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Development and Evaluation of Microemulsion Based Topical Gel Containing Oxiconazole Nitrate

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ABSTRACT

The main purpose of the present study was to develop and investigate the potential of a microemulsion based gel for topical delivery of Oxiconazole Nitrate. The pseudo-ternary phase diagrams were constructed to obtain the concentration range of oil phase, surfactant and co-surfactant for microemulsion formulation, composed of Castor oil as the oil phase, Tween 80 as surfactant and propylene glycol as co-surfactant. The optimized microemulsion was subjected to various tests like size distribution study, zeta potential, conductance, drug content, *in-vitro* drug release study etc. The microemulsion-based gel was prepared by adding 1% Carbopol 934P as a gelling agent and was then characterized for pH, viscosity, spreadability, *in-vitro* drug diffusion studies, *ex-vivo* permeation, release kinetic and antifungal activity. The percentage *in-vitro* drug release of an optimized microemulsion based gel was found to be 95.97% + 0.131% whereas the pH, viscosity, and spreadability of optimizes batch was 6.8, 1041 cps and 3.00 gcm/sec respectively. The results suggest that the microemulsion based gel is promising formulation for topical delivery of Oxiconazole Nitrate.

INTRODUCTION:

Fungal infection is a common infection which affects two-third of population among the world and is usually more difficult to treat than bacterial infections because fungal organisms grow slowly and often occur in tissues that are poorly penetrated by antimicrobial agents. A growing number of topical and systemic agents are available for the treatment of these infections like Miconazole, Ketoconazole,Itraconazole etc. [1-3].The selected antifungal agent Oxiconazole Nitrate has a broad spectrum of activities. It is effective against several fungal strains like *Candida albicans* and *Candida tropicalis* responsible for topical infections. It is very slightly soluble in water which leads to low dissolution rate and thus poor therapeutic efficacy if given orally. Low systemic absorption can be overcome by its topical delivery which is widely accepted over solid and liquid dosage forms[4]. Microemulsionsthat are clear, stable, isotropic system of oil, water and surfactant, frequently in combination with a co-surfactant have been studied as drug delivery systems because of their capacity to solubilize poorly water-soluble drugs as well as their enhancement of topical and systemic availability [2]. These systems are currently of interest to the pharmaceutical scientist because of their considerable potential to act as drug delivery vehicles by incorporating a wide range of drug molecules. The ingredients of microemulsion could facilitate permeation rate of the drug by reducing the diffusion barrier of the stratum corneum. They offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, and improved drug solubilization and bioavailability [5]. The low viscosity of microemulsion leads to their less retentive capacity on the skin which is overcome by incorporating gelling agent such as Carbopol934P, Xanthangumetc. base to prolong the local contact to the skin[6].

Thus the aim of the present research work was to formulate Microemulsion gel with good stability, powerful permeation ability and suitable viscosity for the topical delivery of Oxiconazole Nitrate.

MATERIALS & METHODS:

MATERIALS:

Oxiconazole Nitrate was kindly gifted by Harman Finochem Pvt. Ltd. Aurangabad, Castor oil was purchased from Loba Chemie Pvt. Ltd, Tween 80 from National Chemical, Pvt. Ltd, and

Propylene glycol from Merck Pvt. Ltd; Carbopol-934P from the Akhil Healthcare Private Limited, Vadodara, Gujarat. All other chemicals used were of AR grade.

METHODS:

Solubility of Oxiconazole Nitrate

Solubility studies were conducted by placing an excessive amount of Oxiconazole Nitrate individually to the oils, surfactants, and co-surfactant and mixed using magnetic stirrer. After stirring for 48 hours at 37ºC, the equilibrated sample was centrifuged for 10 min at 9000 rpm to remove excess amount of Oxiconazole Nitrate. The supernatant was filtered (0.45µm) and diluted with methanol for quantification of Oxiconazole Nitrate by using UV Spectrophotometer. The oil, surfactant, and co-surfactant that showed high solubility of Oxiconazole Nitrate were used in the preparation of microemulsion[7].

Plotting of ternary phase diagrams

To investigate the microemulsion region, pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature with the help of CHEMIX SCHOOL software. The phase diagrams were prepared using Tween 80 and Propylene Glycol as surfactant and co-surfactant with the ratios 1:1, 2:1, 3:1, 4:1 and 5:1 respectively. For each phase diagram at specific surfactant/co-surfactant weight ratio, the ratios of Castor oil to the mixture of surfactant and co-surfactant (Smix) were varied from 1:9 to 9:1 (i.e. 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) at room temperature with continuous stirring. Water was added dropwise to the oil and Smix mixture under gentle stirring until the mixture becomes clear at a certain point. The concentrations of components were recorded in order to complete the pseudo-ternary phase diagrams. After equilibrium was reached, the mixtures were checked visually for transparency. Clear and isotropic water-rich samples were the oil-in-water microemulsion region. The contents of oil, Smix, and water at appropriate weight ratios were selected based on these results[8].

Preparation of o/w Microemulsion loaded with drug

Based on the solubility studies and the ternary phase diagram, few microemulsions were selected with the weight ratio 1:1, 2:1 and 5:1 of oil: Tween 80/propylene glycol. Different formulae were selected from the Microemulsion region. Oxiconazole Nitrate (1% w/w) was

dissolved in oil (Castor oil). The Oxiconazole Nitrate solution was then mixed with the mixture of surfactant and co-surfactant. (Tween 80 and Propylene Glycol), Finally, an appropriate amount of water was added to this solution mixture drop by drop to get clear Microemulsion [6].

