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Original Research Article

FORMULATION AND EVALUATION OF MICROEMULSION BASED IN SITU GEL FOR NASAL DELIVERY OF FLUCONAZOLE

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ABSTRACT

Fluconazole (FLZ) being drug of choice is slightly water soluble (5.1mg/ml) azole efficiently treating fungal infection if available locally in therapeutic dose. In present work intranasal system is developed as a microemulsion based in-situ gel containing 1.5% w/v fluconazole. Cinnamon oil was used as an antifungal oil, surfactants (Tween-80,20) and co-surfactants (PEG-400, IPA, Propylene glycol) was evaluated. After that Construction of Pseudo-Ternary Phase Diagram was done to study the phase behaviour of microemulsion. Pseudo ternary phase diagrams were developed using the aqueous titration method. The microemulsion system was optimized through D-optimal design and an equivalent amount of microemulsion was incorporated in poloxamer 407: poloxamer 188 (2:1) to form a thermosensitive gel. D optimal design was used to optimise the microemulsion based on a pseudoternary phase diagram with three variables: oil(X1), Smix(X2), and water(X3). The results of optimized batch (batch 11) of microemulsions shows 106.2% transmittance, -0.9 mV zeta potential, 208 nm globule size, 0.458 PDI, 28.2 cP viscosity and 99.91% drug content. Also, the optimized batch of microemulsion based in-situ gel shows 34° C gelation temperature with 110 sec as gelling time and having 181cP, 99.1% drug content with 25.5 gm mucoadhesion strength. The *in vitro* drug release study and *ex vivo* permeation study shows 95.75% and 85.45% at 4 hours

respectively, which initially gives burst release and as it turns into gel release rate decreases. The microemulsion based in-situ gel shows larger zone of inhibition. The microemulsion and microemulsion based in-situ gel were stable at both 4 °C and room temperature.

KEYWORDS: Microemulsion, Fluconazole, cinnamon oil, antifungal, in- situ gel.

1 INTRODUCTION:

Sinusitis is an inflammation or swelling of the tissue lining-the sinuses. Sinuses are responsible for making mucus and thus result in cleaning and making the nose bacterial free. When this sinus gets blocked it results in infection. This is also called rhino sinusitis-'rhino' means "nose". One such sinusitis is fungal rhino sinusitis. Symptoms of sinusitis include decreased sense of smell or a bad smell in nose, fever, nasal congestion, sinus headache and pain in sinus area. The prevalence rate of fungal rhinosinusitis is 35.06%. Occurrence ratio of fungal rhinosinusitis in Male to female is higher which ranges from 1.5-2.6. Microorganism responsible for development of disease is Yeast (like Candida albicans) and Mold (Aspergillus fumigatus, Aspergillus flavus)

FLZ is bis-triazole antifungal medication used to treat various fungal infections. It is well reported that oral administration of FLZ results in disturbance in the Gastrointestinal tract which is accompanied by vomiting, bloating, and abdominal pain and significant hepatotoxicity (1). To overcome these adverse effects other formulations of FLZ are preferred which include gel, cream and lotion, yet there are drawbacks of these formulations-insufficient residence time and inaccurate dose which results in variable therapeutic effects. Another limitation is inconveniency of application of these formulation in nasal cavity. This problem can be overcome by formulations such as microemulsion based in-situ gel. FLZ is slightly soluble in water (0.9mg/ml) which makes it a good candidate for microemulsion. Cinnamon oil shows antifungal activity by inhibition of ergosterol, which results in disrupts membrane function and hence reducing growth of fungus (2). Many trials have been conducted to improve bioavailability of FLZ following local administration including development of different drug delivery system such as microsomes and liposomes.

Microemulsions are clear, thermodynamically stable, isotropic mixtures of oils, water, surfactant and co-surfactant. There are various types of microemulsions exist: water in oil(w/o), oil in water(o/w) and bicontinuous emulsions. Microemulsions as delivery systems provide a number of benefits, including the capacity to transport both hydrophilic and hydrophobic molecules, target medications, boost bioavailability, increase penetration through biological membranes, and shield molecules from oxidation (3-5).

The main drawback of nasal drug delivery is lesser contact time of the medication with the nasal epithelium due to mucociliary clearance. This can be overcome by developing microemulsion based in situ gels. Addition of carrageenan polymer in in situ gel improves contact time without affecting ciliary function.

2 MATERIALS AND METHODS

2.1. Materials

Fluconazole was purchased from Redson pharmaceuticals, Ahmedabad. India. Cinnamon oil (Heilen Biopharma), Tween 80, Tween 20, Span 80, Labrasol, PGE 400 were purchased from National chemicals (India), Cremaphor RH 40 was purchased from BASF, Mumbai, India, Transcutol P (Abitech corporation), Poloxamer 147, Poloxamer 188, Carrageenan, Methanol, Double distilled water.

