IJBPAS, November, 2016, 5(11): 2986-3002

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

OPTIMIZATION, EVALUATION AND ANTIFUNGAL ACTIVITY DETERMINATION OF OXICONAZOLE LOADED TRANSFERSOMES GEL BY USING TEA TREE OIL

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Received 27th July 2016; Revised 3rd Sept. 2016; Accepted 28th Sept. 2016; Available online 1st Nov. 2016

ABSTRACT

The main goal of present research work was to evaluate the oxiconazole (OXC) loading transfersomes gel by using tea tree oil. OXC loaded transfersomes formulation was optimized using central composite designs; where the effects of soya lecithin (SL): span80 (S80) molar ratio and the drug amount were evaluated by using variables; including vesicle size and drug entrapment efficiency (%EE). The optimized transfersomes formulation was ready with 85:15 SL: S80 molar ratio and 10 mg drug amount by using vortexing- sonication method with vesicle size of 1.47µm and %EE of 80.47±0.777%. The %Cumulative drug release of the optimized gel formulation (OXCG-6) through the cellophane membrane was excellent (87.891±0.314%) compared with other formulations. The drug release kinetic obeys Higuchi model (R^2 = 0.998 and K_h value 38.87) with fickian diffusion mechanism. The zone of inhibition of OXC transfersomes gel formulation (A) and liposome gel (B), plain gel (C) and marketed formulation (D) were found to be 28.6 ± 0.68, 20.2±0.39, 15.7 ± 0.41 and 19.4 ± 0.54 mm respectively against candida species. Results show that 'A' exhibits most antifungal activity after 24 h, So it can be concluded that the tea tree oil containing formulation 'A' exhibited better anticandidal activity than the other formulations. Results suggested transfersomes gel

containing tea tree oil to be the most conversant carrier system for transdermal delivery of OXC.

Keywords: transfersomes, oxiconazole, tea tree oil, fungal infections

INTRODUCTION

Oxiconazole(1) (OXC) is 2,4-dichloro-2-(imidazol-1-yl) acetophenone O-(2,4 dichlorobenzyl) oxime is a synthetic imidazole derivative of a broad-spectrum antifungal activity and is used for topical treatment of topical fungal infections(2). Ergosterol shows a hormone as role in fungal cells, which promote growth; the net efficacy of OXC is the inhibition the fungal growth(3). The OXC antifungal agents block the synthesis of ergosterol is a major part of fungal cytoplasmic membranes. Now today many OXC formulations are available in the market in the form of creams and lotions but they show no retention properties in the treatment of athlete's foot, tinea infection, jock itch, candida(4) and in vaginal fungal infections.

Transfersomes(5) formulation is a good way to reduce above limitation. Ultraflexible drug carriers(6) mainly retained at the place of administration longer than liberated drug and efficient to improve the dispersion of an concerned substances and increase the efficacy and reduce toxicity. Gregor cevc was first introduced the word transfersomes (elastic or ultraflexible liposomes)(7). Transfersomes can breach the skin barrier, even through the pores that would be confining for other particulates of comparable size(8). This capability is due to the extremely high deformability and ultraflexible of the transfersomes membrane(7). Because transfersomes are consisting of surfactant, they have better elasticity and hydration properties, which are credit for their superior skin penetration ability(9). Traditional liposomes are less deformable vesicles, are applied to the skin surface where they dry completely and link, therefore they have less access power than transfersomes(10). Transfersomes are combining of phospholipids, surfactant, and water, make by thin film hydration(11), vortexing-sonication method(12) and high pressure homogenization method(13).

The focus of this research is to optimize, develop and evaluate the transfersomes vesicular carrier of OXC so as to provide the stable and to retain the anticandidal activity of the drug by tea tree oil. Tea tree oil is here may be increases the antifungal properties of OXC. It takes as important role of natural penetration enhancer for improvement of antifungal properties of drug. Further, they can be incorporated into

commonly used dermal vehicles, such as carbopol gels, in order to have a right semisolid consistency to facilitate easy skin application producing high patient acceptance.

MATERIALS AND METHODS:

Material: Oxiconazole (OXC) was provided as a gift from A.S. Joshi and company Pvt. Ltd (Maharashtra, India). Tea tree oil was purchased from Kanta Enterprises Pvt. Ltd (Noida, India), Carbopol 940; Glycerin; and Cholesterol (CL) was purchased from Hi-media labt. Pvt Ltd, (Mumbai). Span 80 (S80), Ethanol (E) and Triethanolamine were purchased from S.D. fine Chem Ltd (Mumbai). Methanol and Dialysis membrane were purchased from Hi-media labt. Pvt. Ltd (Mumbai). Soya lecithin (SL) was purchased Titan biotech Ltd (Bhiwadi, Rajasthan).Propylene glycol was purchased Central Drug House Ltd (New Delhi).