Characterization of microemulsion systems

pH Determination

The pH of microemulsion was determined at room temperature using digital pH meter, model NLG-333, which was calibrated using 9.2 and 4.0 pH buffer solutions. Then about 25 ml of microemulsion was taken in a small glass beaker and the electrode of pH meter was dipped into it for a minute and the pH was noted [2].

Viscosity Determination

The viscosity of Oil in water (o/w) microemulsion was measured by Brookfield viscometer model (DV-E) using spindle no. 63. The rheograms were plotted between the viscosity and rpm for the further study purpose [9].

Refractive Index

Refractive index was measured by Abbe's refractometer [10].

Electrical conductivity determination

Electrical conductivity measurements can provide valuable information concerning the structure and phase behavior of microemulsion systems. The conductivity measurement divides microemulsions into o/w and w/o type. Conductivity measurements rely on the poor conductivity of oil compared with water and give low values for water in oil microemulsion where oil is the continuous phase. The reverse happens for oil in water microemulsion. An electrical conductivity of microemulsion was measured using conductivity meter, based on it, the phase system of microemulsion system was determined [10].

Drug Content Determination

1 ml of each selected formulation of oil in water (o/w) microemulsion (equivalent to 10 mg of Oxiconazole Nitrate) was placed in a 10 ml of volumetric flask and diluted to the mark with

methanol and after making further dilutions it was analyzed spectrophotometrically at 233 nm, using spectrophotometer (Model UV JASCO) [2].

In vitro Drug Diffusion Studies

Franz diffusion cell (with effective diffusion area 2.8cm2) was used for the drug release studies. Microemulsion (1 ml) was applied to the surface of the membrane evenly which was then clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared mixture of phosphate buffer solution (pH 7.4) and methanol (80:20%, v/v) as a dialyzing medium, temperature was maintained at 32 ± 0.5°C and was stirred at 400 rpm throughout the experiment. 1ml of aliquot was taken after an interval of 1 hr for 12 hrs. Samples were analyzed for drug content by UV visible spectrophotometer at 215 nm after appropriate dilutions. The cumulative amount of drug released across the membrane was determined as a function of time [11].

Zeta Potential Determination

It must be negative or neutral, which indicate that droplets of microemulsion having no charge that means the system is stable. Zeta potential was determined by Zetasizer. Zeta potentially is essentially useful for assessing flocculation since electrical charges on particles influence the rate of flocculation [12].

Particle Size Distribution Analysis

A particle size of prepared microemulsion was analyzed on motic microscope. A drop of microemulsion was placed on glass slide which was then placed on the stage and observed for the particle size on 10X [13].

In vitro Antifungal Activity

In vitro antifungal activity was carried out by cup plate method. The overnight grown culture of *Candida albicans* was inoculated into the sterilized agar media plates. After solidification, wells were cut into the media and fixed with formulation of specific concentration. The plates were incubated at room temperature and the diameter of zone of inhibitions was measured [14].

Thermodynamic stability studies of drug loaded microemulsion

Sample was kept at three different temperature ranges (4ºC, room temperature, and 45ºC) and observed for any pieces of evidence of phase separation, flocculation or precipitation for 28 days [14].

Formulation of Microemulsion-based gel

Carbopol 934P as a gel matrix was used to construct the microemulsion-based gel for improving the viscosity of microemulsion for topical administration. For preparation of microemulsion based gel, different concentrations of Carbopol 934P was added in 80 ml of water, stirred till homogenous mixture was formed, kept it aside for overnight. Then 10 ml of Microemulsion was added drop by drop to the homogenous mixture of water. Then polyethylene glycol was added with continuous stirring till clear viscous solution was obtained, finally, pH was adjusted by adding fixed amount of triethanolamine to form semisolid gel [6].

Evaluation of Microemulsion-based gel

Physical Appearance

The prepared microemulsion based gel formulations were inspected visually for their color, homogeneity, consistency [15].

Determination of pH

The pH of microemulsion based topical gel was determined at room temperature using digital pH meter, model NIG-333which was calibrated using 9.2 and 4.0 pH buffer solutions. All the studies were repeated in triplicate with good agreement being found between measurements [13].

Viscosity Studies

The viscosity of microemulsion based gel formulation was measured by Brookfield viscometer model (DV-E) using spindle no. 63 at 30 rpm. [13].

Spreadability Studies

To determine the spreadability of microemulsion based gel, 0.5 g of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate, over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted. The spreadability was determined by special apparatus and it was calculated using the following formula [3].

$$S=M.L/T$$

Drug Content determination

1gm of Gel formulations was dissolved in methanol and filtered and the volume was made to 100 ml of methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 233 nm using Shimadzu-1700 UV visible spectrophotometer [16].

***In-vitro* Drug Release Study**

Franz diffusion cell (with effective diffusion area 2.8cm2) was used for the drug release studies. Microemulsion-based gel (1 g) was applied to the surface of the membrane evenly which was then clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared mixture of phosphate buffer solution (pH 7.4) and methanol (80:20%, v/v) as a dialyzing medium, temperature was maintained at 32 ± 0.5°C and was stirred at 400 rpm throughout the experiment. 1ml of aliquot was taken after an interval of 1 hr for 12 hrs. Samples were analyzed for drug content by UV visible spectrophotometer at 215 nm after appropriate dilutions. The cumulative amount of drug released across the membrane was determined as a function of time [16].

