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"Development and Characterization of Microemulsion based Insitu gel for Vaginal Delivery of Fenticonazole Nitrate"

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ABSTRACT
Fenticonazole Nitrate is widely used in the treatment of vaginal fungal infection. The present work describes the development and characterization of Microemulsion based in situ gel-forming systems for localized vaginal drug delivery system containing Fenticonazole nitrate.A stable microemulsion (ME) system was prepared and optimized through D-optimal design and an equivalent amount of ME was incorporated in a polymer mixture of poloxamers 188:407 in the ratio of (2:1) to form temperature triggered insitu gelling system.The results of the optimized batch of microemulsion ME-8 Showed 99.88 % transmittance, 36.27 mV Zeta potential, 0.101mm globule size, 0.99 PDI, 0.824 cp viscosity and 99.020 % of drug content. The optimized batch of microemulsion based insitu gel showed gelation temperature, gelation time, drug content uniformity, Viscosity and mucoadhesive force of 36.2°C, 2 min, 402.500 cp, 99.95 %, 0.88 (mJ) respectively. The in-vitro drug release profile study and vaginal mucosa permeation study showed 97.42 % and 97.2 at 8 hours respectively with better drug release profile as compared to tween 80 Insitu gel. The antifungal study of microemulsion based insitu gel showed a larger zone of inhibition and the vaginal mucosa irritation study showed no irritation. The microemulsion and microemulsion based insitu gel was found stable at room temperature. In this study microemulsion based Insitu gel of Fenticonazole nitrate for vaginal delivery was prepared by microemulsion component with insitu polymer as a gelling agent mainly including poloxamer 407 and 118. Optimized in situ gel shows better in vitro drug release study compared to tween 80 in situ gel, it shows mucoadhesion and in vitro mucosal permeation study at 8 hours. The vaginal mucosa irritation study of microemulsion based in situ gel study compared with tween 80 insitu gel and this shows there is no irritation in microemulsion based vaginal insitu gel. The zone of inhibition of Fenticonazole nitrate with insitu gel shows a good zone of inhibition.

KEYWORDS: Fenticonazole Nitrate, Microemulsion based Insitu Gel, Mucoadhesive insitu gel, D optimal design, Vaginal drug delivery system

I. INTRODUCTION

Vaginitis or vaginal candidiasis is one of the most common problems found in women. It is a common fungal infection that occurred under predisposing conditions such as, diabetes, antibiotic therapy, oral contraception, and pregnancy. Candida albicans is recognized as the most frequent etiologic agent of vaginal candidiasis. Treatment of Candida infections is still challenging and is restricted to a small number of antifungal drugs, mainly azole derivatives. The vaginal delivery was used for a long time as a route for drug delivery with the purpose to obtain a local and systemic pharmacological effect. Many newer carriers are evolving with the advent of technology and the demand for targeted delivery like microemulsions. Microemulsions are clear, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a cosurfactant. They offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, and improved drug solubilization with enhancing the drug permeation. Recently, insitu gel formulations have been proved as more convenient dosage forms for vaginal applications. Insitu gels offer several advantages such as easy to administer into the desired body cavities, high spreadability at certain pH and reduction in the frequency of administration, improve patient compliance and comfortable in comparison to conventional dosage forms. Fenticonazole nitrate an imidazole derivative, has a broad spectrum of antimycotic activity against dermatophytes and yeast. Intravaginal administration of Fenticonazole is associated with a high rate of microbiological efficacy in patients with vaginal candidiasis,

International Journal of Pharmaceutical Research and Applications
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trichomoniasis, mixed infection, and bacterial vaginosis. Fenticonazole has a rapid onset of action and clinical efficacy is generally observed within days of commencing treatment. Fenticonazole has been shown to be equal or superior in efficacy to commonly used imidazole compounds such as miconazole, econazole, clotrimazole, and bifonazole. Topical Fenticonazole is very well tolerated; adverse events are generally mild to moderate in severity and transient.

MATERIALS

FNTZL was obtained as a gift sample from Optimus drug private limited, Hyderabad, India. Oleic acid and Tween 80 from Ases chemical work, Jodhpur. Transcutol P from Gattefosse Sas. Methanol procured from Avanror performance material India ltd, thane-Maharashtra. HPMC E5 procured from Corel pharma, Ahmedabad. Poloxamer 407 was obtained from Rakesh chemicals, Gota, Ahmedabad. Poloxamer 188 was from a chemo dyes Corporation, Ahmedabad, India.

II. MATERIALS AND METHOD

1. Solubility study of drug [1]

The solubility of FNTZL was carried out to select appropriate oil (OA, RO, Olive oil, sunflower oil, soybean oil), surfactant (Tween 80, tween 20, Chremophore-Labrafil,) and co-surfactant (PG, Span 80, caproyl 90, Transcutol P. An excess amount of FNTZL was added to 5 ml of oil or surfactant or co-surfactant and the resulting mixture was shaken reciprocally at 37 °C for 72 h followed by centrifugation for 10 min at 10,500 Rpm. The supernatant was filtered through a membrane filter paper (0.45 mm) and the filtrate was analyzed in a UV-visible spectrophotometer (UV-1700, Shimadzu) at 252 nm with suitable dilution. The oil or surfactant or co-surfactant that showed high solubility of FNTZL was used in the preparation of ME containing the drug.

2. Screening and selection of ratio for Oil, Surfactant, and Co-surfactants. [2]

Based on the solubility study of the drug in different oil, surfactant, cosurfactant the final component was decided. These Oil, surfactant, and co-surfactant were compatible with each other. And give transparent microemulsion was obtained. So, these were selected for the pseudo ternary diagram.

