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Optimization and Evaluation of Oleic Acid Based Unsaturated Fatty Acid Liposomes Gel

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Abstract

Oxiconazole loaded Unsaturated Fatty Acid Liposomes (UFL) was used as a transdermal formulation in treatment of chronic fungal infections. Oxiconazole has low oral bioavailability of drug and short half-life that's why preferring this transdermal route for proper treatment against fungal infections. UFL was made by using oleic acid and tween 80 in different proportion, batches were developed by using a central composite design. The optimal UFL-6 formulation has shown maximum entrapment efficiency (61.05%) and good vesicle size (215 nm). The oxiconazole release from UFLG-6 gels, follow Higuchi model and drug permeation via a cellophane membrane was maximum in comparison to other gel formulations. The UFLG-6 formulation also shows maximum antifungal activity against *Candida albicans* fungus.

Keywords: Antifungal; Fatty acid; Gel; Oxiconazole; Transdermal

Introduction

Oxiconazole is most frequently drug which is used in chronic fungal infection such as candida infections, athlete's foot, tinea and vaginal fungal infection treatment because of its antifungal property [1]. The antifungal property of oxiconazole is due to inhibition of synthesis of ergosterol, which is the responsible in fungal cytoplasmic membrane growth. Several formulations have been developed for antifungal transdermal administration [2] due to poor oral bioavailability of drugs, the drug causes several systematic toxicity and gastrointestinal disturbance occur. The oxiconazole is widely administered dermal in the form of cream and lotion. The use of carbopol gel is beneficial because this show good viscosity properties so in long time spend at the site of administration and drug amount on the skin. Now today more approaches have been used to improve the cutaneous release of drugs to overcome the little skin permeability [3]. Unsaturated Fatty Acid Liposomes (UFL) is the new approach to enhance the drug passage via the skin. Unsaturated fatty acids 3 like linolic acid and oleic acids are used as natural permeation enhancers which are like a colloidal suspension with a closed related to the lipid bilayer membrane which is consist of fatty acids and their anionic fatty acids [4,5]. Surfactant is also used in combination of fatty acid which enhanced the flexibility of skin [6] and improves the passage of drug via a skin membrane. UFL enhanced the drug retention properties of drugs within the cell of the skin membrane to treat fungal infections for long period of time.

The objective of the present study is to develop oxiconazole loaded unsaturated fatty acid liposomes gel formulation by modifying vortex-sonication method [7] for transdermal use. The central composite design expert [7] was used to optimize and measured the effect of two formulation variables such as oleic acid and tween 80. The effect of independent variables was evaluated by vesicle size and entrapment efficiency of formulation. The optimized UFL with tea tree oil 8 gel formulations was reported to permeation studies via a cellophane membrane and antifungal studies comparing with marketed and other gel formulation was evaluated.

Material and Methods

Materials

Oxiconazole (OXZ) was available as a gift sample from A.S. Joshi and company Pvt. Ltd. (Maharashtra, India). Tea tree oil was purchased from Kanta Enterprises Pvt. Ltd. (Noida, India), Oleic Acid (OA); Carbopol 940; Glycerin; and Cholesterol (CL) were purchased from Hi-media Lab. Pvt Ltd. (Mumbai). Tween 80, Ethanol (E) and Triethanolamine were purchased from S.D. Fine Chem Ltd. (Mumbai). Dialysis membranes were purchased from Hi-media labt. Pvt. Ltd. (Mumbai). Propylene glycol was purchased Central Drug House Ltd. (New Delhi).

Method of preparation of unsaturated fatty acid liposomes

Unsaturated fatty acid liposomes were prepared by a vortex method [7,8] using central composite design was applied to study the effect of independent variables on the dependent variable as described in the tables. Eight formulations were prepared according to the experimental design shown in Table 1. The exact weight of drug (200 mg), oleic acid and tween 80 in different ratios as listed in tables these all ingredients were dissolved in 2 ml of ethanol in a beaker that cover with aluminum foil. The buffer solution of pH 7 was added in the above mixture with continuous vortex shaking to obtain a cloudy suspension of vesicle which was sonicated for 15 min to get small vesicles. These sonicated vesicles were extruded via a polycarbonate membrane to get uniform sized of vesicles.