***Ex-vivo* Skin Permeation Study**

The excised skin samples (dorsal side) of rat were clamped between the donor and the receptor compartment of Franz diffusion cells with the Stratum corneum facing the donor compartment. Then, 1 g of microemulsion gel containing 1% (w/w) Oxiconazole Nitrate was applied to the donor compartment. The receptor compartment was filled with 20 ml of mixture of phosphate buffer solution (pH 7.4) and methanol (80:20%, v/v), maintained at 32 ± 0.5°C and was stirred at 400 rpm throughout the experiment. At a predetermined time

intervals (1hr), 1 ml receptor medium was withdrawn and the same volume of pure medium was immediately added to the receptor compartment. The procedure was repeated for 12 hrs. All samples were filtered through Whatman filter paper and analyzed by UV spectrophotometer at 215 nm, with suitable dilution. The cumulative amount (% w/w) of Microemulsion based gel permeated through rat skin was plotted as a function of time [17].

Skin Irritation Studies

As per OECD guideline (404), a sample of the test group (Optimized batch of Oxiconazole Nitrate MEG) was applied to on rat by introduction under a double gauze layer to an area of skin approximately 1" x 1" (2.54 x 2.54 cm) square. The sample was re-applied on the skin of Rat. Animals were returned to their cages. After a 60 min, 24hr, 48hr, and 72hr exposure, the samples were removed. And observation was carried out [17].

***In vitro* Antifungal Activity**

In vitro antifungal activity was carried out by cup plate method. The overnight grown culture of *Candida albicans* was inoculated into the sterilized agar media plates. After solidification, wells were cut into the media and fixed with 100 mg of the specimens to be tested using MEG of Oxiconazole Nitrate. The plates were incubated at room temperature and the diameter of zone of inhibitions resulting from drug diffusion into media was measured [3].

Stability Study of Optimized batch

Stability studies were carried out on gel formulation according to ICH guidelines. A sufficient quantity of gel in glass bottles was stored in stability chamber at 25°C (60%RH±5%) and 45°C (65%RH±5%) and samples were withdrawn at 0 and 30 days. The physical stability of gel was observed along with pH and viscosity [18].

RESULT AND DISCUSSION:

Screening of oils, surfactants, and co-surfactants for microemulsion

In order to screen appropriate solvents for the preparation of microemulsion, the solubility of Oxiconazole Nitrate in various oils, surfactants, and co-surfactants was estimated as shown in Table 1. Among various oily phases that were screened castor oil showed the highest solubility of Oxiconazole Nitrate and hence it was selected for further studies. Choosing a

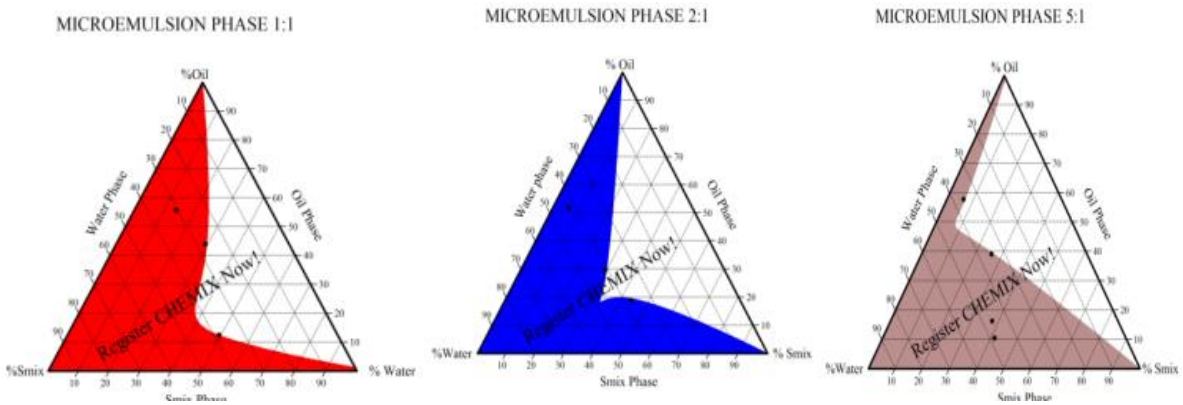
surfactant from safety point of view is important because a large number of surfactants may cause skin irritation. Non-ionic surfactants are less toxic than ionic surfactants. High HLB value surfactants form stable o/w microemulsion. So Tween 80 was selected as surfactant which showed the highest solubility among others under investigation. Similarly, Propylene glycol was selected as a co-surfactant for the study. So Castor oil, tween-80 and propylene glycol were selected as the oil phase, surfactants and co-surfactants respectively.

Table 1: Solubility of Oxiconazole Nitrate in different oils, surfactants, and co-surfactants.

Vehicle	Solvent	Solubility (mg/ml)
Oils	**Castor oil**	**53.1**
	Arachis oil	21.8
	Linseed oil	12.3
	Oleic acid	7.8
Surfactants	**Tween 80**	**20.7**
	Span 80	10.9
	Tween 60	8.2
	Tween 20	7.9
Co-surfactants	**Propylene Glycol**	**24.6**
	PEG-400	11.4

Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams were constructed to determine the concentration range of components in the existence range of microemulsion. The Pseudo-ternary phase diagrams of o/w microemulsion composed of Castor oil, tween 80, Propylene glycol and distilled water with the different ratios of oil&Smix like 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9. With the different ratios of oil phase and surfactant mixture (Smix), system changed from translucent water-in-oil (w/o) region to oil-in-water (o/w) region after dropwise addition of water. The stable area of o/w microemulsion region was observed at the following ratios1:1, 2:1 and 5:1 as shown in Figure 1 (a, b, c). These three ratios were selected for further studies.



a **b** **c**

Figure 1 (a,b, c) The pseudo-ternary phase Diagrams of the Oil-Surfactant/Co-surfactant mixture-water system at the 1:1, 2:1 and 5:1.