3. Construction of pseudo ternary phase diagram: [2] Pseudo-ternary phase diagram was constructed to find the area of ME existence and to study the effect of different surfactant/co-surfactant weight ratios on the extent of stable ME region. The weight ratio of surfactant/co-surfactant varied as 1:1,1:2,1;3,1:5,2:1 and 3:1. For each phase diagram a specific surfactant/co-surfactant weight ratio was selected, water was added dropwise to each oily mixture with continuous stirring at 37 °C until the mixture became clear at a certain point. The pseudo-ternary phase diagrams were prepared by considering three components (oil, Smix, water). The number of components required for the formulation of ME was chosen from the phase diagram.

4. Preparation of drug-loaded microemulsion: [2]

From the phase diagrams, FNTZL- loaded MEs were selected at different component ratios. ME systems were prepared by mixing oil with the mixture of surfactant and co-surfactant and, water was added precisely drop by drop into oily phases with magnetic stirring at 37 C. The systems were equilibrated with gently magnetic stirring for 30 min followed by dissolving of an appropriate amount of FNTZL. The final concentration of FNTZL in ME formulation was 100 mg/ml.

5. Optimization of drug-loaded microemulsion
D-optimal design:

The experimental mixture design technique is applied for the optimization of variables when experimental responses are dependent only on the proportions of the ingredients of the mixture. It is a type of response surface optimization technique in which design factors are the components of a mixture and responses are a function of the proportions of each component. The mixture components cannot range in an independent way since their sum has to be equal to 100%. D-optimal mixture design is commonly used to reveal the main effects and interaction effects between the independent variables of the experiment.

Design-Expert 7.1 (Stat-Ease Inc., Minneapolis, USA) software program was used in the present studies for the experimental design. A single block D-optimal mixture design with 6 model points and 4 points to estimate the lack was employed to construct polynomial models. Different design constraints, i.e. A (oil), B (Smix), and C (water) were taken at high levels (86,56,36) and low levels(8,9,3). The Independent variables[Amount of oil (X1), Amount of Smix (X2), Amount of water (X3)] and dependent

International Journal of Pharmaceutical Research and Applications
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variables[Globule Size (Y1),% Drug content (Y2)]were selected.

Characterization of drug loaded microemulsion.

1) **Percentage transmittance:** A total of 1 ml of ME formulation was diluted with 100 ml distilled water. Percentage transmittance was then measured spectrophotometrically at 252 nm using distilled water as a blank by UV-spectrophotometer.
2) **Zeta potential:** Zeta potential is used to identify the charge of the droplets. In conventional ME, the charge on an oil droplet is negative due to the presence of free fatty acids. Zeta potential determined by Zeta meter was monitored at 25°C at a scattering angle of 173°C.
3) **Globule size distribution and PolyDispersity Index(PDI):** The globule size distribution of oil droplets in the microemulsion was analyzed using a Dynamic Light Scattering (DLS) technique by Malvern -Zeta sizer (Nano ZS 90) with dilution at 25℃.
4) **The conductivity of microemulsion:** Electrical conductivity of microemulsion was measured using a conductivity meter. Based on electrical conductivity, the phase system of the ME system was determined.
5) **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.
6) **Rheological studies:** The prepared formulations were poured into the small volume adaptor of the Brookfield synchrolectric viscometer (LVDVI prime). Viscosities of microemulsions were measured at different angular velocities at a temperature of 25±1℃.
7) **Drug content determination**: ME (1 ml) was dissolved in 10 ml of methanol in a 10 ml volumetric flask separately and then 0.1 ml of the stock solution measured accurately and then transferred to a 10 ml volumetric flask to which 10 ml methanol was added and filtered through Whatman filter paper. The above solution was analyzed by UV spectrophotometer at 252 nm. The amount of drug present in the formulation was determined using the prepared standard calibration curves of a drug in methanol.

Characterization of optimized microemulsion

1. **Thermodynamic stability studies:** ME was centrifuged at 10,000 rpm for 10 min and the formulation was observed visually for phase separation. The formulations that did not show any sign of phase separation after centrifugation were subjected to three to four freeze-thaw cycles, which included freezing at −4°C for 24 h followed by thawing at 40°C for 24 h. Centrifugation was performed at 3,000 rpm for 5 min. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.
2. **Stability study of microemulsion:** Stored microemulsion system was evaluated by visual inspection, % Transmittance, pH, Globule size, zeta potential.

Method of preparation for Insitu gel[3]

Vaginal mucoadhesive insitu gel formulations will be prepared with PLX 407/188 mixture, adding either HPMC E50 or K100M as a mucoadhesive agent.PLX mixtures ratio should be decided on the bases of gelation temperature and gelation time. Optimization of the dispersion of poloxamer and HPMC. The optimized formulation of microemulsion will be mix with the sol-gel dispersion.

Screening and selection of poloxamer ratio[4]

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

Screening and selection of HPMC grade[4]From the result of poloxamer ratio selection the further study was done on a 2:1 ratio. For HPMC selection different grade was used for the same concentration and check their adhesiveness and viscosity.

Screening and selection of HPMC k 100[4]For the final concentration of HPMC k100 was selected based on the Mucoadhesion study.

Characterization of insitu vaginal gel[5-7]

1.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.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

2.Gelation time and gelation temperature

Gelation temperature is a temperature at which the phase of the system was changed from sol to gel state, whereas gel melting temperature is the temperature at which the phase of the system changed to sol state. At gelation temperature, the liquid phase makes the transition into a gel.