Vesicle size: UFL was examined by using a transmission electron microscope was used to view the image capture and analysis the size of vesicles [9].

Entrapment efficiency: The entrapment efficiency [10] of the drug was determined by using ultracentrifugation at 25000 rpm for 3 h at 4°C. The supernatant was separated and the drug amount was calculated

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Independent variables	Level used		
	-1	0	+1
A: A Oleic acid (mg)	100	400	800
B: B Tween 80 (mg)	100	300	400
Dependent variables			
R1: Entrapment efficiency% (EE)	-	-	-
R2: Vesicle size(nm)	-	-	-

Table 1: Variables in central composite designs at different levels.

Formula Code	Independent variable		Dependent variables	
	A	B	EE%	Vesicle size(nm)
UFL-1	0	1	56.14	349
UFL-2	-1	1	34.55	455
UFL-3	-1	0	41.28	350
UFL-4	1	1	47.46	490
UFL-5	-1	-1	40.78	334
UFL-6	0	-1	61.05	215
UFL-7	1	-1	45.22	342
UFL-8	1	0	46.11	390

Table 2: Oxiconazole loaded unsaturated fatty acid liposomes formulation was prepared as per the experimental design.

by using supernatant and which carried out by detection of entrapment efficiency at 257.6 nm with UV spectroscopy. The amount of entrapment drug is determined as a percentage was estimated from the following equation:

$$\text{Entrapment efficiency } \% = (A-B)/A \times 100 \quad (1)$$

Where:

A=Amount of drug added initially;

B=Amount of drug determined in the filtrate by spectrophotometrically;

A-B=Represents the amount of drug entrapped in the formulation.

Unsaturated fatty acid liposomes gel formulation

Carbopol gel was prepared by mixing the 200 mg of powder carbopol 940, propylene glycol in 20 ml of suspension of UFL vesicle and stirring continuously until gel was made. The dispersion was neutralized with triethanolamine to adjust at pH 7.4. Final weight was prepared up to 20 g to obtain a UFL loaded gel formulation [11] was shown in Table 3.

In vitro drug release study of vesicular gel formulation through cellophane membrane

In vitro drug release studies [12] were performed on a Franz diffusion cell by applying dialysis membrane (Himedia laboratory private limited). 50 ml volume of receptor zone was maintained with phosphate buffer of pH 7.4. 1 g of gel formulation was spread on giver compartment. The temperature of receptor cell was maintained at 37°C. Equal volumes of the sample were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 12 h and maintained with equal volume of fresh phosphate buffer solution. Each sample was determined by spectrophotometrically at 257.6 nm and % cumulative drug release was calculated.

Calculation of permeation parameters: Cumulative amount drug release from vesicular gel permeation of unit area was taken like a function of time. The slope of the linear section was used to estimate the flux of the drug [13]. The permeability coefficient (*Kp*) of drug via a dialysis membrane was calculated by Fick's first law of diffusion, which is determined by following equation:

$$Kp = J/C$$

Where, *J* is the flux and *C*is the concentration of the drug in receptor zone.

Stability studies

The stability studies [10,14] of gel formulation were determined at 45 ± 1°C, 37 ± 0.5°C and 4 ± 2°C in glass container for 3 months. The gel formulations were checked in the change in physical appearance and drug content was analyzed by applying a spectrophotometrically at 257.6 nm and phosphate buffer used as blank.

Mathematical modelling of optimized batch

The result of *in vitro* drug release by vesicular gel formulations was estimated kinetically by applying these mathematical models [15] such as Zero-order, First-order, Higuchi and Koresmeyer peppas model.