Characterization of the Oxiconazole Nitrate-Loaded Microemulsion

Table 2 shows the composition of microemulsion. It was observed that the disperse system of nine formulations of microemulsion were macroscopically identical i.e homogeneous, transparent without any precipitates, so they were characterized for different parameters like pH, viscosity, refractive index, conductivity and drug content and the results are shown in Table 3. The pH value for microemulsion was found to be in the range of 6.13±0.040 to 7.41±0.005, indicating its compatibility to the skin. The viscosity was found to be in range 99.6±0.577cps to 441.3±1.527cps, indicates that increasing shear rate decreases the viscosity of formulation. Therefore, all these formulations exhibited Newtonian flow behavior, as expected from microemulsion. Refractive index 1.311±0.01 to 1.445±0.057 and the Electrical conductivity of the Microemulsion was found to be in the acceptable range of 0.293 ±0.0321 to 0.485±0.0230 ms/cm**.** Drug content of F3 ME formulation was found to be maximum i.e. 87.30 ± 0.169 % indicating that the drug was almost completely entrapped into oil globule and uniformly distributed throughout microemulsion.

In-vitro, drug release studies were performed to study the release behavior of drug from the formulation. Table 4and 5shows the percent drug release from microemulsion through dialysis membrane. From the Figure 2 (a, b) it was observed that maximum drug delivery from the microemulsion was achieved in 12 hours. Formulation F3 showed highest drug release 87.46±0.241 as compared to others. The zeta potential of the F3 formulation was (-10.7 mv). The negative zeta potential indicates that no charge on the droplets of

microemulsion i.e. the system is stable. The F-3 formulation had the mean particle size in the range of 0.1-0.7 µm (Table 6). It has been reported that the smaller particle size of the emulsion droplets may lead to more rapid absorption and improve the bioavailability. The polydispersity index (PI) of optimized F-3 was found to be 0.960 (Figure 4). The PI indicated that ME formulation had narrow size distribution.

Table 7 shows the thermodynamic stability studies performed to evaluate the phase separation as well as precipitation of the drug in the excipients mixture. It was found that F-3 formulation was physically stable and no phase separation and precipitation of drug into lipid matrix was observed.

Table 2 Composition of castor oil Microemulsion containing Smix (5:1, 1:1 & 2:1)

Sr. No.	Components (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Castor oil	12 ml	12 ml	12 ml	9 ml	9 ml	9 ml	6 ml	6 ml	6 ml
2	Smix (Tween 80: Propylene glycol)(5:1)	24 ml	27 ml	30 ml	--	--	--	--	--	--
3	Smix (Tween 80: Propylene glycol)1:1)	--	--	--	24 ml	27 ml	30 ml	--	--	--
4	Smix (Tween 80: Propylene glycol)(2:1)	--	--	--	--	---	--	24 ml	27 ml	30 ml
5	Distilled water	24 ml	21 ml	18 ml	27 ml	24 ml	21 ml	30 ml	27 ml	24 ml
6	Drug (Oxiconazole Nitrate)	1g	1g	1g	1g	1g	1g	1g	1g	1g

Table 3 Evaluation parameters of the various formulation of microemulsion

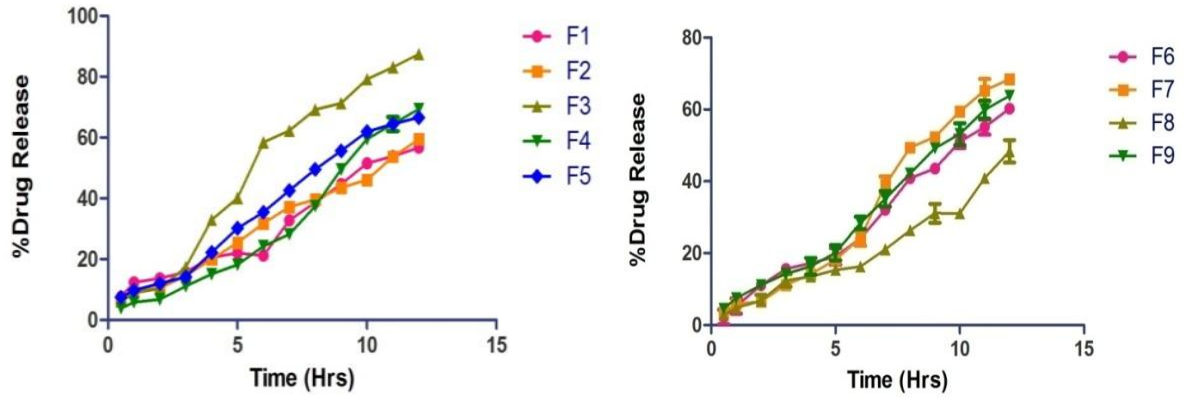
Formulation Code	pH	Viscosity (cps)			Refractive index	Conductivity ms/cm	Drug Content (%)
		30 (rpm)	50 (rpm)	60 (rpm)			
F-1	6.37±0.056	171.3±1.15	144±1.0	120.3±0.57	1.321±0.57	0.435±0.0207	68.41±0.0412
F-2	6.39±0.194	144.6±1.52	121.6±0.57	107±3.46	1.440±0.81	0.371±0.0577	74.4±0.0231
F-3	7.41±0.005	334±1.126	311±1.053	245±1.123	1.445±0.02	0.342±0.0152	**87.30±0.169**
F-4	6.17±0.015	280.3±0.577	200.6±1.154	133±1.0	1.391±0.015	0.485±0.0230	52.18±0.0577
F-5	6.36±0.047	441.3±1.527	351±1.0	251±1.0	1.311±0.01	0.383±0.0264	64.41±0.0115
F-6	6.13±0.040	299.6±0.577	241±1.0	167.6±0.577	1.441±0.05	0.383±0.0264	81.36±0.0243
F-7	6.15±0.01	99.6±0.577	94.9±2.532	87.7±0.642	1.445±0.057	0.446±0.0550	42.13±0.0311
F-8	6.36±0.060	204±6.08	141.6±2.081	114±1.0	1.442±0.015	0.421±0.0115	69.98±0.632
F-9	7.20±0.005	341±1.0	280.3±0.577	245.6±0.577	1.442±0.01	0.293±0.0321	75.37±0.0554