2.2. Methods

Quantification of FLZ

In this study, a UV-visible spectrophotometer was used to scan fluconazole using methanol (Shimadzu UV-1800). Using a UV spectrophotometer, a stock solution of fluconazole was checked for absorbance between 200 and 800 nm ranges (Schimadzu UV-1800). Methanol was utilised as a control. At 261 nm, fluconazole exhibits its peak UV absorption. Fluconazole was dissolved in 100 ml of methanol to create a stock solution with a 1000 g/ml concentration. (Stock-I), From the aforementioned stock, 1, 1.5, 2, 2.5, 3, and 3.5 ml of the solution were taken out and transferred to a 10 ml volumetric flask, where the capacity was then filled with methanol to achieve a concentration of 100-350 µg/ml.

2.2.1 Preparation of standard calibration curve in simulated nasal fluid (SNF)

To create the simulated nasal fluid, 250 ml of double distilled water was mixed with sodium chloride (2.1925 g), calcium chloride (0.145 g), and potassium chloride (0.745 g). Fluconazole solution (300 g/ml) was created in SNF. Using a spectrophotometer, the solution was checked for absorbance in the 200–400 nm range. The UV absorption maximum of fluconazole is at 261 nm. Fluconazole was dissolved in 2 ml of methanol to create the stock solution. Then, SNF was added to the mixture to increase its volume to 100 ml and achieve a concentration of 1000 g/ml (Stock-I). From the aforementioned stock I, 1, 1.5, 2, 2.5, 3, 3.5 were taken out and diluted up to 10 ml to get concentrations of 100, 150, 200, 250, 300, and 350 g/ml. Each solution's UV spectrophotometer absorbance at 261 nm was recorded.

2.2.2 Preliminary Screening of microemulsion components

An excess amount of (FLZ) was dissolved in 2 ml of each solution separately to test the solubility of (FLZ) in cinnamon oil, surfactants (Span 20, Span80, Tween 20, Tween 80, and labrasol), and co-surfactants (PEG 200, PEG 400, Transcutol HP, ethanol, propylene glycol, and isopropyl alcohol). The mixtures were centrifuged at 3000 rpm for 15 minutes after being let to stand for 24 hours. Methanol was used to dilute the supernatant before the concentration of (FLZ) was measured spectrophotometrically at 261nm.Each experiment was performed in triplicate (6).

2.2.3 Construction of pesudoternary phase diagrams

Based on the solubility experiments, the chosen oil (cinnamon oil), surfactant (Tween 20), and co-surfactant (IPA) were employed to make the microemulsion. Figures in result and discussion section shows that the surfactant and co-surfactant were blended in various mass ratios (1:1, 1:2, and 2:1). Different combinations of oil and Smix (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were developed in order to cover the widest range of ratios while creating the pseudoternary phase diagram. Double distilled water was added to the aforementioned mixture as part of the aqueous titration procedure, with turbidity serving as the endpoint. Pseudoternary phase diagrams were drawn using computed component concentrations. Chemix® was used to plot the pseudoternary phase diagram. The phase diagram's microemulsion region serves as a sign of a substance's stability. system. This led to the selection of the Smix ratio for additional research that results in a larger microemulsion zone. The phase diagram's region represents a particular stability of substance (7).

2.2.4 Preparation of Fluconazole loaded microemulsion

Using the established phase diagrams, the proper oils and Surfactant to Co-Surfactant weight ratios for the microemulsions were selected. The chosen percent composition for all microemulsion systems was 5% weight-weight oil, 20.003% weight-weight water, and 74.9% weight-weight S/CoS at a ratio of 2:1. This ratio was chosen since it appeared in all of the phase diagrams made using the various oils employed. Due to the comparatively low weight ratio of oil to water, it was also anticipated that this ratio would produce an O/W microemulsion system. This factor is crucial because fluconazole is a lipophilic medication and should ideally be integrated into the microemulsion's internal phase (8).

2.2.5 Characterization of drug loaded microemulsion (9).

Percentage transmittance: Percentage transmittance of microemulsion was measured spectrophotometrically at 650 nm using distilled water as a blank.

Zeta potential: Zeta potential is used to identify the charge of the droplets. In conventional microemulsion, the charge on an oil droplet is negative due to presence of free fatty acids. Zeta potential determined by Zeta meter was monitored at 25°C at a scattering angle 173°.

Globule size distribution and poly dispersity index (PDI):

The globule size distribution of the oil droplets in the microemulsion was analyzed using a Dynamic Light Scattering (DLS) technique by Malvern-Zeta sizer (Nano ZS90) at 25°C.

Conductivity of microemulsion:

Electrical conductivity of microemulsion was measured using conductivity meter. Based on electrical conductivity, the phase system of microemulsion system was determined.

pH measurements:

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and 7.

Rheological studies:

The prepared formulations were poured into the small volume adaptor of the Brookfield viscometer. Viscosities of microemulsions were measured at 100 rpm with spindle no. 63.