Methods: Transfersomes were prepared by vortexing - sonication method(12). A mixture of OXC, SL and S80 with different ratios was dissolved in 1ml of ethanol to form a solution. This mixture was dispersed in E 7% (v/v) (Ethanol) and vortexed for 5min to attain a milky suspension. The suspension was sonicated by QSONICA 125 watt sonicator (M2 Scientifics Holland,Michigan) for 25 min followed by freezing at 15-20 °C for 12 h and thawing at room temperature for 6 h, for three consecutive cycles. The suspension was extruded through 0.2 µm sartorius membrane filter (weender landstr,goettingen Germany) for five times at 50°C. Then transfersomes formulations were centrifuged in a refrigerated centrifuge (REMI Laboratory instruments, thane, Maharashtra) for three hours at 20,000 rpm and 4°C. OXC transfersomes at the base of the tubes were cleaned with E 7%v/v to remove the free drug and diluted with E 7% (v/v) up to 10 ml. Samples were stored at 2-8°C in tightly closed containers for further evaluations as shown in Table 1 and in Table 2.

Similarly, liposomes(14) (SL:CL, 70:30) of OXC were prepared using cholesterol instead of surfactants in the optimized formula as shown in Table 3.

Experimental design: Central composite designs were employed using Design-Expert Software10.0.3.1.32-bit, using SL and using S80. In these designs, two independent formulation variables were prepensely to evaluate their combined effects and individualistic; SL:S80 molar ratio (A) and amount of OXC added (B). The experimental trials were carrying out at all eight presumable combinations with 3 times replication for each transfersomes system(15). To study the effect of variables used central composite designs for

transfersomes performance and evaluation. The central composite designs including investigated independent and dependent variables are shown in Table 2. The one-way analysis of variance (ANOVA) was applied to estimate the significance of the model ($P < 0.05$) and particular response parameters.

Table 1: Variables in central composite designs

	Level used		
	-1	**0**	**+1**
Independent variables			
A: SL:S80(mg)	**75:25**	**85:15**	**95:5**
B: OXC(mg)	**10**	**15**	**20**
Dependent variables			
R1: Entrapment efficiency%(EE)			
R2: Vesicle size(µm)			

Table 2: Response parameters of transfersomes formulation of OXC prepared as per the experimental design

Formula Code	Independent variable		Dependant variables	
	A	**B**	**EE%**	**Vesicle size(µm)**
OXC-1	**0**	**1**	**74.54±0.362**	**3.89±0.31**
OXC-2	**-1**	**1**	**59.52±0.538**	**4.07±0.930**
OXC-3	**-1**	**0**	**62.45±0.025**	**3.55±0.25**
OXC-4	**1**	**1**	**63.49±0.495**	**5.86±0.39**
OXC-5	**-1**	**-1**	**61.46±0.670**	**2.55±0.662**
OXC-6	**0**	**-1**	**80.47±0.777**	**1.47±0.110**
OXC-7	**1**	**-1**	**64.48±0.320**	**3.02±0.831**
OXC-8	**1**	**0**	**65.51±0.821**	**3.99±0.754**

Each value represents the mean ± SD (N= 3)

Table 3: Formulation of liposomes

Batches	SL: CL (mg)	E (ml)	E 7%v/v (ml)	OXC (mg)	EE (%)	Vesicle size(µm)
OXC-9	70:30	1	10	10	59.54±0.45	5.81±0.0251

Each value represents the mean ± SD (N= 3)

Evaluation of vesicles:

Morphological and size of vesicles: An optical microscope (Suswox 50ptic, sudheer scientific works, ambala cantt.) fitted with a digital camera was used to photograph the prepared formulation before extrusion under magnification 40X. A fine layer of vesicles formulation was spread on a glass slide and examined after placing the cover slip. The mean size of at least 100 particles was calculated.