Table 4 *In vitro* drug release study of microemulsionF1-F5

Sr. No.	Time (Hrs)	Percentage release of drug (%)				
		F1	F2	F3	F4	F5
1	0.5	7.64± 0.076	6.63±0.016	6.16±0.102	3.85±0.0152	7.42±0.043
2	1	12.41±0.043	9.37±0.012	8.95±0.514	5.88±0.023	9.81±0.026
3	2	13.73±0.015	10.51±0.032	10.34±0.127	6.83±0.056	12.08±0.190
4	3	15.80±0.023	14.56±0.055	17.35±1.024	10.14±0.032	14.24±0.238
5	4	20.63±0.054	20.04±0.011	32.95±0.213	15.12±0.026	22.20±0.087
6	5	22.04±0.057	25.46±0.022	40.11±0.109	18.23±0.059	30.19±0.531
7	6	27.17±0.014	31.79±0.025	58.43±0.576	24.32±0.025	35.48±0.261
8	7	32.81±0.023	37.22±0.015	62.32±0.231	28.20±0.234	42.64±0.364
9	8	38.70±0.011	39.62±0.042	69.18±0.364	37.43±1.34	49.12±0.380
10	9	44.67±0.021	43.51±0.058	71.32±0.184	49.52±0.058	55.63±0.486
11	10	51.51±0.054	46.14±0.026	79.19±0.261	59.46±0.026	61.93±0.961
12	11	53.90±0.058	53.72±0.018	83.12±0.163	64.44±2.31	64.43±0.831
13	12	56.69±0.032	59.47±0.024	**87.46±0.241**	69.38±0.326	66.63±0.413

Table 5 *In vitro* drug release study of microemulsionF6-F9

Sr. No.	Time (Hrs)	Percentage release of drug (%)			
		F6	F7	F8	F9
1	0.5	2.26 ± 2.03	3.73±1.06	2.92±1.53	4.55±0.36
2	1	5.33 ± 2.08	5.93±1.53	4.82±0.23	7.53±0.53
3	2	11.12±1.15	6.54±0.57	6.86±1.56	11.15.0.23
4	3	15.63±0.53	11.25±0.53	12.43±1.73	14.29±0.58
5	4	17.25±0.28	14.14±1.54	13.47±1.76	16.37±2.31
6	5	19.13±2.31	18.25±1.00	15.34±0.54	20.14±2.08
7	6	24.15±2.08	24.27±2.08	16.33±3.21	28.43±1.73
8	7	32.08±0.56	39.06±2.31	21.13±1.00	35.17±2.18
9	8	40.91±0.32	49.42±1.53	26.35±0.37	42.18±1.04
10	9	43.54±1.09	52.39±0.58	31.14±2.65	49.26±1.53
11	10	51.06±1.73	59.45±1.53	34.10±0.14	53.15±3.02
12	11	55.15±2.07	65.32±3.21	40.92±0.34	59.93±2.52
13	12	59.20±0.54	68.46±0.21	48.36±3.21	63.82±0.42