2.2.6 Optimization of microemulsion

D-optimal design

Optimization of microemulsion was done using design of experiment (DOE) in which D-Optimal design (mixture) was selected. This type of design is applied for optimization of variables when experimental responses are dependent only on the proportions of the ingredients of mixture. Because the sum of the mixture components must equal 100%, they cannot range independently. The D-optimal mixture design is frequently used to uncover main effects and interaction effects between the experiment's independent variables. Design expert 7.1 (State-Ease Inc., Minnearpolis, USA) software program was used in the present studies for the experimental design. Different design constraints,A (oil), B (Smix) and C (water) were taken at high and low levels (10).

2.2.7 Screening and selection of poloxamer ratio

Screening of polymer ratio is based on its gelation temperature and viscosity parameter for gel preparation. The various concentration of poloxamer 407 and poloxamer 188 was taken for the in-situ gel.

2.2.8 Incorporating microemulsion into in-situ gel

Method of preparation for in-situ gel

- Intranasal mucoadhesive in-situ gel formulations will be prepare with poloxamer (PLX) 407/188 mixture, adding carrageenan as mucoadhesive agent.
- PLX mixtures ratio should be decided on the basis of gelation temperature and gelation time.
- Optimized formulation of microemulsion will be mix with sol-gel dispersion.

2.2.9 Characterization of in-situ nasal gel (11).

Clarity

Clarity is one of the most important characteristics. The clarity of the formulations after and before gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

Gelation time and gelation temperature

Gelation temperature is temperature at which system changes from sol to gel state, whereas gel melting temperature is temperature at which system changes to sol state. At gelation temperature, liquid phase makes transition into gel. In this study simple test tube inversion method was employed. 5 ml aliquot of gel was transferred to a test tube, immersed in a thermostat water bath. The temperature of water bath was increased. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer moves upon tilting through 90°. After attaining the gelation temperature T1, further heating of gel causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature is noted as T2 1.e. gel melting temperature.

pH evaluation

pH is one of the most important parameter involved in the nasal formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of nasal formulation should be such that the formulation will be stable at that pH and at the same time there would be

no irritation to the patient upon administration of the formulation. Nasal formulations should have pH range in between 5.4 to 6.8. The pH of gel (1 ml) was determined using calibrated digital pH meter.

Rheological studies

The prepared formulations were poured into the small volume adaptor of the Brookfield viscometer (DV-II+ Pro). Viscosities of In-situ gel were measured at different angular velocities at a temperature of 25±1°C.

Drug content

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 mL of the formulation to 10 mL with methanol. The absorbance of prepared solution was measured at 261nm by using UV/Visible double beam spectrophotometer. Fluconazole concentration was then determined.

Mucoadhesion strength

Modified pan balance method was used for mucoadhesive strength measurement as shown in Figure 1. The goat nasal mucosal tissue (C) was pasted to the flat glass block (facing the air interface) using cyanoacrylate adhesive. The flat watch glass (D) was fixed with one side of pan. On the opposite side of the balance, small plastic container (B) of same weight as that of the watch glass was fixed. Mucoadhesion test assembly was set as mentioned below. Balance between two side arms was checked. Thermosensitive gel (A) was hydrated for 15 min by immersing it in Buffer before starting the experiment. Freshly cut goat nasal mucosa was fixed to the glass block and put into the petri dish filled with Buffer at about 37°C. Hydrated gel was applied to the watch glass (on the bottom side facing tissue). Pre weight of 20 g was put on the watch glass thus allowing contact between gel and tissue for 10 min. Added water drop wise to the opposite side arm. Water addition was stopped at the detachment point of patch and tissue. Amount of water required was noted from the burette and required weight was calculated. Mucoadhesion strength (gm) = W/p° Where W-Weight of water required for detachment, p-Density of water.

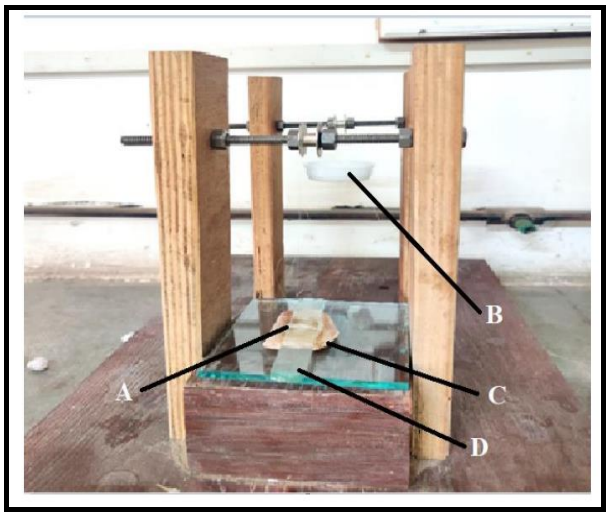

Figure 1 Modified balance

2.2.10 In vitro drug release study

The drug's release profile provides important information about its in vivo behaviour and forecasts how a delivery device would perform. Franz diffusion cells were used to test Fluconazole's in situ gel release profile by in vitro drug release. SNF filled the receptor compartment. In situ gel formulation was put within the donor chamber. A cellulose membrane (0.2 m) separated the donor compartment from the receptor chamber. A 1 ml sample was taken at regular intervals. After the release medium was sampled, fresh release medium was added. After the proper dilution with SNF at 261 nm, the content of the released medication was assessed using a UV spectrophotometer (12).