In this study, a simple test tube inversion method was employed. 5 mL aliquot of the gel was transferred to a test tube, immersed in a thermostat water bath. The temperature of the water bath was increased. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer move 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 i.e. gel melting temperature.

3.pH evaluation

pH is one of the most important parameters involved in the vaginal formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of the vaginal 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. Vaginal formulations should have a pH range between 3.5 to 4.0. The pH of the gel (1 ml) was determined using a calibrated digital pH meter.

4. Rheological studies:

The prepared formulations were poured into the small volume adaptor of the Brookfield synchrolectric viscometer (LVDVI prime). Viscosities of Insitu gel were measured at different angular velocities at a temperature of 25±1℃.

5. 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 the prepared solution was measured at 252 nm by using a UV/Visible double beam spectrophotometer. Fenticonazole concentration was then determined.

6. In-vitro drug release study:

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its in vivo behavior. In vitro drug release was carried out by the use of Franz diffusion cells in order to evaluate the Fenticonazole nitrate insitu gel release profile. The cumulative percentage of drug released as a function of time is shown in Figure. The release of drug from these gels was characterized by an initial phase of high release (burst effect) and as the gelation proceeded, the remaining drug was released at a slower rate followed by the second phase of moderate release. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with an increase in polymer concentration.

7.Mucosal permeation study[8-12]

Sprague Dawley female rat tissue was collected immediately after sacrificing the animal. The tissue was frozen in SVF at −20°C. Samples were cut in and defrosted before experiments at ambient temperature in the presence of Simulated vaginal fluid (SVF). Franz cells of an area of 1.0 ± 0.1 cm^2 were used having receptor solution: 11 mL of SVF at 37 °C and 1mL of the sample was collected at the end of 30 min at the first instance and at an interval of 1hour thereafter. The total amount of drug permeation through the unit diffusion surface and into the receptor was calculated and plotted as a function of time (8 hours). This study was compared with the gel formulation with tween 80.

8. Antimicrobial efficacy studies[12-16]

The antimicrobial efficacy was determined by the agar diffusion test employing the cup plate technique. Standard of Fenticonazole (standard preparation) and the developed formulations, diluted suitably with purified water. All the diluted solutions were sterilized and poured into the cups bored into the sterile nutrient agar seeded with test organisms (candida albicance). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37℃ for 24 hours. The zone of inhibition (ZOI) measured around each cup was compared with the control. The entire operation except the incubation was carried out in a laminar airflow unit. Each solution was tested in triplicate. Both positive (with test organisms) and negative controls (without test organisms) were maintained throughout the study.

9. Stability studies

Stability studies were carried out on optimized formulation according to ICH guidelines

International Journal of Pharmaceutical Research and Applications
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to ensure that drug products retain their fitness for use until the end of their expiration dates. Optimized formulation subjected to stability studies at room temperature. The samples were withdrawn after 15 days and were evaluated for drug content and clarity.

III. RESULTS AND DISCUSSION:

Results of solubility of Fenticonazole nitrate:

The solubility of Fenticonazole nitrate in various media was analyzed in order to screen components for ME. Previous reports indicated that the superior dermal flux appeared mainly due to the large solubilizing capacity of the ME. Among four oils, the solubility of Fenticonazole nitrate was highest in oleic acid (2.6 mg/ml). It has been reported that oleic acid is a powerful permeation enhancer for dermal delivery since it could increase the fluidity of the lipid portion of the stratum corneum. So oleic acid was chosen as oil for the preparation of the MEs containing Fenticonazole nitrate.

Fenticonazole nitrate had a higher solubility in Tween 80 (80.82 mg/ml) so, it was selected as a surfactant.

For co-surfactants, the solubility of Fenticonazole nitrate in Transcutol P (120 mg/ml) was more than Propylene glycol.

Table 1: Solubility profile of Fenticonazole nitrate

Name of Excipients	Types	The solubility of the drug (mg/ml)
Oil	Olive oil	0.384 mg/ml
	Sunflower oil	0.311 mg/ml
	Soybean oil	0.209 mg/ml
	Rice bran oil	0.411 mg/ml
	Oleic acid	**2.6 mg/ml**
Surfactant	**Tween 80**	**80.82 mg/ml**
	Tween 20	70 mg/ml
	Labrafil M1944 CS	2.34 mg/ml
	Chremophore EL	18.66 mg/ml
Co-surfactant	Span 80	14.4 mg/ml
	Propylene glycol	25 mg/ml
	Capryol 90	20 mg/ml
	Transcutol P	**120 mg/ml**

Discussion of screening and selection ratio for oil, surfactant, cosurfactant

In this study, oleic acid, Tween 80 and Transcutol P were used as the oil phase, surfactant and co-surfactant respectively found compatible with each other and give transparent microemulsion formulation containing Fenticonazole nitrate.