a **b**

Fig 2 *In-vitro* drug release (a) F1-F5 and (b) F6-F9

Zeta potential and Droplet size of optimized F-3 formulation of Microemulsion

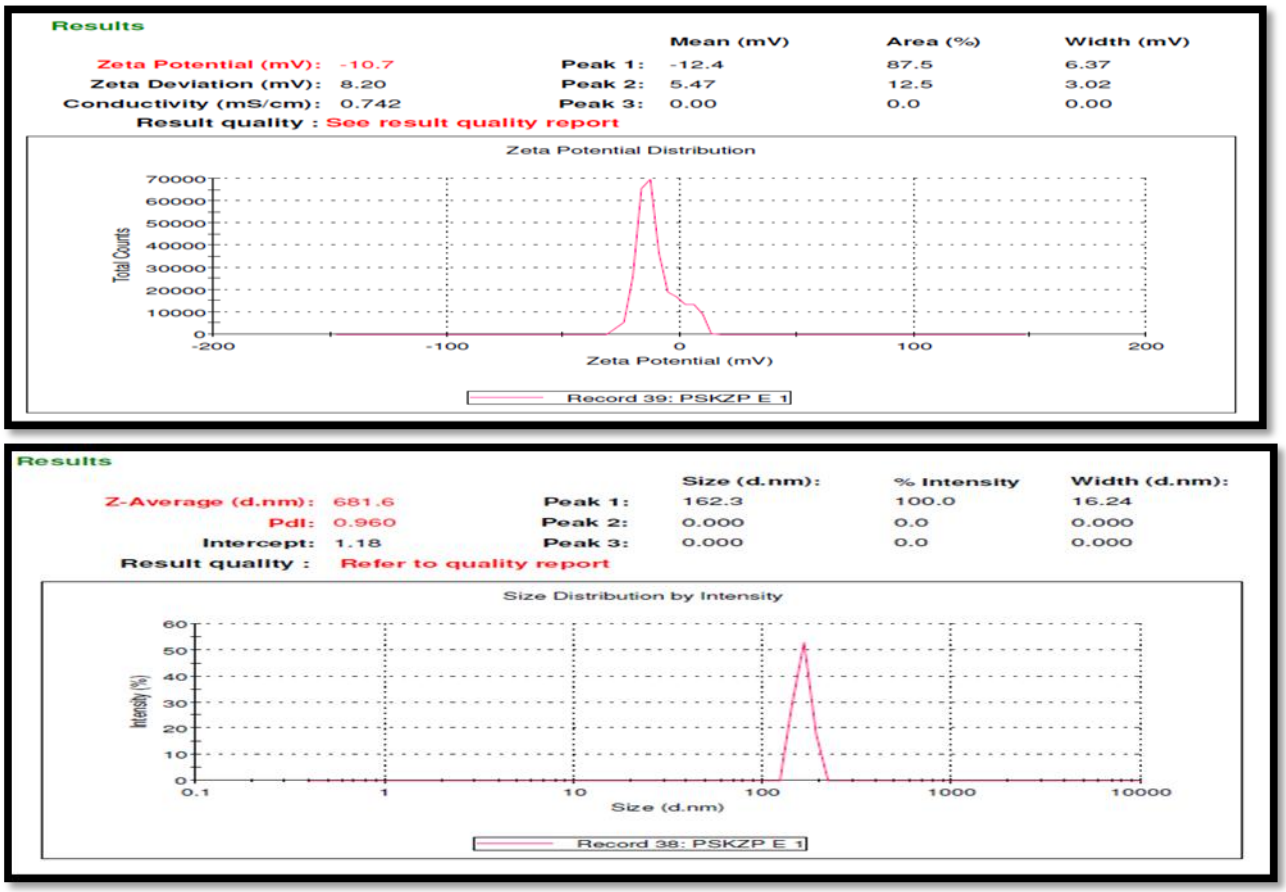


Fig. 4 Zeta potential and Droplet size of optimized F-3 formulation of microemulsion

Table 6: Particle size of microemulsion by Motic microscope.

Micro emulsion	Average Particle size (mean diameter)
F3 batch	0.1-0.7 µm

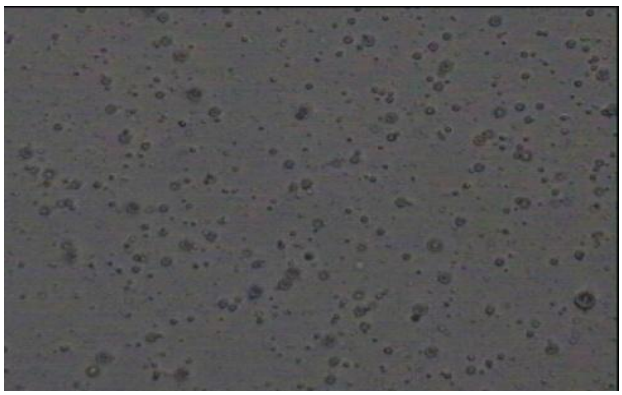


Fig. 3 Motic image of particle size of microemulsion

Table 7: Thermodynamic stability studies of microemulsion at different temperatures

Formulation	Time period	Room temp. (25°C±3°C)	Cool temp. (At 4°C)	Elevated temp. (At 45°C) At RH of 75% 45°C	Remark
Micro emulsion F3 batch	Day 0	No separation of phase	No separation of phase	No separation of phase	Stable
	Day 2	No separation of phase	No separation of phase	No separation of phase	Stable
	Day 5	No separation of phase	No separation of phase	No separation of phase	Stable
	Day 7	No separation of phase	No separation of phase	No separation of phase	Stable
	Day 9	No separation of phase	No separation of phase	No separation of phase	Stable
	Day 12	No separation of phase	No separation of phase	No separation of phase	Stable
	Day 28	No separation of phase	No separation of phase	No separation of phase	Stable

Characterization of microemulsion based gel formulation

Table 8 shows the composition of microemulsion based gel formulation. All the prepared gels were clear and transparent in appearance. Table 9showsthe evaluation parameters of microemulsion based gel like pH, viscosity, spreadability and drug content. The pH of microemulsion gel was in the range of 6.54±0.04 to 6.95± 0.02, which are considered acceptable to avoid the risk of irritation after topical application. The viscosity was in the range of 1041±0.149cps to 2192±0.028cps. Spreadability of the gel was found to be 2.5, 2.7, and 3.0 respectively which indicates that the polymer used as a gelling agent spread by small amount of shear. The drug content of the formulated gel was in the official limits within the range of 80% to 99%. The drug content of MEG1 batch was 87.04±0.05 highest among others which showed that the drug was uniformly distributed throughout the gel.