Entrapment efficiency: The entrapment capacity of transfersomes vesicles was determined by an indirect method. The vesicle solution was centrifuged (Remi Equipments, Mumbai, and India) at 10,000 rpm at 4°C for 10 min. The combined supernatant was analyzed for the drug content after suitable dilution with blank solution by measuring absorbance at 257 nm using UV Spectrophotometer (Systronics 2202, Ahmedabad). The supernatant was filtered and the entrapment efficiencies of the OXC-loaded formulation were calculated by (1).

$$\text{Entrapment efficiency\%} = \frac{(X_1 - X_2)}{} \times 100 \quad \ldots\ldots\ldots (1)$$

X_1

Where,
X_1= Amount of OXC added initially,
X_2= Amount of OXC determined in the filtrate by spectrophotometrically,
($X_1 - X_2$) = represents the amount of OXC entrapped in the formulation.

Preparation of vesicular gel: Vesicular gel was prepared by adding 200mg Carbopol 940, propylene glycol and triethanolamine portion wise by sprinkling to 10 ml of the previously prepared suspension of vesicles and stirring until a gel was formed (Table 4). Finally weight was made up to 20 gm to get final viscous gel formulation.

Table 4: Formulation Of Vesicular Gel

Batches	Carbopol 940 (mg)	Triethanolamine (q.s)	Tea Tree oil (ml)	Propylene glycol (ml)	10ml of selected batches (shown in Table 2 and 3)	Water (gm) Up to
OXCG-6	200	q.s	5	1	OXC-6	20
OXCG-9	200	q.s	5	1	OXC-9	20
OXCG-10	200	q.s	5	1	-	20
OXCG-11	200	q.s	-	1	OXC-6	20

Evaluation of vesicular gel:

Physical appearance and Homogeneity: The physical feature and homogeneity of the ready gels were tested by visual observations after the gels have been put in the glass container. They were tested for their appearance and presence of any aggregates(16).

Clarity: The clarity of different formulations was determined by sight inspection under black and white background and it was marked as follows; turbid: +, clear: ++, very clear (glassy): +++.

Viscosity: The viscosity of the vesicular gel was determined using a viscometer. The equipment was knitted to a thermostatically monitored circulating water bath preserved at 25°C. The formulation whose viscosity was to be demarcated was added to a beaker off by thermostatic jacket. Spindle was permitted to run freely into the gel formulation and the measurements was noted(17).

Measurement of pH: The pH of various gel formulations was determined by applying digital pH meter (Systronics 802, Ahmedabad). 1 gm of gel was diluted in 100 ml distilled water and stored for 2 h. The measurement of pH of each formulation was done in three times and mean values are calculated(18).

In vitro drug release studies through cellophane membrane: The invitro release studies of OXC from all transfersomes gel, liposomes gel transfersomes gel without tea tree oil and the plain gel formulation were evaluated using vertical franz diffusion cells (Harco scientific works,Ambala) with an effective diffusion area of 1 cm^2. The receptor's compartment volume was 50 ml (methanol 50%v/v)(19), maintained at 37ºC± 0.5 and convulse by a magnetic bar at 500 g. The donor compartment was

parted from the receptor compartment by cellophane membrane (molecular weight cut of 12000-14000, high media Ltd, mumbai, India). Sample (1 g) of the gel was placed in the giver compartment. Four hundred lL aliquots were withdrawn from the sampling port at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 24 h and substituted with fresh methanol 50%v/v, to maintain a equal volume and then examined spectrophotometrically at 257 nm. The experiment was carried out in three times and the average cumulative percentage show from three batches was calculated. The rate of OXC release was numerated from the slope of the initial portion of the graph(20).

Mathematical modeling of optimized batch: Mathematical model access important in research and development because of it may decrease the number of trials in ultimate optimization, thus improving the formulation evaluation process. The permeation profile of the improved batch was fitted to the various kinetic models. The data of in vitro drug release from various vesicular gels were characterized kinetically using various mathematical models (Zero-order, First-order, Higuchi and Koresmeyer - peppas model) (19).

Stability Studies: The stability studies were directed according to ICH guidelines by storing the formulation at 45 ± 1˚C / 75% RH, 37 ± 0.5°C and 4 ±2˚C/ 30% RH in stability chamber for three months. The formulation was analyzed for the change in appearance and drug content by procedure stated earlier spectrophotometrically at 257 nm using methanol 50% v/v as blank(21).

Antifungal activity of optimized formulations: The antifungal efficacy studies were carried out to ascertain the biological activity of transfersomes gel, in comparison with plain OXC gel, liposomes gel and marketed OXC formulation against Candida albicans (MTCC 3017) as the test fungus. This is determined by agar diffusion test employing 'Cup plate' technique. A layer of Sabourauds dextrose agar media (20 ml) inseminated with the test microorganisms were allowed to solidify in the petri dishes. Cups were formed on the solidified agar layer with the help of sterile borer (5 mm). 0.5 ml of transfersomes gel solution (1 µg of drug) is filled into one cup (bore) which is marked with 'A' and 0.5 ml of marketed gel solution (1 µg of drug) is filled into the second cup (bore) which is marked with 'D'. Like wise the third 'B' (Plain gel) and fourth 'C' (liposome gel). Subsequently abidance the petri dishes at room temperature to 1 h, the Petri dishes were incubated at 37 °C for 24 h. The zones of

inhibition were measured around each cup(22).