Antifungal activity of optimized formulations: Vesicular gel formulations were evaluated by using antifungal activity [16-18] studies against *Candida albicans* (MTCC 3017) [19] as the test microorganisms. The cup, plate method is applied in which a sabourauds dextrose agar media (20 ml) used for activation of the test fungus was allowed to solidify in the petri dishes. 5 mm borer was made on cups of the solidified agar layer. 0.5 ml of vesicular gel solution (1 µg of drug) is placed into one bore which is noted with '1' and 0.5 ml of marketing gel solution (1 µg of drug) is kept in the bore cup which is named with '4' similarly the third '2' and fourth '3' bore for other two formulations. The petri dish was maintained at room temperature for 1 h and incubated at 37°C for 24 h. The zones of minimum inhibition were recorded in every pore [18,20,21].

Results and Discussion

UFL was manufactured easily by modifying the vortex shaking method, because it yields small uniform size vesicles. The polynomial equations consist of mathematical sign and coefficient signs. A synergistic effect shows positive sign, while the antagonistic effect shows negative sign of the factor. The results of dependent variables are shown in the Table 2.

Vesicle size increases (215 nm to 490 nm) with increases the concentration of the edge activator from 100 mg to 400 mg, while increasing the amount of oleic acid from 400 mg to 800 mg which increases the size of vesicle from 215 nm to 490 nm. The best formulation of UFL-6 has a good vesicle size with spherical in shape over the other batches. Equation 1 represents the linear regression models for vesicle size using oleic acid and tween 80 UFL as obtained from the central composite design study.

$$\text{Vesicle size} = 246.75 + 13.83 \times A + 67.17 \times B + 6.15 \times A2 + 35.25 \times B2 \quad (1)$$

Positive sign of coefficient A indicate that the increases in concentration of oleic acid, which positively increase in vesicle size. A similarly positive sign of coefficient B indicates that the increases in concentration of tween 80 which positively enlarge the vesicle size.

Batches	C 940 (mg)	TA (q.s.)	TO (ml)	OXZ (mg)	PG (ml)	SB	Water (g) Up to
UFLG-6	200	q.s.	5	-	1	UFL-6	20
OXG-10	200	q.s.	5	200	1	-	20
UFLG-9	200	q.s.	-	-	1	UFL-6	20

Note: TO: Tea Tree Oil; C940: Carbopol 940; TA: Triethanolamine; PG: Propylene Glycol; OXZ: Oxiconazole; SB: 20ml of selected batches.

Table 3: Formulation of gels.

42.513 ratios indicate the signals are adequate. The F-value 170.64 implies the model was significant. There was only a 0.58% chance that an F-value this high could occur due to noise. The value of the model was 0.0058 (Prob>F less than 0.0500 indicates model values were significant) indicate model was significant (Tables 4 and 5). Figures 1 and 2 was explained the result of vesicle size responses in contour plot and in 3D response surface.

S. No.	Terms	Vesicle size(nm)	%EE
1	Std. Dev.	7.56	1.97
2	Mean	365.63	46.57
3	C.V. %	2.07	4.23
4	PRESS	2055.00	139.69
5	-2 Log Likelihood	43.97	22.46
6	R-Squared	0.9977	0.9848
7	Adj R-Squared	0.9918	0.9467
8	Pred R-Squared	0.9579	0.7261
9	Adeq Precision	42.513	14.874
10	BIC	56.45	34.94

Table 4: Results summary of response for fitting to quadratic model.

Source	Sum of squares	df	Mean square	F value	p-value Prob>F
%EE					
Model	502.31	5	100.46	25.89	0.0376
A-A	81.99	1	81.99	21.13	0.0442
B-B	13.20	1	13.20	3.40	0.2064
AB	17.94	1	17.94	4.62	0.1645
A^2	367.08	1	367.08	94.60	0.0104
B^2	3.82	1	3.82	0.98	0.4257
Vesicle size					
Model	48703.71	5	9740.74	170.64	0.0058
A-A	1148.17	1	1148.17	20.11	0.0463
B-B	27068.17	1	27068.17	474.19	0.0021
AB	182.25	1	182.25	3.19	0.2159
A^2	20254.08	1	20254.08	354.82	0.0028
B^2	1656.75	1	1656.75	29.02	0.0328

Table 5: The model coefficients estimated the central composite design measured the dependent variables by using two independent variables.