2.2.11 Ex-vivo permeation study

The franz-diffusion cell was used to study the drug release from the improved microemulsion. SNF was placed inside the receptor compartment (6.8). Goat nasal mucosa separated the donor compartment from the receptor compartment. A 1ml sample was taken at predefined intervals. Following the sampling of the release medium, new release medium was added. Following a sample of the release medium, the oil that had been discharged was refilled. After the required dilution with SNF, a UV spectrophotometer was used to quantify the amount of released medication at 261 nm (13-15).

2.2.12 Antifungal studies

This was discovered through an agar diffusion test using the cup plate method. Both the new formulations and the standard fluconazole dosage were used. All of the aforementioned preparations were sterilised before being placed into plates containing potato dextrose agar and test organisms (Candida albicans). Agar plates were incubated at 37°C for 24 HRS after allowing solutions to diffuse. Each plate's zone of inhibition (ZOI) was evaluated, and the results were compared to controls. With the exception of incubation, the entire procedure was completed in a laminar airflow unit (16).

3. RESULTS AND DISCUSSION

3.1. Analytical method

The range of clotrimazole's absorbance in methanol is reported to be between 0.2-0.8 at concentrations between 100 and 350 g/mL. The calibration equation, y=0.0021x+0.0045 has R2 value of 0.9985.

3.2 Screening of microemulsion components

Among surfactants, fluconazole showed higher solubility in Tween-20 and Labrafil. As Tween-20 gave clear microemulsion compared to Labrafil, it (Tween-20) was chosen. Among co-surfactants IPA had highest solubilizing power compared to other co-surfactant so it was selected as co-surfactant.

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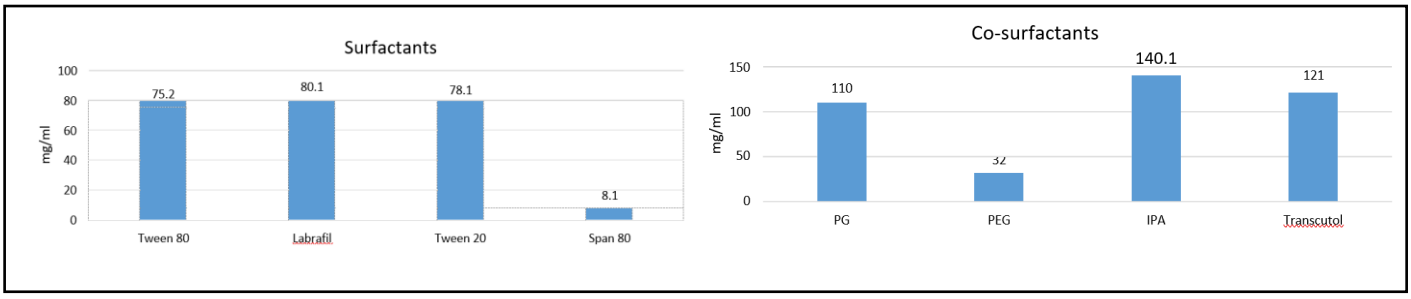

Figure 2 screening of surfactants and co- surfactants

3.3 Construction of pesudoternary phase diagrams

When compared to 1:1 and 1:2 ratios, it was found that the majority of phase diagrams' percentage area of the microemulsion zone was highest at a S/CoS weight ratio of 2:1. This can be explained by the improvement in micelle formation brought on by a rise in the S/CoS ratio, which in turn raises the microemulsion's solubilizing capacity. Additionally, at a S/CoS weight ratio of 2:1, cinnamon oil was seen to generate the biggest microemulsion.