Table 10 and Fig. 5shows that % drug release from the prepared gel formulation that was 95.97±0.131, 73.19±0.462, 69±0.372 respectively. An increased drug release rate was achieved in MEG195.97±0.131 as compared to MEG273.19±0.462and MEG369±0.372. The enhanced dissolution rate of Oxiconazole Nitrate from the gel could be attributed to the small size of microemulsion incorporated in the gel, which permitted a faster rate of drug dissolution into the aqueous phase.

Table 11 shows the correlation coefficient value (r^2)**0.9746**, 0.7879, 0.8808, 0.8757, 0.9709. The best-fitted model was Zero Order because correlation coefficient obtained by zero order kinetics was more near to 1 as compared to other kinetics, suggested prepared formulation exhibit zero order kinetics release. The 'n' value for MEG1 batch was 0.82 which were in the range of 0.5-1.0 showing on Fickian diffusion release mechanism which state that drug is released from microemulsion gel by diffusion manner.

Table 12 and Fig. 6 shows the results of ex-vivo permeation profile of drug through rat skin from optimized MEG1 in increasing order for 12 hours and % permeation rate was found to be 80.49 ± 0.21.Skin irritation study was shown in Table 13. No irritation was observed on the skin of rat at 60 min, 24Hrs, 48Hrs and 72 Hrs. The stability studies performed showed no significant change in viscosity and pH, also no phase separation was observed during 30 days at variable temperature condition as shown in table 14.

Table 8 Composition (% w/w) of microemulsion based gel formulation

Batch code	Carbapol 934p	Distilled Water	Microemulsion	PEG 400	Triethanolamine
MEG1	1	80	10	10	0.5
MEG2	1.5	80	10	10	0.5
MEG3	2	80	10	10	0.5

Table 9 Evaluation parameters of MEG

Formulation code	pH	Viscosity at 30 rpm	Spreadability (g.cm/sec)	Drug Content (%)
MEG1	6.80 ± 0.18	1041± 0.149	3.0 ± 0.05	87.04 ± 0.05
MEG2	6.54 ± 0.04	1382± 0.053	2.7 ± 0.09	64.42± 0.08
MEG3	6.95 ± 0.02	2192 ± 0.028	2.5 ± 0.06	59.54 ± 1.07

Table 10: Percent Drug release of microemulsion based gel formulation

Time (Hrs)	Release of drug (%)		
	MEG1	MEG2	MEG3
0.5	7.64±0.431	7.73±0.123	6.71±0.054
1	9.54±0.123	9.95±0.015	7.28±0.152
2	13.61±0.567	11.70±0.152	8.04±0.312
3	18.28±0.312	14.27±0.512	13.85±0.192
4	24.81±0.872	19.82±0.862	18.21±0.052
5	30.66±1.142	24.86±0.912	22.26±0.032
6	34.79±0.021	29.87±0.612	26.17±0.419
7	43.21±1.084	35.37±0.01	30.56±0.293
8	45.33±0.213	41.90±0.021	36.41±0.132
9	51.28±0.143	45.75±1.16	42.83±0.327
10	65.71±0312	50.34±0.327	49.52±0.419
11	81.20±0.012	54.62±0.018	62±0.912
12	**95.97±0.131**	73.19±0.462	69±0.372

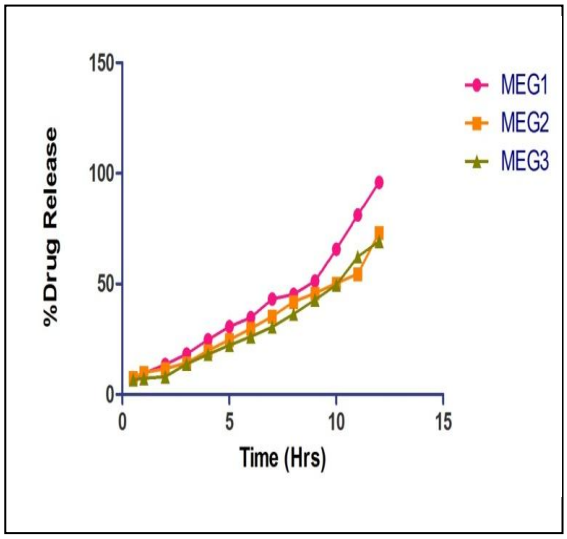


Fig. 5 %Drug release of MEG1, MEG2 and MEG3 batch

Table 11 Result of kinetic model fitted for microemulsion-based gel (MEG1)

Batch	Zero Order	1st Order	Matrix	Hixson Crowell	Korsmeyer Peppas	
MEG1	R^2	R^2	R^2	R^2	R^2	N
	0.9746	0.7879	0.8808	0.8757	0.9709	0.82

Table 12 Cumulative drug release of MEG1 formulation

Sr. No	Time (Hrs)	Cumulative drug release (%)
		MEG1
1	0.5	4.72 ± 0.34
2	1	7.93 ± 0.81
3	2	11.31± 0.12
4	3	16.23± 1.14
5	4	19.82± 0.05
6	5	24.21±0.82
7	6	28.54 ± 0.93
8	7	35.12 ± 1.14
9	8	42.29 ± 1.18
10	9	59.35 ± 0.82
11	10	67.48 ± 0.69
12	11	75.51 ± 0.92
13	12	80.49 ± 0.21