Table 2: Result of the pseudo ternary diagram

1:1			**1:2**			**1:3**		
oleic acid	**Smix**	**water**	oleic acid	Smix	water	oleic acid	Smix	water
0.1	**0.9**	**0.6**	0.1	0.9	0.64	0.1	0.9	0.65
0.2	**0.8**	**0.3**	0.2	0.8	0.28	0.2	0.8	0.55
0.3	**0.7**	**0.2**	0.3	0.7	0.16	0.3	0.7	0.3
0.4	**0.6**	**0.2**	0.4	0.6	0.1	0.4	0.6	0.3
0.5	**0.5**	**0.15**	0.5	0.5	0.15	0.5	0.5	0.2
0.6	**0.4**	**0.17**	0.6	0.4	0.1	0.6	0.4	0.2

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

0.7	**0.3**	**0.09**	0.7	0.3	0.14	0.7	0.3	0.15
0.8	**0.2**	**0.04**	0.8	0.2	0.07	0.8	0.2	0.1
0.9	**0.1**	**0.04**	0.9	0.1	0.09	0.9	0.1	0.1
1:5			**2:1**			**3:1**		
oleic acid	**Smix**	**water**	**oleic acid**	**Smix**	**water**	**oleic acid**	**Smix**	**water**
0.1	0.9	0.6	0.1	0.9	0.6	0.1	0.9	0.55
0.2	0.8	0.42	0.2	0.8	0.62	0.2	0.8	0.5
0.3	0.7	0.35	0.3	0.7	0.6	0.3	0.7	0.4
0.4	0.6	0.3	0.4	0.6	0.2	0.4	0.6	0.15
0.5	0.5	0.25	0.5	0.5	0.15	0.5	0.5	0.16
0.6	0.4	0.2	0.6	0.4	0.13	0.6	0.4	0.15
0.7	0.3	0.15	0.7	0.3	0.1	0.7	0.3	0.1
0.8	0.2	0.05	0.8	0.2	0.12	0.8	0.2	0.08
0.9	0.1	0.05	0.9	0.1	0.03	0.9	0.1	0.05

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

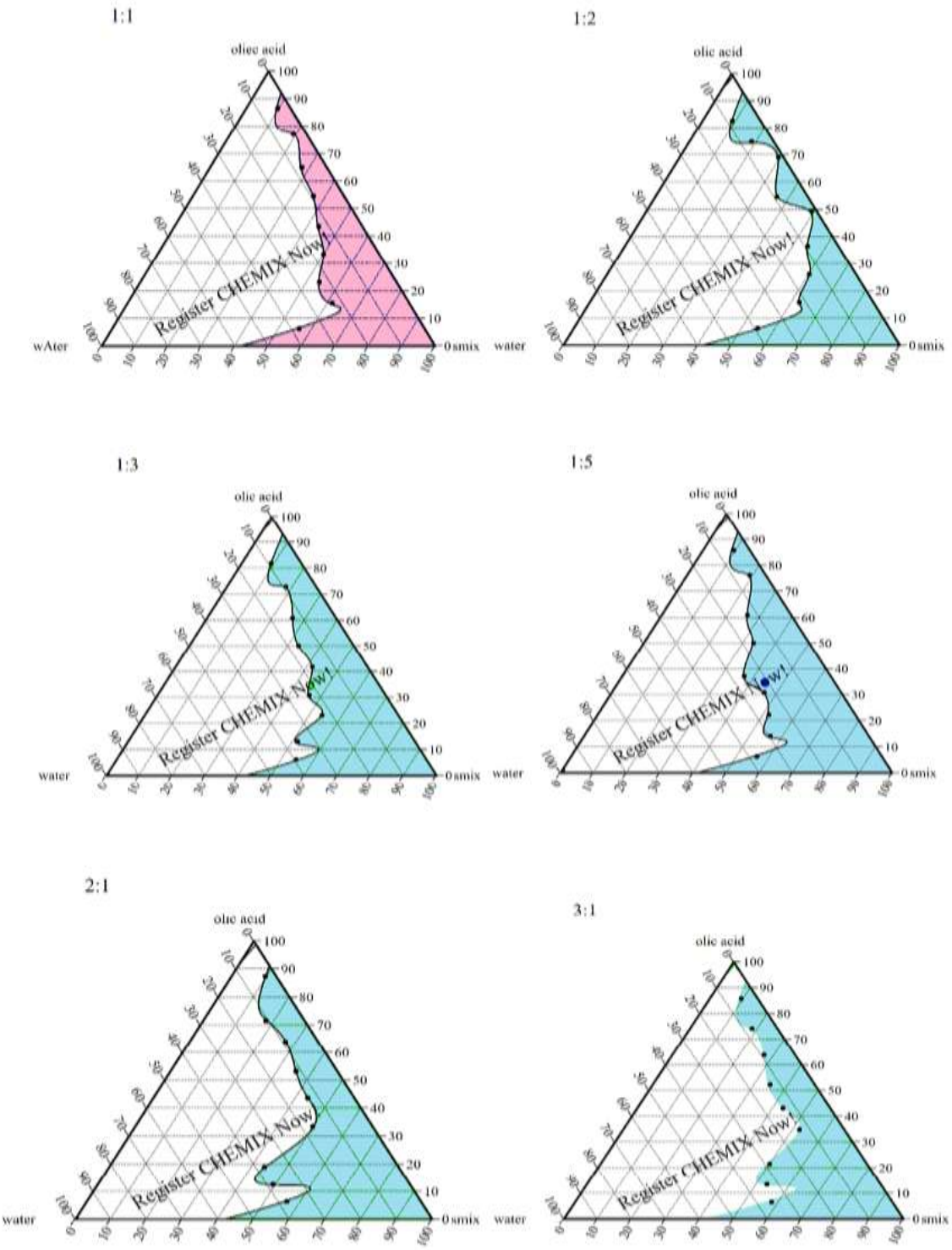

Figure 1 Pseudo ternary diagram of oleic acid, Smix, and water in the ratio of a) 1:1, b) 1:2, c) 1:3, d) 1:5, e) 2:1, f) 3:1 of Tween 80:Transcutol P respectively.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

Pseudo ternary diagram:

The studied systems composed of safe constituents including oleic acid, Tween 80, Transcutol P, and water. The construction of phase diagrams makes it easy to find out the concentration range of components for the existence range of ME. The pseudo-ternary phase diagrams with various weight ratios of Tween 80 to Transcutol P are described in Figure 1.