Table 1 pesudoternary phase diagrams

Smix:Oil	Percentage of components		
	2:1		
	%Smix	%Oil	%Water
9:1	8.18	0.91	90.91
8:2	7.27	1.82	90.91
7:3	16.67	7.14	76.19
6:4	40.00	26.67	33.33
5:5	41.67	41.67	16.67
4:6	33.90	50.85	15.25
3:7	25.86	60.34	13.79
2:8	17.70	70.80	11.50
1:9	8.93	80.36	10.71

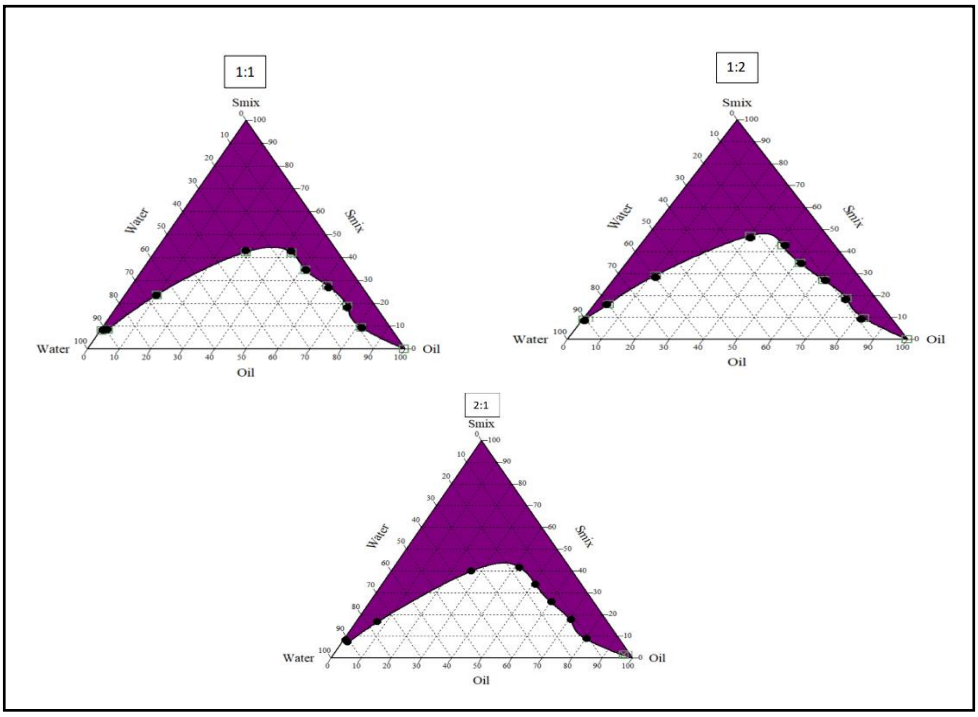

Figure 3 pseudo ternary phase diagram of 1;1, 1:2 and 2:1

Dark color represents microemulsion area. As 2:1 had larger microemulsion area it was selected for further optimization.

3.4 Optimization of microemulsion

Overlay plot

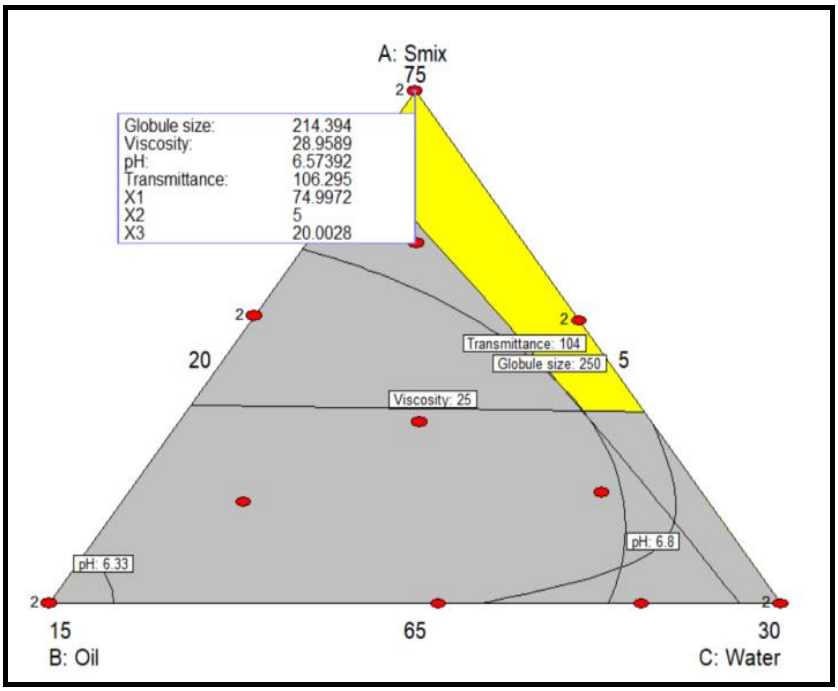

Figure 4 Overlay plot

A flag batch was generated and formulation was prepared and further evaluation was done.