EE% was increased from 46.11% to 61.05% with decreasing in the concentration of tween 80 from 300 mg to 100 mg respectively. Similarly, on increasing the amount of tween 80 from 300 to 400 mg the entrapment efficiency decreases from 46.11% to 34.55%, this was decreased due to fusion of drug from the big size vesicles. While on decreasing the amount of oleic acid from 800 mg to 400 mg which increasing the entrapment efficiency of drug from 45.22% to 61.05%. Equation 2 represents the linear regression models for EE% using tween 80 (B) and oleic acid (A) of UFL as obtained from the central composite design study (Figures 1 and 2).

$$EE\% = 60.29 + 3.70 \times A - 1.48 \times B + 2.12 - 16.59 \times A2 - 1.69 \times B2 \quad (2)$$

Positive sign of coefficient A indicate that the increases in concentration of oleic acid (to 400 mg) with positively increase in EE%. Similarly, negative sign of coefficient B indicates that the decrease in concentration of tween 80-100 mg which positively enhanced the EE% of drug loaded vesicles. 14.87 ratios indicate the signals are adequate. The F-value 25.89 implies the model was significant. There was only a 3.76% chance that an F-value this high could occur due to noise. The value of the model was 0.0376 (prob>F less than 0.0500 indicates model values were significant) indicate model was significant (Tables 4 and 5). Figures 3 and 4 was explained the result of EE% responses in contour plot and in 3D response surface.

The formulation UFL with OA (400 mg), Tween 80 (100 mg), and OX (200 mg) was found to be a better formulation (i.e., UFL-6). The UFL-6 formulation has the EE% to 61.05% and vesicle size of 215 nm (spherical in shape, uniform particle structure was shown in Figure 5) compare to other formulations, which shown the less EE% and big size of the vesicle.

The UFL-6 gel formulation was formed by using the optimized batch of vesicle formulation UFL-6 in suspension, which can be converted into gel form by using a carbopol 940 which improve the application of drug loaded UFL-6 gel formulation on the skin.

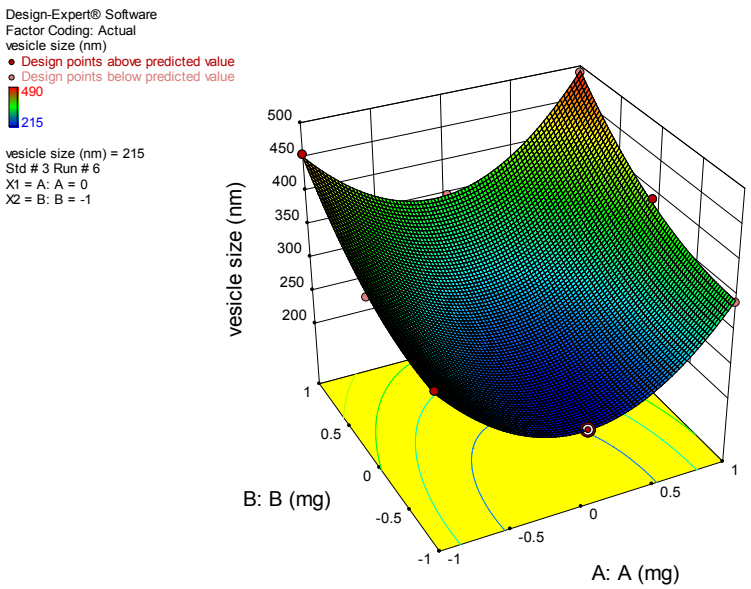

Figure 1: 3D Response surface plot showing effect of independent variables on vesicle size.