RESULT AND DISCUSSION:

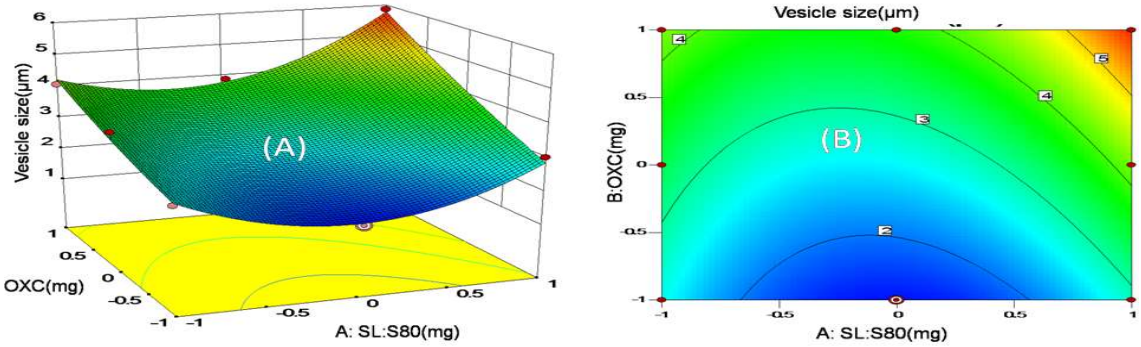

Fig. 1: Response surface plot {(a) 3d response surface plot and (b) contour plot} showing effect of independent variables on vesicle size.

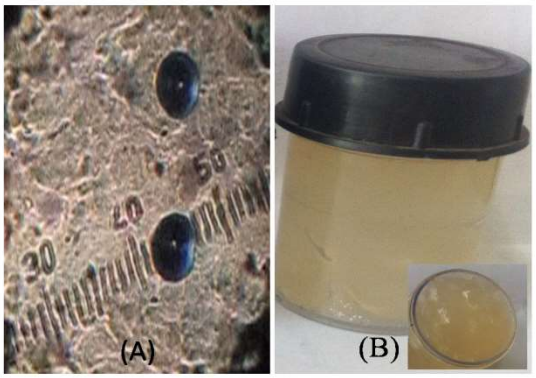

Fig. 2: Photomicrograph representing best batches (a) microscope of OXC-6 at 40x magnification and (b) OXCG-6 formulation.

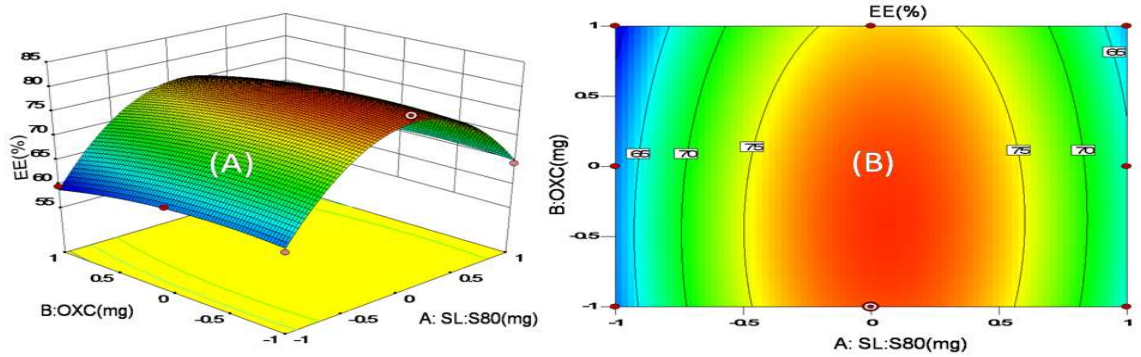

Fig. 3: Response surface plot {(a) 3d response surface plot and (b) contour plot} showing effect of independent variables on percent entrapment efficiency.