Model summary statistics:

Table 2 Model summary statistics

Response	Model	Std Dev.	R2	Adjusted R2	Predicted R2
Globule size	Quadratic	6.65	0.9890	0.9835	0.9709
Viscosity	Linear	1.09	0.8363	0.8112	0.7477
pH	Linear	0.1302	0.7072	0.6622	0.5965
Transmittance	Quadratic	0.2806	0.9690	0.9535	0.9152

Table 3 Evaluation parameters of optimized microemulsion

Batch no.	%Smix	%Oil	%Wat er	Globule size (nm)	Viscos ity (cP)	pH	Transmitta nce (%)	Conducti vity (ms/cm)
1	65.005	14.995	20.000	361	21.6	6.25	102.1	0.289
2	65.000	5.002	29.998	238	21.3	6.94	105.5	0.321
3	70.611	9.389	20.000	313	24.3	6.76	103.2	0.313
4	70.520	5.000	24.480	225	26.1	6.85	104.2	0.351
5	65.000	9.677	25.323	332	22.3	6.83	103.1	0.336
6	68.544	8.165	23.291	289	25.6	6.58	103.5	0.323
7	70.520	5.000	24.480	231	26.2	6.6	104.8	0.289
8	72.033	6.462	21.506	258	28.4	6.58	104.1	0.310
9	67.164	6.364	26.472	262	24.1	6.71	103.8	0.381
10	65.000	5.002	29.998	240	22.4	6.88	104.7	0.338
11	74.997	5.000	20.003	208	29.3	6.59	106.5	0.343
12	74.997	5.000	20.003	220	27.4	6.55	106.2	0.361

13	65.005	14.995	20.000	358	22.4	6.2	102.3	0.311
14	66.983	11.355	21.663	349	24.8	6.31	102.5	0.380
15	70.611	9.389	20.000	301	27.5	6.61	103.4	0.330
16	65.000	6.904	28.096	271	23.8	6.82	104.2	0.322

All microemulsion formulations were clear and transparent. The average droplet size ranges between 208 to 361 nm and viscosity between 21.3-29.3 cP. The pH value was in between 6.25 to 6.88 and transmittance ranges from 102.1-106.5. Conductivity of microemulsion ranges between 0.289-0.381 ms/cm, this shows that microemulsion were O/W type. Thus, Batch 11 was selected.

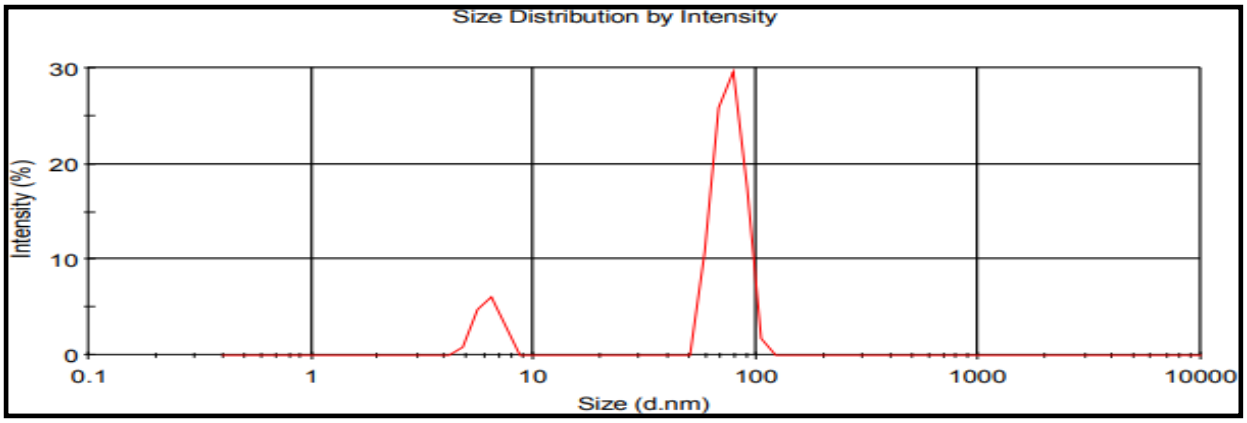

Figure 5 Globule size and PDI (Polydispersity index

Zeta potential of optimized formulation was found to be -0.9 mV

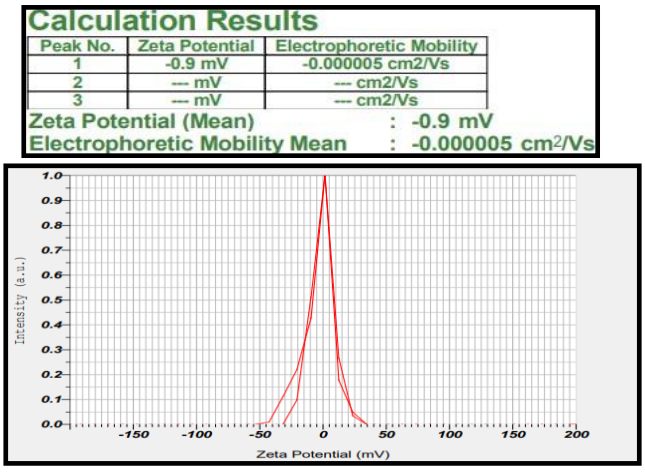

Figure 6 Zeta potential

3.5 Characterization of CTM Microemulsion System

The optimised microemulsion, which had a PDI of 0.458 and the smallest globule size of 208 nm, contained 70% Smix, 5% oil, and 20% water. The measured zeta potential was -0.9 mV. Microemulsion and in-situ gel were discovered to have viscosities of 33 cP and 181 cP, respectively, and a pH of 6.71. According to the results of conductivity, which is 0.343 ms/cm, all microemulsions were of the o/w type.