The translucent ME region is presented in phase diagrams. No distinct conversion from w/o to o/w MEs was observed.

The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. The area of the ME isotropic region changed slightly in size with the increasing ratio of surfactant to cosurfactant.

Dose Calculation:

As per the reference of "Fenticonazole vaginal cream 2% Formulation," the Fenticonazole dose is set as 200 mg per 5 ml of Insitu gel (dispersion). [21-25]

Drug Content: Each ml of Fenticonazole loaded microemulsion contains 100 mg of Fenticonazole.

Dose: Apply approximately 5 ml of Insitu gel on the infection once a day at bedtime.

Table 3: Coded values for batches used in the design of the experiment

Independent Variables	**Real Values**		
	Low (-1)	**Medium (0)**	**High (+1)**
Amount of oil required (mL)	8	47	86
Amount of Smix required(ml)	9	32.5	56
Amount of water required(ml)	3	19.5	36
Batches	**Coded Values**		
	X1	**X2**	**X3**
ME1	1	-1	-1
ME2	1	-1	1
ME3	1	1	0
ME4	-1	1	1
ME5	0	1	-1
ME6	-1	1	0
ME7	0	1	-1
ME8	0	0	0
ME9	0	1	0
ME10	-1	1	1
ME 11	0	0	1

International Journal of Pharmaceutical Research and Applications
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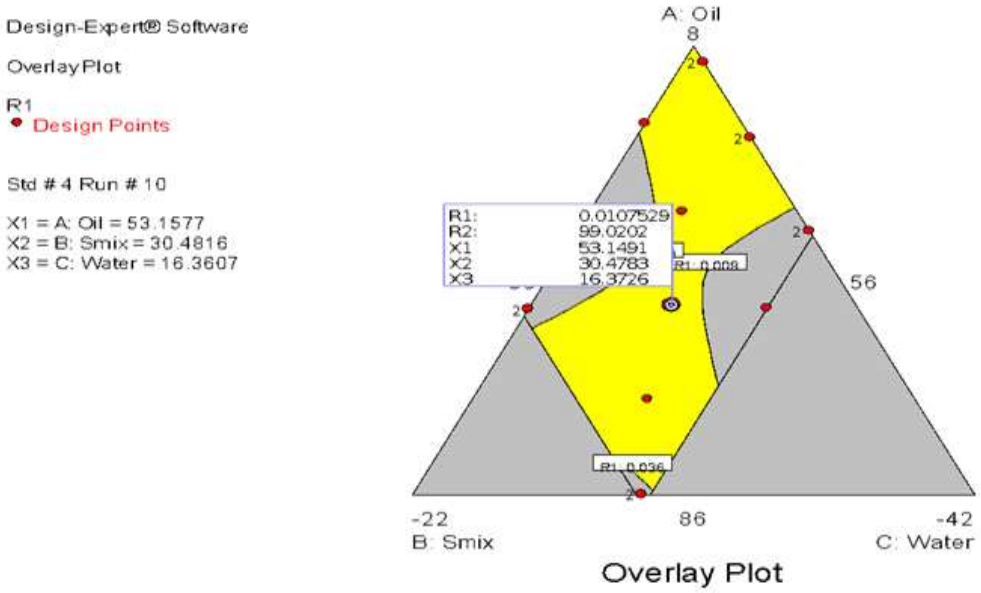
Figure 2 D- optimal Design



Result of D- optimal Design

Adjusted Predicted for Quadratic and Cubic source
STDV= 0.00648,0.00441
R-Squared=0.88013,0.96673
R-Squared=0.8202,0.91683
R-Squared=0.73438,-50.354
PRESS=0.00093,0.18004

Result of D- optimal Design equation:

EQUATION R1=0.000614638* Oil
0.00135824* Smix
-0.002156592 * Water
-4.147E-05 * Oil * Smix
2.58012E-05* Oil * Water
2.17457E-05 * Smix * Water

Table 4: Result of evaluation parameters

Batch No:	% Transmittance	Zeta potential (mv)	Globule size(mm)	PDI	Conductivity (uS/cm)	pH	Viscosity(cp)	Drug content
ME 1	99.8	12.45	0.354	0.53	1,168	2.1	0.832	98.06
ME 2	99.85	0.71	0.043	3.49	54	2.86	0.829	98.2
ME 3	99.75	-2.65	1.061	5.27	300	2.97	0.818	98.4
ME 4	98.9	0.7	0.012	6.42	101	3.42	0.82	99.2

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

ME 5	98.85	25.7	0.342	1.9	248	3.76	0.838	99.4
ME 6	98.8	9.22	0.022	0.6	344	3.06	0.823	98.2
ME 7	98.86	22.22	0.426	1.9	517	3.07	0.815	99.42
ME 8	**99.88**	**36.27**	**0.101**	**1.26**	**802**	**3.9**	**0.824**	**99.8**
ME 9	99.86	1.16	0.004	0.15	531	2.94	0.818	98.26
ME 10	98.96	8.47	5.91	0.18	292	3	0.813	99
ME 11	98.95	1.19	0.24	1.29	749	2.2	0.828	98

Discussion of evaluation parameter:

All ME formulations were clear and transparent. The percentage of transmittance falls within the range of 98.00 % to 100%. The drug content in ME was within the range between 98.00% and 100%. Polydispersity index is a measure of particle homogeneity and it varies from 0.50 to 6.42 ME which is suitable for topical application. The pH value was between 2.00 to 3.90 and the average droplet size 0.004 mm to 1.061nm, respectively. The drug content was within the variation of 98 to 99 %. The viscosity was lies between the range of 0.810cp and 0.380cp. The conductivity of the microemulsion range between 101 us/cm to 1168 us/cm result shows the MEs were o/w type.