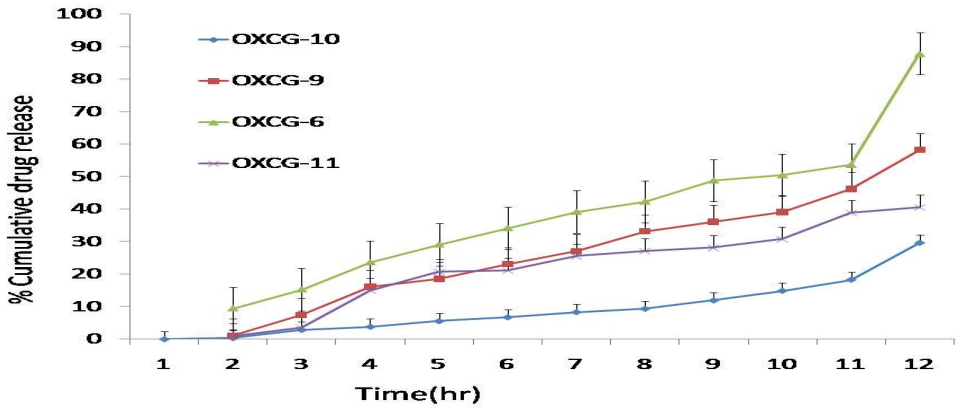

Fig. 4: Graphical representation of % CDR for OXCG-10, OXCG-9, OXCG-6 and OXCG11.

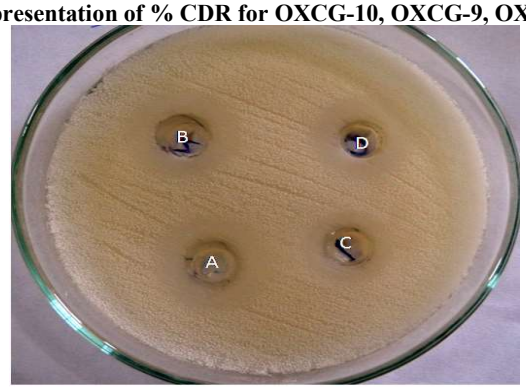

Fig. 5: In vitro antifungal activity against candida albicans of compounds {a (transfersomes gel), b (liposome gel), c (plain gel) and d (marketed formulation)} through agar well diffusion method

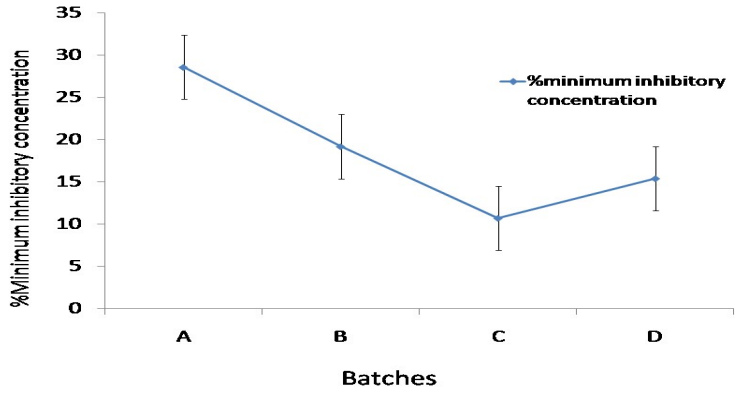

Fig. 6: Comparison of % minimum inhibitory concentration of optimized formulations.

Table 5: Sum of squares, degree of freedom, mean squares, f-values and p-values for the model coefficients estimated from the central composite design for the measured dependent variables using s80

Source	Sum of squares	df	Mean square	F value	p-value Prob > F
%EE					
Model	357.74	5	71.55	21.33	0.0454
A-SL:S80	16.83	1	16.83	5.02	0.1544
B-OXC	13.08	1	13.08	3.90	0.1870
AB	0.23	1	0.23	0.067	0.8196
A^2	310.80	1	310.80	92.66	0.0106
B^2	4.05	1	4.05	1.21	0.3865
Vesicle size					
Model	11.35	5	2.27	25.51	0.0382
A-SL:S80	1.22	1	1.22	13.66	0.0660
B-OXC	7.66	1	7.66	86.13	0.0114
AB	0.44	1	0.44	4.90	0.1574
A^2	1.90	1	1.90	21.41	0.0437
B^2	0.015	1	0.015	0.17	0.7237

Table 6: Summary of results of regression analysis for response for fitting to quadratic model