3.6 Incorporating microemulsion into in-situ gel

Table 4 Incorporating microemulsion into in-situ gel

Final formulation of in-situ gel	
Ingredient	Quantity
In situ gel	2.5 ml
Microemulsion	2.5 ml
Final formulation	5ml

Prepared microemulsion based in-situ gel:

3.7 Characterization of in-situ nasal gel

Table 5 Characterization of in-situ nasal gel

Sr.no.	Evaluation parameter	Result of microemulsion based in situ gel
1	Clarity	Light yellow (clear)
2	Gelation temperature	34°C
3	Gelation time	115 secs
4	pH	6.71
5	Viscosity (after gelation)	182 cP
6	Drug content	99.1

Figure 7 2:1 polymer ratio

As 2:1 polymer ratio was selected gelation temperature decreased to 34° C due to increase in poloxamer 407.

3.8 Mucoadhesion strength was found to be 25.5 gm.

3.9 In vitro drug release study:

Table 6 in vitro drug release study

Time (min)	In-situ gel (%CPR)	Microemulsion (%CPR)
15	5.14	13.43
30	20.20	22.34
60	31.47	42.95

90	37.21	62.81
120	58.96	84.56
150	76.92	97.74
180	83.19	
210	88.81	
240	94.75	

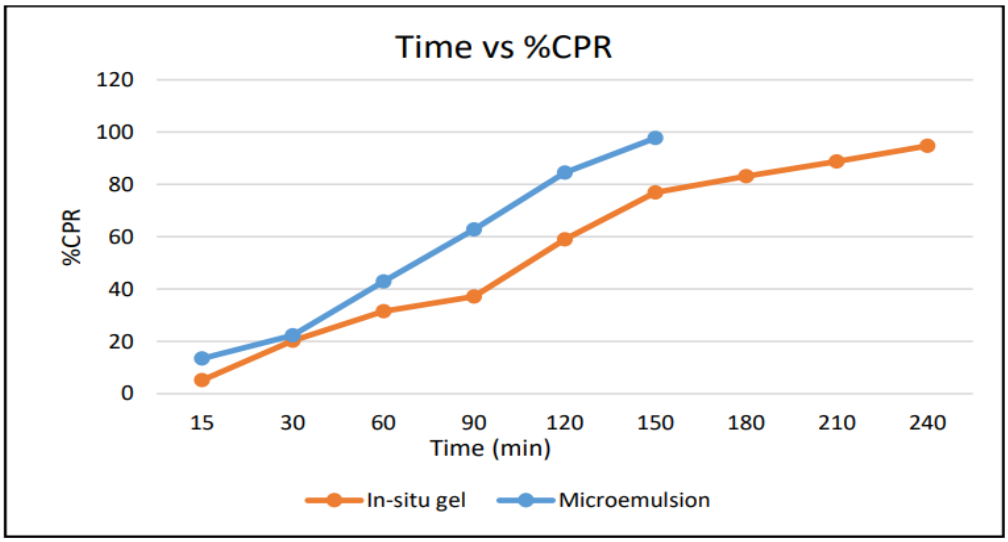

Figure 8 In vitro drug release

In vitro permeation study performed on cellulose membrane showed %CPR of 97.74% and 94.75% of microemulsion at 150 minutes and microemulsion based in situ gel at 240 minutes respectively.