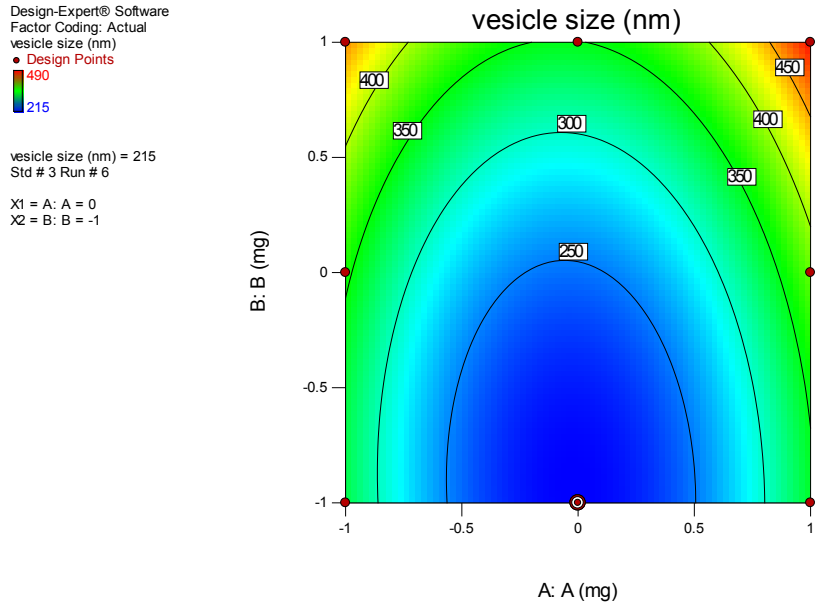

Figure 2: Contour response surface plot describing impact of independent variables on vesicle size.

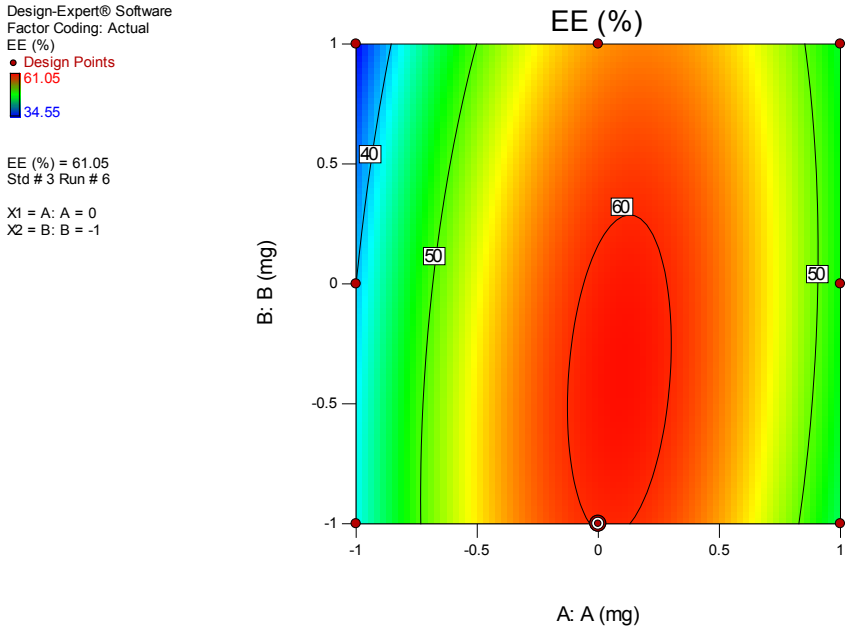

Figure 3: Contour response surface plot showing the effect of independent variables on percent entrapment efficiency.

Cellophane membrane permeation of oxiconazole in UFLG-6 with tea tree oil gel, gel, and UFLG-9 gel without tea tree oil was compared with each other at the equal concentration. The Figure 6 shown cumulative amount of oxiconazole permeated via the cellophane membrane unit versus time. From the results, it was clear that the oxiconazole loaded UFLG-6 gel shown a higher release compared to other gel formulations. Tea tree oil contains formulation shown maximum release because it acts as a good natural permeation enhancer which enhances the release of drug via a cellophane membrane to provide a maximum antifungal activity.