Result of thermodynamic stability study

Results showed that Batch number 8 was clear and has no phase separation.

Discussion of thermodynamic stability study

Microemulsion formulation is found to be stable in these conditions. microemulsion was stable and did not show any phase separation. No changes in the visual description of samples after freeze-thaw cycles were observed.

Result of stability study

The formulated microemulsion stored at room temperature and after 1 month, it showed 99.86% Transmittance, 3.90 pH,0.100 Globule size,36.20 Zeta potential.

From the results, it was observed that there was no change in the physical appearance of the formulation such as consistency, pH, globule size, etc. This clearly indicates that the formulation was stable at room temperature stated as per ICH guidelines.

Characterization of fenticonazole loaded microemulsion with insitu gel

Table 5: Result of the selection of poloxamer ratio

RATIO	PLX407 (g)	PLX188 (g)	WATER (ml)	TEMPERATURE (ºc)	VISCOSITY
1:1	1	1	Up to 10 ml	70 ºc	+
1:2	1	2	Up to 10 ml	65 ºc	+
1:3	1	3	Up to 10 ml	45 ºc	++

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

2:1	**2**	**1**	Up to 10 ml	**37 ºc**	**+++**

Viscosity symbolic representation: '+' very less viscous, '++' less viscous, '+++' viscous

Screening and selection of polymer ratio

From the result, a 2:1 ratio was selected for further study because their gelation temperature and viscosity are within the range.

Result of the selection of HPMC grade

The result showed that HPMC K 100 (0.5%) found to exhibit high mucoadhesiveness and viscose properties compared to HPMC K 50(0.5%)

From the adhesiveness and viscosity, the HPMC k100 was selected for further study because of their viscosity was higher than HPMC k 50.

Result of screening and selection

Polaxamer ratio 2:1 with different HPMC K 100 was taken i.e 0.2, 0.5, 1, 1.5 their mucoadhesive force was 0.043,0.088,0.125,0.165 respectively.

Discussion on screening and selection of HPMC concentration.

From the above result, 0.5% of HPMC was selected for final Insitu gel formation because it gives mucoadhesion in the range.

FINAL FORMULATION OF IN SITU GEL WITHOUT MICROEMULSION.

In the final formulation the poloxamer 407 and poloxamer 188 were taken 2 gm and 1 gm respectively, the HPMC K 100 was taken 0.5 gm with 10 ml water

FINAL FORMULATION OF IN SITU GEL WITH MICROEMULSION.

the formulated microemulsion was taken 2 ml with 3 ml of in situ gel which gave 5 ml of the final formulation.

Figure 3 Prepared microemulsion based insitu gel and simple insitu gel.

Table 6: Result of the evaluation parameter of microemulsion based Insitu gel

Sr no:	Evaluation Parameter	Result of microemulsion based Insitu gel	Result of tween 80 with Insitu gel
1	Clarity	Yellowish color clear gel	Yellowish color clear gel
2	Gelation temperature	36.2 °c	34.3
3	Gelation time	2 min	3 min
4	pH	6.5	6.3

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

5	Viscosity	402.500 cp	395.45 cp
6	Drug content	99.95%	99.45

Discussion on Result of Evaluation parameter.

The microemulsion based Insitu gel compared with tween 80 Insitu gel. The microemulsion based Insitu gel shows greater gelation temperature and gelation time compared to tween 80 Insitu gel. Also, it shows that tween 80 Insitu gel having a decrease in pH, viscosity compares to microemulsion based Insitu gel.

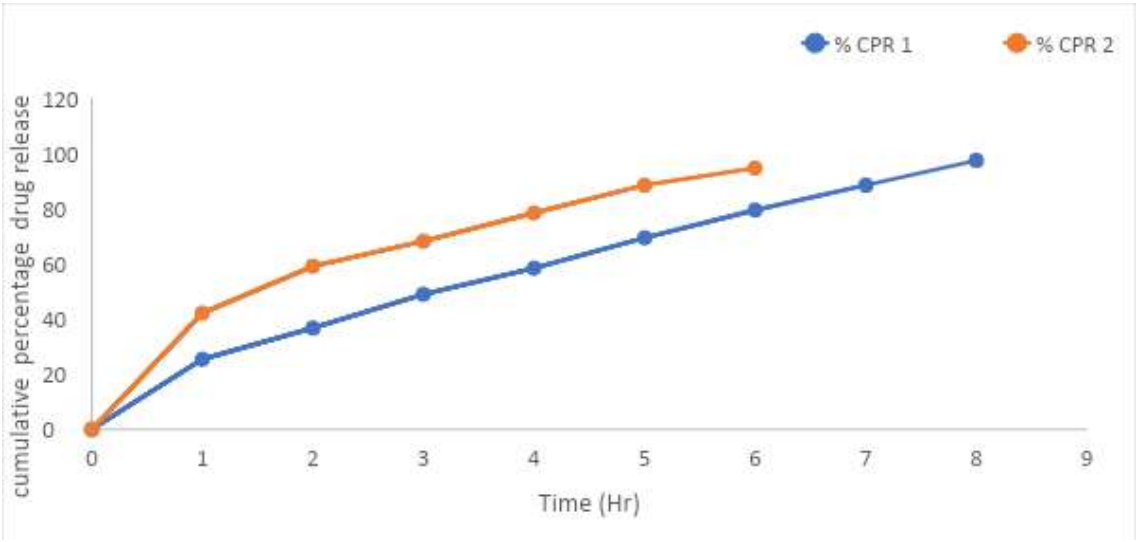


Figure 4 : In Vitro Drug Release Study of fenticonazole nitrate ME based insitu gel

