content was found within the range(98 to 102%) there was no drug degradation found within the formulation.

5.5 Particle size: The particle size of the clotrimazole in situ gel formulation was found to be 8.8 to 12.30 micrometer. This is within the ophthalmic range(20-25µm). The lesser the particle size more stable the formulation and it doesn't cause any irritation to the eye.

5.6 Gelling capacity: Immediate in situ gelation of the prepared clotrimazole suspension formulation was observed visually at 37^0C on addition to STF. Gelation of gellan gum is induced by the presence of positive ions (NA^+, K^+,CA^+). They exhibit a conformational transformation from the disordered state (single chain) to the ordered state (double helix) . The gelation is considered to be mediated by the double-helix formation and the aggression of such helices into a three-dimensional structure. Metallic cations are needed to electrically shield the carboxyl groups and to allow a tighter aggregation of the helices.Because of the phase transition properties, the crosslinking reaction forms an immediate gel within 10-sec, that retained the drug for longhours.

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5.7 Viscosity: According to the literature, formulations should have a viscosity of 5 to 1000 cps before gelling and a viscosity of 50-50,000 cps after gelling in the eye. The viscosity ofprepared formulation before gelling was found to be 600 cP and after gelling was 4000 cP at 100 RPM and 50.2 % torque. Viscosity of the formulation is adequate for easy application and retention of the in-situ gel

5.8 Texture analysis: The hardness of in situ gel formulation was found to be 10gm.

5.9 In vitro release studies: A combination of STF (7.4 pH) and PEG 400 (10%) was used as a diffusion medium which showed a drug release of 12.80% after 1h and sustained the release showing 97.77% at 8 hrs. This is due to the presence of HPMC K4M which is a hydrophilic matrix former polymer. It absorbs the water and drug entrapment take place hence it caused a decrease in the diffusion of the drug over the period of 8 hrs.

5.10 Sterility Testing: The prepared in situ gelling systems were evaluated for sterility. Till 14 days of incubation, the results showed no microbial growth in the formulation and were found to be a sterile formulation and it is suitable for ophthalmic. (Table 3)
(NOTE: '–' sign indicates no Growth)

TABLE 3: RESULT OF STERILITY TEST DATA OF IN SITU GELS

Medium	**Incubation Days**						
	1	2	3	4	5	6	7
Fluid thioglycolate medium	-	-	-	-	-	-	-
Soybean casein digest medium	-	-	-	-	-	-	-

Figure 2. Fluid Thioglycolate MediumFigure 3. Soybean Casein DigestMedium

5.11 Antifungal Efficacy Studies: The final formulation showed the highest zone of inhibition which is 97 % of the standard zone of inhibition. Placebo-containing formulation showed the least zone of inhibition which is 1 % of the standard ZOI. This is due to sodium chloride that acts by altering osmatic gradients forcing organisms to expend energy in Osmo regulation, diverting it away from growth. The observations are indicating that the formulation is having antifungal efficacy. The results of the antifungal efficacy test are shown in (Table 4).

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Table 4: Antifungal Efficacy Study Result

Sr. no	Formulation No.	Zone of Inhibition (mm)	% Zone of inhibition
1	Standard	18	100 %
2	Formulation	17.6	97.32 %
3	Placebo	1.6	1%

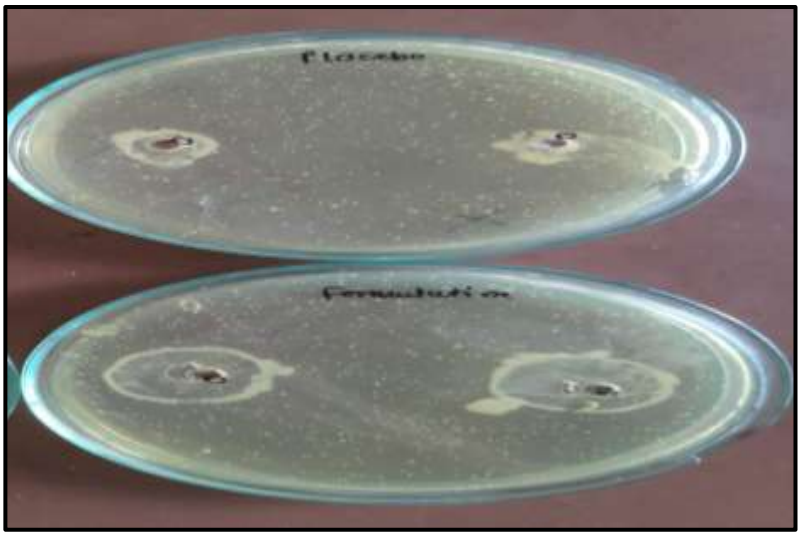

Figure4. Antifungal efficacy (Zone of inhibition)

5.12 Accelerated stability studies: Formulations showed no change in appearance, clarity, and pH.Further, it wasobserved that the gelling capacity of theformulations was not affected. Depending on this result we can conclude that formulation was stable.

VI. CONCLUSION:

A novel and stable in situ gelling ophthalmic suspension of antifungal drug clotrimazole were successfully formulated and evaluated for the treatment of Fungal keratitisa sight-threatening ocular infection that occurs as an infection of candida species. Conventional ocular drug delivery exhibitpoor bioavailability and therapeutic response due to rapid precorneal elimination of drug that could be overcome by using in situ gel-forming systems. As an additional advantage, due tosustained drug release through the formed in situ gelthe frequency of dosing is also reduced.

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