Sr.no	Terms	%EE	Vesicle size
1.	Std. Dev.	1.83	0.30
2.	Mean	66.49	3.55
3.	C.V. %	2.75	8.40
4.	PRESS	120.75	3.20
5.	-2 Log Likelihood	21.29	-7.74
6.	R-Squared	0.9816	0.9846
7.	Adj R-Squared	0.9356	0.9460
8.	Pred R-Squared	0.6687	0.7221
9.	Adeq Precision	12.694	16.396
10.	BIC	33.77	4.73
11.	AICc	117.29	88.26

Table 7: Evaluation of the prepared formulation of vesicular gels

Sr. no	Evaluation parameters	OXCG-10	OXCG-9	OXCG-6	OXCG-11
1.	Clarity	++	++	+++	++
2.	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
3.	pH	6.3±0.014	6.5±0.014	6.8±0.073	6.7±0.014
4.	Viscosity (centipoises)	8,099±0.415	10,900±0.83	13,500±0.92	14,105±0.60
5.	Spreadibility (cm)	3.9±0.449	2.5±0.106	3.6±0.290	2.8±0.519
6.	Drug Content analysis	95.83±0.416	97.60±0.064	99.08±0.029	98.90±0.152

Each value represents the mean ± SD (N= 3)

Table 8: Results of drug release data fitting to different models (dissolution parameters and regression coefficients)

Formulation Code	Parameter	Types of batches			
		OXCG-10	OXCG-9	OXCG-6	OXCG-11
Zero order	r^2	0.934	0.773	0.913	0.608
	Ko	2.8543	5.2647	7.2818	3.5006
First order	r^2	0.954	0.866	0.997	0.662
	K1	-0.006	-0.015	-0.036	-0.008
Higuchi model	r^2	0.964	0.940	0.998	0.833
	K_h	7.164	14.33	38.87	10.12
Korsmeyer	r^2	0.910	0.872	0.995	0.836
Peppas model	n	1.068	0.482	0.576	0.976
Hixson & crowel	r^2	0.934	0.773	0.913	0.608
	K_H	0.0092	0.0161	0.023	0.0115

Where, K =Constant K= 2.3 X Slope

Table 9: Accelerated stability studies parameters of optimized batch OXCG-6

		2-8ºC		37 ± 0.5°C		45±1ºC	
Batch	Months	A	Drug content	A	Drug content	A	Drug content
OXCG-6	0	Clear	99.08±0.029	Clear	99.08±0.029	Clear	99.08±0.029
	1	Clear	98.77±0.82	Clear	98.90±0.289	Clear	89.56±0.310
	2	Clear	98.47±0.36	Clear	98.13±0.608	Clear	87.92±0.481
	3	Clear	98.95±0.11	Clear	98.05±0.213	Clear	85.53±0.63

Appearance –A; values represented as mean ± SD, N=3

In the present work OXC transfersome were prepared using surfactant (S80) along with SL in different proportions by a modified vortexing - sonication method. This process is easily, fast manufacturer and reduced production cost. The prepared OXC transfersomes were characterized for various test parameters like particle size, shape and entrapment efficiency.

Central composite designs are designed to calculate the coefficients of a quadratic model. All point descriptions are in terms of coded values of the factors. These are provided very good predictions in the middle of the design space. The effect of the process variable like molar ratio of lipid and surfactant (A), and amount of drug (B) added and their interactions using a suitable

statistical tool (Design-Expert Software10.0.3.1.32-bit) by applying one-way ANOVA at $P < 0.05$ levels (Table 2).

Microscopically photograph of the vesicles showed that prepared vesicle were spherical in shape. The mean vesicle size using SL:S80 was ranged from 1.47±0.110 to 5.86±0.39µm (Fig. 1). Equation (2) represent the linear regression models for vesicle size using SL:S80 transfersomes obtained from central composite design study.

Vesicle size $=+2.57+0.45*A+1.13*B+0.33*AB+1.20*A^2+0.11*B^2$----------(2)

R^2=0.9846, F value=25.51, P-value= 0.0382

From equation-3 it was found that as increasing the concentration of SL the vesicle size increased, where as decreasing the concentration at optimal value of surfactant (15mg) was expected to decrease the vesicle size. The vesicle size was increased by increasing the SL:S80 molar ratio as can be deduced from the positive coefficient of A. This might be attributed to the decreases in the S80, which lead to incomplete maturation of vesicles and thus reduction in their sizes(23). On the contrary, by amplifying in the drug amount, the vesicle size was increased, which may be due to the increases in drug loading. Adequacy Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 16.396 indicates an adequate signal. The Model F-value of 25.51 implies the model is significant shown in Table 5. There is only a 3.82% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant shown in Table 5 and in Table 6. This model can be used to navigate the design space. This was further revealed by the response surface plots that shown in Fig. 1 and in Fig. 2.