In vitro study:

Figure 4 shows the **cumulative drug release of microemulsion with gel (%CPR 1) was 97.42% at 8 hours and tween 80 in situ gel (%CPR 2) was 94.6 % at 6 hours.**cumulative amount of Fenticonazole nitrate released vs. time profiles for two different formulations.

From the in vitro drug release studies, it was observed that the microemulsion and mucoadhesive polymers affected the drug release from the formulations.

The addition of mucoadhesive polymers retarded the drug release from the formulations. An increase in the overall product viscosity might contribute to the retarding effect of these mucoadhesive polymers as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses thereby delaying the release process. The microemulsion based Insitu gel shows a 96.99% drug release at 8 hours wherever the tween 80 Insitu gel shows higher drug release.

Vaginal mucosa study:

Figure 5 showed that the cumulative drug release of microemulsion with gel (%CPR 1) was 94.22%at 8 hours and tween 80 in situ gel (%CPR 2) was 92.56% at 8 hours.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

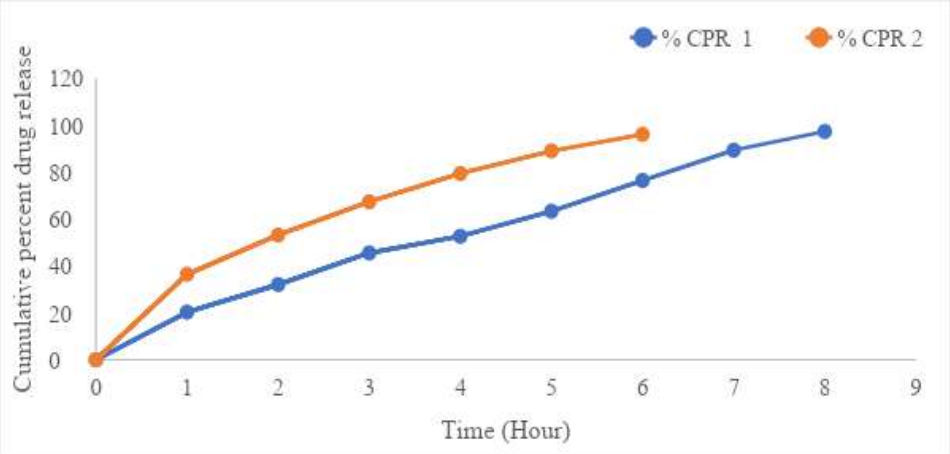


Figure 5: Vaginal Mucosa Permeation Study

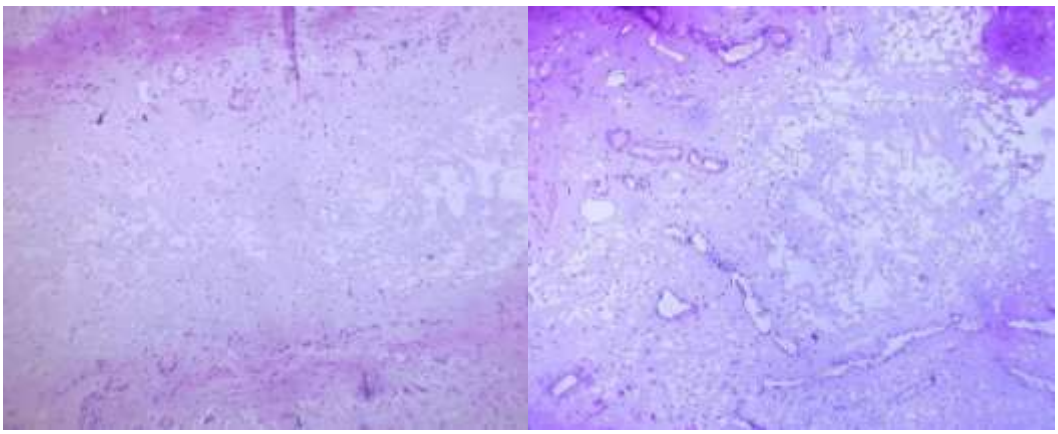
The in vitro drug release conditions may be very different from those likely to be encountered in the vagina. From the result, the vaginal mucosa permeation study shows that the cumulative percentage drug release of microemulsion based Insitu gel was 94.22% at 8 hours and tween 80 Insitu gel was 92.56% at 8 hours. From this result, the microemulsion based Insitu gel retards the drug release and tween 80 gives faster drug release.

Result of vaginal mucosa irritation study

Figure 6 shows the vaginal mucosa irritation study with 4 different formulations.