Mathematical modelling

The *in vitro* drug release from UFL gel formulation release oxiconazole via a cellophane membrane was evaluated by these mathematical models like zero order, first order, higuchi, hixson and crowel and korsmeyer peppas model. From r2 data for higuchi model was found to be higher than other gel formulations shown in Table 6. However, the UFLG-6 gel formulation shows n value is <0.5 showing fickian diffusion of OXZ release as specially intermediated through vesicles due to chemical potential gradient.

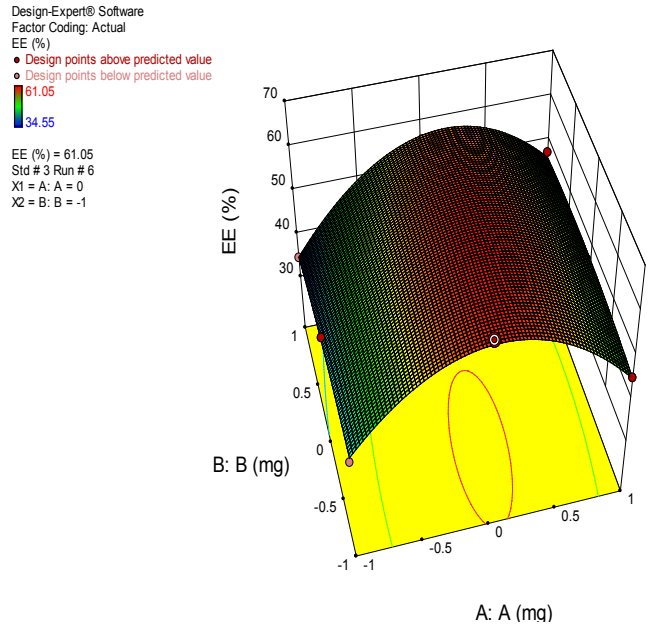

Figure 4: 3D response surface plot showing effect of independent variables on percent entrapment efficiency.

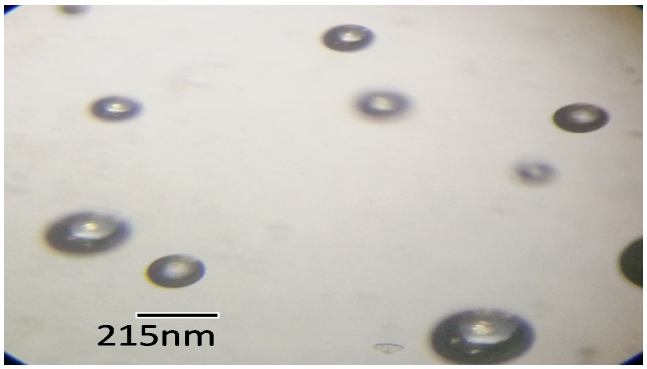

Figure 5: TEM representing best batches of UFL-6.

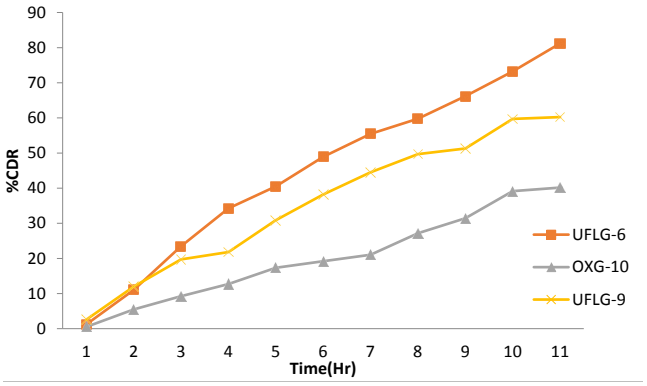

Figure 6: Graphical representation of % Cumulative drug release for OXG-10, UFLG-9 and UFLG-6.