Fig. 1: Response surface plot {(A) 3D response surface plot and (B) Contour plot} showing effect of independent variables on vesicle size.

The mean size of the various transfersomes formulations was from 1.47±0.110 to 5.86±0.39 µm (Table 2). Particle size of vesicles containing transfersomes at optimal ratio SL:S80(85:15) was 1.47±0.110µm, on increasing in ratio of SL:S80(95:25) led to larger particles size3.02±0.831 µm which could be due to the higher concentration of S80 that reaches its critical micelle concentration (CMC)(24). Microscopy studies show that transfersomes appeared as unilamellar vesicles, with the vesicles of evenly spaced to the core (Fig.2).

Equation (3) represent the linear regression models for EE% using SL:S80 transfersomes obtained from central composite design study.

EE% = +79.25+1.67*A-1.48*B+0.24*AB-15.27*A^2-1.74*B^2----------(3)

The negative coefficient of B reveals the increases in the OXC amount, which leads to a decrease in the %EE. While an increase in the %EE was observed with the decreased in the drug amount could be attributed to drug actuation to encapsulated vesicles(25). As can be remarked, from the positive coefficient of the interaction between A and AB, the %EE is synergistically increased by any interaction. A ratio of 12.694 indicates an adequate signal (Table 5 and Table 6).

The largest and smallest EE% values acquired were 80.47±0.777% for OXC-6 and 59.52±0.538% for OXC-2, respectively (Table 2). It was concluded that while increasing OXC concentration from 10 mg to 15 mg in the transfersomes formulation, the EE % increased from 65.51±0.821% (OXC-8, with least concentration SL and S80) to 80.47±0.777% (OXC-6). However, a further increase in drug concentration to 20 mg led to a decrease in EE % to 59.52±0.538% (OXC-2); this may be due to the leakage of large amount of drug from the vesicular design. Effects of independent variables on EE % are presented by 3D response surface and Contour plot in Fig. 3. Finally, the EE % increased significantly with increasing surfactant concentration from 5 to 15 mg (w/w). Further increase in surfactant concentration from 15 up to 25 mg (w/w) showed a decrease in EE % (Table 2). The ratio (85mg SL/15mg S80) showed good EE %. On incorporation of S80 in low concentration, growth in vesicle size occurred, whereas further increase in the content of S80 may have led to pore formation in the bilayers of vesicles(23). It was observed that with increased S80 concentration in the lipid components of the vesicles, the EE % of the OXC decreased.

The optimum formulation of OXC-loaded transfersomes systems was selected based on the maximum value of EE % and minimum value the vesicles size by applying the point prediction method of the design expert software 10.0.3.1.32-bit. The formulation composition with SL (85 mg), S80 (15 mg), and OXC (10 mg) was found to be an optimum formulation (i.e., OXC-6). The optimized formulation has the EE % of 80.47±0.777% and vesicles size of 1.47±0.110 µm (spherical in shape, displaying sealed particle structure) is presented in Fig. 1, Fig. 2 and in Fig. 3.

The liposomes formulation showed EE% of 59.54±0.45%, having a mean vesicles size of 5.81±0.0251µm shown in Table 3. The OXC-6 of OXC showed EE % of 80.47±0.777% and vesicles size of 1.47±0.110 µm over the liposomes formulation(26), which produced the less EE% and large vesicle size. On the basis of

central composite designs approach, the transfersomes formulation (OXC-6) was selected for further in they can be incorporated into commonly used dermal vehicles, such as carbopol gel in order to have a proper semisolid viscosity to facilitate convenient skin application(27), and evaluated by in vitro drug release, antimicrobial activity and stability studies were done and compared with liposomes gel, marketed gel and OXC plain gel.

All optimized gels were successfully prepared and evaluated shows maximum drug content in the range 99.78±0.029 to95.83±0.416%, viscosity in the range 8,099±0.415 to 14,105±0.60 centipoises, pH in the range 6.3±0.014 to 6.8±0.073, good spreadability and homogenous shown in Table 7.

The cumulative amount of drug release was numerated for four formulations. The cumulative percentage drug release (% CDR) at different time intervals are shown in Fig. 4. The result obtained from in-vitro drug release study showed the following pattern of drug release OXCG-6 > OXCG-9 > OXCG-11> OXCG-10. Thus from the above study, it was concluded from that tea tree oil was the best permeation enhancer. Results revealed that the OXCG-6 had the highest cumulative amount of drug release (87.891±0.314%) up to 24 h as compared to other gel formulations (OXCG-9, OXCG-11 and OXCG-10). Formulation OXCG-6 was optimized and found to be suitable for further studies (Fig. 2B).