A. Simple vaginal mucosa
B. vaginal mucosa with tween 80 insitu gel
C. vaginal mucosa with microemulsion based insitu gel
D. vaginal mucosa with IPA

A B



C D

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

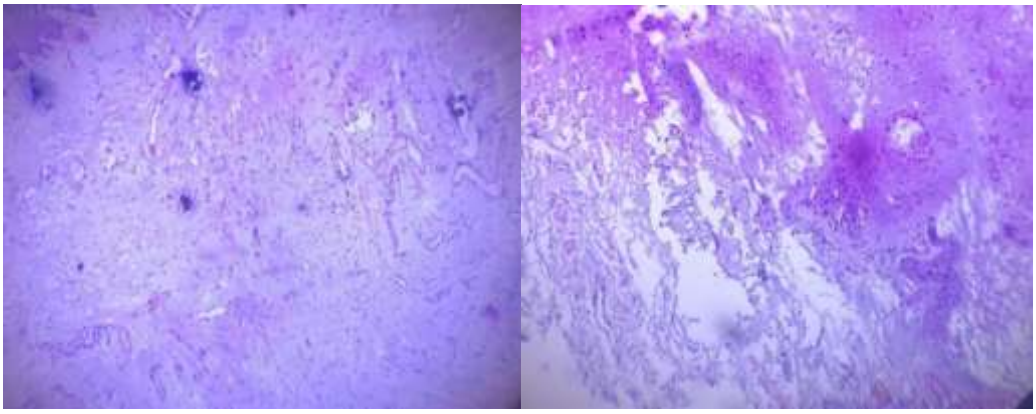


Figure 6 Result of Vaginal mucosa irritation study

From the above result, it was observed that microemulsion based Insitu gel showing no vaginal mucosa irritation when Vaginal mucosa with IPA showing more irritation than vaginal mucosa with tween 80 Insitu gel showing.

Antifungal study

Figure 7 shows the antimicrobial study with 4 different formulations.

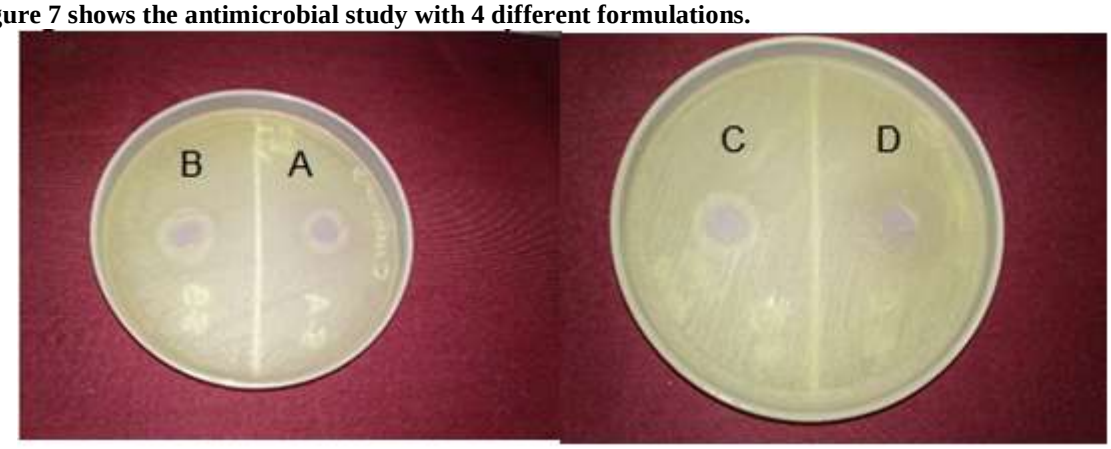


Figure 7: Antimicrobial studies of 4 different formulation

Description:
A: Drug + Microemulsion
B: Drug + Microemulsion +Insitu Gel
C: Drug + Tween 80 + Insitu Gel
D: Microemulsion + Gel

Antimicrobial efficacy study:

From the above results, the formulation shown in figure 7 in which microemulsion based Insitu gel showed better zone of inhibition compared to other formulations. Here, the microemulsion based Insitu gel (without drug) showed no zone of inhibition.

Table 7: Result of stability study

Storage condition	After 15 days parameter	
Temperature	Drug content	Clarity
(25°C) Room temperature	97.73	Clear
Storage condition	**After 15 days parameter**	

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 6 Nov-Dec 2021, pp: 1042-1059 www.ijprajournal.com ISSN: 2249-7781

Temperature	Drug content	Clarity
(25°C) Room temperature	97.73	Clear

Stability study: Optimized formulation showed a slight decrease in drug content at 25°C (97.73%) after 15 days of storage. From the stability studies, it was confirmed that insitu gelling formulations of Fenticonazole remained stable at ambient temperature (25°C) and humidity.

IV. CONCLUSION

In this study microemulsion based Insitu gel of Fenticonazole nitrate for vaginal delivery was prepared by microemulsion component with insitu polymer as a gelling agent mainly including poloxamer 407 and 118. The microemulsion was 99.98% transmittance and globule size 101nm, zeta potential 36.28mv, and drug content was 99.88%. Optimized in situ gel shows better in vitro drug release study compared to tween 80 in situ gel, it shows mucoadhesion and in vitro mucosal permeation study at 8 hours. The vaginal mucosa irritation study of microemulsion based in situ gel study compared with tween 80 insitu gel and this shows there is no irritation in microemulsion based vaginal insitu gel. The zone of inhibition of Fenticonazole nitrate with insitu gel shows a good zone of inhibition.

V. FUTURE SCOPE

The developed formulation of Fenticonazole which gives promising in vitro performance can be treated further with statistically significant no of animal tissue models. Further, the formulation can be clinically tested for patient compliance, effectivity and toxicity /irritation. Microemulsion based vaginal insitu gel is one of the probable systems which is applicable to near antifungal analogs belonging to BCS class II and IV.

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