Models	Parameter	Types of batches		
		UFLG-6	UFLG-9	OXG-10
Zero order	r^2	0.944	0.962	0.927
	K_o	6.941	3.59	5.255
First order	r^2	0.996	0.962	0.958
	K_1	-0.062	-0.02	-0.036
Higuchi model	r^2	0.996	0.959	0.976
	K_h	29.75	14.95	22.5
Korsmeyer Peppas model	r^2	0.891	0.901	0.934
	N	1.171	1.207	0.922
Hixson and crowel	r^2	0.944	0.962	0.927
	K_H	0.023	0.012	0.017

Table 6: Results of mathematical modeling drug release data fitting to different models.

Batches	Permeated amount at 12 h ($\mu g/cm^2$)	Flux($\mu g/cm^2/h$)	Permeability coefficient (Kp) × 10^{-3} (cm/h)
UFLG-9	120.48	10.04	1.004
OXG-10	80.34	6.695	0.6695
UFLG-6	162.4	13.53333	1.353333

Table 7: Permeation parameters of optimized formulation.

Table 7 indicates that the flux and permeability coefficient of UFLG-6 gel was higher than the gel and without tea tree oil UFLG-9 formulation. A result of zone of inhibition of (1) UFL with tea tree oil, (UFLG-6) (2) UFL without tea tree oil (UFLG-9), (3) gel (OXG-10) and (4) marketed formulations. Results of 1, 2, 3, and 4 were explained to be 20.5 mm, 18.74 mm, 12.45 mm and 8.76 mm respectively. Formulation UFLG-6 shown maximum, minimum inhibitory concentration up to 24 h as compared to other formulations because tea tree oil containing 1,8-cineole group which have the property of inhibiting the fungal infection shown in Figure 7. UFLG-6 gel formulation shows good stability after 3 months at 4 ± 2°C and at 37 ± 0.5°C and do not change in drug concentration and in physical appearance (Table 8).

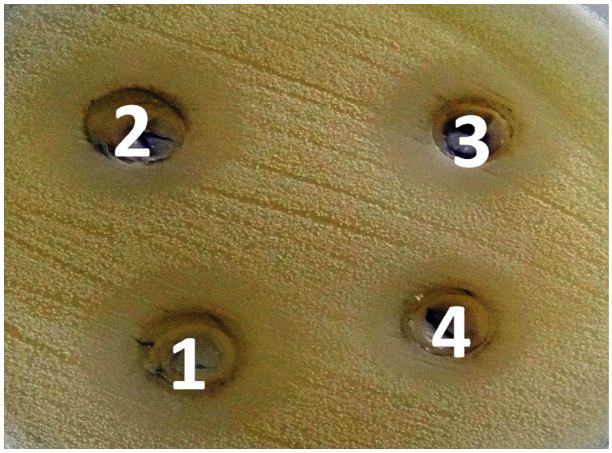

Figure 7: *In vitro* antifungal activity against candida albicans of sample. (1) UFLG-6 gel, (2) UFLG-9 gel, (3) plain gel and (4) marketed formulation via agar well diffusion process.

Stability Parameters							
		2-8˚C		37 ± 0.5˚C		45 ± 1˚C	
Batch	Months	A	Drug content	A	Drug content	A	Drug content
UFLG-6	0	Clear	98.92	Clear	98.92	Clear	98.92
	1	Clear	98.75	Clear	98.36	Clear	90.24
	2	Clear	98.41	Clear	98.06	Clear	76.20
	3	Clear	97.94	Clear	97.20	Clear	50.88

Table 8: Accelerated stability study's parameters of optimized batch UFLG-6.

Conclusion

UFL formulations were prepared successfully by using modified vortex-sonication method which was low production cost and easy method for manufacturing of UFL formulations. Eight batches of UFL formulation were easily optimized by central composite design. The UFL-6 formulation was shown good entrapment efficiency and vesicle size as compared to other batches. This UFL-6 formulation was further used in carbopol gel which has the right viscosity to enhance the easy skin application. With results, it was clear that the oxiconazole loaded UFLG-6 gel shown a higher drug release; flux and permeability coefficient compared to other gel and UFLG-9 formulations. Tea tree oil based UFL gel formulation shown higher antifungal activity and stability up to 3 months as compared to other formulations.

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