3.10 Ex-vivo drug permeation

Table 7 Ex-vivo drug permeation

Time(min)	%CPR
15	4.77
30	14.21
60	29.36
90	33.63
120	55.81

150	70.00
180	75.26
210	80.35
240	85.45

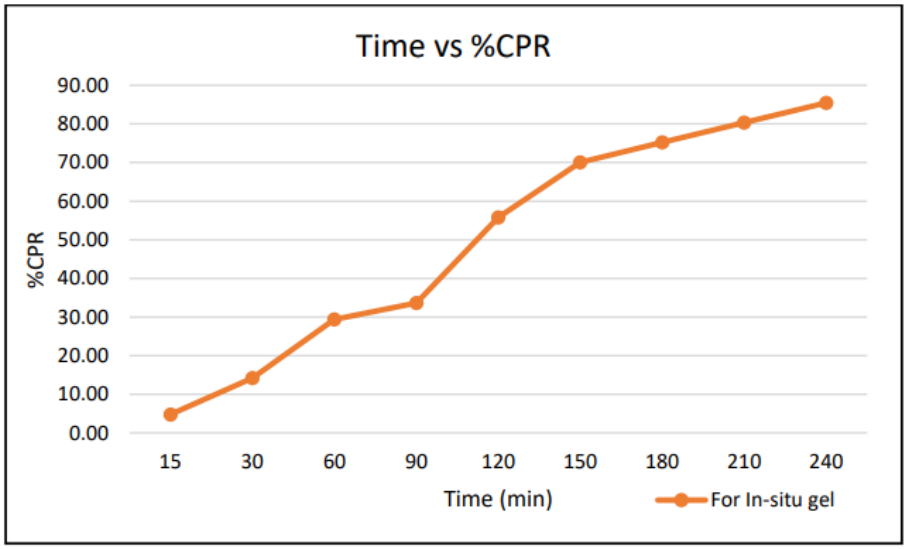

Figure 9 Ex- vivo permeation study

Ex vivo permeation study of microemulsion based in situ gel performed on goat nasal mucosa showed 85.45% CPR at 240 minutes.

3.11 Antifungal studies

A- Marketed formulation B- In-Situ gel C-Water D-Microemulsion without drug

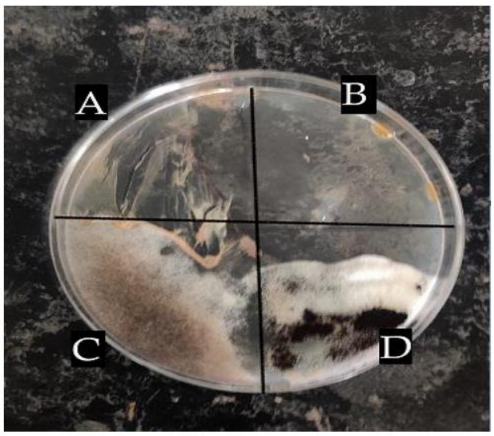

Figure 10 Antifungal study: A- Marketed formulation B - In-Situ gel C-Water D-Microemulsion without drug

The above results show that In-situ gel and marketed formulation both provide better zone of inhibition compared to microemulsion without drug.

3.12 Stability studies:

Table 8 Stability study

Storage condition	After 15 days	
Temperature	Drug content	Clarity
30 °C	97.38%	Clear
4 °C	97.61%	Clear

Optimized formulation showed slight decrease in drug content at 30° C (97.38%) after 15 days of storage. From the stability studies it was confirmed that in-situ gelling formulation of fluconazole remained stable at ambient temperature and humidity.

4. CONCLUSION

The amount of fluconazole, an antifungal drug, that can be made into gel (% 0.5) is constrained by its extremely poor solubility in water. In this study, fluconazole in-situ gel based on microemulsion was created for nasal delivery by dispersing microemulsion into the in-situ gel. Fluconazole is present in the formulation as a consequence in %1.5. As a surfactant and co-surfactant, tween 20 and IPA were utilized. In conjunction with carrageenan, which served as a mucoadhesive polymer, poloxamer 407 and poloxamer 188 were utilized as thermosensitive gelling polymers. Studies on the pseudo ternary phase diagram using various Smix ratios came to the conclusion that 2:1 produces a better microemulsion region. Using D-optimal design, the composition of the microemulsion was optimized with the responses of globule size, pH, viscosity, and transmittance. Because Batch 11 obtained in overlay plot. Replies covered all features of responses, it was considered the most optimal batch.

The optimized microemulsion, which had a PDI of 0.458 and the smallest globule size of 208 nm, contained 70% Smix, 5% oil, and 20% water. The measured zeta potential was -0.9 mV. Microemulsion and in-situ gel were discovered to have viscosities of 33 cP and 181 cP, respectively, and a pH of 6.71. According to the results of conductivity, which is 0.343 ms/cm, all microemulsions were of the o/w type. The stability of the microemulsion was confirmed by thermodynamic stability studies on the optimized formulation, which revealed no phase separation.

Based on a screening of the ratio of in-situ gel polymers based on gelation temperature and gelation time, it was found that poloxamer 407, whose effect on gelation time is directly proportional to its amount, and poloxamer 188, whose effect on gelation time is also directly proportional to its amount, both have an impact on gelation time. It led to the selection of a 2:1 ratio of poloxamer 407 and poloxamer 188. Using modified balance, a mucoadhesion test was conducted, and the mucoadhesion strength was discovered to be 25.5 gm. According to an in vitro permeation investigation, the microemulsion without in-situ gel released drugs more quickly than in-situ gel did. The gelling polymer may be the cause of the drug's slower release in gel. Since it is an in-situ gel, the medication releases more quickly at first—a process known as burst release—but as the gelling process progresses, the rate of release slows. Ex-vivo testing for the in-situ gel revealed 4% drug release after 15 minutes, 14% after 30 minutes, and 29% after 60 minutes. At 4 hours, 85% of the medication had been released. According to antifungal research, in-situ gel provided a superior zone of inhibition (ZOI) than a microemulsion without a medication. Drug content in stability trials decreased to 97.38% after 15 days, at 30° C room temperature.

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