In order to correlate the in vitro OXC permeation behavior from these vesicular gels through cellophane membrane, it is necessary to fit into a suitable mathematical model. The in vitro drug permeation data from various vesicular gel formulations containing OXC through cellophane membrane were evaluated kinetically through different mathematical models like Zero-order, First-order, Hixson and crowel, Higuchi, and Korsmeyer-Peppas model. The outcome of the curve fitting into these up described mathematical models reveal the in vitro OXC permeation behavior of gels (OXCG-6,OXCG-9, OXCG-11, OXCG-10). The in-vitro drug release revealed that Higuchi equation was the very convenient model to depict the release kinetics of OXCG-6 from the examined transfersomes formulations (Table 8). This indicates that, OXCG-6 release from transfersomes is square root of time dependent process. The n value of OXCG-6 is >0.5 <1.0, indicating non-fickian anomalous drug release that is mainly intermediated by diffusion and matrix erosion(19). In variant words, there is a significant outcome ($P<0.05$) of carrier fabrication on the mechanism of OXCG-6 release from the transfersomes. From the

results of data fitting to various models, it was found that the optimized batch OXCG-6 showed maximum r^2 value (0.998) and K_h value 38.87 as compared to other formulations.

The *in vitro* characterization studies of OXC transfersomes gel formulations were further evaluated for antifungal activity by cup-plate method. A result of zone of inhibition of OXC transfersomes gel formulation (A) was compared to liposome gel(B), plain gel (C) and marketed formulation (D). Results of A, B, C, and D were found to be 28.6 ± 0.68, 20.2±0.39, 15.7 ± 0.41 and 19.4 ± 0.54 mm respectively (Fig. 5 and Fig. 6). Results indicate that A exhibits higher antifungal activity after 24 h, So it can be concluded that the tea tree oil containing formulation 'A' exhibited better anticandidal activity than the other formulation, probably due to the extra potential of tea tree oil to kill organisms by denaturing their proteins and diluents their lipids, asunder from skin fluidity and penetration. Tea tree oil include a 1,8-cineole, terpinen-4-ol, a terpinolene which can enhance penetration of drug through skin membrane(28). These components are responsible for antimicrobial and antinflammatory properties, and bind to the subcutaneous membrane and are thought to enhance lipophilic drug penetration by growing the partition coefficient and hydrophilic drug penetration by increasing the diffusion coefficient(29). 1,8-cineole has been found to rise skin infiltration by commute intercellular lipids in subcutaneous and to change subcutaneous membrane fluidity at low concentrations(30).

The optimized transfersomes formulation OXCG-6 was selected for stability studies as shown in Table 9. It was found that the transfersomes gel did not alter after 3 months and no drug particle come out even after 3 months at room temperature (37 ± 0.5°C) and at refrigerated temperature (4 ± 2 °C). It was observed that increase in vesicle size and loss of entrapped drug in vesicular carrier systems were found more in the formulations those were stored at 45±1ºC than at 4±2ºC. Accelerated study showed that our formulation is stable and does not show any remarkable change in appearance and their drug content. However, after 3months formulation OXCG-6 was much stable during refrigerated stage than at room temperature.

CONCLUSION:

In the present research work, OXC-loaded transfersomes were successfully prepared by a vortexing - sonication method that is fast manufacturer and reduced production cost. S80 based transfersomes formulation (OXC-6) showed good entrapment efficiency and uniform vesicle size as

compared with other formulations. OXCG-6 showed good drug content, its viscosity, homogeneity, stability, in vitro drug release and antifungal activity was also found to be better as compared to liposomes gel, plain gel and marketed formulations. Tea tree oil based transfersomes gel formulation exhibits maximum antifungal activity after 24 hr, so it can be concluded that the tea tree oil containing transfersomes gel formulation exhibited better anticandidal activity.

ACKNOWLEDGEMENTS:

Author are thankful to microbiology department, kurukshetra university, kurukshetra for their cooperation and providing me help for antifungal activity support for this work and to director, Amar shaheed baba ajit singh jujhar singh memorial college of pharmacy, Bela 140111, Punjab, India; I.K. gujral punjab technical university, kapurthala, Jalandhar, Punjab-144601 and Faculty of Pharmacy, RP Educational Trust Group of Institution, Bastara, Karnal for providing laboratory, instrument and all other facilities required for this work.

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