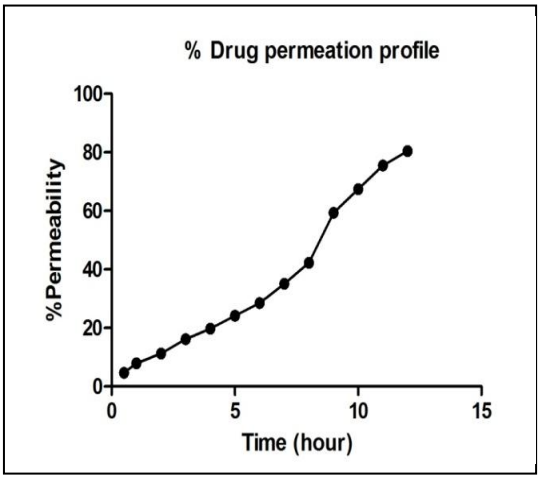


Fig. 6 *Ex-vivo* permeation profile of drug through rat skin from optimized MEG1Formulation

Table 13 Skin Irritation Study MEG1

Rat	Erythema				Edema			
Time	60 min.	24 Hrs	48 Hrs	72 Hrs	60 Min.	24 Hrs	48 Hrs	72 Hrs
	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Erythema means redness of skin, Edema means inflammation observed on the skin

Where +ve means No irritation -ve means Irritation

Table 14 Stability study of optimized MEG1 formulation

Parameter	Initial	Storage temperature for MEG1at 30 days	
		25°C/60%RH ± 5%	45°C/65%RH ± 5%
Appearance	Transparent gel	No change	No change
Phase separation	No phase separation	No phase separation	No phase separation
Consistency	Good	Good	Good
pH	6.80	6.79	6.81
Viscosity	1041	1039	1042

***In vitro* antifungal activity of ME and optimized MEG1 formulation**

Table 15 and 16 shows the antifungal activity of microemulsion and microemulsion based gel formulation (MEG1) respectively whereas figure 7 (a,b) shows the zone of inhibition indicating significant antifungal activity of Oxiconazole Nitrate gel formulation.

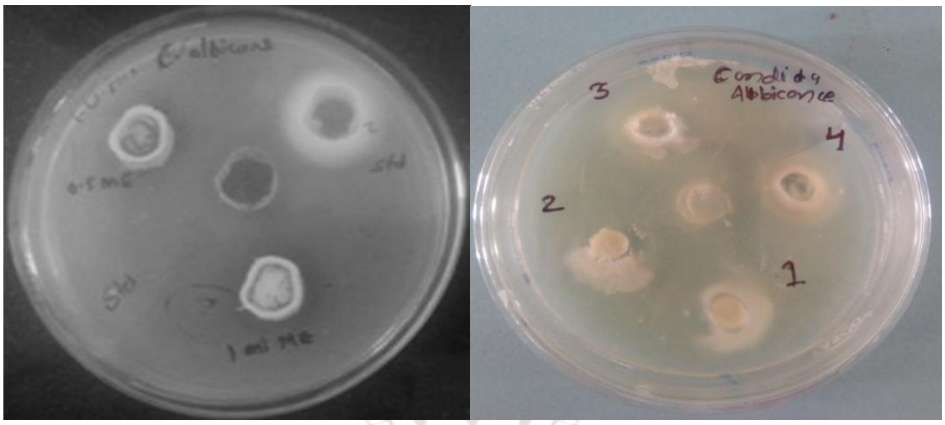
Table 15 Zone of inhibition of optimized F-3 microemulsion formulation

Formulation	Name of Micro-organism	Zone of inhibition (mm)		
F3 batch	*Candida albicance*	Oxiconazole Microemulsion (A) 0.5 ml	Oxiconazole Microemulsion (B) 1 ml	Standard Oxiconazole Nitrate (C)
		19.4±1.40 mm	27.5±0.52mm	28.9±0.84mm

Table 16 Zone of inhibition of optimized MEG1 formulation

Formulation	Name of Micro-Organism	Zone of inhibition (mm)			
MEG1	*Candida albicans*	Gel (A) 0.5gm	Gel (B) 0.6gm	Gel (C) 1gm	Oxiconazole Nitrate (D) (10mg/ml
		15.2 ± 0.73mm	20.5 ± 0.62mm	27.5 ± 0.31mm	28.9 ± 0.59 mm

Candida albicans



a **b**

Fig. 7: Antifungal activity of ME and MEG1

CONCLUSION:

In this study, several microemulsions and microemulsion-based gels were formulated and evaluated for their potential as topical delivery systems for Oxiconazole Nitrate, a hydrophobic drug with very poor aqueous solubility. The results showed that the content of microemulsion based gel components (oil, Smix, and water) had significant effect on their physical, rheological and *in-vitro* drug release characteristics.

The most optimum and desirable formulation of the microemulsion-based gel of Oxiconazole Nitrate was found to be containing Castor oil as oil phase, Smix (5:1) Tween 80 &Propylene Glycol as surfactant:cosurfactant, and Carbopol 934p (1%) as gelling agent, since it exhibited zero order kinetic release, very good release rate (95.97±0.131%), good spreadability values (3.0 g.cm/sec). The formulations also possessed the globule size in the acceptable range of 0.1-0.7 µm and zeta potential of -10.7 mV respectively. The developed oxiconazole nitrate

microemulsion-based gel showed good in-vitro antifungal activity against Candida albicans when compared with the standard. It also showed better retention of the skin and minimal irritation. Thus, the drug-loaded microemulsion based Gel could be a successful alternative dosage form to deliver poorly soluble Oxiconazole Nitrate and also a promising formulation as a topical delivery for antifungal activity.

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